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(Research Paper)

Physiological and biochemical responses of lilac sage (*Salvia verticillata* L.) to methyl jasmonate and multi-walled carbon nanotubes: A comparative study

Nosrat Rahmani[1], Tayebeh Radjabian[1]*

[1] Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran

Abstract

Lilac sage (*Salvia verticillata* L.) a medicinal plant from the Lamiaceae family, is rich in bioactive compounds such as flavonoids and phenolic acids, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. This study aims to compare the effects of multi-walled carbon nanotubes (MWCNTs) and methyl jasmonate (MJ) on the physiological and biochemical responses of *S. verticillata*. At the twenty-two-leaf stage, plants were subjected to foliar application of MJ (100 µM) and MWCNTs (100 mg L^{-1}), and some physiological and biochemical traits were assessed at multiple time points. The application of MJ enhanced the accumulation of phenolic and flavonoid compounds. It increased the activities of antioxidant enzymes while simultaneously causing a reduction in chlorophyll content, likely due to stress-related responses. In contrast, the application of MWCNTs initially promoted chlorophyll and protein synthesis, followed by a delayed increase in oxidative stress markers and the activation of key enzymes involved in the biosynthesis of phenolic acids. Principal component analysis provided strong evidence for the distinct mechanisms of action of MJ and MWCNTs. While MJ primarily functions through stress signaling pathways, MWCNTs combine growth stimulation with stress induction, impacting metabolic processes in different ways. These findings offer insights for optimizing elicitation strategies for enhancing the production of bioactive compounds in medicinal plant biotechnology.

Keywords: Methyl Jasmonate, Multi-Walled Carbon Nanotubes, Oxidative Stress, Phenolic Compounds, *Salvia verticillate* L.

*Corresponding author: rajabian@shahed.ac.ir

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1. Introduction

Lilac sage (*Salvia verticillata* L.) is a perennial herbaceous plant from the Lamiaceae family. It grows as wild populations in the mountainous regions of northern Iran (Jamzad, 2020). This species is recognized for its diverse array of bioactive compounds, including phenolic acids (particularly rosmarinic acid), flavonoids, and terpenoids. These compounds exhibit various therapeutic properties, including antioxidant, anti-inflammatory, antibacterial, and anticancer effects. These characteristics make *S. verticillata* a promising candidate for applications in the food and pharmaceutical industries (Ivanova et al., 2024; Karakaya et al., 2020; Stanković et al., 2020).

The investigation into how various stimuli affect the physiological characteristics and growth of plants, particularly concerning the production of secondary metabolites, is a topic that captures the interest of scientists. (Guru et al., 2022; Humbal & Pathak, 2023; Siahmansour et al., 2018). Methyl jasmonate (MJ), a biotic elicitor, is a lipid-derived signaling molecule that plays a crucial role in regulating plant responses to both abiotic and biotic stresses. Methyl jasmonate modulates various biochemical pathways, prompting the production of secondary metabolites, including phenolics, flavonoids, and terpenoids. These compounds play essential roles in plant defense mechanisms and have significant applications in industries like pharmaceuticals and cosmetics (Ghorbel et al., 2021; Ho et al., 2020; Nabi et al., 2021). Several studies have demonstrated MJ's ability to enhance the expression of key biosynthetic genes, thereby increasing the yield of medicinally important metabolites in species like *Salvia* (Hou et al., 2021; Pesaraklu et al., 2021; Rahmani & Radjabian, 2024; Zhou et al., 2021).

Nanotechnology has emerged as a transformative tool in agricultural and plant sciences, offering innovative solutions for enhancing plant growth and productivity (Okey-Onyesolu et al., 2021). Researchers have recently utilized metallic and carbon-based nanoparticles as elicitors to enhance nutrient uptake, stimulate growth, and increase secondary metabolite production. Among these, multi-walled carbon nanotubes (MWCNTs) have garnered attention due to their unique properties, including a high surface area, excellent conductivity, and biocompatibility (Chen et al., 2021). Due to their unique properties, MWCNTs can effectively interact with plant cells, altering physiological and biochemical processes. This interaction may enhance plants' ability to cope with stress and increase the accumulation of metabolites (Heydari et al., 2020; Mahmoud & Abdelhameed, 2023; Tardast et al., 2023). We demonstrated that foliar application of low concentrations of MWCNTs on *S. verticillata* stimulates the production of phenolic acids by affecting the expression levels of key enzymes involved in the phenolic acid biosynthesis pathway (Rahmani et al., 2020).

Despite extensive research into their potential to enhance plant secondary metabolites, our understanding of how MJ and nanomaterials, such as MWCNTs, affect plants like *S. verticillata* remains limited. The specific mechanisms by which MWCNTs influence physiological and biochemical processes, particularly in medicinal plants, are poorly understood, despite MJ being commonly studied as a signal inducer. Additionally, there is a lack of comparative studies examining these two elicitors on the same species under similar conditions, which presents a significant gap in our understanding of their relative effectiveness and mechanisms of action. This study aims to compare the effects of MJ and MWCNTs on the physiological and biochemical traits of *S. verticillata*. The objective is to clarify how these compounds affect plant growth and productivity, and to propose strategies for enhancing their cultivation and utilization. We hypothesized that MJ and MWCNTs would differentially influence phenolic biosynthesis through distinct enzymatic pathways.

2. Materials and Methods

2.1. Plant

Mature seeds were harvested from wild-grown *S. verticillata* plants at the flowering stage in the Alamut region (Qazvin province, Iran) at approximately 2250 meters above sea level. A voucher herbarium specimen was deposited in the central herbarium of Bu-Ali Sina University (BASU33996). The seeds were placed in Petri

dishes with a diameter of 10 cm, lined with wet Whatman No. 1 filter paper, and incubated at 25 °C in the dark. After two weeks, healthy seedlings were transferred to plastic pots filled with a soil mixture of loamy sand, cock peat, and perlite (3:1:1). The seedlings were then allowed to grow for two months under greenhouse conditions, maintained at a temperature of at 23±2 °C, with a photoperiod of 16 h and a photon flux density of 450-600 µmol m^{-2} s^{-1}. Finally, plants at the twenty-leaf stage were used for treatment with MWCNTs and MJ.

2.2. Treatment with elicitors

Aqueous solutions of COOH-functionalized MWCNTs were prepared at a concentration of 100 mg L^{-1} using a product from US Research Nanomaterials (USA, US4311). Additionally, MJ (Sigma-Aldrich, USA) was prepared at a storage concentration of 100 µM. The physicochemical characteristics of the MWCNTs, including scanning electron microscope (SEM) images, X-ray diffraction (XRD) patterns, Raman spectra, and Fourier transform infrared (FTIR) spectra, were analyzed as described in our previous publication (Rahmani et al., 2020).

Leaves of cultivated *S. verticillata* were treated by spraying them with a solution containing 100 mg L^{-1} of MWCNTs and 100 µM of MJ. The control plants were treated with deionized water. Samples of the treated and control leaves were harvested at various time points: 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours after treatment. Each treatment was replicated three times. Half of each plant sample was stored at -80 °C, while the other half was dried in an oven at 40 °C for further analysis.

2.3. Photosynthetic pigments

A 0.1 g sample of fresh plant tissue was thoroughly ground in 3 mL of 80% acetone to extract photosynthetic pigment. The resulting suspension was then filtered through the Whatman No. 2 filter paper, and the final volume was adjusted to 5 mL using 80% acetone. The absorbance of the extract was measured at wavelengths 645, 663, and 480 nm using a spectrophotometer (Chromophore, Germany). The concentrations of the photosynthetic pigments were calculated using Lichtenthaler's (1978) formula and expressed in milligrams per gram fresh weight (FW).

2.4. Protein content and enzyme assays

Frozen leaf samples (250 mg) were homogenized in 1 mL of cold phosphate buffer (100 mM, pH 6.0) containing 2 mM EDTA, four mM dithiothreitol, and 2% (w/w) polyvinylpyrrolidone for 15 minutes using a pestle and mortar on ice. The homogenates were centrifuged at 13,000 × g for 25 minutes at 4 °C to obtain a solid-free extract. The protein extracts were stored at −80 °C until needed. The total protein content in the extracts was quantified using the Bradford method (1976), with bovine serum albumin as the standard.

The activity of peroxidase (POD, EC 1.11.1.7) in the leaf extracts was measured using guaiacol as the substrate, based on the procedure described by Abeles and Biles (1991). The results were expressed as micromoles of substrate converted (µkat) per milligram of protein.

Catalase (CAT, 1.11.1.6) activity in the samples was evaluated based on the decomposition of hydrogen peroxide, as outlined by Cakmak and Horst (1991), with results reported as µkat per milligram of protein.

Superoxide dismutase (SOD, EC 1.15.1.1) activity in the leaf extracts was measured according to the method described by Giannopolitis and Ries (1977). The results were expressed in units per milligram of protein.

