**JASMONIC ACID AS A NATURAL ENHANCER OF NUTRACEUTICAL QUALITY IN SWEET BASIL LEAVES**

**ABSTRACT**

 Sweet basil (Ocimum basilicum L.) is more than a culinary herb — it’s a powerhouse of bioactive compounds with immense nutraceutical value. In this study, we explored how jasmonic acid (JA), a naturally occurring plant signal, can be used as a bio-elicitor to amplify the phytochemical and antioxidant potential of basil leaves across three distinct growth stages (35, 50 and 65 DAS). Foliar applications of JA at three concentrations {0.3 mM (JA 1), 0.6 Mm (JA 2) and 0.9 mM (JA 3) in 2% ethanol} were evaluated against control (2% ethanol) plants to measure their effect on key biochemical traits, including phenolics, flavonoids, antioxidants, ascorbic acid, alkaloids, pigments, and free amino acids. Results revealed a clear dose- and stage-dependent response, with the highest accumulation of beneficial compounds observed at 50 DAS under 0.9 mM JA (JA 3) treatment. Antioxidant activity and total phenolic content showed striking increases, while flavonoid and alkaloid levels were also significantly enhanced. With minimal intervention and strategic timing, jasmonic acid application emerges as a promising approach to elevate the phytochemical value of sweet basil in a sustainable and commercially viable manner.

**Key words:** Sweet basil, Jasmonic acid, Bio-elicitor, Phytochemicals, Antioxidant

**INTRODUCTION**

 Sweet basil (Ocimum basilicum L.), a widely cultivated aromatic herb of the Lamiaceae family, is esteemed globally not only for its culinary value but also for its rich nutraceutical profile (Ilic *et al*., 2019). Native to Southeast Asia and cultivated extensively across tropical and subtropical regions, sweet basil is a vital ingredient in traditional medicine systems and functional foods. Its leaves are a natural reservoir of bioactive phytochemicals including essential oils (eugenol, linalool, methyl eugenol), phenolic acids, flavonoids, carotenoids, chlorophylls, and vitamins such as ascorbic acid and tocopherols, which collectively confer potent antioxidant, antimicrobial, and anti-inflammatory properties (Labra *et al*., 2004; Simon, 1985).

 The increasing demand for high-value medicinal and aromatic plants has highlighted the need for sustainable strategies to enhance the biosynthesis of secondary metabolites without genetic modification. One such strategy is the application of natural elicitors like jasmonic acid (JA), a plant-derived hormone known to regulate plant defence, growth, and metabolism (Miclea *et al*., 2020). JA plays a critical role in stimulating the production of phytochemicals through activation of the phenylpropanoid pathway (Ebrahim *et al*., 2017; Koeduka *et al*., 2006; Reddy *et al*., 2021).

 Previous studies have shown that foliar application of JA significantly elevates the levels of total phenolics, flavonoids, chlorophylls, and antioxidant capacity in sweet basil leaves (Zlotek *et al*., 2016; Kim *et al*., 2006; Malekpoor *et al*., 2016). JA also modulates chloroplast function, enhances proline and amino acid synthesis, and improves physiological resilience under stress conditions (Miclea *et al*., 2020; Kianersi *et al*., 2022). The elicitor-induced upregulation of secondary metabolism not only improves the therapeutic value of basil but also supports eco-friendly cultivation practices aligned with consumer demands for plant-based health products (Thounaojam *et al*., 2020).

 Despite these promising results, limited research has been conducted on how JA influences the nutraceutical quality of sweet basil **leaves** across different developmental stages. Since phytochemical synthesis is growth-phase dependent, understanding the dynamic response of leaf constituents to JA application is essential to optimize elicitor-based enhancement strategies in basil production.

**2. MATERIALS AND METHODOLOGY**

 The present investigation, “Jasmonic Acid as a Natural Enhancer of Nutraceutical Quality in Sweet Basil Leaves” experimental field was carried out during the *Kharif* season of 2023-24 at the Medicinal and Aromatic Plants Research Station, AAU, Anand. Phytochemical analysis was performed at Department of Biochemistry, B. A. College of Agriculture, AAU, Anand.

**2.1 Experimental design and treatments**

 The study aimed to evaluate the effect of exogenous JA application on phytochemical accumulation in sweet basil leaves at different growth stages. The experiment was conducted using a Randomized Block Design (RBD) with five replications. Gujarat Anand Basil-1 (GAB-1), a high-yielding sweet basil cultivar, was selected as the experimental material. Four treatments were imposed: control (2% ethanol), 0.3 mM (JA 1), 0.6 mM (JA 2), and 0.9 mM (JA 3), with JA dissolved in 2% ethanol for improved foliar absorption. The foliar application was carried out once at 20 days after transplanting, and phytochemical responses were evaluated at three critical growth stages—35 DAS, 50 DAS and 65 DAS. Fresh leaf samples were collected at each growth stage from all treatments for biochemical analyses. All procedures were carried out using high-purity reagents and standardized protocols to ensure the accuracy and reproducibility of results. The experiment was designed to determine both the optimal dose and timing of JA application for maximizing nutraceutical quality in sweet basil leaves.

