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Research Article

Improvement of morphological, physiological, and antioxidant potential in purple basil (*Ocimum basilicum* L.) through exogenous treatments of salicylic acid and jasmonic acid

Zeynep E. DUVARCI[1], Huseyin TURKER[2]*, Bengu TURKYILMAZ UNAL[2]

[1]*Nigde Omer Halisdemir University, Graduate School of Natural and Applied Sciences, Biotechnology Department, Nigde, 51240, Turkiye; dytebruduvarci@gmail.com*
[2]*Nigde Ömer Halisdemir University, Science Faculty, Biotechnology Department, Nigde, 51240, Turkiye; huseyinturker@ohu.edu.tr (*corresponding authors); bturkyilmaz@ohu.edu.tr*

Abstract

The effects of exogenous jasmonic acid (JA) and salicylic acid (SA) treatments on purple basil, which is used in the food industry and traditional medicine for its aromatic properties, were investigated. Purple basil (*Ocimum basilicum* L.) seeds were sown in pots containing peat moss and germinated in a plant growth chamber at 25 ± 2 °C, under a photoperiod of 16 hours light/8 hours dark, at a light intensity of 27 µmol $m^{-2}s^{-1}$, and at 45 ± 5% humidity. When the seedlings reached the two-leaf stage, different concentrations (0, 0.5, and 1 mM) of SA and/or JA were treated foliarly. Fifteen days after the second application, the seedlings were harvested. Morphological measurements (root-shoot length, fresh and dry weight) and physiological-biochemical analyses were performed. Root-shoot length, fresh and dry weights, photosynthetic pigment, anthocyanin, and total phenolic content, along with 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, phenylalanine ammonia-lyase (PAL), and catalase (CAT) enzyme activities, increased in all treatment groups compared to the control, while total protein content decreased. It was determined that exogenously treated SA and JA elicitors positively affected the increase in morphological and physiological parameters, as well as the antioxidant potential, in purple basil.

Keywords: anthocyanin; basil; carotenoid; elicitor; growth and development; phenolic compound; signaling molecule

Introduction

The *Ocimum* species belonging to the Lamiaceae family are known as basil or sweet basil in Turkiye. Basil (*Ocimum basilicum* L.) is widely distributed in tropical and temperate regions of South Asia and is native to India. The *Ocimum* genus has over 65 species and is naturally distributed in Africa, Asia, and Central America (Dudai *et al*., 2020; Türkmen, 2021). It is cultivated as a crop plant in Italy, France, Spain, and the

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western and southern regions of Anatolia (Ceylan, 1997). It is a plant that is quite sensitive to cold and is more prevalent in dry and warm environments. It is a herbaceous, annual plant 20-60 cm tall. Its leaves are soft, light to dark green in color, 1-5 cm long, and 1-3 cm wide. Its flowers are white, pink, or purple. Purple basil gets its color from the accumulation of cyanidin, peonidin, and anthocyanin in its leaves and flowers.

Basil species are rich in various natural products (secondary metabolites) such as phenolic compounds, flavonoids, and anthocyanins. The importance of basil essential oils stems from their biological effects, including antimicrobial, antifungal, nematicidal, insecticidal, and antioxidant properties (Baydar, 2009). Purple basil has a higher antioxidant effect (Telci *et al*., 2005; Flanigan and Niemeyer, 2014; Szymanowska *et al*., 2015). Thanks to the volatile oils in purple basil, it is a valuable medicinal and aromatic plant for the pharmaceutical industry due to its insecticidal and antifungal biological properties. Purple basil is a potential natural food preservative, additive, or coloring agent for the food industry due to its high anthocyanin content (Simon *et al*., 1999; Adıgüzel *et al*., 2005).

The purpose of using medicinal and aromatic plants is to protect human health, prevent diseases, and treat illnesses. The pharmaceutical industry uses medicinal and aromatic plants for this purpose (Marshall, 2011). One such plant, purple basil, is also used in the fields of pharmacology, cosmetics, and the food industry due to its high secondary metabolite content (Telci *et al*., 2006).

Purple basil acts as a natural food preservative in the food industry during the preservation stage of foods due to its anthocyanin content. The addition of essential oils from medicinal and aromatic plants to ready-to-eat foods increases the shelf life of these foods by exhibiting antimicrobial effects (Telci, 2005). Basil is a powerful natural antioxidant due to its phenolic compounds, flavones, and tannins. Phenolic compounds constitute a large portion of natural antioxidants. Phenolics exhibit antioxidant effects by protecting substances in the structure of foods that are easily oxidized from oxidation (Çelebi, 2010).

Salicylic acid (SA), a signaling molecule involved in defense against biotic and abiotic stresses and recognized as a plant growth regulator, was first isolated in small quantities as salicin from the bark of the willow tree by a German pharmacologist named Johann Andreas Buchner in 1828. The word salicylic acid comes from the plant *Salix* (willow). It was first used by Raffaele Piria in 1838 (Raskin, 1992). Studies conducted on different tissues and organs of plants have revealed that salicylic acid can be found everywhere and at all times in plants. It plays many important roles in plant growth and development. The most common known effects of SA and its analogue aspirin are to inhibit ethylene biosynthesis and delay aging. SA also has a thermogenic effect and plays a regulatory role in the flowering process of odor-producing plants. Furthermore, exogenous SA treatments stimulate the synthesis of pathogen-associated proteins in plants, thereby promoting the development of resistance to diseases (Raskin, 1992; Turkyılmaz *et al*., 2005).

Jasmonates, which were first obtained from the jasmine plant (*Jasminum grandiflorum*) in the mid-1960s and are classified as signaling molecules and plant growth regulators, contain jasmonic acid (JA) and methyl jasmonate (MeJA) esters. MeJA, the methyl ester of JA, is synthesized from JA via the jasmonic acid methyl transferase enzyme (JMT). JA and MeJA play an active role in plant growth and development (Tsai *et al*., 1996; Linkies and Leubner-Metzger, 2012). JA was first isolated from the fungus *Lasidiplodia theobromae* in 1971. Its growth-accelerating and enhancing effect was demonstrated in the 1980s (Tsai *et al*., 1996). In recent years, JA and MeJA have been considered important plant signaling molecules. It has also been stated that it significantly increases the formation of certain specific plant genes in damaged plants (Staswick *et al*., 1992; Staswick *et al*., 1995). Jasmonates are synthesized by plant organs such as leaves, roots, flowers, or fruits. The most important benefit of jasmonates is that they increase the resistance of plants to diseases and pests, thereby ensuring their resilience. Exogenous jasmonate treatments promote adventitious root formation, stomatal closure, seed germination during dormancy, protein synthesis, abscission, ethylene synthesis, and thus the ripening of the plant's fruits. However, it has been reported that jasmonates inhibit embryogenesis, photosynthesis, root growth, plant elongation, bud formation in flowers, and pollen germination (Baktır, 2010).