The activity of L-phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) in the MWCNTs-elicited leaf samples was measured by producing trans-cinnamic acid in a reaction mixture, using a spectrophotometer according to the methodology established by Heide et al. (1989). Specific activities were expressed as nkat per milligram of protein.

Tyrosine aminotransferase (TAT, EC 2.6.1.5) activity in the protein samples was evaluated following the method established by Diamondstone (1966), utilizing an extinction coefficient of 4-hydroxybenzaldehyde (24,900 L

mol^{-1} cm^{-1}) as a product. The specific activities of the enzymes were expressed as nkat per milligram of protein.

The activity of rosmarinic acid synthase (RAS, EC 2.3.1.140) in the protein extracts was determined based on the production of RA, following the protocol outlined by Peterson and Alfermann (1988). The quantity of RA produced during the RAS enzymatic reaction was measured using Smartline HPLC instrument (Kenuer, Germany), equipped with a C18 MZ-Analysentechnik (5 µm particle size, 250 mm length and 4.6 mm diameter), with a solvent mixture of methanol and water (1:1), to which 100 µL of 85% H_3PO_4 was added per liter. A detection wavelength of 333 nm was employed for RA measurement for 20 minutes. A calibration curve was created using standard RA solutions at varying concentrations (0–1 mg L^{-1}), and the enzyme-specific activity in the samples was expressed as nkat per milligram of protein.

2.5. Proline content

A 0.25 g of fresh plant tissue was homogenized for proline extraction using 5 mL of 3% sulfosalicylic acid. The resulting suspension was then filtered through the Whatman No. 2 filter paper. Next, 2 mL of the suspension was combined with 2 mL of glacial acetic acid, 1 mL of 3% sulfosalicylic acid, and 2 mL of ninhydrin solution. This mixture was incubated at 100°C for one hour. After incubation, the samples were rapidly cooled on ice to reach ambient temperature (Bates et al., 1973). Then, 1 mL of toluene was added to the samples, and the mixture was thoroughly agitated for 30 seconds. The upper toluene layer, which contained the chromophore-proline complex, was carefully separated from the aqueous phase. The absorbance of the toluene layer was measured at 520 nm. The proline content of the plant samples was quantified using a standard curve and expressed in µg per g of FW.

2.6. Total phenol and flavonoid content

A total of 0.5 g of powdered dried leaves was extracted with 5 mL of methanol using ultrasonic-assisted extraction for 1 hour at 25°C. The mixture was then centrifuged at 13,500 rpm for 5 minutes. The supernatant was collected and used for further phytochemical assays.

A 150 µL of the methanolic extract was mixed with 1350 µL of deionized water and 150 µL of Folin-Ciocalteu reagent. After thoroughly mixing, the solution was allowed to react for 5 minutes, followed by the addition of 1,500 µL of 7% sodium carbonate. The final volume of the mixture was adjusted to 3500 µL using deionized water. After incubating for 90 minutes at room temperature, the absorbance was measured at 750 nm using a spectrophotometer (Singleton et al., 1999). The total phenolic content of each treated sample was quantified using a standard curve generated with gallic acid and reported as mg GA per g dry weight (DW).

The total flavonoid content in the extracts from treated seedlings was measured using the method described by Zhishen et al. (1999). A 200 µL of the methanolic extract was thoroughly mixed with 200 µL of aluminum chloride and 100 µL of 33% acetic acid. The mixture was then adjusted to a final volume of 5 mL using 90% ethanol. After a 30-minute incubation, the absorbance of the solution was measured at 414 nm. The total flavonoid content in the treated leaves was calculated using a quercetin standard curve and expressed as mg quercetin (QE) per g DW.

2.7. Statistical analysis

The study was a factorial experiment utilizing a randomized complete block design. The SPSS software (Version 22) was used to analyze the data, presented as the mean and standard deviation of three replications (n = 3). A one-way ANOVA revealed significant differences among the means, as determined by the Duncan test at $P < 0.05$. Principal component analysis (PCA) was applied using the PAST program (Version 3.15) to reveal the pattern and link between the used treatments and the researched parameters (Hammer & Harper, 2024).

3. Results

3.1. Photosynthesis pigments

The influence of MJ and MWCNTs on photosynthetic pigment levels, specifically chlorophyll *a*, chlorophyll *b*, and carotenoids, were assessed in the leaves of *S. verticillata* (Fig. 1).

The obtained data displayed that treatment with MJ generally led to a decrease in chlorophyll *a* and *b* content in the leaves of the treated plants compared to the control group. The leaves of plants harvested 72 hours after treatment showed the most significant impact of MJ on chlorophyll *a* level, measuring 1.1 times higher than those of the control. Our findings also revealed a significant decrease in chlorophyll *b* content 96 hours after MJ exposure, with levels approximately half of those measured in the control plants. The variations in carotenoid levels in the MJ-treated leaves followed a similar trend; compared to the control plants, carotenoid concentrations in the leaves of plants harvested 1, 48, and 96 hours after MJ treatment showed a substantial reduction ($P < 0.05$).

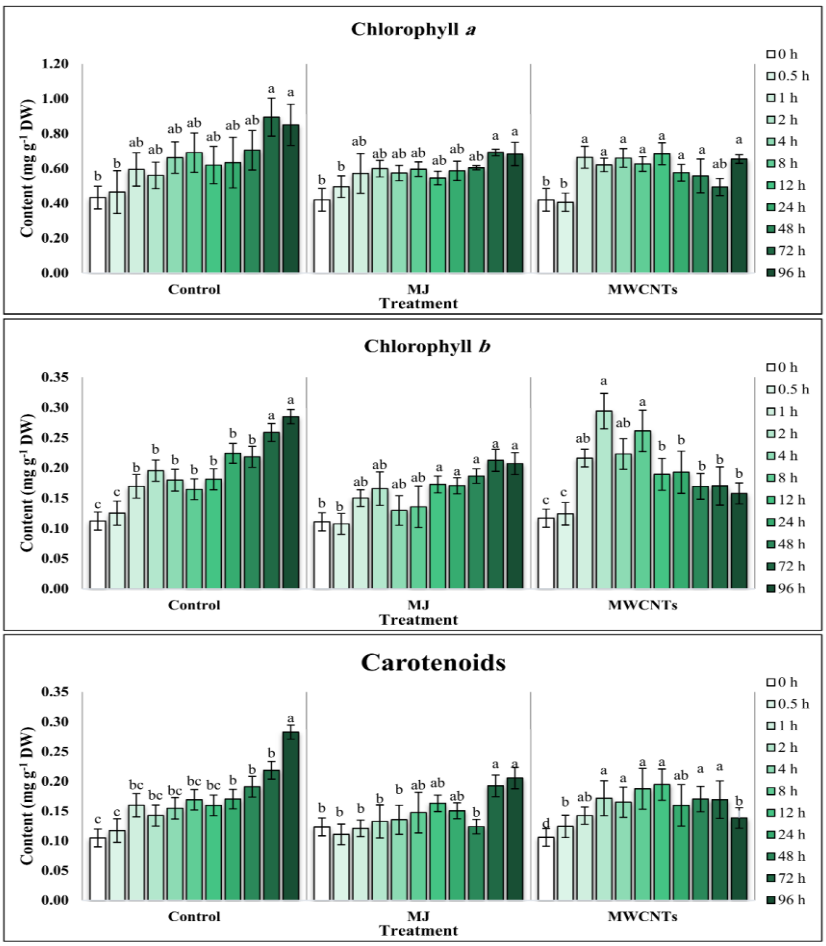

Fig. 1. Changes in the contents of photosynthetic pigments in the leaves of the *Salvia verticillata* after treatment with MWCNTs and MJ. Based on Duncan's multiple-range test, bars not sharing the same superscript letter(s) are significantly different at $P < 0.05$ within each treatment group. All data are means of three replicates, with error bars indicating SE. MJ: methyl jasmonate, MWCNTs: multi-walled carbon nanotubes.

3.2. Proline and total protein

As illustrated in Fig. 2, exposure to MJ and MWCNTs significantly affected the proline and total protein content in the leaves of *S. verticillata*.

Plants treated with MJ showed a higher protein content than the control plants at all measured time points. The highest protein concentration occurred 2 hours after treatment, reaching a level 2.65 times greater than that of the control plants. Following this peak, the protein content began to

decline, and by 72 to 96 hours after treatment, no significant difference was observed between the treated and control plants. In contrast, the treatment with MWCNTs exhibited a delayed yet measurable effect on protein content. The total protein content increased from 1 to 72 hours post-treatment, peaking at 8 hours after treatment, with a 2.55-fold increase in protein concentration recorded at that time.

