Plant Research Journal, 02(02), 50-64 (2026) ISSN: 3135-9728 (E), 3135-971X (P)

RESEARCH ARTICLE - PHYTOCHEMICALS

Enhancing phytochemical production in *Ocimum species* using Methyl jasmonate and Salicylic acid as elicitors

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Received: 02-07-2026 / Accepted: 04-08-2026 / Published: 08-08-2026

Abstract

Ocimum species, commonly known as basil, are economically and medicinally significant aromatic herbs belonging to the family Lamiaceae. They are widely recognized for their rich phytochemical profiles, encompassing potent antioxidant compounds, phenolics, flavonoids, and essential oils with well-documented pharmaceutical and nutraceutical value. Despite their therapeutic potential, the enhancement of secondary metabolite accumulation in *Ocimum* species through safe and sustainable agrochemical strategies remains insufficiently explored. This study investigated the effects of two well-known signal molecule elicitors, methyl jasmonate (MeJA) and salicylic acid (SA) on the morphological characteristics and biochemical composition of four *Ocimum* species: *O. basilicum* (sweet basil), *O. sanctum* (holy basil), Lemon basil, and *Cinnamon* basil. Experiments were conducted under controlled greenhouse conditions. Seeds were sown in seedling trays at one seed per cell and maintained for 40 days under natural light and ventilation. Seedlings with four fully expanded leaves were transplanted into pots and treated with MeJA at 1 µM and SA at concentrations of 0.5, 1.0, and 1.5 mM. Results revealed that elicitor application significantly enhanced both growth parameters and phytochemical production across all four *Ocimum* species relative to untreated controls. Treated plants exhibited elevated chlorophyll accumulation, increased antioxidant enzyme activities (SOD and POD), higher soluble protein content, and greater concentrations of phenolic compounds and flavonoids. These findings establish elicitor application as a promising and sustainable strategy for improving the medicinal, pharmaceutical, and nutraceutical value of *Ocimum* species through targeted stimulation of antioxidant defense mechanisms and secondary metabolite biosynthesis.

Keywords: Aromatic herbs, methyl jasmonate, *Ocimum*, peroxidase activity, reactive oxygen species

Abbreviations: SOD (Superoxide dismutase), POD (Peroxidase), SA (Salicylic acid), MeJ (Methyl Jasmonates), TPC (Total Phenolic Contents), TFC (Total Flavonoids Contents), DPPH, NBT (Nito blue tetrazolium), CRD (Completely randomised design), BSA (Bovine serum albumin)

1. Introduction

The genus *Ocimum* is among the most important groups of medicinal and aromatic herbs, recognized for its extensive therapeutic potential. It comprises more than 150 species that are widely distributed across tropical and subtropical regions of the world. Several species, including *O. basilicum* (sweet basil), *O. sanctum* (holy basil), C*innamon* basil, and Lemon basil, are of significant commercial importance. These species are valued for their medicinal, aromatic, and culinary properties and are rich in bioactive compounds with antioxidant potential. The genus belongs to the family *Lamiaceae*, which includes many plants traditionally used as herbs and in folk medicine. Members of this family have long been utilized for their therapeutic properties, and several species of *Ocimum* are collectively referred to as basil (Rewers et al., 2016).

Holy basil, *O. tenuiflorum* (syn. *Ocimum sanctum*), is a highly valued medicinal plant belonging to the family *Lamiaceae*. It is widely known as "Tulsi" in the Indian subcontinent and holds significant religious and cultural importance. This species is native to tropical regions and is characterized by its erect, highly branched growth habit, reaching a height of 30–60 cm. The plant bears green or purple leaves depending on the variety and is well known for its therapeutic applications. It is often referred to as the "Queen of Herbs" due to its extensive medicinal properties (Pattanayak et al., 2010). Other important basil varieties include cinnamon basil (*O. basilicum* cv. 'Cinnamon') and *lemon* basil (*O. × citriodorum*).

Cinnamon basil is known for its distinctive spicy aroma and flavor and is commonly used in baked goods, infusions, and culinary dishes. It is also cultivated for its essential oil, which has applications in the perfumery industry (Wesołowska et al., 2016). Lemon basil, a natural hybrid between *O. basilicum* and *O. americanum*, is widely used in Mediterranean and Asian cuisines due to its characteristic lemon-like aroma. It also has industrial significance in the pharmaceutical and food sectors (Paulus et al., 2019).

Phytochemicals are bioactive compounds produced through the secondary metabolism of plants and include flavonoids, phenolic acids, and essential oils. These compounds possess significant biological activities, including antioxidants, antimicrobial, and anticancer properties (Jermini et al., 2019). In *Ocimum* species, key phytochemicals such as eugenol, rosmarinic acid, apigenin, and ursolic acid are responsible for various therapeutic effects, including anti-inflammatory, hepatoprotective, and hypoglycemic activities. These species have been traditionally used to treat a wide range of ailments, including fever, respiratory disorders, kidney diseases, and metabolic conditions (Shahrajabian et al., 2020). Furthermore, different *Ocimum* species have demonstrated strong antioxidant activity in various in vitro models, including DPPH, ABTS, hydrogen peroxide scavenging, and lipid peroxidation inhibition assays (Rezaie et al., 2022).

Despite their medicinal importance, the natural accumulation of phytochemicals in plants is often limited and influenced by environmental conditions, and developmental stage and genetic factors. This variability poses a challenge for large-scale production and standardization of bioactive compounds. To address this issue, elicitation has emerged as an effective strategy for enhancing secondary metabolite production in medicinal plants.

Elicitation refers to the stimulation of plant defense responses through the application of biotic or abiotic agents, resulting in increased production of secondary metabolites. Chemical elicitors such as methyl jasmonate and salicylic acid mimic stress conditions and activate complex signaling pathways, including calcium influx, reactive oxygen species generation, and mitogen-activated protein kinase cascades. These signaling events lead to the activation of genes involved in the biosynthesis of phenolic and terpenoid compounds (Jain & Singh, 2024). Elicitors are broadly classified into biotic (e.g., microbial extracts) and abiotic (e.g., chemical compounds) categories.