**2.3 Phytochemical Screening**

 Ten phytochemical parameters were analysed using the following standard protocols:

**2.3.1 Moisture Content**

 Moisture content was estimated by oven-drying 5 g of sweet basil leaf samples at 105°C for 6 hours, following A.O.A.C. (2000). Samples were cooled in a desiccator and weighed. Moisture percentage was calculated based on the weight loss using the formula:

Moisture (%) = [(Fresh weight – Dry weight) / Fresh weight] × 100.

**2.3.2 Total Phenol Content**

 Total phenol content was estimated using the method of Malick and Singh (1980). One gram of leaf tissue was extracted with 80% methanol, centrifuged, and pooled to a final volume of 10 ml. For analysis, 0.2 ml of the extract was reacted with Folin-Ciocalteu reagent and 20% sodium carbonate. After 30 minutes of incubation, absorbance was recorded at 620 nm. Phenolic content was calculated using a catechol standard curve and expressed as mg per 100 g sample.

**Total phenol (mg 100g-1)** = $\frac{Reading × Graph factor × Total volume ×10¯⁶}{Taken volum ×Sample weight (g) }$

**2.3.3 Total Antioxidant Content (FRAP Assay)**

 Antioxidant activity was determined using the FRAP assay (Blois, 1958). One gram of sweet basil leaf was extracted in 60% methanol with 0.1% HCl and centrifuged. The extract (1 ml) was mixed with 3 ml of FRAP reagent and incubated at 37°C for 10 minutes. Absorbance was recorded at 593 nm, and antioxidant capacity was expressed as mg ascorbic acid equivalent per gram fresh weight.

**Total antioxidant content (%) =**

Graph factor$×$ $\frac{Sample reading}{Weight of sample}×\frac{Total volume}{Taken volume}×$ 10-4

**2.3.4 Ascorbic Acid Content**

 Ascorbic acid content in sweet basil leaves was estimated using the titrimetric method of Sadasivam and Balasubraminam (1987). Fresh leaf tissue was homogenized in 4% oxalic acid, centrifuged, and the supernatant was titrated with 2,6-dichlorophenol indophenol dye. The endpoint was indicated by a persistent pink color. Ascorbic acid content was calculated using the dye factor and expressed as mg 100 g⁻¹ fresh weight.

**Amount of ascorbic acid mg 100g-1 sample=**

Dye factor $×$ Reading of sample$ ×$ $\frac{Total volume}{Taken volume}× \frac{1}{Sample weight}$ $×$ 100

**2.3.5 Chlorophyll Content**

 Chlorophyll a, b, and total chlorophyll were estimated by extracting fresh leaves in dimethyl sulfoxide (DMSO), and absorbance was read at 645 and 663 nm (A.O.A.C., 2000). Equations were used for calculation.

Chlorophyll content (in mg g-1 fresh weight):

**Chlorophyll a** = (12.7 × A663) − (2.69 × A645)

**Chlorophyll b** = (22.9 × A645) − (4.68 × A663)

Total Chlorophyll = Chl a + Chl b

Where;

A663 and A645 = Absorbance values at 663 nm and 645 nm

**2.3.6 Total Alkaloid Content**

 Alkaloid content was estimated following Mishra (1996). One gram of fresh sweet basil leaf was extracted with chloroform and ammonia, allowed to stand overnight, and filtered. The combined chloroform extracts were evaporated, redissolved in ethyl alcohol, and treated with 0.01 N H₂SO₄. After warming and cooling, excess acid was titrated with 0.01 N NaOH. Alkaloid content was calculated using the formula:

**Total alkaloid (mg 100 g⁻¹)** = 0.415 × volume of acid consumed × 1000

**2.3.7 Anthocyanin Content**

 Anthocyanin content in sweet basil leaves was estimated following the method of Stanciu *et al*. (2009). Leaf tissue (0.1–0.5 g) was extracted with an ethanol:HCl (85:15) solution and kept overnight at room temperature. The extract was filtered, diluted, and incubated in the dark for 2–3 hours. Absorbance was recorded at 535 nm using a spectrophotometer. Anthocyanin content was calculated using the formula;

**Total anthocyanin content (mg 100 g-1) =**

$$\frac{Reading ×Total volume ×100}{Aliquot ×98.2}$$

**2.3.8 Free Amino Acids**

 Free amino acid content was estimated using the ninhydrin-based colorimetric method described by Toghrol and Daneshpejouh (1974). Fresh leaf tissue (100–200 mg) was extracted with 80% ethanol, centrifuged, and the volume adjusted to 25 ml. For the assay, 1 ml of extract was mixed with 1 ml distilled water and 5 ml ninhydrin reagent, then heated in a boiling water bath for 12–15 minutes. After cooling, absorbance was measured at 570 nm. Glycine was used as the standard.