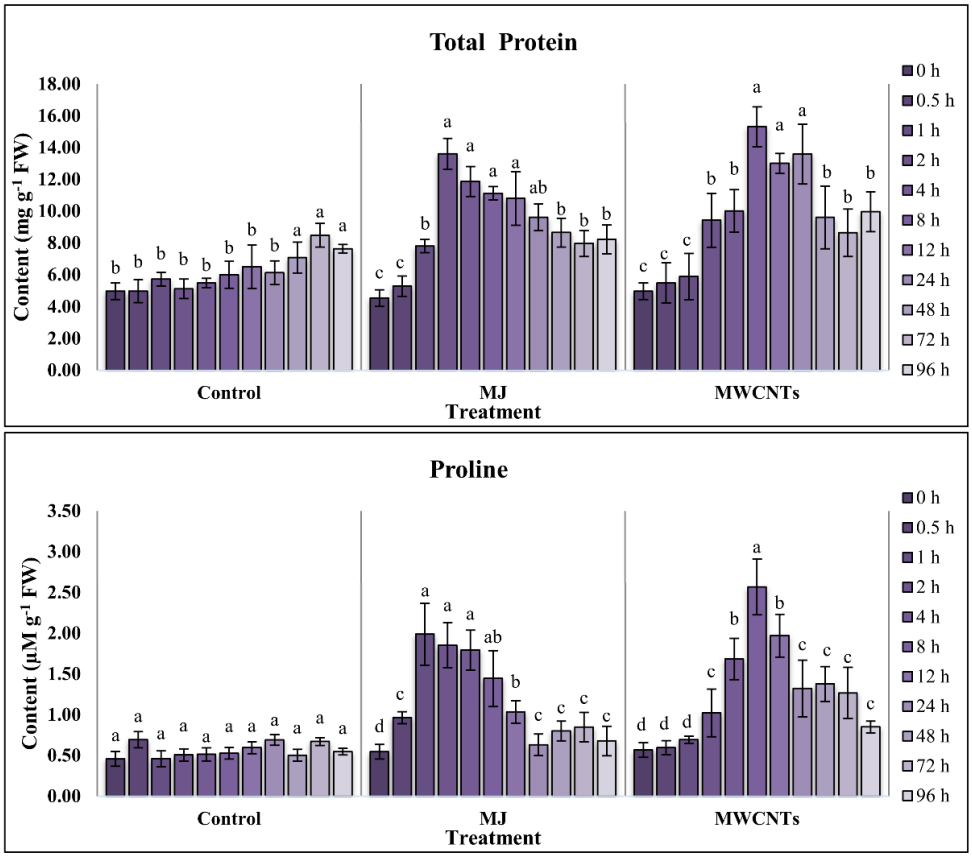

Fig. 2. Changes in the contents of total protein and proline in the leaves of the *Salvia verticillata* after treatment with MWCNTs and MJ. Based on Duncan's multiple-range test, bars not sharing the same superscript letter(s) are significantly different at $P < 0.05$ within each treatment group. All data are means of three replicates, with error bars indicating SE. MJ: methyl jasmonate, MWCNTs: multi-walled carbon nanotubes.

Data indicated that treatment with MJ resulted in higher levels of proline compared to the control group. In the leaves of the treated plants, proline content showed a significant increase within the first hour, followed by a decline over the next 24 hours. A notable 3.4-fold increase in proline content was observed in leaves collected 2 hours after treatment compared to the control group. However, the proline levels in leaves collected between 24 and 96 hours did not differ significantly from those of the control.

The research revealed no significant difference in proline content between the early and later time points after treatment with MWCNTs, compared to the control plants. While proline content significantly increased following carbon nanotube treatment at 2, 48, and 96-hour intervals, the highest proline concentration was recorded in leaves collected 8 hours after treatment.

3.3. Antioxidant enzymes activity

To investigate the effects of MJ and MWCNTs on antioxidant enzymes, we measured the activity levels of SOD, CAT, and POD in the leaves of the treated plants. A comparative analysis of the mean data showed significant variations ($P < 0.05$) in enzyme activity levels among the leaves of treated plants at different harvesting times (Fig. 3).

The results indicated a significant increase in SOD activity after treatment with MJ, peaking at 2 hours, where it was approximately 2.1-fold higher than the control group. Although SOD activity experienced a slight decline after 72 hours, it remained elevated compared to the control at the 96-hour mark. In contrast, following exposure to MWCNTs, SOD activity gradually increased during the initial period, reaching a peak at 24 hours, approximately 1.8-fold higher than the control levels. After this peak, SOD activity progressively decreased, returning closer to control levels by the 96-hour point.

The findings also indicated that MJ significantly increased CAT enzyme activity in the leaves of treated plants compared to those of the control plants. An initial increase in CAT activity was observed up to 8 hours after treatment with MJ, followed by a subsequent decrease. The highest level of CAT activity occurred in the leaves harvested 8 hours after treatment, which was 3.9-fold greater than that of the control plants. By 96 hours after treatment with MJ, CAT activity levels were similar to those in the control plants.

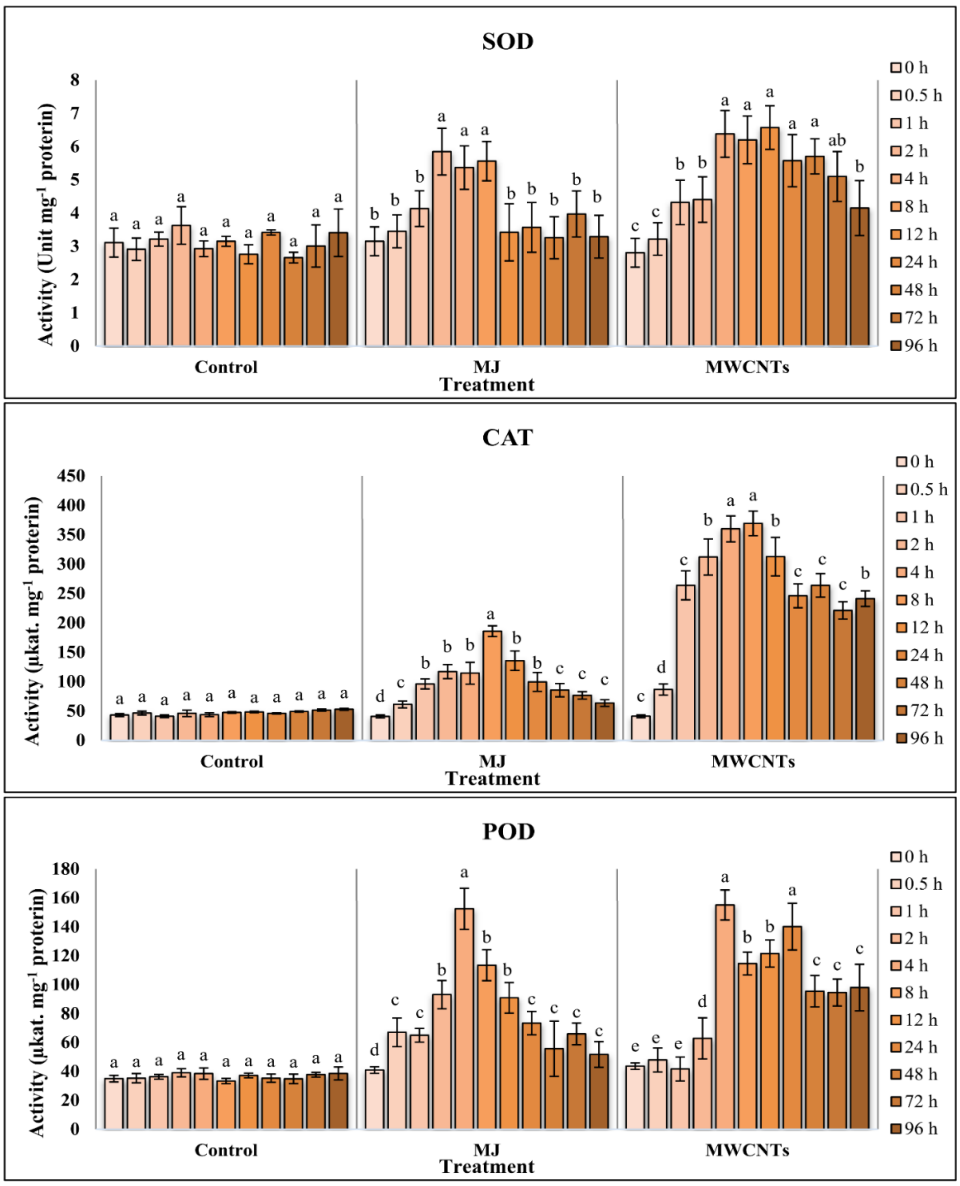

Fig. 3. Changes in the activities of antioxidant enzymes in the leaves of *Salvia verticillata* after treatment with MWCNTs and MJ. Based on Duncan's multiple-range test, bars not sharing the same superscript letter(s) are significantly different at $P < 0.05$ within each treatment group. All data are means of three replicates, with error bars indicating SE. MJ: methyl jasmonate, MWCNTs: multi-walled carbon nanotubes, SOD: superoxide dismutase, POD: peroxidase, CAT: catalase.