Methyl jasmonate (MeJA) is a key signaling molecule involved in plant defense and stress responses. It plays a crucial role in regulating secondary metabolism and has been widely reported to enhance the accumulation of phenolic compounds, flavonoids, and essential oils in medicinal plants (Jain & Singh, 2024). Recent studies have demonstrated that exogenous application of MeJA significantly increases flavonoid biosynthesis and antioxidant activity in plants (Ye et al., 2025). Additionally, MeJA treatment has been shown to improve essential oil yield and biochemical characteristics in basil under controlled conditions (Chutimanukul et al., 2025). Similarly, salicylic acid (SA) is another important phytohormone that regulates plant defense mechanisms and systemic acquired resistance. It influences various physiological processes, including photosynthesis, stress tolerance, and secondary metabolite production. SA signaling enhances phenolic and flavonoid synthesis through activation of the phenylpropanoid pathway (Ali, 2021). Foliar application of salicylic acid has been reported to significantly increase phenolic content and antioxidant activity in medicinal plants (Olennikov, 2025). Comparative studies indicate that methyl jasmonate primarily enhances terpenoid and volatile compound production, whereas salicylic acid is more effective in stimulating phenolic metabolism. Interaction between these signaling pathways is complex and may exhibit synergistic or antagonistic effects depending on environmental and physiological conditions. Considerable progress has been made in understanding the role of elicitors in plant metabolism. Limited studies have investigated the comparative and combined effects of methyl jasmonate and salicylic acid specifically in *Ocimum* species under greenhouse/glasshouse conditions. Furthermore, there is a lack of standardized protocols regarding optimal concentrations, timing, and methods of elicitor application. In addition, detailed studies focusing on specific phytochemicals such as phenolics, flavonoids, and essential oils in different *Ocimum* species are still limited. Therefore, the present study aims to address these gaps by evaluating the effects of methyl jasmonate and salicylic acid on selected phytochemicals in different *Ocimum* species under controlled conditions. The objectives of the present study were to assess the impact of methyl jasmonate and salicylic acid on growth and morphological traits and their effects on the biochemical properties including antioxidant enzyme activities, phenolic and flavonoid contents, protein content, and chlorophyll content in *Ocimum* species.

2. Materials and Methods

2.1 Plant material

The present study was conducted on four selected species of the genus *Ocimum*, namely *O. basilicum*, *O. sanctum*, *Lemon* basil (*Ocimum × citriodorum*), and *cinnamon* basil (*O. basilicum* var. *cinnamon*). Healthy, disease-free seedlings of uniform size were selected to ensure experimental accuracy and consistency. The plant material was obtained from a nursery and carefully transported to the greenhouse of the Botanical Garden, University of the Punjab, Lahore. All plants were acclimatized under greenhouse conditions prior to the application of elicitor treatments. The greenhouse was provided with controlled temperature, humidity, and light conditions, minimizing environmental variations and ensuring uniform plant growth.

2.2 Soil preparation and planting of seedlings

The soil used in the experiment consisted of garden soil, sand, and well-decomposed organic compost mixed in a ratio of 1:1:1. This mixture ensured proper aeration, adequate drainage, and sufficient nutrient availability for optimal plant growth. The soil was thoroughly mixed to achieve homogeneity and sterilized (if applicable) to eliminate pathogens and contaminants. A total of 96 earthen pots (with three drainage holes the bottom) of equal size were filled with the prepared soil mixture. Each pot was labeled according to species and treatment. Uniform seedlings were transplanted into each pot, ensuring proper root coverage and firm establishment. All pots were placed in the greenhouse under natural light conditions. Regular irrigation was carried out to maintain optimal soil moisture, and standard agronomic practices were followed to ensure healthy plant growth.

2.3 Preparation of stock and working solutions

Stock solutions of Salicylic acid (SA) and Methyl Jasmonate (MeJA) were prepared using analytical-grade chemicals and distilled water. The required quantities were accurately weighed using a digital balance and dissolved before making desired volume. Working solutions were prepared by diluting the stock solutions. Salicylic acid solutions were prepared at concentrations of 0.5, 1, and 1.5 mM. Similarly, methyl jasmonate solutions were prepared according to the experimental design. All solutions were freshly prepared prior to application to ensure effectiveness. Proper labeling and storage were maintained to avoid contamination and degradation.

2.4 Treatment and layout of experiment

The experiment was conducted using a Completely Randomized Design (CRD) under greenhouse conditions. A total of 96 pots were used, comprising four *Ocimum* species, eight treatments, and three replicates per treatment. The treatments were T0: Control (distilled water only), T1: Salicylic acid (0.5 mM), T2: Salicylic acid (1.0 mM), T3: Salicylic acid (1.5 mM), T4: Methyl jasmonate (1µM), T5: MeJA + SA (0.5 mM+1µM), T6: MeJA + SA (1.0 mM+1µM), T7: MeJA + SA (1.5 mM+1µM).

Plants were allowed to grow for two months before elicitors application. Each pot received 100 mL of the respective treatment solution twice at an interval of 15 days. Control plants received distilled water instead of elicitors. A second application was carried out after 15 days to enhance treatment effects. The layout ensured uniform exposure of all plants to greenhouse conditions.

2.5 Manual thinning

Manual thinning was performed after seedling estab and weak seedlings were carefully removed, leaving healthy and vigorous plants. This practice reduced competition for nutrients, water, and light and improved air circulation, thereby minimizing disease risk.

2.6 Data collection for morphological parameters

Data collection was conducted after completion of treatment applications at 30 day of experiment. Morphological parameters were recorded to evaluate the effects of salicylic acid and methyl jasmonate on plant growth. All measurements were taken using standard instruments to ensure accuracy and reproducibility.

2.6.1 Number of side branches

The number of side branches per plant was recorded by counting all fully developed lateral branches emerging from the main stem.

2.6.2 Plant height

Plant height was measured from the soil surface to the tip of the main shoot using a measuring scale and recorded in centimeters (cm).

2.6.3 Shoot and root length

After harvesting, plants were carefully uprooted. Shoot length and root length were measured using a ruler.

2.7 Data collection for biochemical parameters

2.7.1 Estimation of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was determined using a photochemical method based on the inhibition of nitro blue tetrazolium (NBT) reduction by following methodology of Maral et al., (1977). The reaction mixture consisted of phosphate buffer, methionine, NBT, riboflavin, and enzyme extract. The reaction was initiated by exposing the mixture to light, which resulted in the formation of blue formazan due to NBT reduction. The presence of SOD inhibits this reaction by scavenging superoxide radicals. After a defined illumination period, absorbance was measured at 560 nm using a spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT under assay conditions. The enzyme activity was expressed units per gram of fresh weight (U mg^{-1} FW). This assay is important for evaluating the antioxidant defense mechanism of plants under stress conditions.

2.7.2 Estimation of peroxidase (POD) activity

Peroxidase (POD) activity was measured using guaiacol as a substrate by following the method of Luck (1974). The reaction mixture included phosphate buffer, guaiacol, hydrogen peroxide, and enzyme extract. The reaction was initiated by the addition of hydrogen peroxide, leading to the oxidation of guaiacol and formation of a brown-colored product. The increase in absorbance was monitored at 470 nm using a spectrophotometer over a fixed time interval. Peroxidase activity was calculated based on the rate of change in absorbance. One unit of enzyme activity was defined as the amount of enzyme required to cause a change of 0.01 in absorbance per minute. The results were expressed as units per gram of fresh weight (U mg^{-1} FW). This method reflects the role of peroxidase in detoxifying reactive oxygen species and enhancing plant stress tolerance.

2.7.3 Determination of total phenolic content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method with slight modifications Jakobek et at. (2007). Fresh leaf extract was used for the analysis. An aliquot of the extract was mixed with diluted Folin–Ciocalteu reagent and allowed to react for a few minutes. Subsequently, sodium carbonate solution was added to the mixture to develop the color. The reaction mixture was incubated at room temperature for proper color development. The absorbance was then measured at 765 nm using a spectrophotometer. Gallic acid was used as a standard to construct a calibration curve. The total phenolic content was expressed as milligrams of Gallic acid equivalents per gram of fresh weight (mg GAE g^{-1} FW). This method provides a reliable estimation of phenolic compounds, which play an important role in plant defense and antioxidant activity.