**Free amino acid (%)** = Graph factor$ ×$ $\frac{Sample reading}{Weight of sample}×\frac{Total volume}{Taken volume}×$ 10-4

**2.3.9 Total Carotenoids**

 Total carotenoid content was estimated following the method of Mahadevan and Shridhar (1981) with slight modifications. Fresh sweet basil leaves (0.1 g) were extracted 4–5 times using a petroleum ether:acetone (1:1 v/v) mixture until colourless, aided by adsorbents like magnesium oxide and hyflo-supercel. The pooled extract was concentrated and the absorbance measured at 450 nm using a UV-Visible spectrophotometer against a petroleum ether blank.

**Total carotenoid (mg 100g-1)** = $\frac{Reading × 100 × Total volume}{196 ×0.1 × Sample weight}$

**2.3.10 Total Flavonoid Content**

 Total flavonoid content in sweet basil leaves was estimated using the method of Sorease (2015). Leaf tissue (0.5 g) was extracted in 80% methanol, centrifuged, and 0.2 ml of the supernatant was diluted with distilled water. After sequential addition of 5% NaNO₂, 10% AlCl₃, and 1 M NaOH, the absorbance was recorded at 510 nm. Quercetin was used as the standard for calibration.

**Total flavonoid (mg 100g-1)** = $\frac{Reading ×Graph factor × Total volume ×10¯⁶}{Taken volum ×Sample weight (g) }$

**2.3.11 Qualitative Phytochemical Screening**

 Methanol extracts of fresh sweet basil leaves were screened for alkaloids (Mayer’s and Wagner’s tests), flavonoids, tannins, saponins, steroids, glycosides, and terpenoids using standard procedures (Fadil *et al*., 2007; Panchal and Parvez, 2019).

### 2.4 Statistical Analysis

 The experimental data were statistically analysed using two-way analysis of variance (ANOVA) under a RBD to evaluate the effects of treatments and growth stages. Data analysis was conducted by the Department of Statistics, AAU, Anand. Treatment means were compared to determine the significance of differences at the 5% probability level (p < 0.05). All assumptions of ANOVA, including homogeneity of variances and normality of residuals, were ensured prior to interpretation.

**3. RESULTS AND DISCUSSION**

**3.1 Moisture content**

 Moisture content in sweet basil leaves showed a consistent decline with plant maturity and increasing concentrations of JA. At 35 DAS, the highest moisture was recorded in the control (92.86%), while JA treatments significantly reduced moisture, with JA3 showing the lowest value (80.88%). This trend continued at 50 DAS and 65 DAS, where moisture content decreased across all treatments, reaching a minimum of 68.85% in JA3 at 65 DAS.

 The observed reduction in moisture at early stages under JA treatments is likely due to JA-induced partial stomatal closure, which limits transpiration and water retention (Wasternack & Hause, 2013; Chaves *et al*., 2009). By 50 DAS, JA may have further induced osmotic adjustment and secondary metabolite accumulation, both contributing to reduced tissue hydration (Wasternack & Song, 2017). At 65 DAS, advanced plant aging and lignification naturally decreased moisture, while higher JA concentrations likely enhanced stress signalling and accelerated senescence through interaction with abscisic acid (Lorenzo *et al*., 2004; Leon-Reyes *et al*., 2009). Moreover, JA has been shown to promote cuticle thickening and restrict water uptake by altering root functions, which may have further contributed to the moisture decline (Wasternack & Hause, 2013). Notably, reduced moisture levels may be beneficial for post-harvest stability by limiting microbial growth and concentrating bioactive compounds (Valero & Serrano, 2010).

**3.2 Total phenol content**

 **Total phenol content in sweet basil leaves increased significantly with JA application in a dose-dependent manner.** At 35 DAS, JA 3 showed nearly double the phenol content of the control. The highest values were recorded at 50 DAS, especially in JA 3, followed by JA 2 and JA 1. By 65 DAS, phenol levels declined slightly, but JA-treated plants still maintained higher content than the control, consistently following the order JA 3 > JA 2 > JA 1 > Control.

 JA significantly enhanced total phenol content in sweet basil, with the highest accumulation at 50 DAS under JA 3, due to activation of the phenylpropanoid pathway via enzymes like PAL (Dong & Lin, 2021). This aligns with peak vegetative growth and increased secondary metabolism (Zlotek *et al*., 2016). Although phenol levels declined at 65 DAS, JA-treated plants retained higher levels than the control (Scagel & Lee, 2012). The dose-dependent increase highlights JA’s role in defence activation, antioxidant build-up, and stress adaptation (Kim *et al*., 2006; Pirbalouti *et al*., 2017).