Çetiner (2022) reported that exogenously applied 0.5 and 1 mM JA and SA increased antioxidant content and yield in carrots. These signaling molecules have been reported to enhance vegetative growth, accelerate above-ground organ senescence, and promote storage root development. It has been determined that exogenously treated SA at different concentrations increases the amount of secondary metabolites and peroxidase activity in purple basil (Karalija and Parić, 2017). Złotek *et al*. (2017) found that exogenously applied arachidonic acid and JA increased the amount of phenolic compounds and anthocyanins in purple basil.

Hashmi *et al.* (2012) found that applying exogenous SA to fennel plants resulted in significant changes in plant growth, yield, and essential oil composition. In the study, solutions with concentrations of 0.01 mM, 0.1 mM, and 1 mM were prepared for exogenous SA application and applied to fennel plants. The results of the study indicated that the 0.1 mM concentration of salicylic acid was the most effective dose in promoting root and stem development, plant height, dry and fresh weight, and the amount of carotenoids, chlorophyll a (chla), chlorophyll b (chlb), and total chlorophyll (total chl).

In a study conducted by Conceição *et al*. (2006), the effects of exogenous SA and MeJA treatments on phenolic accumulation and synthesis in cell cultures of St. John's wort (*Hypericum perforatum* L.) were investigated. The study reported that SA and MeJA treatments increased phenolic accumulation and synthesis in cells.

The literature reports the use of SA and/or MeJA to increase bioactive metabolites and yield in purple basil and different plant species (Nazir *et al*., 2021), the alleviation of stress effects by promoting secondary metabolites and the photosynthesis mechanism against drought stress (Lopes *et al*., 2025), enhancing tolerance to salinity stress (Lopes *et al*., 2024; Meftahizadeh *et al*., 2025), and increasing biochemical potential with methyl jasmonate treatment (Chutimanukul *et al*., 2025). Although there are a few studies investigating the separate effects of SA and JA on the growth-development and/or antioxidant potential of basil plants, there is no study examining their combined effects. The lack of research on the specified topic is a significant gap, and this study aims to fill that gap.

Materials and Methods

Plant material

Material procurement

The plant material used was purple basil (*Ocimum basilicum* L.) seeds, procured from the commercial seed company Arzuman.

Plant cultivation and treatment with growth regulators

Basil seeds that were firm, plump, and of similar size were selected and planted in pots containing peat moss, spaced 5 cm apart. Three pots were used per treatment. Twenty seeds were planted in each pot. Seed germination and seedling development were carried out in a plant growth chamber at 25 ± 2 °C, long-day photoperiod (16 hours light/8 hours dark), 45 ± 5% humidity, and light intensity of 27 μmol $m^{-2}s^{-1}$. salicylic acid (SA) and/or jasminoic acid (JA) (obtained from Sigma-Aldrich) at different concentrations (0, 0.5, and 1 mM) were applied foliarly for the first time when the purple basil seedlings were in the two-leaf stage and for the second time two weeks after the first application. Harvesting was performed 15 days after the second application.

Determination of fresh and dry weights of roots and shoots from developing seedlings

Following harvest, 10 seedlings were randomly selected from the experimental design, and their root and shoot lengths were measured. The roots and shoots were then separated at the root-shoot junction. Fresh weights were determined for roots and shoots, followed by dry weights after drying.

Quantities of photosynthetic pigment substances

The procedure developed by Witham *et al*. (1971) was applied to determine the quantities of chlorophyll (chlorophyll a, chlorophyll b, total chlorophyll). Two milliliters of 80% acetone were added to 1 g of leaves randomly selected from the control and experimental groups at the same level, and the leaves were crushed in a mortar. The extracts were filtered using Whatman No. 2 filter paper, and 80% acetone was added to each to make up 10 mL. The chlorophyll pigment absorption values (645 nm and 663 nm) of the extracts were then measured using a spectrophotometer. The amounts of chlorophyll a, chlorophyll b, and total chlorophyll present in 1 g were calculated in mg by substituting the values into the equations below.

mg chlorophyll a/g tissue = [12.7 (D663) – 2.69 (D645)] (V/ 1000.W)

mg chlorophyll b/g tissue = [22.9 (D645) – 4.68 (D663)] (V/1000.W)

mg total chlorophyll/g tissue = [20.2 (D645) + 8.02 (D663)] (V/1000.W)

In the equations: V represents the final volume of 80% acetone; W represents the fresh weight of the extracted plant in grams; D represents the optical density (absorbance value) of the chlorophyll extract at the specified wavelengths.

Determination of total protein content

To determine the total protein content (TPC), 1 g of fresh purple basil leaf sample from each experimental group was subjected to extraction in 5 mL of 0.05 M Sodium-Phosphate Buffer at pH 7.6 in an ice bath. These extracts were then centrifuged at 13,000 rpm for 20 minutes. The resulting supernatants were used to determine protein content as well as catalase enzyme activity.

Bovine Serum Albumin (BSA) standards provided by Bradford (1976) were used to determine total protein content. One milliliter of reaction solution containing Coomassie Brilliant Blue protein dye was added to the obtained supernatants. The absorbance of the plant samples, incubated at room temperature for 10 minutes, was measured at 595 nm using a spectrophotometer. These measured absorbance values were applied to the calibration curve obtained from BSA standards (0.02-0.2 mg Ml^{-1}), and the soluble TPC in the plant samples was determined in mg.g fresh $weight^{-1}$.

Antioxidant capacities

Antioxidant substance quantities

Determination of carotenoid quantity

According to the method of Witham *et al*. (1971), the absorbance value measured at 450 nm wavelength in a spectrophotometer for extracts prepared from purple basil leaves was substituted into the following formula to calculate the carotenoid content in mg per 1 g of fresh leaf weight.

mg total carotenoid/tissue = 4.07 x D450 - (0.0435 x Cla amount + 0.367 x Clb) amount

Determination of anthocyanin content

Samples were extracted from purple basil leaves according to the procedure given by Mancinelli (1984) in 18 mL of methanol acidified to 1% with acid. The extracts were left to stand in the refrigerator (4°C) for two days, shaken occasionally, and then filtered using filter paper. The anthocyanin content of these solutions was measured at a wavelength of 530 nm using a spectrophotometer and expressed as optical density. Considering that chlorophyll degradation products in the extracts would also absorb at this wavelength, the values at 657 nm were also measured and substituted into the formula (A530 – 0.33 x A657) to determine the amount of anthocyanin per unit fresh weight.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity determination

The DPPH radical scavenging activity determination was performed using the method of Brand-Williams *et al*. (1995). A series of dilutions was prepared using 0.1 mM DPPH solution, plant extracts, and

methanol to determine the antioxidant activities of the extracts. 100 µl of each dilution was added to 2.9 mL of DPPH solution and incubated at room temperature for 15 minutes. After incubation, the absorbance values of the plant samples were measured at a wavelength of 517 nm. A 0.1 mM DPPH solution was used as a blank tube, and the % DPPH scavenging effect of the samples was calculated (100 × % DPPH AB-AS/AB).