2.7.4 Determination of total flavonoid content (TFC)

Total flavonoid content was estimated by following the method of Ivanova et al. (2011) using the aluminum chloride colorimetric method. A measured volume of leaf extract was mixed with distilled water followed by the addition of sodium nitrite solution. After a short incubation period, aluminum chloride solution was added to the mixture. Subsequently, sodium hydroxide was added to develop the characteristic color, and the total volume was adjusted with distilled water. The mixture was thoroughly mixed, and absorbance was recorded at 510 nm using a spectrophotometer. Quercetin was used as a standard for calibration, and the flavonoid content was expressed as milligrams of quercetin equivalents per gram of fresh weight (mg QE g^{-1} FW). This method is widely used to quantify flavonoids, which contribute significantly to antioxidant properties in plants.

2.7.5 Determination of total soluble protein content

Total soluble protein was estimated by following the method of Racusen and Johnstone (1961) using the Bradford assay. Fresh leaf extracts were prepared in phosphate buffer and centrifuged to obtain clear supernatant. The extract was mixed with Bradford reagent containing Coomassie Brilliant Blue dye and incubated for color development. Absorbance was measured at 595 nm, using bovine serum albumin (BSA) as the standard. Protein content was expressed as mg per gram fresh weight (mg g^{-1} FW). This method is widely used for rapid and sensitive protein quantification in plant tissues.

Protein content: CV × TE / EU × WT × 1000

Where, CV= Curve value
TE= Total extract
EU= Extract used
Wt = Fresh weight of sample tissue

2.7.6 Determination of total chlorophyll content

Total chlorophyll content was measured following Arnon's (1949) method. Fresh leaves were homogenized in 80% acetone and centrifuged to obtain the extract. Absorbance was recorded at 645 nm, 653nm and 663 nm. Chlorophyll concentration was calculated using Arnon's equations and expressed as mg per gram fresh weight (mg g^{-1} FW). Chlorophyll content serves as an-important indicator of photosynthetic efficiency and plant health under varying environmental conditions.

3. Results

3.1 Effect of methyl jasmonate and salicylic acid on morphology of *O. basilicum*

The effect of methyl jasmonate and salicylic acid on the morphological traits of *O. basilicum* grown in field conditions was assessed. Measurements of shoot length, leaf count, and stem diameter were recorded at the end of the treatment period.

3.1.1 Stem diameter

The effect of methyl jasmonate and salicylic acid on stem diameter of *O. basilicum* is presented in Table 1 and Figure 1. Results indicate that elicitor supplementation significantly ($P \leq 0.05$) increased stem diameter compared to the untreated control. The control treatment (T0) recorded the lowest stem diameter (0.20 cm). Among the treated cultures, T1, T2, T3, T4, T5, and T6 exhibited the highest stem diameter (0.30 cm), while T7 showed a comparatively lower stem diameter (0.27 cm).

3.1.2 Shoot length

Results revealed that elicitor treatments significantly ($P \leq 0.05$) enhanced the shoot elongation as compared to the control treatment. The control treatment (T0) recorded a shoot length of 16 cm. Among the treated cultures, T4 exhibited the maximum shoot length (31 cm), followed by T7 (24 cm). Treatments T5 and T6 showed comparable shoot lengths of 19 cm, while T1 recorded 19 cm. Treatments T2 and T3 exhibited comparatively lower shoot lengths of 12 cm and 13 cm, respectively.

3.1.3 Number of leaves

Results revealed significant differences ($P \leq 0.05$) among the treatments. The control group (T0) showed the lowest number of leaves, measuring 16 cm. Among the treated cultures, T4 showed the highest number of leaves (25 cm), followed by T7 (23 cm) and T1 (21 cm). Treatments T5 and T6 recorded 20 cm leaves, while T2 and T3 showed comparatively lower values of 18 cm each.

Total chlorophyl (mg/g) = 20.2(A645) + 8.02(A663) × V / 1000 × W

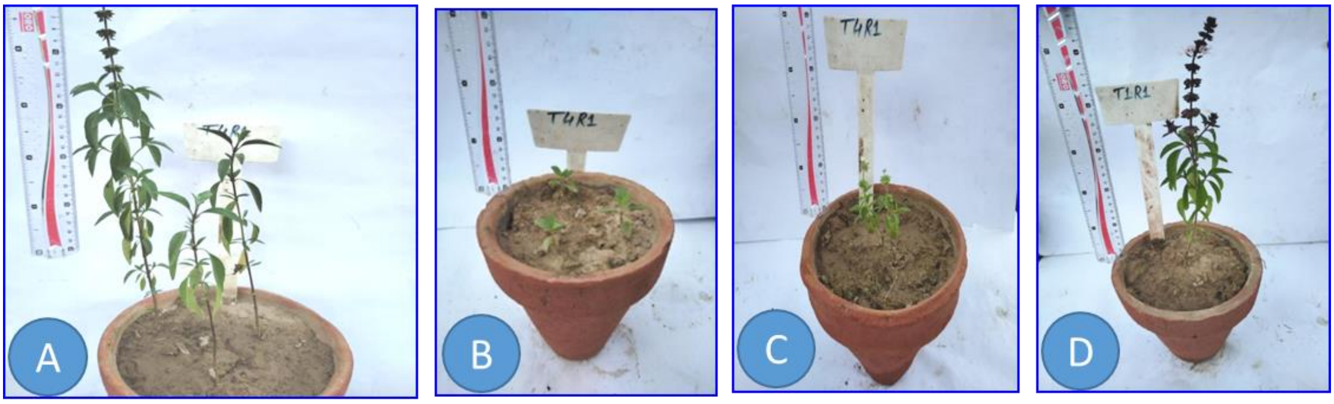

Figure 1. Representative photographs showing the effect of Methyl Jasmonate (T4: 1 µM) on the morphological characteristics of basil plants. Images represent one replicate from each treatment (T0–T7) recorded at the end of the experimental period (30 days).

Table 1. Effect of Methyl Jasmonate and Salicylic acid on different shoot parameters of basil species after 30 days of experimentation.

Treat-ments	*O. basilicum*				*O. sanctum*			*Lemon* basil			*Cinnamon* basil		
	SD (cm)	SL (cm)	NL		SD (cm)	SL (cm)	NL	SD (cm)	SL (cm)	NL	SD (cm)	SL (cm)	NL
T0	0.21	15.63	16.00		155.87	0.20	21.29	0.20	04.80	07.00	0.20	18.00	12.33
T1	0.32	18.60	21.00		140.23	0.30	13.99	0.20	05.20	06.33	04.63	25.00	14.00
T2	0.33	11.40	17.33		175.45	0.30	23.21	0.30	06.20	10.33	0.30	18.33	14.00
T3	0.32	12.60	17.00		116.80	0.23	13.07	0.30	10.20	14.00	0.30	28.00	16.33
T4	0.33	31.00	25.00		133.53	0.30	17.92	0.30	09.80	11.00	0.30	19.00	14.00
T5	0.34	18.30	20.00		165.08	0.20	9.88	0.20	07.33	11.00	0.40	31.00	19.00
T6	0.32	19.00	20.33		96.628	0.20	17.92	0.30	06.70	10.00	0.30	21.00	14.00
T7	0.2667	24.00	22.33		147.64	0.30	10.92	0.20	07.10	11.00	0.40	26.00	15.00

T0= Control (No Treatment); T1, T2 and T3 = different concentrations of salicylic acid, T4 methyl jasmonate and T5, T6, T7 = different concentrations of salicylic acid + methyl jasmonate. SD (stem diameter), SL (shoot length), NL (number of leaves). ± (SE). Different letters along the means represent statistical analysis according to Duncan's Multiple Range Test at $p > 0.05$.