**Figure 1: Effect of different concentration of JA on moisture and total phenol content of sweet basil at different growth stages**

**3.3 Total Antioxidant content**

 **Total antioxidant content in sweet basil increased significantly under JA treatments across all growth stages.** At 35 DAS, JA 3 and JA 2 showed the highest antioxidant levels, both notably higher than the control. The peak occurred at 50 DAS, with JA 2 recording the maximum content, followed closely by JA 3 and JA 1. Although levels declined by 65 DAS, JA-treated plants still maintained higher antioxidant content than the control, with JA 2 consistently performing best.

 JA enhanced antioxidant activity in sweet basil at all growth stages, with JA 2 showing the highest effectiveness, especially at 50 DAS. This peak is linked to increased activity of antioxidant enzymes and secondary metabolites (Wasternack & Song, 2017; Scagel & Lee, 2012). Although JA 3 induced more metabolite build up, its antioxidant effect was lower, likely due to redox imbalance or enzyme inhibition (Salimi *et al*., 2016; Sirhindi *et al*., 2016). Declines at 65 DAS were attributed to senescence, yet JA treatments maintained higher activity overall. JA 2 emerged as the optimal dose for balancing accumulation and functionality.

**3.4 Ascorbic acid**

 Ascorbic acid content in sweet basil leaves increased significantly with JA treatment across all growth stages. JA 3 consistently showed the highest values, peaking at 50 DAS (69.00 mg 100 g⁻¹), followed by JA 2 and JA 1. Although levels slightly declined at 65 DAS, all JA treatments maintained significantly higher ascorbic acid than the control throughout the growth period.

 Ascorbic acid content in sweet basil increased in a dose- and stage-dependent manner under JA treatment, with JA 3 consistently inducing the highest levels. This enhancement is attributed to JA-mediated activation of the L-galactose pathway and ROS-scavenging genes (Wang *et al*., 2013; Qiu *et al*., 2014). The peak at 50 DAS aligns with heightened metabolic activity, while the slight decline at 65 DAS may result from senescence and hormonal interactions. Interestingly, despite high ascorbate levels under JA 3, antioxidant activity was lower, suggesting possible redox feedback or bioactivity loss (Sirhindi *et al*., 2016).

**Figure 2: Effect of different concentration of JA on antioxidant and ascorbic acid content of sweet basil at different growth stages**

**3.5 Chlorophyll content**

 Chlorophyll content in sweet basil leaves increased significantly with JA treatment at all growth stages. JA 3 consistently showed the highest levels, peaking at 50 DAS (3.74 mg g⁻¹), followed by JA 2 and JA 1. Although, chlorophyll content reduced at 65 DAS due to plant aging.

 JA significantly enhanced chlorophyll content in sweet basil in a dose- and stage-dependent manner. JA 3 consistently showed the highest values, especially at 50 DAS, aligning with peak vegetative growth and improved chloroplast activity. This effect is attributed to JA’s role in delaying senescence, boosting antioxidant defence, and stabilizing chloroplast structures (Wang *et al*., 2013; Per *et al*., 2016). Even at 65 DAS, JA-treated plants maintained higher chlorophyll than controls, likely due to reduced degradation and better membrane protection (Bajguz & Hayat, 2009; Danish *et al*., 2024).

**3.6 Total Alkaloid content**

 JA treatments significantly enhanced total alkaloid content in sweet basil leaves at all growth stages. JA 3 consistently led to the highest alkaloid accumulation, followed by JA 2 and JA 1, with all treatments outperforming the control. Peak levels were observed at 50 DAS, while elevated alkaloid content was maintained through 65 DAS.

 JA significantly boosted alkaloid content in sweet basil in a dose-dependent manner, with JA 3 being most effective-especially at 50 DAS, the likely optimal stage for accumulation. This is attributed to JA’s activation of secondary metabolism via transcription factors (MYC2) and enzymes (TDC and STR) (Wasternack & Hause, 2013; Goossens *et al*., 2017). Similar responses have been reported in other medicinal plants, and JA’s interaction with other hormones may further enhance alkaloid biosynthesis (Erb & Reymond, 2019), supporting its role as a potent elicitor of plant secondary metabolites (Bari & Jones, 2009).

**Figure 3: Effect of different concentration of JA on chlorophyll and alkaloid content of sweet basil at different growth stages**

**3.7 Anthocyanin content**

 JA significantly enhanced anthocyanin content in sweet basil leaves across all growth stages, with JA 3 consistently yielding the highest levels. Anthocyanin accumulation peaked at 50 DAS, particularly under JA 3 (9.84 mg 100 g-1), followed by a slight decline at 65 DAS. The overall trend showed a dose- and stage-dependent increase, highlighting JA’s strong influence on anthocyanin biosynthesis.

 JA significantly enhanced anthocyanin accumulation in sweet basil, especially under JA3 at 50 DAS. This peak is linked to JA’s stimulation of key transcription factors (MYB, bHLH, WRKY) and structural genes involved in flavonoid biosynthesis (Kim *et al*., 2006; De Geyter *et al*., 2012). JA’s interaction with developmental and light-regulated pathways (Zhang et al., 2013) likely contributed to the observed pattern. Additionally, studies by Deluc *et al*. (2006), Guo *et al*. (2023), and Sultana *et al*. (2023) support JA’s broader role in enhancing pigment biosynthesis and stress resilience, while Cisneros-Zevallos (2003) highlighted its contribution to antioxidant potential.