AB: Blank absorbance value

AS: Sample absorbance value

Catalase enzyme activities

Catalase (CAT) enzyme activities were determined using the Bergmeyer (1970) method. To the supernatants (200 µl), 2.77 mL of 0.05 M sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 30 µl of 3% H_2O_2 were added, and the change in absorbance due to H_2O_2 consumption per minute was monitored at a wavelength of 240 nm using a Thermo Scientific UV spectrophotometer. Specific enzyme activity was expressed as EU mg protein^{-1}. G fresh weight^{-1}.

Determination of phenylalanine ammonia lyase (PAL) activity

0.1 g samples of fresh purple basil leaves taken from the experimental groups were homogenized in a 50 mM sodium phosphate buffer at pH 6.5 containing 1% polyvinylpyrrolidone (PVP) and 1 mM phenylmethylsulfonyl (PMSF). The resulting homogenate was centrifuged at 10,000 g for 25 minutes at 4 °C. The supernatant obtained from this homogenate was used to determine PAL activity. A spectrophotometric method was used to determine PAL activity (Pascholati *et al*., 1986). 100 µl of enzyme extract and 1000 µl of 0.2% phenylalanine solution were reacted at 37 °C for one hour, and the conversion of L-phenylalanine to trans-cinnamic acid was measured at a wavelength of 290 nm. PAL was determined using a cinnamic acid standard and expressed as µmol cinnamic acid hour $^{-1}$.

Determination of total phenolic content

0.1 g samples of fresh purple basil leaves taken from the experimental groups were extracted with 5 mL of 80% methyl alcohol, incubated at 80 °C for 15 minutes, and centrifuged at 500 g for 10 minutes. Then, 2.5 mL of 80% methyl alcohol was added, and the mixture was incubated at 80 °C for another 15 minutes and centrifuged again at 500 g for 10 minutes. The supernatant was dried at 80 °C, and the dried supernatant was resuspended in 1 mL of 80% methyl alcohol. This suspension was used to determine the total phenolic content (Gayoso *et al*., 2004). The Folin-Ciocalteu method was used to determine the total phenolic content (Singleton and Rossi, 1965). 100 µl of extract was incubated with 10-fold diluted Folin Ciocalteu at room temperature for 5 minutes, then 750 µl of sodium bicarbonate solution was added to this solution and incubated again at room temperature for 90 minutes. The amount of phenolic substances was calculated by recording the absorbance change at 765 nm using a spectrophotometer with a gallic acid standard.

Statistical evaluation

All measurements and analysis data were evaluated using Variance analysis (Tukey, 1954) in the SPSS 16.0 program at a significance level of $p<0.05$. A one-way ANOVA was performed for each parameter, followed by Tukey's HSD post hoc test ($p<0.05$). The standard error and standard deviation values of the means were also calculated using the same program.

Results and Discussions

Fresh and dry weights of roots and shoots in developing seedlings

Data on the growth parameters of purple basil seedlings treated with exogenous jasmonic acid (JA) and SA are shown in Tables 1 and 2.

Table 1. Effects of exogenous JA and SA treatments on root development (n:10)

Treatment groups	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Control	13.3 ± 1.125[a]	0.206 ± 0.038[a]	0.025 ± 0.001[a]
0.5 mM JA	14.8 ± 1.320[a]	0.326 ± 0.078[a]	0.047 ± 0.019[ab]
1.0 mM JA	15.6 ± 1.557[ab]	0.528 ± 0.046[b]	0.060 ± 0.013[b]
0.5 mM SA	15.0 ± 1.620[ab]	0.434 ± 0.084[bc]	0.050 ± 0.013[ab]
1.0 mM SA	15.9 ± 0.825[b]	0.591 ± 0.081[c]	0.067 ± 0.003[b]
½ 0.5 mM JA+½ 0.5 mM SA	14.1 ± 0.994[a]	0.411 ± 0.071[b]	0.047 ± 0.009[ab]
½ 1.0 mM JA+½ 1.0 mM SA	15.3 ± 1.547[ab]	0.579 ± 0.122[bc]	0.066 ± 0.015[b]

* Same letters indicate that the difference is not statistically significant ($p<0.05$)

Table 2. Effects of exogenous JA and SA treatments on shoot development (n:10)

Treatment groups	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Control	22.960 ± 1.376[a]	1.496 ± 0.153[a]	0.165 ± 0.009[a]
0.5 mM JA	29.675 ± 3.331[b]	2.481 ± 0.975[ab]	0.369 ± 0.083[b]
1.0 mM JA	31.940 ± 3.737[b]	3.654 ± 0.603[b]	0.504 ± 0.134[b]
0.5 mM SA	30.720 ± 3.434[b]	3.886 ± 0.864[bc]	0.470 ± 0.113[b]
1.0 mM SA	33.520 ± 3.854[b]	4.951 ± 0.596[c]	0.579 ± 0.016[c]
½ 0.5 mM JA+½ 0.5 mM SA	30.725 ± 2.208[b]	3.694 ± 0.349[bc]	0.410 ± 0.059[b]
½ 1.0 mM JA+½ 1.0 mM SA	32.950 ± 4.054[b]	4.147 ± 0.582[c]	0.518 ± 0.051[bc]

* Same letters indicate that the difference is not statistically significant ($p < 0.05$)

Root length, fresh weight, and dry weight increased in all treatment groups compared to the control group. The increases in root length were not statistically significant. The highest increases in root fresh and dry weight (186.89% and 168%, respectively) were observed in the 1.0 mM SA treatment and were statistically significant ($p < 0.05$) (Table 1).

In a study by Kahveci *et al*. (2021), where 0.05 mM SA was treated to basil seeds to prepare them for germination, root and shoot lengths and weights were found to increase compared to the control group. Another study conducted on purple basil indicated that 1 mM MeJA was treated, and this treatment resulted in increases in root dry weight (Lopes *et al*., 2024). It was stated that SA applied foliar to the plant species *Echinacea purpurea* caused increases in root volume and root weight (Abbaszadeh, 2019). In another study, SA and MeJA were applied to Chinese chives (*Allium tuberosum)*, and it was reported that 150 µM SA elicitor treatments caused an increase in root and shoot fresh-dry weight in Chinese chives (Wang *et al*., 2022). It has been determined that growth and development can be stimulated by elicitor treatment. It is thought that SA, JA, and their derivatives applied to aromatic plants with medicinal properties stimulate significant increases in growth and development parameters. For this reason, it is thought that elicitor treatments, rather than chemical fertilizers, could be a more environmentally friendly and alternative method for increasing the yield of aromatic plants that may also contain essential oils.