Plants treated with MWCNTs showed a significant increase in CAT enzyme activity ($P < 0.05$). Similar to the plants treated with MJ, the pattern of CAT activity in response to MWCNTs treatment demonstrated an increasing trend up to 8 hours post-treatment, after which it began to decline. The highest enzyme activity was recorded in plants harvested 8 hours after treatment, reaching 7.7 times the levels observed in the control group.

The pattern of POD enzyme activity in plants treated with MJ exhibited fluctuations up to 4 hours post-treatment, with the highest activity levels observed in leaves harvested 4 hours after treatment, nearly four times higher than the control. After 96 hours, POD enzyme activity in treated plants was similar to that of the control plants.

Interestingly, there were no noticeable changes in POD activity levels during the first hour after treatment with MWCNTs; however, after this period, POD activity significantly increased compared to control plants. The leaves of plants treated with carbon nanotubes exhibited the highest POD activity level, which was 4 times higher than that of the control plants. Following an initial decrease, POD activity levels rose again until 24 hours post-treatment, after which they remained constant. The study concluded that POD enzyme activity levels in leaves remained stable after 48 hours of treatment with MWCNTs.

3.4. Total phenols and flavonoids

To investigate the effect of MJ and MWCNTs on the production of biochemical compounds in the leaves of *S. verticillata*, we measured total phenol and total flavonoid content (Fig. 4).

The results indicated no significant difference in total phenol content between the control and treated plants up to two hours after MJ treatment. However, the levels of these compounds increased over time after harvest. Plants harvested 24 hours post-treatment exhibited the highest total phenol content, which was twice that of the control group. After this peak, the total phenol content in treated plants began to decline, and by 96 hours after treatment, their levels were comparable to those of the control plants. Regarding flavonoid content, the treated plants exhibited higher levels than the control plants for up to 8 hours after treatment. However, the flavonoid levels decreased between 12 and 48 hours post-treatment, approaching those found in the control plants. Notably, the total flavonoid content in the leaves of plants harvested 72 and 96 hours after MJ treatment decreased significantly ($P < 0.05$).

In general, treatment with MWCNTs increased the total phenolic content of the leaves in treated plants compared to the control plants. Two peaks in the total phenol content were observed in the leaves of these treated plants. The first peak occurred 0.5 to 2 hours after treatment, while the second peak was recorded between 12 to 96 hours after treatment. The highest total phenol content was measured in the leaves of plants that were harvested 72 hours post-treatment. The highest total phenol content was found in the leaves of plants harvested 72 hours after treatment, which was 2.4 times higher than that of the control plants. Additionally, the pattern of changes in total flavonoid content in MWCNT-treated plants mirrored that of the total phenolic content. The highest total flavonoid content was measured in plants harvested 24 hours after treatment, which was 1.7 times higher than in the control group.

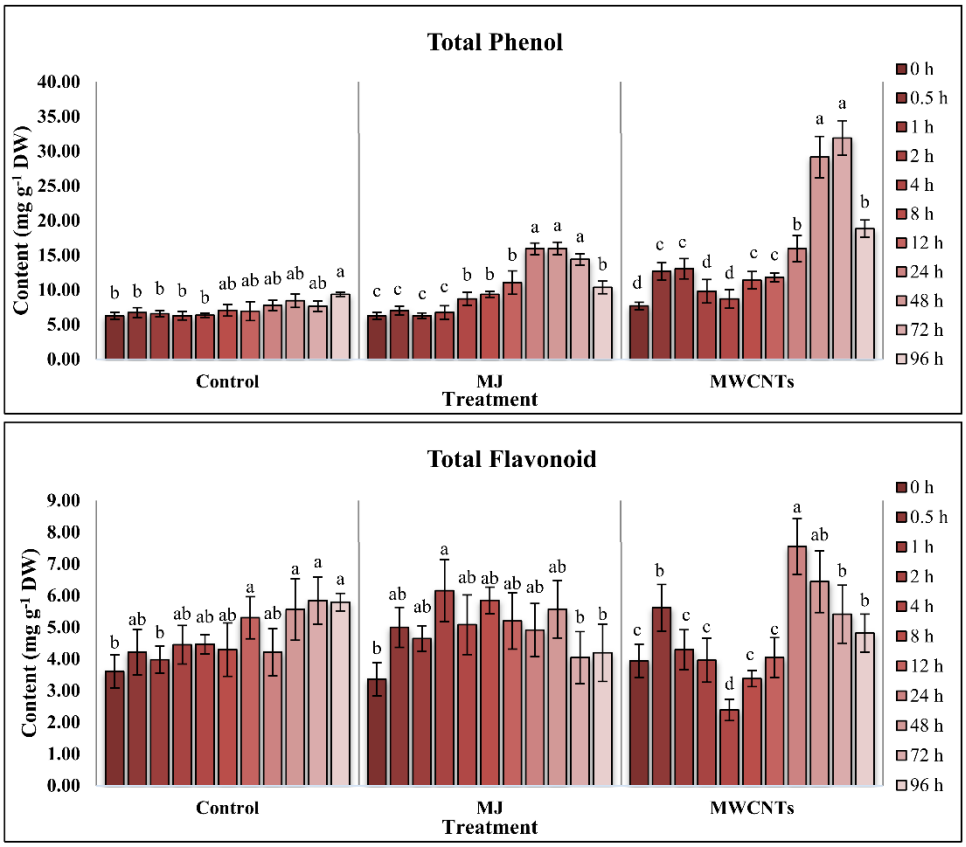

Fig. 4. Changes in the total phenol and flavonoid content of the leaves of *Salvia verticillata* after treatment with MWCNTs and MJ. Based on Duncan's multiple-range test, bars not sharing the same superscript letter(s) are significantly different at $P < 0.05$ within each treatment group. All data are means of three replicates, with error bars indicating SE. MJ: methyl jasmonate, MWCNTs: multi-walled carbon nanotubes.

3.5. Activities of PAL, TAT, and RAS

The activity levels of the enzymes PAL, TAT, and RAS were analyzed to investigate the effect of MJ and MWCNTs on crucial enzymes in the phenolic acid biosynthesis pathway (Fig. 5).

Treatment with MJ did not result in significant changes in PAL activity during the first two hours compared to the control group ($P < 0.05$). However, PAL activity increased significantly over the following four hours, peaking at levels 7.4-fold higher than the control at four hours post-treatment. After reaching this peak, PAL activity gradually declined, returning to baseline levels by 24 hours. Plants harvested 48- and 96 hours post-treatment showed no significant differences in PAL activity compared to the control group.

PAL activity in plants treated with MWCNTs was similar to that of the control group during the first two hours after treatment. However, significant increases in activity were noted from 4 to 48 hours post-treatment, peaking at 48 hours, when it was 6.8-fold higher than the control. A decrease in PAL activity was observed at 72- and 96 hours after treatment.

MJ treatment significantly reduced TAT activity during the first four hours after treatment, demonstrating a marked decrease compared to the control. However, TAT activity displayed a significant increase from 4 to 96 hours post-treatment, except at the 72-hour time point. By 96 hours, TAT activity levels in MJ-treated plants were similar to those in the control group.