**3.9 Free Amino Acid**

 JA significantly increased free amino acid content in sweet basil leaves at all growth stages, with JA 3 showing the highest levels throughout. The maximum accumulation (0.202%) was observed at 50 DAS under JA 3, indicating this stage as the most responsive. JA 2 and JA 1 also enhanced amino acid levels compared to the control, though to a lesser extent. Overall, both JA concentration and plant maturity influenced amino acid accumulation, with JA 3 at 50 DAS proving most effective.

 JA treatment significantly increased free amino acid content in sweet basil leaves in a concentration- and stage-dependent manner. JA 3 consistently showed the highest levels, with a peak at 50 DAS, indicating enhanced nitrogen metabolism and stress response during active growth. This aligns with reports that JA promotes amino acid biosynthesis and mobilization for defence (Chen *et al*., 2022; Wasternack & Hause, 2013). The decline at 65 DAS reflects nutrient redistribution and reduced metabolic activity in mature tissues (Hildebrandt *et al*., 2015; Araujo *et al*., 2011).

**Figure 4: Effect of different concentration of JA on anthocyanin and free amino acid content of sweet basil at different growth stages**

**3.9 Total Carotenoid**

 JA treatments significantly enhanced carotenoid content in sweet basil leaves across all stages, with JA 3 showing the highest levels throughout. At 35 DAS, carotenoids increased with JA concentration, peaking at 69.69 mg/100 g under JA 3. The highest accumulation occurred at 50 DAS under JA 3 (101.79 mg/100 g), indicating this stage as the most responsive. Even at 65 DAS, JA 3 maintained superior carotenoid levels. Overall, carotenoid content followed the trend: JA 3 > JA 2 > JA 1 > Control, with peak accumulation at mid-growth.

 JA enhanced carotenoid accumulation in sweet basil by upregulating key biosynthetic genes like phytoene synthase and lycopene β-cyclase (Lu & Li, 2008), especially at 50 DAS, when photo protective demands are highest. The parallel rise in chlorophyll and carotenoids aligns with their shared isoprenoid pathway and roles in photosynthesis (Rodriguez-Concepcion, 2010). JA-induced oxidative stress likely triggered antioxidant pigment accumulation (Wasternack & Hause, 2013; Sharma *et al*., 2018). The slight decline at 65 DAS reflects natural senescence and pigment breakdown (Toledo-Ortiz *et al*., 2010), but JA treatments still maintained higher levels, confirming JA’s role in pigment biosynthesis and stress tolerance.

**3.10 Total Flavonoid content**

 JA treatments significantly increased total flavonoid content in sweet basil leaves across all growth stages. JA 3 consistently showed the highest flavonoid levels, followed by JA 2 and JA 1, all significantly higher than the control. The peak accumulation occurred at 50 DAS, with JA 3 reaching 104.00 mg/100 g. Although flavonoid content slightly declined at 65 DAS, the overall trend of JA 3 > JA 2 > JA 1 > Control remained consistent throughout.

 JA enhanced flavonoid content in sweet basil by activating key biosynthetic genes (CHS, CHI, FLS) and related transcription factors (MYB, bHLH), boosting antioxidant defenses (Zhang *et al*., 2013; Wasternack & Hause, 2013). The highest flavonoid levels at 50 DAS reflect optimal biosynthesis conditions due to mature leaves and precursor availability (Ghasemzadeh & Ghasemzadeh, 2011). Although levels declined at 65 DAS, they remained above control, likely due to resource shifts during flowering (Tattini *et al*., 2004). JA 3 consistently showed the strongest effect, supporting a dose-dependent response, similar to findings by Zlotek *et al*. (2016) and Kim *et al*. (2006).

**Figure 5: Effect of different concentration of JA on total carotenoid and flavonoids content of sweet basil at different growth stages**

**3.11 Qualitative test of phytochemicals**

 A qualitative analysis of sweet basil leaves revealed that all major phytochemical groups—alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides—were consistently present across all JA treatments and growth stages, while saponins were absent throughout. This suggests these secondary metabolites are constitutively present in sweet basil, regardless of jasmonic acid application or plant maturity. While JA may influence their concentration, their presence appears unaffected, indicating the need for quantitative analysis to assess treatment effects more precisely.