The length of purple basil shoots, as well as their fresh and dry weights, increased in all treatment groups compared to the control group, similar to the root data. The highest increases compared to the control were observed in shoot length (45.99%), shoot fresh weight (230.95%), and shoot dry weight (250.91%) in the 1.0 mM SA application ($p < 0.05$) (Table 2).

Consistent with our study, exogenous 0.5 and 1.0 mM SA treatments to purple basil increased plant height, fresh and dry weights, compared to the control group. The 1.0 mM SA treatment was found to be more effective (Elhindi *et al*., 2017). It was also found that the application of 1.4 mM SA in purple basil significantly increased plant height, fresh and dry weights, compared to the control group (Amer *et al*., 2021). In another

study conducted by Gharib and Abed (2006), foliar treatments of 10-5, 10-4, and 10-3 M SA were found to increase shoot fresh and dry weights in basil plants.

In a study conducted on the plant species *O. sanctum*, 100 µM MeJA was applied, and decreases in shoot fresh and dry weights were observed compared to the control group (Autaijamsripon *et al*., 2023). It is thought that differences in parameters related to shoot development may occur depending on the plant species, the elicitor applied, and the dose. In a study conducted by Lopes *et al*. (2024), increases in fresh and dry weight were observed in purple basil plants treated with 1 mM MeJA. In a study conducted by Złotek *et al*. (2016a), the authors reported treating 0.01 µM JA to sweet basil plants. It was stated that the treated 0.01 µM JA concentration caused plant to increase their fresh and dry weights compared to the control (Złotek *et al*., 2016a). The results obtained from our study are generally consistent with the literature data, and it is thought that SA and JA treatments have positive effects on yield and quality.

Quantities of photosynthetic pigment substances

Significant increases in chlorophyll a (Chla), Chlorophyll b (Chlb), and total chlorophyll (total chl) quantities were determined in all treatment groups compared to the control group (Figure 1A, 1B, and 1C). The highest increases were observed in the ½ 0.5 mM JA+½ 0.5 mM SA treatment, with a 148.72% increase in chla, a 444.66% increase in chlb, and a 217.80% increase in total chl ($p < 0.05$) (Figure 1A, 1B, and 1C).

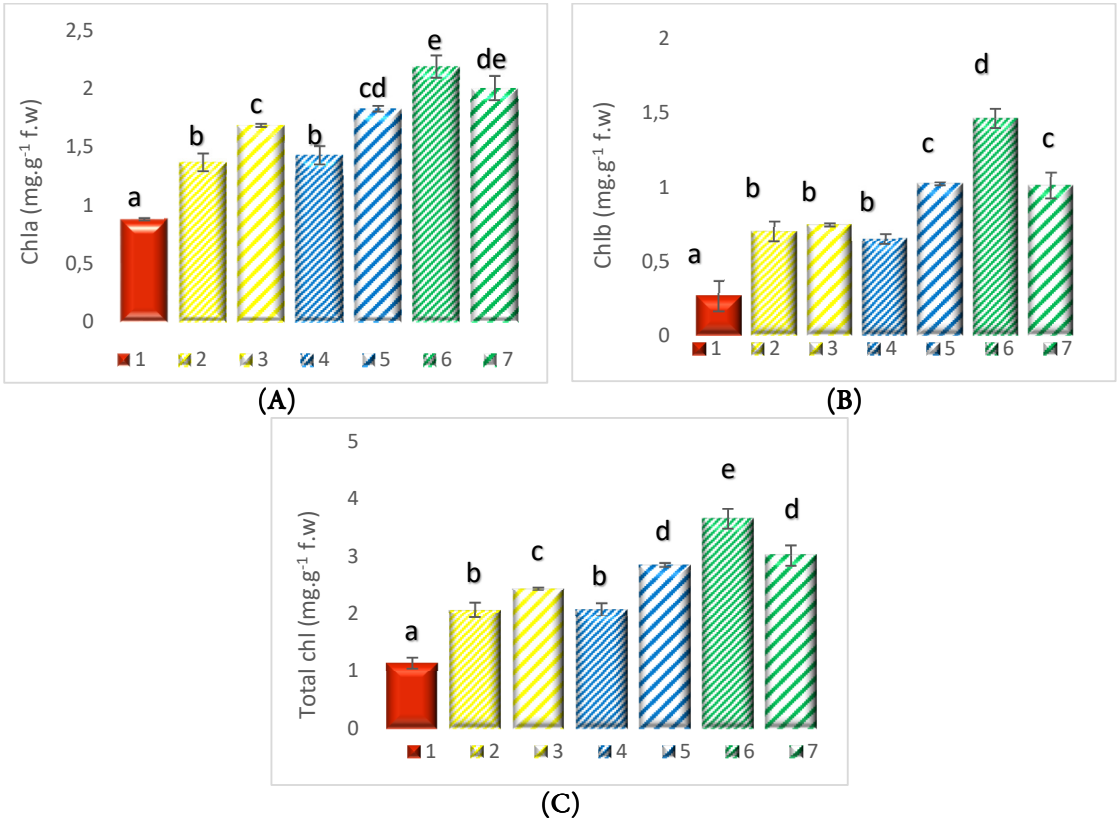

Figure 1. Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on chlorophyll content in purple basil: Cla (A), Clb (B), and total cl (C)
(n=3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA. Same letters indicate that the difference is not statistically significant ($p<0.05$)

In the studies by Elhindi *et al*. (2017), it was determined that 0.5 and 1.0 mM SA treatments increased the Cla and Clb values of basil. Similarly, it was found that a 1.4 mM SA treatment significantly increased the cla, clb, and total cl content in basil (Amer *et al*., 2021). Compared to the control, JA treatments at all concentrations (10^{-4}, 10^{-5}, 10^{-6}) were found to have a significant positive effect on increasing the chlorophyll content of basil leaves (Al Timman and Kamil, 2019). Another study reported that JA applied to purple basil plants under drought stress resulted in increases in chlorophyll values compared to the control group (Sorial *et al*., 2010). Kovac and Ravnikar (1994) reported that the increase in chlorophyll values with JA treatments was due to an increase in active cytokinin concentration. Another study indicated that 500 µM MeJA treatment caused increases in chlorophyll a and total chlorophyll amounts in Chinese chives. It has been reported that 150 µM SA treatment caused increases in chlorophyll b and total chlorophyll values (Wang *et al*., 2022). In a study conducted by Villamarin (2017), 1 mM MeJA was applied to Genovese basil plants, and the study indicated that the applied MeJA dose caused increases in chlorophyll values. Previous studies are considered to be consistent with our data. It is predicted that the increase in photosynthetic substance amounts with SA and JA treatments may also cause changes in morphological, physiological, and antioxidant content. The basic life activities of plants, their growth, development, and yield, depend on the mechanism of photosynthesis. In this context, it is thought that the amount of pigments used in photosynthesis directly affects the rate of photosynthesis. For the reasons stated, since chlorophyll levels are directly related to yield and quality values, it is thought that SA and JA applications can be used as an alternative method to increase the growth and development of purple basil plants.