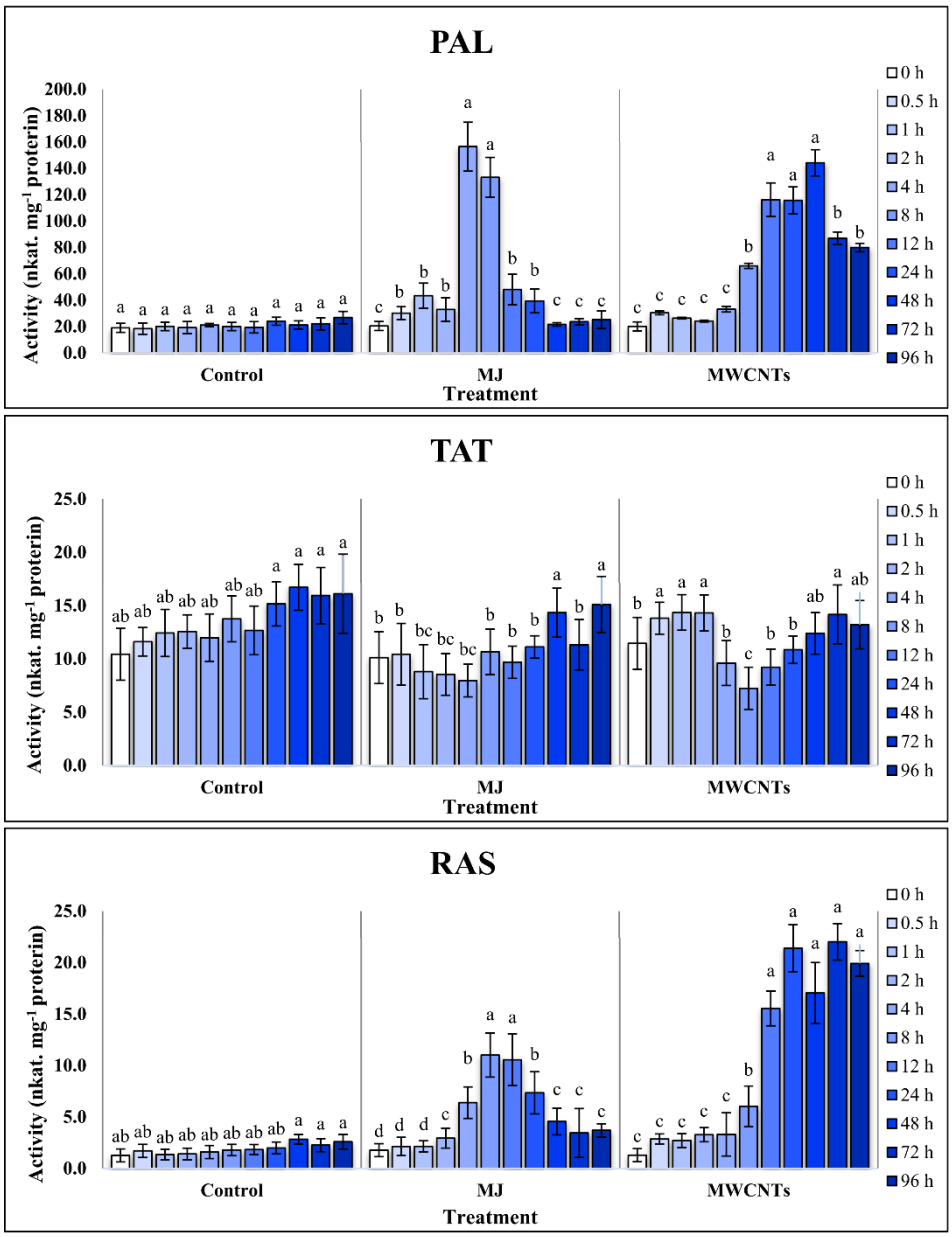

Fig. 5. Changes in the total phenol and flavonoid content in the leaves of *Salvia verticillata* after treatment with MWCNTs and MJ. Based on Duncan's multiple-range test, bars not sharing the same superscript letter(s) are significantly different at $P < 0.05$ within each treatment group. All data are means of three replicates, with error bars indicating SE. MJ: methyl jasmonate, MWCNTs: multi-walled carbon nanotubes, PAL: phenylalanine ammonia-lyase, TAT: tyrosine aminotransferase, RAS: rosmarinic acid synthase.

In plants treated with MWCNTs, TAT activity initially increased within two hours of treatment. However, compared to the control group, TAT activity consistently declined from 4 to 96 hours post-treatment. Treatment with MJ did not significantly affect RAS activity during the first two hours post-treatment. However, a pronounced increase in RAS activity was observed after two hours, reaching a peak 1.6 times higher than that of the control group at 8 hours post-treatment. Following this peak, RAS activity began to decline, and by 48 hours, the activity levels were comparable to those of the control group. No significant changes in RAS activity were noted between 48 and 96 hours compared to the control group.

In plants treated with MWCNTs, RAS activity remained unchanged during the first 0.5 to 4 hours after treatment. However, a significant increase in activity was observed between 8 and 96 hours post-treatment, with the peak activity recorded at

72 hours, reaching 9.7 times higher than the control group. A transient decline in activity was observed at 48 hours; however, overall, activity levels remained significantly elevated.

3.6. Correlation analyses

The PCA analysis of plants treated with MJ (Fig. 6A) showed distinct clustering of samples at various time intervals, indicating significant physiological changes over time. In the initial stages, from 0 to 0.5 hours post-treatment, no substantial variations in the biochemical parameters were observed compared to the control group.

However, between 1 and 4 hours after treatment, the plants exhibited an increase in proline content and antioxidant enzyme activities, including POD, CAT, and SOD. These changes indicated the beginning of oxidative stress and the activation of plant's defense mechanisms. The PCA biplot also correlated these biochemical changes with specific principal components during this period. At 8 to 12 hours post-treatment, a decrease in chlorophyll *a* and *b* levels was observed, likely due to the oxidative stress induced by the MJ treatment. During this period, there was a simultaneous increase in total protein and phenolic compounds. These observations suggest an enhanced defense response characterized by the synthesis of secondary metabolites. The samples collected during this time were distinctly separated on the PCA plot, strongly correlating with the vectors for phenolic compound synthesis and total protein content.

In the later stages, from 24 to 96 hours post-treatment, phenolic compounds prominently accumulated. The PCA vectors associated with these compounds were closely aligned with the samples from this period, highlighting their significant contribution to the observed variability. This prolonged defense response highlights the crucial role of phenolic metabolism in protecting plants against stress.

Principal component 1 (PC1) and PC2 accounted for 43.66% and 28.14% of the total variance, respectively, cumulatively explaining 71.80% of the observed variability in the data. This high cumulative variance indicates that the PCA model provides a reliable representation of the underlying biochemical changes induced by MJ treatment.

In comparison, the PCA analysis for plants treated with MWCNTs (Fig. 6B) showed gradual changes in biochemical parameters over time. In the early stages (0 to 2 hours), there were no significant variations in biochemical content, as indicated by the clustering of samples close to the control group. Between 2 to 8 hours post-treatment, the plants began to show increased proline content and promoted (or boosted) activity of antioxidant enzymes, such as POD and SOD. Unlike the treatment with MJ, the chlorophyll content remained relatively stable during this period, suggesting that MWCNTs had a less detrimental (harmful) effect on the photosynthetic pigments.

From 8 to 12 hours post-treatment, there was a gradual increase in total protein content and total phenol content. The PCA plot indicated a moderate correlation between these parameters and the samples from this period, reflecting a steady activation of defense responses. In the later stages, specifically from 24 to 96 hours post-treatment, the accumulation of defensive metabolites became more prominent. The levels of phenolic compounds and total protein content significantly contributed to the variability observed, as evidenced by their strong alignment with the PCA vectors associated with these biochemical traits.

In this analysis, PC1 and PC2 explained 51.97% and 25.68% of the total variance, respectively, together accounting for 77.65% of the data variability. This suggests that the selected principal components effectively capture the major trends and treatment effects in the biochemical responses to MWCNTs.