**Table.1: Qualitative analysis of phytochemical of sweet basil at all growth stages and treatments**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Phytochemical** | **Test Name** | **Control** | **JA 1** | **JA 2** | **JA 3** |
| Alkaloids | Mayer’s/Wagner’s | Present | Present | Present | Present |
| Flavonoids | Yellow color test | Present | Present | Present | Present |
| Tannins | Ferric chloride | Present | Present | Present | Present |
| Saponins | Foam test | Absent | Absent | Absent | Absent |
| Steroids | Salkowski test | Present | Present | Present | Present |
| Glycosides | Keller–Killani test | Present | Present | Present | Present |
| Terpenoids | Salkowski test | Present | Present | Present | Present |

 Alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides were consistently detected in leaves across all treatments and growth stages, indicating their constitutive presence. Flavonoids and tannins play antioxidant and antimicrobial roles (Nadeem *et al*., 2022), while terpenoids and steroids support defence functions (Javanmardi *et al*., 2002). Saponins were absent, possibly due to genotypic traits or methanol extraction inefficiency (Sankhalkar & Vernekar, 2016). Although JA is a known metabolic elicitor, it induced no qualitative changes, suggesting a quantitative effect, as reported by Khataee *et al*. (2019).

**CONCLUSION**

 Harnessing the power of plant signalling pathways, this study demonstrates that JA acts as a dynamic bio-elicitor capable of reprogramming the phytochemical landscape of sweet basil across its developmental continuum. Among the tested concentrations, 0.9 mM JA (JA 3) emerged as the most effective in stimulating a robust accumulation of key secondary metabolites—including phenolics, flavonoids, alkaloids, anthocyanins, carotenoids, and ascorbic acid—with peak enhancement observed at 50 DAS. This phytochemical surge was paralleled by a significant increase in antioxidant capacity and pigment integrity, reflecting the intricate regulatory influence of JA on the phenylpropanoid and isoprenoid pathways. Although the qualitative presence of major phytochemicals remained unchanged, their quantitative elevation underscores JA’s potential to modulate biosynthetic intensity without altering core metabolic signatures. Altogether, these findings position JA as a strategic agronomic tool—offering a sustainable, non-genetic means to elevate the nutraceutical and commercial value of basil under modern cultivation systems.

**REFERENCES**

**Association of Official Analytical Chemists (AOAC) (2000).** Official methods of analysis (15th ed., pp. 11–62) *Journal of the Association Official Agricultural Chemists,* Virginia, USA.

Araujo, W. L., Tohge, T., Ishizaki, K., Leaver, C. J., & Fernie, A. R. (2011). Protein degradation – an alternative respiratory substrate for stressed plants. *Trends in Plant Science, 16*(8), 489–498. https://doi.org/10.1016/j.tplants.2011.05.008

Bajguz, A., & Hayat, S. (2008). Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiology and Biochemistry, 47*(1), 1–8.

**Bari, R., & Jones, J. D. G. (2009).** Role of plant hormones in plant defence responses. Plant Molecular Biology, 69(4), 473–488. https://doi.org/10.1007/s11103-008-9435-0

Blois, M. S. (1958). Antioxidant determination by use of stable free radicals. *Nature*,181(4617), 1199-1200.

Chaves, N. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, 103(4), 551–560.

**Chen, L.-H., Cheng, Z.-X., Xu, M., Yang, Z.-J., & Yang, L.-T. (2022).** Effects of nitrogen deficiency on the metabolism of organic acids and amino acids in Oryza sativa. Plants, 11(19), 2576.

**Cisneros-Zevallos, L. (2003).** The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. Journal of Food Science, 68(5), 1560–1565.

Danish, S., Sana, S., Hussain, M. B., Dawar, K., Almoallim, H. S., Ansari, M. J., Hareem, M. & Datta, R. (2024).Effect of methyl jasmonate and GA₃ on canola (Brassica napus L.) growth, antioxidants activity, and nutrient concentration cultivated in salt‑affected soils. BMC Plant Biology, 24, 363. <https://doi.org/10.1186/s12870-024-05074-9>

**De Geyter, N., Gholami, A., Goormachtig, S., & Goossens, A. (2012).** Transcriptional machineries in jasmonate‑elicited plant secondary metabolism. Trends in Plant Science, 17(6), 349–359.

**Deluc, L., Barrieu, F., Marchive, C., Lauvergeat, V., Decendit, A., Richard, T., Carde, J.-P., Merillon, J.-M., & Hamdi, S. (2006).** Characterization of a grapevine R2R3‑MYB transcription factor that regulates the phenylpropanoid pathway. Plant Physiology, 140(2), 499–511.

**Dong, N-Q., & Lin, H-X. (2021).** Contribution of phenylpropanoid metabolism to plant development and plant–environment interactions. Journal of Integrative Plant Biology, 63(1), 180–209.

Ebrahim, B. M., Babak, A. M., & Kheradmend, F. (2017). The effect of methyl jasmonate on the expression of phenylalanine ammonia lyase and eugenol-o-methyl transferase genes in basil. *Philippine Agricultural Scientist*, 100(2), 163-67.

**Erb, M., & Reymond, P. (2019).** Molecular interactions between plants and insect herbivores. Annual Review of Plant Biology, 70, 527–557. https://doi.org/10.1146/annurev-arplant-050718-095910

Fadhil, S., Reza, M. H., Rouhollah, G., & Reza, V. R. M. (2007). Spectrophotometric determination of total alkaloids in *Peganum harmala* L. using bromocresol green. *Research Journal of Photochemistry*, 1(2), 79-82.