Total protein content

TPC decreased in all treatment groups compared to the control group. These decreases were significant in all treatments except for the 0.5 mM SA treatments ($p < 0.05$). The lowest protein content was observed in the 1.0 mM JA treatment at 0.098 mg.g^{-1} t.a ($p < 0.05$) (Figure 2).

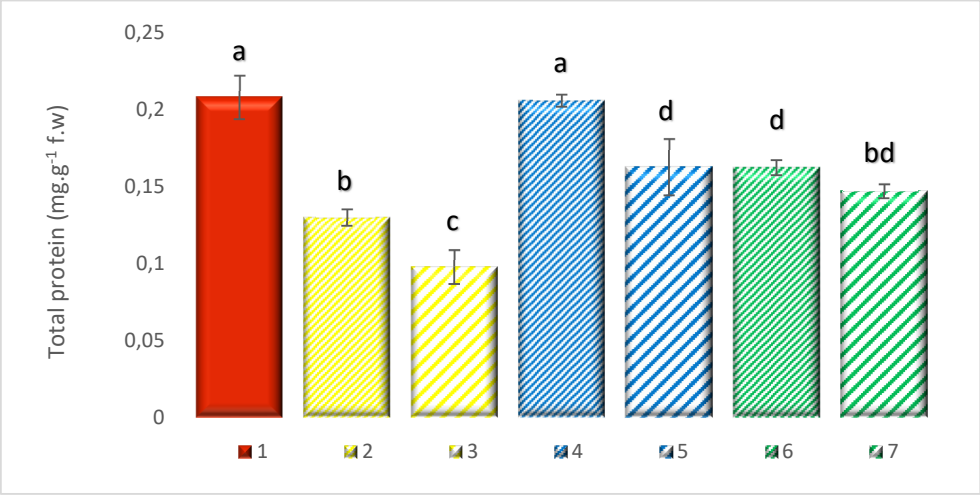

Figure 2. Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on purple basil protein levels
(n=3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA). Same letters indicate that the difference is not statistically significant ($p < 0.05$)

Bagheri (2014) found that when salicylic acid was applied to corn at various concentrations (1, 2, 3 mM), total protein amounts decreased. It was also found that when 1.0 mM JA was applied to soybeans, the total protein content decreased compared to the control group (Farhangi-Abriz and Ghassemi-Golezani, 2016). It has been stated that the treatment of 2.40 and 4.80 mM JA to sweet basil plants caused an increase in total

amino acid content (Sorial *et al*., 2010). It is thought that this occurred because the application of 2.40 and 4.80 mM JA triggered an increase in amino acid content by causing protein degradation. Karalija and Parić (2017) applied different concentrations of SA to basil plants and reported that the application of 1.0 mM SA caused an increase in total protein content compared to the control group. Since JA and SA are signaling molecules involved in responding to different stress factors, it is predicted that the marjoram plant reduces the amount of soluble proteins by activating the response mechanism against any stress through these elicitor applications. Compared to the groups where JA was applied alone, increases in protein levels were observed in the groups where SA was applied alone and in combination with JA. This is thought to be related to the activation of the plant's defense mechanism. When the limited data available in the literature are evaluated together, it is thought that SA and JA treatments may cause a decrease in TPC. In this context, the data obtained from our study are consistent with the literature.

Antioxidant capacities

Antioxidant substance quantities

Carotenoid quantity

The carotenoid quantity in purple basil leaves increased in all JA and SA treatments. The greatest increase (134.39%) was observed in the ½ 0.5 mM JA+½ 0.5 mM SA treatment ($p<0.05$) (Figure 3).

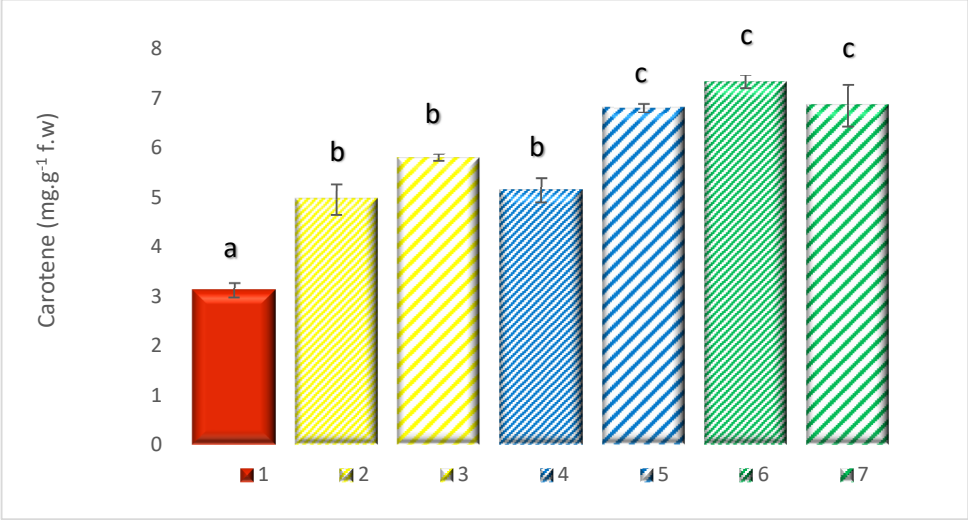

Figure 3. Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on purple basil carotenoid levels
(n=3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA). Same letters indicate that the difference is not statistically significant ($p<0.05$)

When SA was applied at different concentrations to purple basil, it was found to increase the amount of carotenoids compared to the control (Mahmoudi *et al*., 2022). It was found that photosynthetic pigments (chlorophyll and carotenoids) increased in *Cajanus cajan* treated with 1 µM JA (Poonam *et al*., 2013). Another study conducted on purple basil reported that the treatment of 1 mM MeJA caused an increase in carotenoid content (Lopes *et al*., 2024). It has been stated that the treatment of 1 mM MeJA to Genovese basil plants caused increases in carotenoid content (Villamarin, 2017). It has been reported that MeJA triggers secondary metabolism, increasing the biosynthesis of anthocyanins, phenolics, and carotenoids (Kim *et al*., 2019; Taheri *et al*., 2020). Tajik and Niknam (2015) applied SA to saffron plants. The study found that SA treatment increased the amounts of carotenoids and carotenoid-derived phytochemicals (Tajik and Niknam, 2015).

When studies conducted on purple basil and other plant species in the literature are evaluated together, it is thought that SA and JA elicitor treatments may play an important role in increasing carotenoid amounts. It is also thought that the dose applied in elicitor treatments is quite important in increasing phytochemical content.