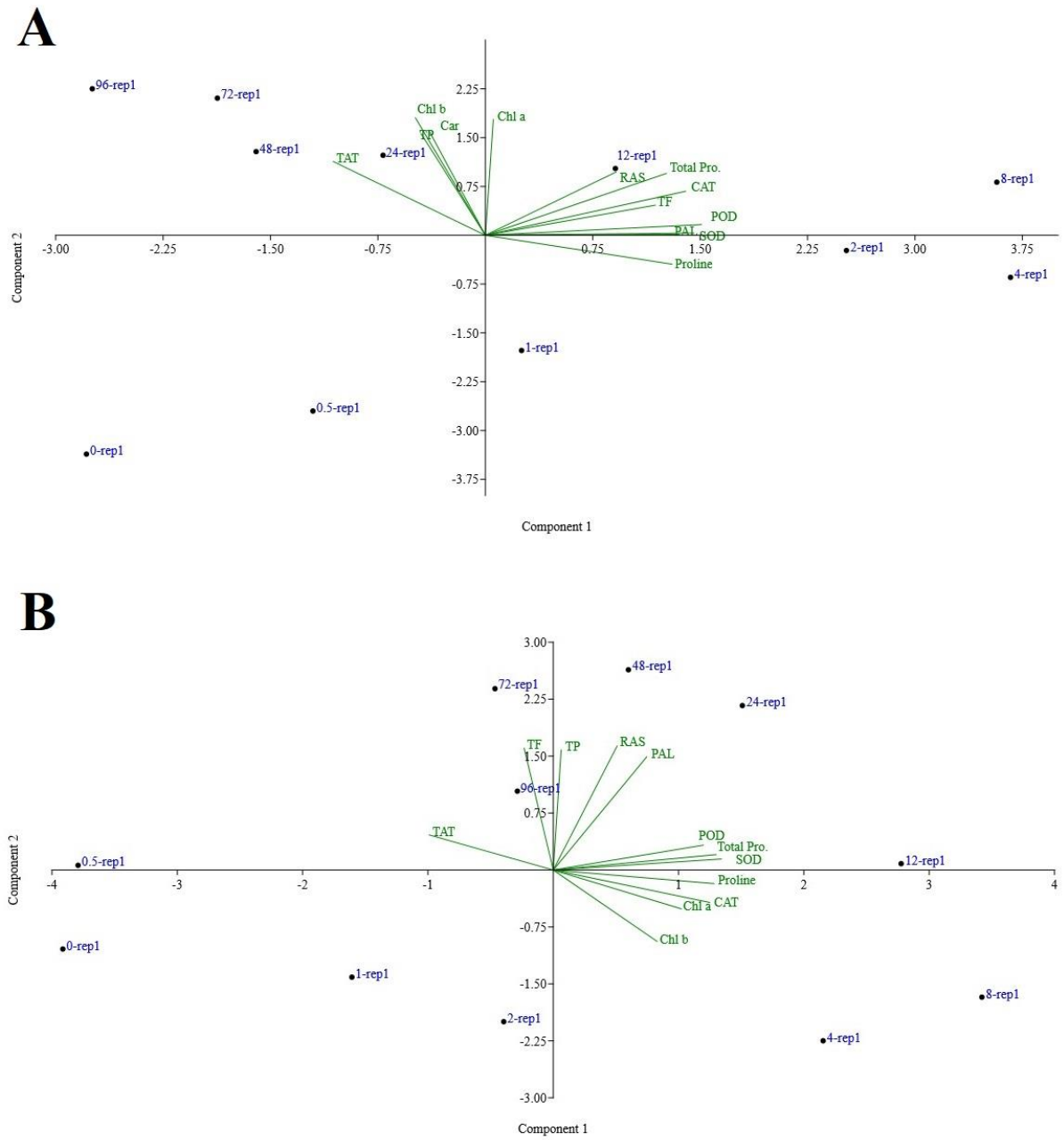

Fig. 6. Principal component analysis (PCA) biplot of the analyzed parameters in *Salvia verticillata* leaves after exposure to MJ (A) and MWCNTs (B). MJ: methyl jasmonate, MWCNTs: multi-walled carbon nanotubes, Chl a: chlorophyll *a*, Chl b: chlorophyll *b*, PAL: phenylalanine ammonia-lyase, TAT: tyrosine aminotransferase, RAS: rosmarinic acid synthase, SOD: superoxide dismutase, POD: peroxidase, CAT: catalase, TP: total phenol, TF: total flavonoid, Total Pro: total protein.

4. Discussion

The current study investigated the effects of MJ and MWCNTs on the physiological and biochemical characteristics, as well as the secondary metabolite production of *S. verticillata.* The obtained results enhance our understanding of how these elicitors influence plant stress responses and secondary metabolism.

4.1. Effect on photosynthetic pigment content

The study revealed that MJ and MWCNTs have a significant impact on the photosynthetic pigment content of S. verticillata plants, resulting in notable physiological changes. Plants treated with MJ showed considerable reductions in chlorophyll *a*, chlorophyll *b*, and carotenoid content, particularly in the later stages of the experiment.

This decrease aligned with previous research suggesting that MJ induces oxidative stress, contributing to pigment degradation as part of the plant's defense mechanism (Nabi et al., 2021). Studies have demonstrated that the impact of MJ on chlorophyll content and photosynthesis varies based on the plant species, concentration, and method of application (Ho et al., 2020). Notably, high concentrations of MJ (greater than 100 µM) lead to increased production of abscisic acid and ethylene in plants. This process activates mitogen-activated protein kinases (MAPKs) and protein kinase cascades, leading to stomatal closure, increased stomatal resistance, and a subsequent decrease in the rate of photosynthesis (Gomi et al., 2003).

Additionally, MJ has been shown to promote the degradation of chlorophylls *a* and *b* by enhancing the expression of the stay-green (SGR) enzyme gene in *Arabidopsis* leaves. This enzyme plays a crucial role in removing Mg^{2+} from the chlorophyll structure during the degradation of these essential photosynthetic pigments (Ono et al., 2019). Furthermore, Liu et al. (2016) demonstrated that the exogenous application of MJ increases lipid peroxidation and membrane leakage while stimulating the expression of cell death-related genes (*CDRGs*) and *SGRs* genes. These events ultimately lead to chlorophyll degradation and senescence in rice plants.

In contrast, treatments involving MWCNTs demonstrated a more complex effect on pigment content. Initially, the levels of chlorophyll a and b showed slight increases, but these levels later declined. This biphasic response suggests that MWCNTs may initially enhance photosynthesis through improved light absorption or nutrient delivery. However, prolonged exposure could lead to stress-induced declines (Chen et al., 2015).

The observed reduction in carotenoid content across both treatments aligns with their role in alleviating oxidative damage, as carotenoids help to quench ROS generated during stress (Marslin et al., 2017). Previous research on *Thlaspi arvense* L. and *Calendula officinalis* L. has also reported varied effects of nanomaterials on chlorophyll content, highlighting the intricate relationship between nanoparticle properties and plant physiology (Ghanati & Bakhtiarian, 2014; Khalifa, 2018). Interestingly, while the oxidative stress induced by MJ is well-documented, the mechanisms through which MWCNTs influence pigment content are less understood. It has been hypothesized that MWCNTs might interfere with the efficiency of photosystems, possibly through oxidative damage or by disrupting chloroplast structure (Chen et al., 2021).

4.2. Oxidative stress caused by elicitors

The significant increases in the activities of antioxidant enzymes (SOD, CAT, and POD) observed in both MJ- and MWCNT-treated plants underscore their crucial role in mitigating oxidative damage. MJ induced rapid and temporary spikes in enzyme activity, peaking between 2 to 8 hours. This finding is consistent with previous research demonstrating MJ's role in activating ROS-mediated defense pathways (Ho et al., 2020; Pesaraklu et al., 2021). These elevated enzyme activities likely helped decrease oxidative stress and allow for the reallocation of metabolic resources toward synthesizing phenolics and flavonoids (Hanaka et al., 2018; Siboza et al., 2017; Yu et al., 2019).

MWCNTs, on the other hand, triggered a gradual yet sustained increase in the activities of antioxidant enzymes. This delayed response may suggest a progressive buildup of oxidative stress, likely resulting from the interaction between the nanoparticles and plant cells, which leads to the generation of ROS and subsequent activation of antioxidant defenses (Ghorbanpour & Hadian, 2015; Hatami et al., 2017). The extended antioxidant response observed in MWCNTs-treated plants might offer a more enduring protective mechanism, thereby minimizing cellular damage over time. Some studies also indicate that other carbon nanoparticles, such as single-walled carbon nanotubes and graphene oxide, can induce oxidative stress in plants. The intensity of this stress depends on the concentration of nanocarbon (Verma et al., 2019).

The contrasting effects of MJ and MWCNTs on proline and total protein content indicate that they operate through different mechanisms. Treatment with MJ led to a rapid increase in proline levels,

which reached its peak within the first few hours. This spike in proline aligns with its established role as an osmoprotectant and antioxidant under stress conditions, as documented in studies involving *Glycine max* L. and *Mentha arvensis* L. (Sirhindi et al., 2016; Zaid & Mohammad, 2018). The pattern of proline accumulation suggests that MJ triggers an acute stress response, which diminishes as the plant adapts to the elicitor.

MWCNTs caused a delayed but sustained increase in proline levels, indicating a gradual activation of the plant's stress response. Similar patterns have been observed in plants treated with other nanoparticles, such as silver and zinc oxide, which have also been shown to influence stress-related metabolites over extended periods (Faizan et al., 2018; Moazzami Farida et al., 2020). The delayed response to MWCNTs may suggest that they interact with plant cells more slowly or elicit a less intense stress signal than MJ.

The total protein content in plants treated with MJ initially increased and then declined, indicating a temporary upregulation of stress-related proteins and a return to baseline levels. In contrast, plants treated with MWCNTs showed a more sustained increase in protein content, suggesting a longer-lasting metabolic adjustment. This observation is consistent with findings from Verma et al. (2019), who reported that nanomaterials can enhance protein synthesis by improving nutrient uptake and cellular metabolism.

4.3. Enhanced secondary metabolite production

Metabolic patterns in plants are influenced by a variety of environmental conditions, as well as by biotic and abiotic stresses. In response to these changes, plants often produce secondary metabolites. Like many other stress factors, the exogenous application of MJ and nanoparticles can produce ROS in plant cells. This ROS production stimulates defense mechanisms, ultimately leading to increased production and accumulation of secondary metabolites (Ho et al., 2020; Rahmani et al., 2020). The data obtained indicate that both elicitors significantly impact the accumulation of secondary metabolites, including total phenolics and flavonoids. This effect is mediated through their influence on key enzymes involved in the phenolic acid biosynthesis pathway, specifically PAL, TAT, and RAS.

MJ treatments resulted in a rapid but temporary increase in specific metabolites, with peak levels observed within 24 hours following treatment. This response aligns with MJ's established capacity to enhance the expression of genes involved in the biosynthesis of secondary metabolites, particularly those in the phenylpropanoid pathway (Pesaraklu et al., 2021). MJ induced a quick and transient spike in PAL activity, which later returned to baseline levels. This suggests a direct regulatory role for MJ in phenylpropanoid metabolism. In contrast, the activities of TAT and the RAS exhibited a delayed but sustained pattern, indicating their involvement in downstream metabolic processes. Overall, cyclic derivatives of jasmonic acid, such as MJ, mediate the transcriptional regulation of secondary metabolism in plant cells. MJ plays a crucial role in several steps, including signal transduction, activation of biosynthetic pathways at the transcriptional level, and the accumulation of metabolites. Consequently, numerous reports highlight the positive effect of MJ on the production of secondary metabolites (Sadeghnezhad et al., 2020).