3.2 Effect of methyl jasmonate and salicylic acid on morphological parameters of *O. sanctum*

The effect of methyl jasmonate and salicylic acid on the morphological traits of *O. sanctum* was assessed under greenhouse conditions following the treatment period. Measurements of stem diameter and shoot length were taken. Results indicated that elicitor application significantly enhanced the vegetative growth of *O. sanctum* plants compared to the untreated controls (Table 1).

3.2.1 Stem diameter

Results revealed significant ($P \leq 0.05$) variations among treatments. The control treatment (T0) recorded the minimum stem diameter of 0.10 cm. Among elicitor treated plants, T1, T2, T3, T4, T5 and T6 showed relatively higher stem diameter values, ranging from 0.20 to 0.30 cm. However, treatment T7 exhibited the maximum stem diameter of 0.40 cm.

3.2.2 Shoot length

Results revealed that elicitor treatments significantly ($P \leq 0.05$) enhanced shoot elongation compared to the control treatment. The control treatment (T0) recorded the lowest shoot length of 8 cm. Among treated plants, T4 exhibited the maximum shoot length of 17 cm, followed by T5 and T6 with shoot lengths of 15 cm and 14 cm, respectively. Treatments T1 and T2 recorded shoot lengths of 10 cm and 11 cm, whereas T3 showed comparatively lower shoot length of 9 cm. Treatment T7 recorded shoot length of 12 cm.

3.2.3 Number of leaves

Results revealed that elicitor treatments significantly (P ≤ 0.05) enhanced the number of leaves compared to the control treatment. The control treatment (T5) recorded the lowest leaf number. Among treated plants, T2 showed the maximum leaf number of 23 cm, followed by T0 and T4 with numbers of leaves of 21 cm and 17cm, respectively. Treatments T1 and T3 recorded shoot lengths of 14 cm and 13 cm, whereas T5 showed a comparatively lower shoot length of 9 cm. Treatment T7 recorded shoot length of 10 cm.

3.3 Effect of methyl jasmonate and salicylic acid on morphological parameters of *Lemon* Basil

The effect of methyl jasmonate and salicylic acid on morphological parameters of the *Lemon* Basil was evaluated after completion of the treatment period. Data of number of leaves, shoot length and stem diameter were recorded. Results revealed that elicitor application significantly influenced vegetative growth compared to untreated control plants.

3.3.1 Stem diameter

Results showed significant differences among treatments (P ≤ 0.05). The control treatment (T0), T1, T5 and T7 recorded stem diameter values of 0.20 cm. Treatments T2, T3, T4 and T6 exhibited a maximum stem diameter of 0.30 cm (Table 1).

3.3.2 Shoot length

Results revealed that elicitor treatments significantly (P ≤ 0.05) affected shoot elongation compared to the control treatment. The control treatment (T0) recorded the minimum shoot length of 5 cm. Among treated plants, T3 exhibited the maximum shoot length of 10 cm, followed by T4 with 9 cm. Treatments T5, T6 and T7 recorded shoot lengths ranging from 6 to 7 cm, whereas T1 and T2 showed comparatively lower values of 5 and 6 cm, respectively.

3.3.3 Number of leaves

Results revealed significant variations among treatments (P ≤ 0.05). The control treatment (T0) and T1 recorded the minimum number of leaves with values of 6 and 5 leaves, respectively. Among treated plants, T3 exhibited the maximum number of leaves (14), followed by T4, T5 and T7 with 11 leaves each. Treatments T2 and T6 recorded 10 leaves each.

3.4 Effect of methyl jasmonate and salicylic acid on morphological parameters of *Cinnamon* basil

The effect of methyl jasmonate and salicylic acid on morphological parameters of *Cinnamon* basil was evaluated after completion of the treatment period. Data regarding shoot length, number of leaves and stem diameter were recorded. Results revealed that elicitor supplementation significantly influenced vegetative growth compared to untreated control plants.

3.4.1 Stem diameter

Results revealed significantly (P ≤ 0.05) enhanced stem diameter among treated plants compared to the control treatment. The control treatment (T0) recorded the minimum stem diameter of 0.20 cm. Among treated plants, T1 exhibited the maximum stem diameter of 0.60 cm. Treatments T5 and T7 recorded stem diameter values of 0.40 cm, whereas T2, T3, T4 and T6 showed comparatively similar values of 0.30 cm (Table 1).

3.4.2 Shoot length

Results revealed significant (P ≤ 0.05) variations among treatments. The control treatment (T0) recorded shoot length of 20 cm. Among treated plants, T5 exhibited the maximum shoot length of 31 cm, followed by T3 with 28 cm and T7 with 26 cm. Treatments T1 and T6 recorded shoot lengths of 25 cm and 21 cm, respectively, whereas T2 and T4 showed comparatively lower values of 20 cm each.

3.4.3 Number of leaves

Results revealed significant (P ≤ 0.05) differences among treatments. The control treatment (T0) recorded the minimum number of leaves (12). Among treated plants, T5 exhibited the maximum number of leaves (19), followed by T3 with 16 leaves. Treatments T1, T2 and T7 recorded 14 leaves each, whereas T4 and T6 showed 13 leaves each plants.

3.5 Biochemical parameters of *O. basilicum*

3.5.1 Superoxide dismutase (SOD) activity

Table 2 illustrates the effect of methyl jasmonate and salicylic acid on superoxide dismutase (SOD) activity in *O. basilicum*. Results indicated that treatment with these elicitors significantly (P ≤ 0.05) increased SOD activity compared to the untreated control. The control treatment (T0) recorded the lowest SOD activity (10 U mg^{-1}). Among the treated cultures, T2 exhibited the highest SOD activity (74 U mg^{-1}), followed by T6 (30 U mg^{-1}). Treatments T1, T3, and T7 recorded comparable SOD activities of 18 U mg^{-1}, while T5 showed 15 U mg^{-1}. Treatment T4 exhibited comparatively lower SOD activity (11 U mg^{-1}).

3.5.2 Peroxidase (POD) activity

Results revealed that treatment with these elicitor significantly (P ≤ 0.05) increased POD activity as compared to the control treatment. The untreated cultures (T0) recorded the lowest POD activity (47 U mL^{-1}). Among the treated cultures, T4 exhibited the highest POD activity (168 U mL^{-1}), followed by T2 (156 U mL^{-1}) and T1 (141 U mL^{-1}). Treatments T3, T5, and T6 recorded comparable POD activities of 117 U mL^{-1}, whereas T7 showed comparatively lower POD activity (94 U mL^{-1}).