Anthocyanin content

As shown in Figure 4, the anthocyanin content increased with SA and JA treatments compared to the control. The increases in the treatments were significant ($p < 0.05$). The highest anthocyanin content was 3.148 $mg.g^{-1}$ t.a. with the 0.5 mM JA treatment. An approximately two-fold increase was observed compared to the control group. Szymanowska *et al*. (2015) reported applying 10^{-6} M JA to *O. basilicum* L. cv. Dark Opal plants. The study indicated that the treatment of 10^{-6} M JA caused increases in anthocyanin content compared to the control group (Szymanowska *et al*., 2015). *Brassica rapa* treated with SA and JA showed increased anthocyanin content compared to the control (Thiruvengadam *et al*., 2016). It is also known that exogenous SA treatment increases the carotenoid and anthocyanin content in shoots and storage roots of carrots (Eraslan *et al*., 2007). Numerous scientific studies have indicated that the use of jasmonate derivatives and other elicitors causes an increase in the synthesis and accumulation of anthocyanins in plant tissues. Studies conducted on *Malus sylvestris* L. (Rudell *et al*., 2002) and *Vitis vinifera* L. (Mihai *et al*., 2010) can be cited as examples. The combined use of SA and MeJA has been reported to cause an increase in anthocyanin content in in vitro cultures of *Daucus carota* (Sudha and Ravishankar, 2003). It has been stated that MeJA treatments were made at different concentrations (250, 500, 750, 1000 ppm) on the plant species *O. tenuiflorum* L. (Chutimanukul *et al*., 2025). The study indicated that the highest increase in anthocyanin content was observed with the 1000 ppm MeJA treatment. Anthocyanins and phenolic compounds found in purple basil are among the most important phytochemicals for health. Therefore, elicitor treatments may become increasingly important in the future for growing plants with high levels of phytochemicals safely and inexpensively without the use of chemical fertilizers.

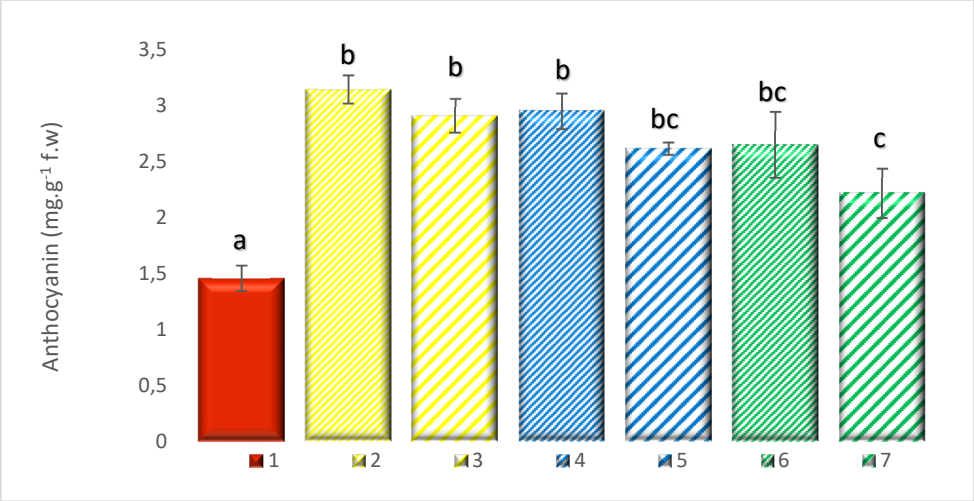

Figure 4. Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on anthocyanin content in purple basil

(n=3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA). Same letters indicate that the difference is not statistically significant ($p<0.05$)

Antioxidant activity

DPPH radical scavenging activity

DPPH scavenging activity, which indicates antioxidant efficacy, increased significantly in all treatment groups compared to the control. The highest increases were observed in the 0.5 mM JA (41.28%) and 0.5 mM SA (31.80%) treatments ($p < 0.05$) (Figure 5).

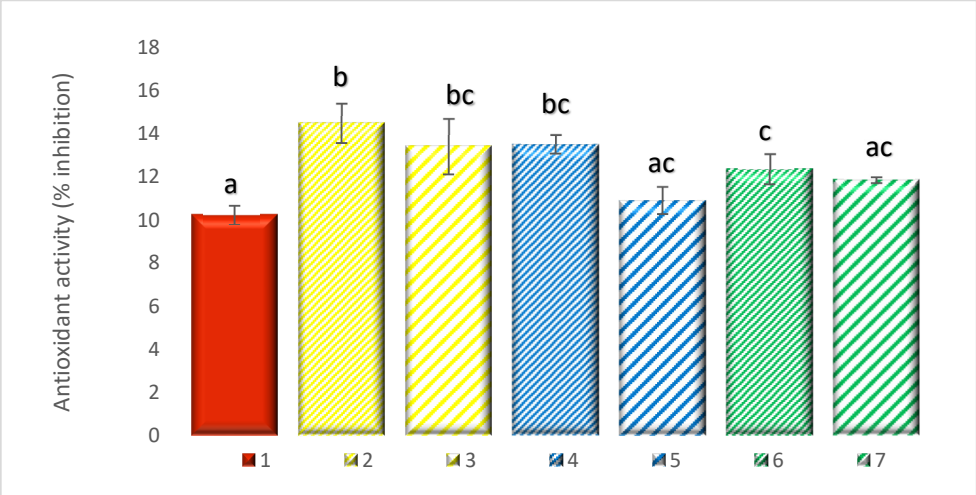

Figure 5. Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on the antioxidant activity of purple basil
(n=3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA). Same letters indicate that the difference is not statistically significant ($p<0.05$)

The DPPH method has been described as an electron-accepting method that utilizes color change to measure quantity via absorbance, and it has been noted as a highly reliable method for determining radical scavenging effects (Hasan *et al*., 2009). Kulak *et al*. (2021) found that SA treatment increased DPPH percentages and that 0.05 mM SA had a greater effect than 0.1 mM SA. It was determined that 0.01 µM and 1 µM JA treatments to purple basil increased DPPH percentages (Złotek *et al*., 2016). It was reported that the DPPH radical scavenging activity increased in basil plants treated with 10^{-6} M JA compared to the control group. It was also stated that exogenously treating SA and JA to *Brassica rapa* plants increased the radical scavenging value compared to the control (Thiruvengadam *et al*., 2016).