**Ghasemzadeh, A., & Ghasemzadeh, N. (2011).** Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research, 5(31), 6697–6703.

**Goossens, J., Mertens, J., & Goossens, A. (2017).** Role and functioning of bHLH transcription factors in jasmonate signalling. Journal of Experimental Botany, 68(6), 1333–1347. https://doi.org/10.1093/jxb/erw440

**Guo, J., Wang, Y., Li, J., Zhang, J., Wu, Y., & Wang, G. (2023).** Overview and recent progress on the biosynthesis and regulation of flavonoids in Ginkgo biloba L. International Journal of Molecular Sciences, 24(19), 14604. <https://doi.org/10.3390/ijms241914604>

**Hildebrandt, T. M., Nesi, A. N., Araujo, W. L., & Braun, H.-P. (2015).** Amino acid catabolism in plants. Molecular Plant, 8(11), 1563–1579. https://doi.org/10.1016/j.molp.2015.09.005

Ilic, A. S., Antic, M. P., Jelacic, S. C., & Solevic-Knudsen, T. M. (2019). Chemical composition of the essential oils of three *Ocimum basilicum* L. cultivars from Serbia. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*,47(2), 347-351.

**Javanmardi, J., Khalighi, A., Kashi, A., Bais, H. P., & Vivanco, J. M. (2002).** Chemical characterization of basil (Ocimum basilicum L.) found in local accessions and used in traditional medicines in Iran. Journal of Agricultural and Food Chemistry, 50(21), 5878–5883.

**Khataee, E., Karimi, F., & Razavi, K. (2019).** Alkaloids production and antioxidant properties in Catharanthus roseus (L.) G. Don. shoots and study of alkaloid biosynthesis-related gene expression levels in response to methyl jasmonate and putrescine treatments as eco-friendly elicitors. Acta Biologica Hungarica, 70(1), 102–114.

Kianersi, F., Azarm, D. A., Fatemi, F., Pour-Aboughadareh, A., & Poczai, P. (2022). Methyl jasmonate induces genes involved in linalool accumulation and increases the content of phenolics in two Iranian coriander (*Coriandrum sativum* L.) ecotypes. *Genes*, 13(10), 1717.

Kim, H., Chen, F., Wang, X., & Rajapakse, N. C. (2006). Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). *Journal of Agricultural and Food Chemistry*, 54(6), 2327-32.

Koeduka, T., Fridman, E., Gang, D. R., Vassao, D. G., Jackson, B. L., Kish, C. M., Orlova, I., Spassova, S. M., Lewis, N. G., Noel, J. P., Baiga, T. J., Dudareva, N.,. & Pichersky, E. (2006). Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proceedings of the National Academy of Sciences*, 103(26), 10128–10133.

Labra, M., Miele, M., Ledda, B., Grassi, F., Mazzei, M., & Sala, F. (2004). Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. *Plant Science*, 167, 725-731.

Leon-Reyes, A., Spoel, H S., De Lange, E. S., Abe, H., Kobayashi, M., Tsuda, S., Millenaar, F. F., Welschen, R. A. M., Ritsema, T., & Pieterse, C. M. J. (2009). Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. *Plant Physiology*, 149(4), 1797–1809.

Lorenzo, O., Chico, M. J., Sanchez-Serrano, J. J., & Solano, R. (2004). JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. *The Plant cell*, 16(7), 1938-1950.

**Lu, S., & Li, L. (2008).** Carotenoid metabolism: Biosynthesis, regulation, and beyond. Journal of Integrative Plant Biology, 50(7), 778–785. https://doi.org/10.1111/j.1744-7909.2008.00708.x

**Mahadevan, A., & Shridhar, R. (1981).** Methods in Physiological Plant Pathology (pp. 183–184). Sivakami Publications.

**Malekpoor, F., Salimi, A., & Pirbalouti, A. G. (2016).** Effect of jasmonic acid on total phenolic content and antioxidant activity of extract from the green and purple landraces of sweet basil. Acta Poloniae Pharmaceutica – Drug Research, **73**(5), 1229–1234.

**Mallick, E. P., & Singh, M. B. (1980).** Plant enzymology and histoenzymology (1st ed., Pp. 280). New Delhi: Kalyan Publisher.

Miclea, I., Suhani, A., Zahan, M., & Bunea, A. (2020). Effect of jasmonic acid and salicylic acid on growth and biochemical composition of *in-vitro*-propagated *Lavandula angustifolia* (Mill.). *Agronomy*, 10(11), <https://doi.org/10.3390/agronomy10111722>

Mishra, N. S. (1996). Quick method for estimation of total alkaloid in ashwagandha *Withania somnifea* L. In: Biennial Report of All India Coordinated Research Project on Medicinal and Aromatic Plants, NRCM & AP (Anand), pp. 254 (1996).