3.5.3 Total phenolic contents

Figure 4.16 shows the effect of methyl jasmonate and salicylic acid on the total phenolic content (TPC) of *Ocimum* basil*icum*. Results demonstrated that elicitor treatment significantly (P ≤ 0.05) increased total phenolic content compared to the untreated control. The control group (T0) had a total phenolic content of 1.68 mg GAE g^{-1}, while among the treated samples, T1 showed the highest phenolic content at 1.81 mg GAE g^{-1}, followed by T2 with 1.74 mg GAE g^{-1}. Treatments T4, T5, and T6 recorded comparable phenolic contents ranging from 1.71 to 1.73 mg GAE g^{-1}, while T3 and T7 showed comparatively lower values of 1.69 mg GAE g^{-1} and 1.64 mg GAE g^{-1}, respectively.

3.5.4 Total flavonoid contents

Table 3 illustrates the influence of methyl jasmonate and salicylic acid on the total flavonoid content (TFC) of *O.* basil*icum*. The findings showed that elicitor treatment significantly (P ≤ 0.05) increased flavonoid content in treated plants as compared to the control. The control group (T0) had a flavonoid content of 0.0098 mg QE g^{-1}. Among the treated groups, T7 showed the highest flavonoid content at 0.0102 mg QE g^{-1}, followed by T2 with 0.0099 mg QE g^{-1}. Treatments T1, T4, T5, and T6 had similar flavonoid contents ranging from 0.0089 to 0.0095 mg QE g^{-1}, while T3 recorded the lowest value of 0.0088 mg QE g^{-1}.

3.5.5 Total soluble protein contents

The impact of methyl jasmonate and salicylic acid on total soluble protein content in *O.* basil*icum* is presented in Figure 4.18. Results indicated that the treatment with these elicitor significantly (P ≤ 0.05) enhanced protein accumulation compared to untreated plants. The control treatment (T0) registered a protein content of 14.2 mg g^{-1}. Among treated cultures, T1 recorded the highest protein content at 15.8 mg g^{-1}, followed by T5 with 15.0 mg g^{-1}. Treatments T3, T4, T6, and T7 showed similar protein levels between 14.8 and 15.0 mg g^{-1}, while T2 had a lower protein content of 14.3 mg g^{-1}.

3.5.6 Total chlorophyll contents

The effect of methyl jasmonate and salicylic acid on total chlorophyll content in *O.* basil*icum* is also shown in figure 5.9. Results revealed that elicitor application significantly (P ≤ 0.05) increased chlorophyll content as compared to the untreated control. The control group (T0) had a chlorophyll content of 1.02 mg g^{-1}. Among treatments, T7 showed the highest chlorophyll content at 1.58 mg g^{-1}, followed by T4 and T5, each with 1.20 mg g^{-1}.

Treatments T1 and T2 had similar chlorophyll contents of 1.12 mg g^{-1}, whereas T3 and T6 showed slightly lower values of 1.15 mg g^{-1} and 1.16 mg g^{-1}, respectively.

3.6 Biochemical parameters of *O. sanctum*

3.6.1 Superoxide dismutase (SOD) activity in *O. sanctum*

The effect of methyl jasmonate and salicylic acid on superoxide dismutase (SOD) activity of *O. sanctum* under greenhouse conditions is presented in Table 3. Results revealed that elicitor application significantly (P ≤ 0.05) enhanced SOD activity as compared to untreated control plants. The control treatment (T0) recorded the lowest SOD activity of 12 U mg^{-1}. Among treated plants, T2 exhibited the highest SOD activity of 80 U mg^{-1}, followed by T6 with 36 U mg^{-1}. Treatments T1, T3 and T7 showed comparatively similar SOD activities ranging from 18 to 22 U mg⁻¹, whereas T4 and T5 recorded lower values of 14 and 17 U mg⁻¹, respectively.

Table 2. Effect of Methyl Jasmonate and Salicylic acid on different biochemical parameters of *O. basilicum* after 30 days of experimentation.

Treatment	SOD (U/mg)	POD (U/mg)	Flav (mgQE/g)	Phen (mGAE/g)	Pro (mg/g)
T0	09.62	46.67	0.0097	1.66	14.15
T1	17.04	140.23	0.0092	1.80	15.80
T2	73.60	155.87	0.0098	1.72	14.39
T3	17.92	116.80	0.0088	1.68	14.88
T4	10.66	168.13	0.0095	1.71	14.97
T5	14.46	116.80	0.0088	1.69	15.12
T6	30.08	116.80	0.0086	1.70	15.02
T7	00.26	24.00	0.0101	1.63	14.92

T0= Control (No Treatment); T1, T2 and T3 = different concentrations of salicylic acid; T4 methyl jasmonate; and T5, T6, T7 = different concentrations of salicylic acid + methyl jasmonate. SOD (superoxide dismutase), POD (peroxidase), ± (SE). Different letters along the means represent statistical analysis according to Duncan's Multiple Range Test at $p > 0.05$.

Table 3. Effect of Methyl Jasmonate and Salicylic acid on different biochemical parameters of *O. sanctum* after 30 days of experimentation.

Treatment	SOD (U/mg)	POD (U/mg)	Flav (mgQE/g)	Phen (mGAE/g)	Pro (mg/g)
T0	0.0094	1.60	28.40	2.70	5.00
T1	0.0046	1.68	27.93	3.20	9.66
T2	0.0092	1.74	27.83	3.80	11.66
T3	0.0045	1.73	28.31	2.46	6.33
T4	0.0083	1.75	28.31	2.10	7.00
T5	0.0096	1.63	28.78	1.40	5.00
T6	0.0100	1.71	28.59	1.80	7.00
T7	0.0091	1.68	27.55	2.76	10.00

T0= Control (No Treatment); T1, T2 and T3 = different concentrations of salicylic acid, T4 methyl jasmonate and T5, T6, T7 = different concentration of salicylic acid + methyl jasmonate. Different letters along means represents statistical analysis according to Duncan' Multiple Range Test at $p > 0.05$.

3.6.2 Peroxidase (POD) activity in *O. sanctum*

Results revealed significantly ($P \leq 0.05$) enhanced POD activity among elicitor treated plants compared to the control treatment. The untreated control plants (T0) recorded the minimum POD activity of 55 U mL⁻¹. Among treated plants, T4 showed the maximum POD activity of 180 U mL⁻¹, followed by T2 and T1 with values of 165 U mL⁻¹ and 148 U mL⁻¹, respectively. Treatments T3, T5 and T6 recorded comparatively similar POD activities ranging from 120 to 128 U mL⁻¹, whereas, T7 showed comparatively lower POD activity such as 100 U mL⁻¹.