Increases in DPPH radical scavenging activity compared to the control were detected with SA treatments in the plant species *Crocus sativus* (Tajik and Niknam, 2015). Malekpoor *et al*. (2016) conducted a study involving JA treatment on sweet basil plants. In this study, it was determined that the treatment of 200 µL JA increased DPPH radical scavenging activity compared to the control group. Kim *et al*. (2006b) observed increases in the DPPH radical scavenging activity of sweet basil plants with 0.1 and 0.5 mM MeJA treatments. It was stated that the best DPPH radical scavenging activity was exhibited by the basil plant obtained by the in vitro culture method with a 10 µM SA treatment (Nazir *et al*., 2021). It was stated that the highest DPPH radical scavenging activity was observed in *O. tenuiflorum* L. plants with 750 and 1000 ppm MeJA treatments (Chutimanukul *et al*., 2025). When the studies are evaluated together, it is thought that SA and JA treatments in basil plants are quite effective in increasing antioxidant properties. The antioxidant activity in basil plants may vary depending on the plant species, variety, or differences in phenolic fractions. Due to its high antioxidant activity, which is increased by elicitor treatments, basil, with its aromatic and medicinal properties, is thought to be a good raw material for the pharmaceutical industry. It is also anticipated that it could create new alternatives as antioxidant phenolic sources in diets.

CAT activity

CAT enzyme activity increased in the treatment groups compared to the control group. These increases were significant in the 1.0 mM JA and ½ 1.0 mM JA+½ 1.0 mM SA treatments ($p < 0.05$). The highest increase was observed in the 1.0 mM JA treatment (49.08%) (Figure 6).

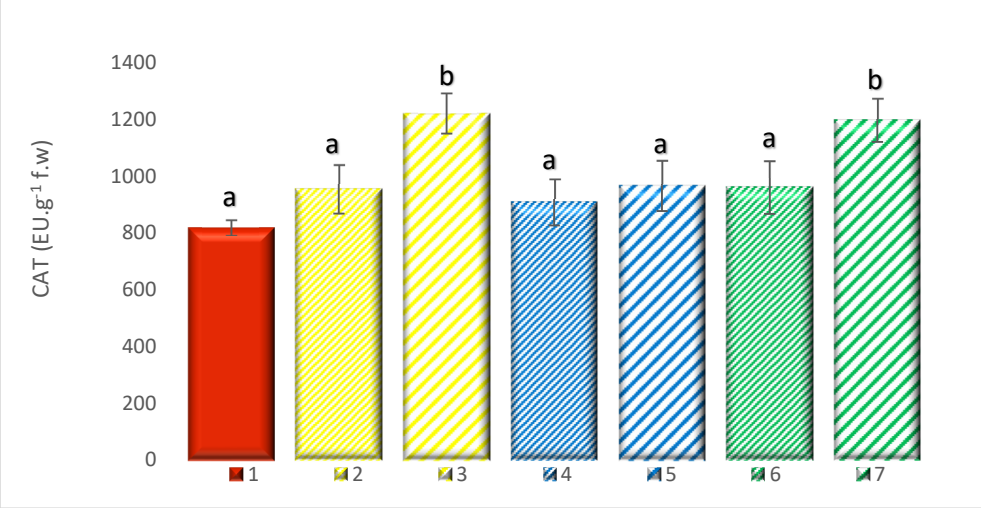

Figure 6 Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on total CAT activity in purple basil
(n=3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA). Same letters indicate that the difference is not statistically significant ($p<0.05$)

Treatments of 50, 100, 150, and 200 mg L^{-1} SA were performed on the *Tagetes erecta* L. plant. The study reported that the treatment of 50 mg L^{-1} SA caused increases in CAT activity (Shalaby *et al*., 2023). Consistent with our data, it has been stated that 1 mM SA and 0.5 mM JA increase CAT activity in colza (Cui *et al*., 2010). In another study, MeJA was applied as an elicitor to cell cultures of the plant *Scrophularia striata*, and increases in CAT activity were observed depending on the elicitor applied (Khanpour-Ardestani, 2015). Karamian *et al*. (2020) stated that MeJA (200 µM) and SA (100 µM) elicitors applied to the plant species *Verbascum sinuatum* led to an increase in enzymatic antioxidants. It has been reported that CAT enzyme activities increased in basil plants with different elicitor treatments (Karataş, 2022). It is thought that exogenously treated growth regulators, such as SA and JA, act as signaling molecules and are effective in reducing the effects of stress factors by increasing CAT activity. The data from our current study are consistent with previous studies.

Plants can produce phenolic compounds and oxidative enzymes with elicitor treatments. It has been reported that total antioxidant activity is a complex process and occurs through different mechanisms (Usman *et al*., 2020). Therefore, to determine antioxidant potential, it may be necessary to perform analyses of multiple types of enzymatic antioxidant activity (such as CAT and PAL) and non-enzymatic antioxidant activity (such as DPPH, phenolic compounds, and carotenoids).

PAL activity

PAL activity increased in all groups treated with growth regulators compared to the control group. The increases observed in the 0.5 mM JA (61.86%), 1.0 mM JA (36.62%), and ½ 0.5 mM JA + ½ 0.5 mM SA (43.10%) treatments were statistically significant ($p < 0.05$) (Figure 7).

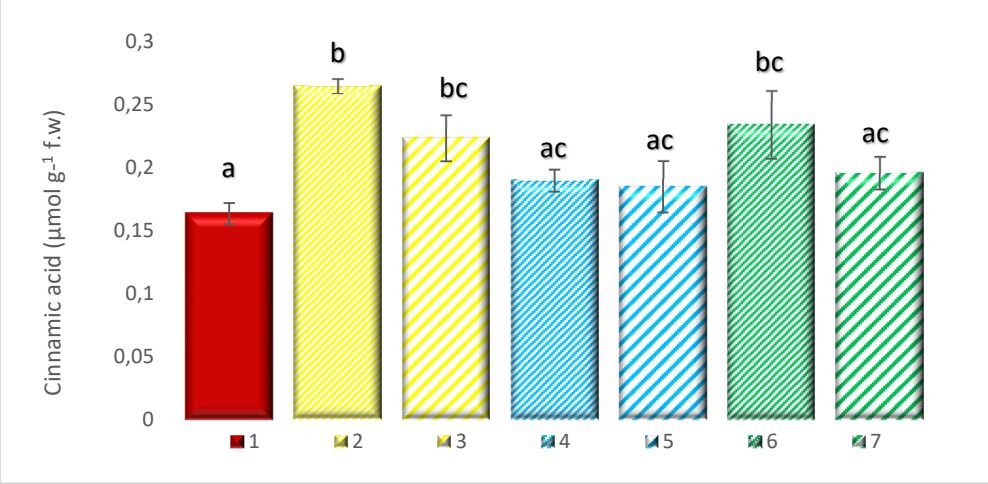

Figure 7 Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on purple basil PAL activity
(n:3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA). Same letters indicate that the difference is not statistically significant ($p < 0.05$)

Koca and Karaman (2015) treated 0.5 mM MeJA on the sweet basil plant in their study. As a result of the study, they observed increases in PAL activity following MeJA treatment (Koca and Karaman, 2015). Consistent with our data, the study by Ali *et al*. (2007) showed that MeJA and SA promote the accumulation of phenolic compounds in ginseng roots by regulating phenolic synthesis enzymes, particularly PAL. Similar results were obtained in the study conducted by Razavi (2012). In another study conducted on sweet basil, MeJA treatment was found to increase PAL enzyme activity (Kim *et al*., 2006b). It has been reported that MeJA treatment of *Raphanus sativus* L. increased PAL activity (Kim *et al*., 2006a). Our results suggest that MeJA and SA are signaling molecules that cause specific changes in gene expression levels that activate PAL activity and phenolic synthesis (Rao *et al*., 2000). Plant phenolics are primarily produced via the phenylpropanoid pathway initiated by PAL and TAL enzymes (Złotek *et al*., 2016). The product of PAL activity is trans-cinnamic acid, formed by the deamination of L-phenylalanine (Boudet, 2007). The results obtained from our study indicate that secondary metabolites in plants can be accumulated by inducing the phenylpropanoid pathway with elicitors such as SA and JA, without any biotic and/or abiotic stress. When the parameters in the literature related to PAL activity are evaluated, our study is considered similar.