In contrast, MWCNTs exhibited a distinct pattern of secondary metabolite accumulation, characterized by two separate peaks. The initial peak occurs within 2 hours of treatment, indicating an immediate stress-induced response. The second, more pronounced peak is observed between 24 and 72 hours, reflecting a sustained activation of metabolic processes. This dual-phase response to MWCNTs may be attributed to their ability to enhance nutrient uptake and stimulate cellular signaling pathways, thereby increasing the biosynthetic capacity of plant cells (Tiwari et al., 2014; Yuan et al., 2011).

One limitation of this study is the lack of assessment of post-treatment recovery or re-equilibration of physiological and biochemical traits after the final sampling time. Although some characteristics showed stabilization or decline in later stages (e.g., 72–96 hours), it remains unclear whether the plant responses fully returned to

baseline levels or entered a new state of homeostasis. Future studies should consider extending the observation period beyond 96 hours to determine the reversibility or long-term stability of elicitor-induced changes.

5. Conclusion

This study highlights the distinct temporal dynamics of biochemical and physiological responses in *S. verticillata* following treatments with MJ and MWCNTs. Methyl jasmonate triggered rapid but transient metabolic changes. In contrast, MWCNTs induced a more gradual and sustained response, making them particularly promising for applications that require long-term modulation of plant metabolism. These differing modes of action suggest that combining both elicitors, either sequentially or simultaneously, could exploit their complementary effects, resulting in a synergistic increase in the biosynthesis of valuable secondary metabolites while minimizing potential adverse impacts on essential processes such as photosynthesis. From a practical perspective, MJ may be well-suited for short-term application in controlled environments to enhance phytochemical production quickly. At the same time, MWCNTs may be more effective for sustained stimulation over extended cultivation periods.

However, despite the promising potential of carbon nanotubes in promoting secondary metabolite accumulation, their environmental and biosafety implications must not be overlooked. The accumulation of nanomaterials in plant tissues may pose risks of bioaccumulation across the food chain and potential leaching into surrounding ecosystems. The long-term fate and ecological impact of these materials remain largely uncertain. Therefore, comprehensive evaluations of their environmental persistence, mobility, and toxicity to non-target organisms are crucial prior to their large-scale implementation in agricultural or medicinal plant systems. Future studies should not only deepen our understanding of the molecular mechanisms involved, particularly regarding signaling pathways and gene regulation, but also integrate environmental risk assessments to ensure the safe and responsible use of nanomaterials in plant biotechnology.

References

Abeles, F. B., & Biles, C. L. (1991). Characterization of peroxidases in lignifying peach fruit endocarp. *Plant Physiology*, *95*(1), 269-273. http://doi.org/10.1104/pp.95.1.269

Bates, L. S., Waldren, R., & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*, 205-207. https://doi.org/10.1007/BF00018060

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Cakmak, I., & Horst, W. J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum*, *83*(3), 463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

Chen, G., Qiu, J., Liu, Y., Jiang, R., Cai, S., Liu, Y., Zhu, F., Zeng, F., Luan, T., & Ouyang, G. (2015). Carbon nanotubes act as contaminant carriers and translocate within plants. *Scientific Reports*, *5*, 15682. https://doi.org/10.1038/srep15682

Chen, X., Wang, J., Hayat, K., Zhang, D., & Zhou, P. (2021). Small structures with big impact: multi-walled carbon nanotubes enhanced remediation efficiency in hyperaccumulator *Solanum nigrum* L.

under cadmium and arsenic stress. *Chemosphere*, *276*, 130130. https://doi.org/10.1016/j.chemosphere.2021.130130

Diamondstone, T. I. (1966). Assay of tyrosine transaminase activity by conversion of p-hydroxyphenylpyruvate to p-hydroxybenzaldehyde. *Analytical Biochemistry*, *16*(3), 395-401. https://doi.org/10.1016/0003-2697(66)90220-X

Faizan, M., Faraz, A., Yusuf, M., Khan, S., & Hayat, S. (2018). Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants. *Photosynthetica*, *56*(2), 678-686. https://doi.org/10.1007/s11099-017-0717-0

Ghanati, F., & Bakhtiarian, S. (2014). Effect of methyl jasmonate and silver nanoparticles on production of secondary metabolites by *Calendula officinalis* L (Asteraceae). *Tropical Journal of Pharmaceutical Research*, *13*(11), 1783-1789. https://doi.org/10.4314/tjpr.v13i11.2

Ghorbanpour, M., & Hadian, J. (2015). Multi-walled carbon nanotubes stimulate callus induction, secondary metabolites biosynthesis and antioxidant capacity in medicinal plant *Satureja khuzestanica* grown in vitro. *Carbon*, *94*, 749-759. https://doi.org/10.1016/j.carbon.2015.07.056

Ghorbel, M., Brini, F., Sharma, A., & Landi, M. (2021). Role of jasmonic acid in plants: the molecular point of view. *Plant Cell Reports*, *40*, 1471-1494. https://doi.org/10.1007/s00299-021-02687-4

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, *59*(2), 309-314. https://doi.org/10.1104/pp.59.2.309

Gomi, K., Yamasaki, Y., Yamamoto, H., & Akimitsu, K. (2003). Characterization of a hydroperoxide lyase gene and effect of C6-volatiles on expression of genes of the oxylipin metabolism in *Citrus*. *Journal of Plant Physiology*, *160*(10), 1219-1231. https://doi.org/10.1078/0176-1617-01177

Guru, A., Dwivedi, P., Kaur, P., & Pandey, D. K. (2022). Exploring the role of elicitors in enhancing medicinal values of plants under in vitro condition. *South African Journal of Botany*, *149*, 1029-1043. https://doi.org/10.1016/j.sajb.2021.10.014

Hammer, Ø., & Harper, D. A. (2005). *Paleontological data analysis*. John Wiley & Sons. https://doi.org/10.1002/9780470750711

Hanaka, A., Lechowski, L., Mroczek-Zdyrska, M., & Strubińska, J. (2018). Oxidative enzymes activity during abiotic and biotic stresses in *Zea mays* leaves and roots exposed to Cu, methyl jasmonate and *Trigonotylus caelestialium*. *Physiology and Molecular Biology of Plants*, *24*(1), 1-5. https://doi.org/10.1007/s12298-017-0479-y

Hatami, M., Hadian, J., & Ghorbanpour, M. (2017). Mechanisms underlying toxicity and stimulatory role of single-walled carbon nanotubes in *Hyoscyamus niger* during drought stress simulated by polyethylene glycol. *Journal of Hazardous Materials*, *324*, 306-320. https://doi.org/10.1016/j.jhazmat.2016.10.064

Heide, L., Nishioka, N., Fukui, H., & Tabata, M. (1989). Enzymatic regulation of shikonin biosynthesis in *Lithospermum erythrorhizon* cell cultures. *Phytochemistry*, *28*(7), 1873-1877. https://doi.org/10.1016/S0031-9422(00)97877-4

Heydari, H. R., Chamani, E., & Esmaielpour, B. (2020). Cell line selection through gamma irradiation combined with multi-walled carbon nanotubes elicitation enhanced phenolic compounds accumulation in *Salvia nemorosa* cell culture. *Plant Cell, Tissue and Organ Culture*, *142*, 353-367. https://doi.org/10.1007/s11240-020-01867-6

Ho, T.-T., Murthy, H. N., & Park, S.-Y. (2020). Methyl jasmonate induced oxidative stress and accumulation of secondary metabolites in plant cell and organ cultures. *International Journal of Molecular Sciences*, *21*(3), 716. https://doi.org/10.3390/ijms21030716

Hou, Z., Li, Y., Su, F., Wang, Y., Zhang, X., Xu, L., Yang, D., & Liang, Z. (2021). The exploration of methyl jasmonate on the tanshinones biosynthesis in hair roots of *Salvia miltiorrhiza* Bunge and *Salvia castanea* f. *tomentosa* Stib. *Industrial Crops and Products*, *167*, 113563. https://doi.org/10.1016/j.indcrop.2021.113563

Humbal, A., & Pathak, B. (2023). Influence of exogenous elicitors on the production of secondary metabolite in plants: A review. *Plant Stress*, *8*, 100166. https://doi.org/10.1016/j.stress.2023.100166

Ivanova, S., Dzhakova, Z., Staynova, R., & Ivanov, K. (2024). *Salvia verticillata* (L.)—Biological Activity, Chemical Profile, and Future Perspectives. *Pharmaceuticals*, *17*(7), 859. https://doi.org/10.3390/ph17070859

Jamzad, Z. (2020). Lamiaceae, a valuable source of medicinal and ornamental plants in the flora of Iran. *Iran Nature*, *4*(6), 29-49. https://doi.org/10.22092/irn.2020.120802 [In Persian].