3.6.3 Total phenolic contents in *O. sanctum*

Results revealed that elicitor supplementation significantly enhanced phenolic accumulation compared to untreated control plants ($P \leq 0.05$). The control treatment (T0) recorded phenolic content of 1.50 mg GAE g⁻¹. Among treated plants, T1 exhibited the highest phenolic content of 1.92 mg GAE g⁻¹, followed by T2 with 1.84 mg GAE g^{-1}. Treatments T4, T5 and T6 recorded comparatively similar phenolic contents, ranging from 1.76 to 1.80 mg GAE g^{-1}, while T3 and T7 showed comparatively lower values of 1.70 and 1.65 mg GAE g^{-1}, respectively.

3.6.4 Total flavonoid contents in *O. sanctum*

The effect of methyl jasmonate and salicylic acid on total flavonoid content (TFC) of *O. sanctum* under greenhouse conditions is shown in Figure 5.22. Results revealed ($P \leq 0.05$) enhanced flavonoid accumulation among elicitor-treated plants compared to the control treatment. The control treatment (T0) recorded flavonoid content of 0.0085 mg QE g^{-1}. Among treated plants, T7 showed the highest flavonoid content of 0.0110 mg QE g^{-1}, followed by T2 with 0.0101 mg QE g^{-1}. Treatments T1, T4, T5 and T6 recorded comparatively similar flavonoid contents, ranging from 0.0090 to 0.0097 mg QE g^{-1}, whereas T3 exhibited the lowest flavonoid content of 0.0087 mg QE g^{-1}.

3.6.5 Total soluble protein contents in *O. sanctum*

Results revealed that elicitor application significantly ($P \leq 0.05$) enhanced protein accumulation compared to untreated control plants. The control treatment (T0) recorded protein content of 13.5 mg g^{-1}. Among treated plants, T1 exhibited the maximum protein content of 16.2 mg g^{-1}, followed by T5 with 15.4 mg g^{-1}. Treatments T3, T4, T6 and T7 showed comparatively similar protein contents, ranging from 14.9 to 15.3 mg g^{-1}, whereas T2 recorded comparatively lower protein content of 14.1 mg g^{-1}.

3.6.6 Total chlorophyll content in *O. sanctum*

Results revealed that elicitor application significantly ($P \leq 0.05$) enhanced chlorophyll accumulation compared to the untreated control plants. The control treatment (T0) recorded chlorophyll content of 0.95 mg g^{-1}. Among treated plants, T7 exhibited the highest chlorophyll content of 1.62 mg g^{-1}, followed by T4 and T5 with values of 1.25 mg g^{-1} and 1.22 mg g^{-1}, respectively. Treatments T1 and T2 recorded chlorophyll contents of 1.15 mg g^{-1} and 1.18 mg g^{-1}, whereas T3 and T6 showed comparatively lower values of 1.12 mg g^{-1} and 1.16 mg g^{-1}, respectively.

3.7 Biochemical parameters in Lemon Basil

3.7.1 Superoxide dismutase (SOD) activity in Lemon Basil

The effect of methyl jasmonate and salicylic acid on superoxide dismutase (SOD) activity of Lemon Basil is presented in Table 4. Results revealed ($P < 0.05$) significantly enhanced SOD activity among elicitor-treated plants as compared to untreated control plants. The control treatment (T0), T4 and T6 recorded the lowest SOD activity of 10 U mg^{-1}. Among treated plants, T5 exhibited the maximum SOD activity of 20 U mg^{-1}, followed by T1 and T3 with values of 19 U mg^{-1} each. Treatment T7 recorded SOD activity of 16 U mg^{-1}, whereas T2 showed comparatively lower SOD activity of 9 U mg^{-1}.

3.7.2 Peroxidase (POD) activity in Lemon Basil

Results revealed significant ($P \leq 0.05$) variations in POD activity among treatments. The control treatment (T0) recorded POD activity of 295 U mL^{-1}. Among treated plants, T4 and T7 exhibited the maximum POD activity of 350 U mL^{-1}, followed by T6 with 330 U mL^{-1} and T5 with 310 U mL^{-1}. Treatments T1 and T2 recorded POD activities of 250 and 265 U mL^{-1}, respectively, whereas T3 showed the minimum POD activity of 220 U mL^{-1}.

3.7.3 Total phenolic contents in Lemon Basil

Results revealed significant ($P \leq 0.05$) enhanced phenolic content among elicitor treated plants compared to the control treatment. The control treatment (T0) recorded phenolic content of 3.30 mg GAE g^{-1}. Among treated plants, T2 exhibited the maximum phenolic content of 3.56 mg GAE g^{-1}, followed by T7 with 3.40 mg GAE g^{-1}. Treatments T1, T4, T5 and T6 showed comparatively similar phenolic contents ranging from 3.28 to 3.35 mg GAE g^{-1}, whereas T3 recorded comparatively lower phenolic content of 3.22 mg GAE g^{-1}.

3.7.4 Total flavonoid content in Lemon Basil

The effect of methyl jasmonate and salicylic acid on total flavonoid content (TFC) of Lemon Basil.. Results revealed significant ($P \leq 0.05$) variations in flavonoid accumulation among treatments. The control treatment (T0) recorded flavonoid content of 0.017 mg QE g^{-1}. Among treated plants, T5 exhibited the maximum flavonoid content of 0.021 mg QE g^{-1}, followed by T7 with 0.019 mg QE g⁻¹. Treatments T1, T6 and T4 recorded flavonoid contents ranging from 0.014 to 0.016 mg QE g⁻¹, whereas T2 and T3 exhibited comparatively lower values of 0.014 and 0.013 mg QE g⁻¹, respectively.

Table 4. Effect of Methyl Jasmonate and Salicylic acid on different biochemical parameters of Lemon basil after 30 days of experimentation.

Treat-ment	SOD (U/mg)	POD (U/mg)	Flav (mgQE/g)	Phen (mGAE/g)	Pro (mg/g)
T0	9.88	295.28	0.0174	3.30	56.81
T1	19.13	254.89	0.0179	3.28	54.15
T2	9.11	267.07	0.0145	3.58	54.15
T3	18.83	224.23	0.0137	3.23	23.21
T4	9.88	350.91	0.0140	3.27	53.96
T5	20.05	311.75	0.0209	3.31	48.65
T6	9.88	330.17	0.0161	3.33	56.81
T7	15.88	350.91	0.0197	3.41	22.83

T0= Control (No Treatment); T1, T2 and T3 = different concentrations of salicylic acid, T4 methyl jasmonate and T5, T6, T7 = different concentration of salicylic acid + methyl jasmonate. Different letters along means represents statistical analysis according to Duncan' Multiple Range Test at p > 0.05.

3.7.5 Total soluble protein contents in Lemon Basil

Results revealed significant ($P \leq 0.05$) differences among treatments. The control treatment (T0) recorded protein content of 56 mg g⁻¹. Among treated plants, T6 exhibited the highest protein content of 56 mg g⁻¹, followed by T1, T2 and T4 with values ranging from 53 to 54 mg g⁻¹. Treatment T5 recorded 48 mg g⁻¹ protein content, whereas T3 and T7 exhibited the lowest values of 23 mg g⁻¹ each.