Total phenolic content

Total phenolic content increased in all exogenous growth regulator treatments compared to the control group. All increases were statistically significant. The highest increase was 151.61% in the 0.5 mM JA treatment (Figure 8) ($p < 0.05$).

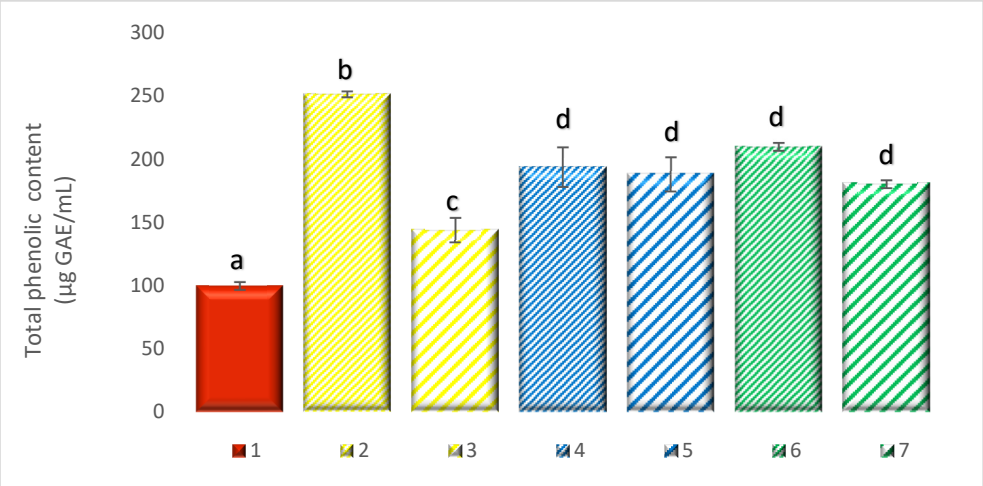

Figure 8. Effects of exogenous jasminoic acid (JA) and salicylic acid (SA) treatments on total phenolic content in purple basil
(n=3) (1: Control, 2: 0.5 mM JA, 3: 1.0 mM JA, 4: 0.5 mM SA, 5: 1.0 mM SA, 6: ½ 0.5 mM JA + ½ 0.5 mM SA, 7: ½ 1.0 mM JA + ½ 1.0 mM SA); Same letters indicate that the difference is not statistically significant ($p < 0.05$)

A study reported that 0.025 and 0.050 M salicylic acid increased the total phenolic content in *Ocimum basilicum* (Kahveci *et al*., 2021). Similarly, it was found that SA, JA, and ABA treatments in radish increased the total phenolic and flavonoid content (Thiruvengadam et al., 2016). Cappellari *et al*. (2019) stated that SA and MeJA treatment of mint increased the total phenolic content. In another study, SA application was reported to positively affect the phenolic and flavonoid content and antioxidant activity in basil leaves (Kulak, 2016). In a study conducted by Malekpoor *et al*. (2016), 200 µL and 400 µL of JA were applied to sweet basil plants. The results showed that the 400 µL JA treatment increased the phenolic content compared to the control (Malekpoor *et al*., 2016). Another study conducted on the *O. sanctum* plant stated that the 100 µM MeJA treatment increased the total phenolic content (Autaijamsripon *et al*., 2023). In a study conducted on sweet basil, 0.5 mM MeJA elicitor was applied, and as a result, an increase in total phenolic content was reported (Kim *et al*., 2006b). *Prunella vulgaris* L. plants were treated with 1 mM JA, and it was stated that the JA treatment resulted in increases in total phenolic content (Tang *et al*., 2023). Chutimanukul *et al*. (2025) stated that they obtained the highest amount of phenolic compounds with a 250 ppm MeJA treatment to the *O. tenuiflorum* plant species. Since phenolic compounds are synthesized from the phenylpropanoid pathway, it is thought that there may be a relationship between PAL enzyme activity and total phenolic content. With elicitor treatment, it is possible to increase secondary metabolites with antioxidant properties by stimulating signaling pathways without performing treatments that could harm human and plant health. The results obtained from our study show that the increase in total phenolic content due to JA treatment causes an increase in antioxidant activity in purple basil.

Conclusions

Today, the rapidly increasing world population and expansion of settlement areas have resulted in a decrease in arable land. Limited cultivation areas and climate change have made food shortages inevitable, leading to the threat of extinction for living species. This situation has required scientists to focus on efforts to increase the yield and quality of plants. At the same time, increasing the amount of secondary compounds and antioxidants in plants has become very important in order to make existing resources more beneficial to health.

In recent years, exogenously treated signaling molecules and growth regulators have been used to serve this purpose. The increase in the quality and yield of purple basil treated with exogenous JA and SA is thought to stem from the fact that JA and SA are compounds that play an internal regulatory role in the plant defense mechanism. Increasing these plant-derived products using abiotic stresses or signaling molecules can provide more antioxidants to diets at a low cost and can be considered as an alternative to genetic modification and breeding studies. Both morphological measurements and physiological-biochemical analyses have shown that SA and JA signaling molecules positively affect yield and quality in purple basil. The findings suggest that exogenous SA and JA treatments can be used as a low-cost and reliable method to increase the yield and quality of purple basil. Based on the data from the *Ocimum* species we worked with, if optimal concentrations are determined for different *Ocimum* species, subspecies, or varieties, these stimulant applications can be integrated and used in agricultural production as well as in the pharmaceutical, perfumery, and food industries. Furthermore, this study will contribute to the literature for future research in this field.

Authors' Contributions

Conceptualization: ZED, HT, BTU; Data curation: BTU, HT; Formal analysis: ZED, HT, BTU; Funding acquisition: BTU; Investigation: ZED, HT; Methodology: BTU; Project administration: BTU; Resources: ZED, HT, BTU; Software: HT, BTU; Supervision: BTU; Validation: HT, BTU; Visualization: ZED, HT, BTU; Roles/Writing - original draft: ZED, HT, BTU; and Writing - review & editing: ZED, HT, BTU.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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