Karakaya, S., Yilmaz, S. V., Özdemir, Ö., Koca, M., Pınar, N. M., Demirci, B., Yıldırım, K., Sytar, O., Turkez, H., & Baser, K. H. C. (2020). A caryophyllene oxide and other potential anticholinesterase and anticancer agent in *Salvia verticillata* subsp. *amasiaca* (Freyn & Bornm.) Bornm.(Lamiaceae). *Journal of Essential Oil Research*, *32*(6), 512-525. https://doi.org/10.1080/10412905.2020.1813212

Khalifa, N. S. (2018). The effect of multi-walled carbon nanotubes on pennycress (*Thlaspi arvense* L.) plant. *Egyptian Journal of Botany*, *58*(3), 529-537. https://doi.org/10.21608/ejbo.2018.3950.1181

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*, 350-382. https://doi.org/10.1016/0076-6879(87)48036-1

Liu, L., Li, H., Zeng, H., Cai, Q., Zhou, X., & Yin, C. (2016). Exogenous jasmonic acid and cytokinin antagonistically regulate rice flag leaf senescence by mediating chlorophyll degradation, membrane deterioration, and senescence-associated genes expression. *Journal of Plant Growth Regulation*, *35*(2), 366-376. https://doi.org/10.1007/s00344-015-9539-0

Mahmoud, N. E., & Abdelhameed, R. M. (2023). Use of titanium dioxide doped multi-wall carbon nanotubes as promoter for the growth, biochemical indices of *Sesamum indicum* L. under heat stress conditions. *Plant Physiology and Biochemistry*, *201*, 107844. https://doi.org/10.1016/j.plaphy.2023.107844

Marslin, G., Sheeba, C. J., & Franklin, G. (2017). Nanoparticles alter secondary metabolism in plants via ROS burst. *Frontiers in Plant Science*, *8*, 832. https://doi.org/10.3389/fpls.2017.00832

Moazzami Farida, S. H., Karamian, R., & Albrectsen, B. R. (2020). Silver nanoparticle pollutants activate oxidative stress responses and rosmarinic acid accumulation in sage. *Physiologia Plantarum*, *170*(3), 415-432. https://doi.org/10.1111/ppl.13172

Nabi, N., Singh, S., & Saffeullah, P. (2021). Responses of in vitro cell cultures to elicitation: Regulatory role of jasmonic acid and methyl jasmonate: A review. *In Vitro Cellular & Developmental Biology-Plant*, *57*, 341-355. https://doi.org/10.1007/s11627-020-10140-6

Okey-Onyesolu, C. F., Hassanisaadi, M., Bilal, M., Barani, M., Rahdar, A., Iqbal, J., & Kyzas, G. Z. (2021). Nanomaterials as nanofertilizers and nanopesticides: an overview. *ChemistrySelect*, *6*(33), 8645-8663. https://doi.org/10.1002/slct.202102379

Ono, K., Kimura, M., Matsuura, H., Tanaka, A., & Ito, H. (2019). Jasmonate production through chlorophyll a degradation by Stay-Green in *Arabidopsis thaliana*. *Journal of Plant Physiology*, *238*, 53-62. https://doi.org/10.1016/j.jplph.2019.05.004

Pesaraklu, A., Radjabian, T., & Salami, S. A. (2021). Methyl jasmonate and Ag^{+} as effective elicitors for enhancement of phenolic acids contents in *Salvia officinalis* and *Salvia verticillata*, as two traditional medicinal plants. *South African Journal of Botany*, *141*, 105-115. https://doi.org/10.1016/j.sajb.2021.04.032

Peterson, M., & Alfermann, A. (1988). Two new enzymes of rosmarinic acid biosynthesis from cell culture of *Coleus blumei*: Hydroxyphenylpyruvate reductase and rosmarinic acid synthetase. *Z Naturforsch, 43*, 501-504. https://doi.org/10.1515/znc-1988-7-804

Rahmani, N., & Radjabian, T. (2024). Integrative effects of phytohormones in the phenolic acids production in *Salvia verticillata* L. under multi-walled carbon nanotubes and methyl jasmonate elicitation. *BMC Plant Biology*, *24*(1), 56. https://doi.org/10.1186/s12870-023-04719-5

Rahmani, N., Radjabian, T., & Soltani, B. M. (2020). Impacts of foliar exposure to multi-walled carbon nanotubes on physiological and molecular traits of *Salvia verticillata* L., as a medicinal plant. *Plant Physiology and Biochemistry*, *150*, 27-38. https://doi.org/10.1016/j.plaphy.2020.02.022

Sadeghnezhad, E., Sharifi, M., Zare-Maivan, H., & Chashmi, N. A. (2020). Time-dependent behavior of phenylpropanoid pathway in response to methyl jasmonate in *Scrophularia striata* cell cultures. *Plant Cell Reports*, *39*(2), 227-243. https://doi.org/10.1007/s00299-019-02486-y

Siahmansour, S., Ismaili, A., & Nazarian Firouzabadi, F. (2018). Effect of different elicitor treatments on hairy root of medicinal plant poppies (*Papaver somniferum* L.). *Journal of Plant Productions*, *41*(1), 29-42. https://doi.org/10.22055/ppd.2018.13548 [In Persian].

Siboza, X. I., Bertling, I., & Odindo, A. O. (2017). Enzymatic antioxidants in response to methyl jasmonate and salicylic acid and their effect on chilling tolerance in lemon fruit (*Citrus limon* (L.) Burm. F.). *Scientia Horticulturae*, *225*, 659-667. https://doi.org/10.1016/j.scienta.2017.07.023

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*, *299*, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sirhindi, G., Mir, M. A., Abd-Allah, E. F., Ahmad, P., & Gucel, S. (2016). Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in *Glycine max* under nickel toxicity. *Frontiers in Plant Science*, *7*, 591. https://doi.org/10.3389/fpls.2016.00591

Stanković, J. S. K., Srećković, N., Mišić, D., Gašić, U., Imbimbo, P., Monti, D. M., & Mihailović, V. (2020). Bioactivity, biocompatibility and phytochemical assessment of lilac sage, *Salvia verticillata* L.(Lamiaceae)-A plant rich in rosmarinic acid. *Industrial Crops and Products*, *143*, 111932. https://doi.org/10.1016/j.indcrop.2019.111932

Tardast, Z., Iranbakhsh, A., Ebadi, M., & Ardebili, Z. O. (2023). Carboxylic acid-

functionalized multiwalled carbon nanotubes (COOH-MWCNTs) improved production of atropine in callus of *Datura inoxia* by influencing metabolism, gene regulation, and DNA cytosine methylation; an in vitro biological assessment. *Plant Physiology and Biochemistry*, *202*, 107975. https://doi.org/10.1007/s00344-023-10924-1

Tiwari, D., Dasgupta-Schubert, N., Villaseñor Cendejas, L., Villegas, J., Carreto Montoya, L., & Borjas García, S. (2014). Interfacing carbon nanotubes (CNT) with plants: enhancement of growth, water and ionic nutrient uptake in maize (*Zea mays*) and implications for nanoagriculture. *Applied Nanoscience*, *4*, 577-591. https://doi.org/10.1007/s13204-013-0236-7

Verma, S. K., Das, A. K., Gantait, S., Kumar, V., & Gurel, E. (2019). Applications of carbon nanomaterials in the plant system: a perspective view on the pros and cons. *Science of the Total Environment*, *667*, 485-499. https://doi.org/10.1016/j.scitotenv.2019.02.409

Yu, X., Fei, P., Xie, Z., Zhang, W., Zhao, Q., & Zhang, X. (2019). Effects of methyl jasmonate on growth, antioxidants, and carbon and nitrogen metabolism of *Glycyrrhiza uralensis* under salt stress. *Biologia Plantarum*, *63*(1), 89-96. https://doi.org/10.32615/bp.2019.011

Yuan, H., Hu, S., Huang, P., Song, H., Wang, K., Ruan, J., He, R., & Cui, D. (2011). Single walled carbon nanotubes exhibit dual-phase regulation to exposed *Arabidopsis* mesophyll cells. *Nanoscale Res Lett*, *6*, 1-9. https://doi.org/10.1007/s11671-010-9799-3

Zaid, A., & Mohammad, F. (2018). Methyl jasmonate and nitrogen interact to alleviate cadmium stress in *Mentha arvensis* by regulating physio-biochemical damages and ROS detoxification. *Journal of Plant Growth Regulation*, *37*(4), 1331-1348. https://doi.org/10.1007/s00344-018-9854-3

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*(4), 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zhou, W., Shi, M., Deng, C., Lu, S., Huang, F., Wang, Y., & Kai, G. (2021). The methyl jasmonate-responsive transcription factor *Sm*MYB1 promotes phenolic acid biosynthesis in *Salvia miltiorrhiza*. *Horticulture Research*, *8*, 10. https://doi.org/10.1038/s41438-020-00443-5