3.7.6 Total chlorophyll contents in Lemon Basil

Results revealed that elicitor application significantly ($P \leq 0.05$) enhanced chlorophyll accumulation compared to untreated control plants. The control treatment (T0) recorded chlorophyll content of 1.20 mg g⁻¹. Among treated plants, T3 exhibited the highest chlorophyll content of 1.27 mg g⁻¹, followed by T1, T2, T5 and T6 with values ranging from 1.21 to 1.24 mg g⁻¹. Treatment T7 recorded chlorophyll content of 1.16 mg g⁻¹, while T4 showed the lowest chlorophyll content among treated plants with 1.15 mg g⁻¹.

3.8 Biochemical parameters of *Cinnamon* basil

3.8.1 Superoxide dismutase (SOD) activity in *Cinnamon* basil

The effect of methyl jasmonate and salicylic acid on superoxide dismutase (SOD) activity of *Cinnamon* basil is presented in Figure 4.31. Results revealed significantly ($P \leq 0.05$) enhanced SOD activity among elicitor treated plants compared to untreated control plants. The control treatment (T0) recorded the minimum SOD activity of 5 U mg⁻¹. Among treated plants, T2 exhibited the maximum SOD activity of 20 U mg⁻¹, followed by T1 with 16 U mg⁻¹ and T7 with 14 U mg⁻¹. Treatments T5 and T6 recorded SOD activities of 11 and 8 U mg⁻¹, respectively, whereas T3 and T4 showed comparatively lower values of 7 U mg⁻¹ each.

3.8.2 Peroxidase (POD) activity in *Cinnamon* basil

The effect of methyl jasmonate and salicylic acid on peroxidase (POD) activity of *Cinnamon* basil is shown in table 5. Results revealed significant ($P \leq 0.05$) variations among treatments. The control treatment (T0) recorded POD activity of 220 U mL⁻¹. Among treated plants, T2 and T3 exhibited the maximum POD activity of 330 U mL⁻¹, followed by T4 with 290 U mL⁻¹. Treatments T1, T5 and T7 recorded POD activities ranging from 260 to 270 U mL⁻¹, whereas T6 showed comparatively lower POD activity of 240 U mL⁻¹.

3.8.3 Total phenolic contents in *Cinnamon* basil

Results revealed ($P \leq 0.05$) enhanced phenolic accumulation among elicitor-treated plants compared to the control treatment. The control treatment (T0) recorded phenolic content of 3.40 mg GAE g⁻¹. Among treated plants, T1 exhibited the highest phenolic content of 3.90 mg GAE g^{-1}, followed by T7 with 3.80 mg GAE g^{-1} and T2 with 3.70 mg GAE g^{-1}. Treatments T3 and T4 recorded phenolic contents of 3.40 mg GAE g^{-1}, whereas T5 showed the lowest phenolic content of 3.20 mg GAE g^{-1}.

Table 5. Effect of Methyl Jasmonate and Salicylic acid on different biochemical parameters of *Cinnamon* basil after 30 days of experimentation.

Treat-ments	SOD (U/mg)	POD (U/mg)	Flav (mgQE/g)	Phen (mGAE/g)	Pro (mg/g)
T0	4.24	215.59	0.0202	3.34	59.85
T1	16.45	254.89	0.0155	3.83	57.95
T2	-19.48	330.17	0.0216	3.73	56.62
T3	6.37	330.17	0.0205	3.41	54.15
T4	6.37	295.28	0.0211	3.40	56.43
T5	11.45	254.89	0.0202	3.26	54.72
T6	7.85	233.60	0.0203	3.72	57.95
T7	13.90	267.07	0.0188	3.78	48.65

T0= Control (No Treatment); T1, T2 and T3 = different concentrations of salicylic acid, T4 methyl jasmonate and T5, T6, T7 = different concentration of salicylic acid + methyl jasmonate. Different letters along means represents statistical analysis according to Duncan' Multiple Range Test at p > 0.05.

3.8.4 Total flavonoid contents in *Cinnamon* basil

Results revealed significant ($P \leq 0.05$) variations in flavonoid accumulation among treatments. The control treatment (T0) recorded flavonoid content of 0.020 mg QE g^{-1}. Among treated plants, T2 exhibited the highest flavonoid content of 0.022 mg QE g^{-1}, followed by T4 with 0.021 mg QE g^{-1} and T3 with 0.020 mg QE g^{-1}. Treatments T5 and T6 recorded flavonoid contents of 0.020 mg QE g^{-1}, whereas T1 and T7 exhibited comparatively lower values of 0.016 mg QE g^{-1} and 0.019 mg QE g^{-1}, respectively.

3.8.5 Total soluble protein contents in *Cinnamon* basil

Results revealed significant ($P \leq 0.05$) differences among treatments. The control treatment (T0) recorded the highest protein content of 60 mg g^{-1}. Among treated plants, T1 exhibited protein content of 58 mg g^{-1}, followed by T6 with 57 mg g^{-1} and T2 and T4 with 56 mg g^{-1} each. Treatment T5 recorded protein content of 55 mg g^{-1}, whereas T7 exhibited the lowest protein content of 49 mg g^{-1}. Findings indicate elicitors accumule proteins in *Cinnamon* basil.

3.8.6 Total chlorophyll content in *Cinnamon* basil

Results revealed significant ($P \leq 0.05$) variations in chlorophyll accumulation among treatments. The control treatment (T0) recorded chlorophyll content of 1.30 mg g^{-1}. Among treated plants, T4 exhibited the highest chlorophyll content of 1.33 mg g^{-1}. Treatments T2 and T3 also showed comparatively higher chlorophyll contents of 1.32 mg g^{-1} each. Treatments T1, T5 and T6 recorded chlorophyll contents ranging from 1.28 to 1.30 mg g^{-1}, whereas T7 exhibited the lowest chlorophyll content of 1.27 mg g^{-1}. T.

4. Discussion

The present study was to determine how methyl jasmonate and salicylic acid effect on morphological, physiological and biochemical attributes of different *Ocimum* species including *Ocimum* basil*icum*, *O. sanctum*, lemon basil and cinnamon basil. Elicitors act as signaling molecules that regulate plant defense mechanisms and stimulate the biosynthesis of valuable secondary metabolites in medicinal plants. In the present investigation, different concentrations of methyl jasmonate and salicylic acid significantly affected growth performance, antioxidant enzyme activities, chlorophyll accumulation, protein content and phytochemical production in all studied *Ocimum* species. The enhancement observed in treated plants indicates that elicitor application successfully activated several physiological and biochemical pathways responsible for improved plant metabolism and secondary metabolite synthesis. Similar findings have been reported in medicinal and aromatic plants where methyl jasmonate and salicylic acid enhanced plant defense responses and phytochemical accumulation through elicitation mechanisms (Ahmad et al., 2021; Hasanuzzaman et al., 2020).

Morphological parameters including shoot length, stem diameter and number of leaves were considerably improved in elicitor treated plants compared to control plants. Enhanced vegetative growth observed in the present study may be associated with improved nutrient uptake, enhanced photosynthetic activity and regulation of endogenous plant hormones induced by salicylic acid and methyl jasmonate. Salicylic acid is widely reported to enhance cell division, cellular elongation and biomass accumulation, whereas methyl jasmonate regulates growth related metabolic pathways under stress and non-stress conditions. Improved shoot growth and stem development observed in the present study indicate that elicitor supplementation positively influenced physiological activities in the studied *Ocimum* species. Similar findings were reported by Elansary et al. (2022), who observed enhanced vegetative growth and biomass accumulation in basil plants treated with salicylic acid and methyl jasmonate. Likewise, Khan et al. (2021) observed improved growth parameters in medicinal plants following exogenous elicitor application due to enhanced metabolic efficiency and hormonal regulation.

Total chlorophyll content was significantly enhanced in response to methyl jasmonate and salicylic acid treatments across all investigated *Ocimum* species. Chlorophyll is an important photosynthetic pigment responsible for light harvesting and energy transfer during photosynthesis; therefore, increased chlorophyll concentration reflects improved photosynthetic efficiency and physiological stability. Salicylic acid and methyl jasmonate are known to protect chloroplast membranes, improve stomatal conductance and stimulate chlorophyll biosynthesis under various environmental conditions. Enhanced chlorophyll accumulation observed in treated plants may have contributed to improved growth and metabolic activities. Jahan et al. (2019) reported similar results, showing the salicylic acid dramatically improved the photosymthetic efficiency and chlorophyll content in aromatic plants. Moreover, methyl jasmonate has also been linked to increased photosynthetic pigments and improved plant productivity in medicinal species (Shahrajabian et al., 2020).

Superoxide dismutase (SOD) and peroxidase (POD) activities, which are antioxidant enzymes, were notably elevated in plants treated with elicitors compared to those that were not treated. Antioxidant enzymes play a vital role in the detoxification of reactive oxygen species produced during stress conditions and metabolic activities. The enhanced SOD and POD activities observed in the present investigation indicate activation of antioxidative defense systems in response to elicitor signaling. Both methyl jasmonate and salicylic acid are known to cause oxidative signaling pathways that stimulate production of antioxidant enzymes, thereby improving stress tolerance and cellular protection. Similar enhancement in antioxidant enzyme activities has been reported previously in medicinal plants following elicitor application. Increased POD and SOD activities may also be linked with enhanced biosynthesis of phenolic compounds and flavonoids because antioxidant metabolism and secondary metabolite production are closely interconnected. These findings suggest that elicitor application strengthened the antioxidative defense mechanism in the studied *Ocimum* species (Hasanuzzaman et al., 2020).

Phenolic compounds ranked as some of the most vital secondary metabolites because of their antioxidant, antimicrobial, and medicinal benefits. In the present study, total phenolic content significantly increased following methyl jasmonate and salicylic acid treatments in all investigated species. Enhanced phenolic accumulation may be associated with activation of the phenylpropanoid pathway and stimulation of phenylalanine ammonia lyase activity by elicitor signaling molecules. Salicylic acid and methyl jasmonate are widely recognized for their role in inducing secondary metabolite biosynthesis through regulation of defense-related pathways. Similar enhancement in phenolic content has been reported in basil and other medicinal plants treated with elicitors (Riaz et al., 2021). Enhanced phenolic accumulation observed in the present study indicates improved antioxidant potential and medicinal value of treated plants. Since phenolic compounds are directly associated with free radical scavenging activities, their enhancement may contribute to better therapeutic properties of *Ocimum* species. Flavonoid content also showed considerable enhancement in response to methyl jasmonate and salicylic acid treatments. Flavonoids are the key bioactive compounds that contribute to antioxidant defense, UV protection and stress adaptation in plants. The increased flavonoid accumulation observed in treated plants may be due to elicitor-induced activation of biosynthetic pathways and enhanced expression of flavonoid synthesizing enzymes. Methyl jasmonate has been extensively used as an effective elicitor for stimulation of flavonoid biosynthesis in medicinal plants. Similarly, salicylic acid enhances flavonoid accumulation by regulating stress responsive metabolic pathways. Comparable findings were reported by Kiani et al. (2022), who observed significant enhancement in flavonoid production in basil species treated with jasmonates and salicylic acid. Increased flavonoid concentration in the present study reflects enhanced phytochemical potential and improved medicinal significance of the investigated *Ocimum* species (Riaz et al., 2021).

Total soluble protein content was also significantly influenced by elicitor supplementation in the studied plants. Protein accumulation is considered an important indicator of physiological adaptation and metabolic activity in plants. Enhanced protein content observed in elicitor-treated plants may be due to increased synthesis of enzymatic proteins, defense-related proteins, and stress-responsive compounds. Salicylic acid and methyl jasmonate are known to regulate the expression of stress-associated genes and protein biosynthesis pathways. Similar enhancement in protein accumulation following elicitor application has been reported in medicinal plants. Increased protein content observed in the current study may also be associated with improved antioxidant defense systems and enhanced metabolic activities induced by elicitor treatments. (Hasanuzzaman et al., 2020).

Variations among different *Ocimum* species were also observed regarding their morphological and biochemical responses to methyl jasmonate and salicylic acid treatments. These differences may be attributed to species-specific genetic makeup, variations in metabolic pathways, and differential sensitivity toward elicitor concentrations. Each *Ocimum* species possesses distinct physiological and phytochemical characteristics that influence its response to external elicitation. However, overall results clearly demonstrated that methyl jasmonate and salicylic acid effectively enhanced growth, chlorophyll accumulation, antioxidant enzyme activities and phytochemical production in all studied species. Similar species-dependent responses have been documented previously in medicinal and aromatic plants subjected to elicitor treatments (Raza et al., 2022).

The current study's observation of increased phytochemical production validates the critical function of elicitors in plant biotechnology. Salicylic acid and Methyl jasmonate serve as signaling molecules that initiates the defence responses and stimulate the production of valuable secondary metabolites. Enhanced production of phenolics and flavinoids in treated plants could greatly boost the pharmaceutical, nutraceutical and therapeutic value of ocimum specie. Basil plants are mainly utilized in traditional medicine for to their antioxidants, antimicrobial, anti-inflammatory and therapeutic properties. Therefore, elicitor mediated enhancement of phytochemicals may contribute to the development of high-quality medicinal plant products and sustainable cultivation strategies. Similar conclusions were also reported by contemporary researchers who emphasized the significance of elicitor application for improving secondary metabolite formation in medicinal plants (Majdi et al., 2020; Riaz et al., 2021).

Conclusion

Overall, the present study demonstrated that methyl jasmonate and salicylic acid significantly enhanced morphological growth, antioxidant enzyme activities, and phytochemical accumulation in different *Ocimum* species. However, further investigations are required to explore the molecular and biochemical mechanisms involved in elicitor-mediated enhancement of secondary metabolites.

Acknowledgements Authors are highly acknowledged the Institute of Botany, University of the Punjab for providing all the necessity facilities required for this research work.

Author(s) contributions M.A & M.U perform the experimental work and wrote down initial draft, and Z.A.S conceived the idea, supervise the experimental work and proofread the final draft.

Conflict of interest The authors declare no conflict of interest.

Data availability All data supporting the findings of this study are available within the paper. We do not have any research data outside the submitted manuscript file.

Declarations

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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