**Nadeem, H. R., Akhtar, S., Sestili, P., Ismail, T., Neugart, S., Qamar, M., & Esatbeyoglu, T. (2022).** Toxicity, antioxidant activity, and phytochemicals of basil (Ocimum basilicum L.) leaves cultivated in Southern Punjab, Pakistan. Foods, 11(9), 1239. https://doi.org/10.3390/foods11091239

Panchal, P., & Parvez, N. (2019). Phytochemical analysis of medicinal herb (*Ocimum sanctum*). *International Journal of Nanomaterials, Nanotechnology and Nanomedicine*, 5(2), 1-5. <http://doi.org/10.17352/2455-3492.00002>

**Per, T. S., Khan, N. A., Masood, A., & Fatma, M. (2016).** Methyl jasmonate alleviates cadmium‑induced photosynthetic damages through increased S‑assimilation and glutathione production in mustard. Frontiers in Plant Science, 7, 1933. <https://doi.org/10.3389/fpls.2016.01933>

Pirbalouti, A. G., Malekpoor, F., Salimi, A., & Golparvar, A. R. (2017). Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (Ocimum ciliatum and Ocimum basilicum) under reduced irrigation*.* ***Scientia Horticulturae*, 217**, 114–122.

**Qiu, Z.‑B., Guo, J.‑L., Zhu, A.‑J., Zhang, L., & Zhang, M.‑M. (2014).** Exogenous jasmonic acid can enhance tolerance of wheat seedlings to salt stress. Ecotoxicology and Environmental Safety, 104, 202–208. https://doi.org/10.1016/j.ecoenv.2014.03.014

Reddy, V. A., Li, C., Nadimuthu, K., Tjhang, J. G., Jang, I-C., & Rajani, S. (2021). Sweet basil has distinct synthases for eugenol biosynthesis in glandular trichrome and roots with different regulatory mechanisms. *International Journal of Molecular Sciences*, 22(2), 681.

**Rodriguez‑Concepcion, M. (2010).** Supply of precursors for carotenoid biosynthesis in plants. Archives of Biochemistry and Biophysics, 504(1), 118–122. <https://doi.org/10.1016/j.abb.2010.06.016>

Sadasivam, S., & Balasubraminam T. (1987). In: Practical Manual in Biochemistry, Tamil Nadu Agricultural University Coimbatore, Pp. 14-15.

**Salimi, F., Shekari, F., & Hamze, J. (2016).** Methyl jasmonate improves salinity resistance in German chamomile (Matricaria chamomilla L.) by increasing activity of antioxidant enzymes. Acta Physiologiae Plantarum, 38, <https://doi.org/10.1007/s11738-015-2023-4>

**Scagel, C. F., & Lee, J. (2012).** Phenolic composition of basil plants is differentially altered by plant nutrient status and inoculation with mycorrhizal fungi. HortScience, 47(5), 660–671.

**Sharma, A., Kumar, V., Yuan, H., Kanwar, M. K., Bhardwaj, R., Thukral, A. K., & Zheng, B. (2018).** Jasmonic acid seed treatment stimulates insecticide detoxification in Brassica juncea L. Frontiers in Plant Science, 9, 1609. <https://doi.org/10.3389/fpls.2018.01609>

Simon, J. E. (1985). The scientific literature on selected herbs, and aromatic and medicinal plants of the temperate zone. *Archon Books*, 770 pp.

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

Soares, L., Silva, L. A., & Pezzini, B. R. (2015). Spectrophotometric determination of the total flavonoid content in *Ocimun basilicum* L. (lamiaceae) leaves. *Pharmaceutical Magazines*, 11(41), 96-01.

Stanciu, G., Lupsor, S., & Sava, C. (2009). Spectrophotometric characterizations of anthocyans extracted from black grapes skin. *Ovidius University Annals of Chemistry*, 20(2), 205-208.

**Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., & Agati, G. (2004).** Differential accumulation of flavonoids and hydroxycinnamates in leaves of Ligustrum vulgare under excess light and drought stress. New Phytologist, 163(3), 547–561.

Thounaojam, A. S., Sakure, A. A., Dhaduk, H. L., Kumar, S., & Mistry, J. G. (2020). Impact evaluation of growth stage and species on morpho-physiological traits and bioactive constituent of essential oil in *Ocimum* through multi-year experiment. *Industrial Crops and Products*, 158(15), 1-10.

Toghrol, F., & Daneshpejouh, H. (1974). Estimation of free amino acids, protein and amino acid compositions of cumin seed (*Cuminum cyminum*) of Iran. *The Journal of tropical Pediatrics and Environmental Child Health*, 20(3), 109-11.

**Toledo‑Ortiz, G., Huq, E., & Rodriguez‑Concepcion, M. (2010).** Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome‑interacting factors. Proceedings of the National Academy of Sciences of the United States of America, 107(25), 11626–11631. https://doi.org/10.1073/pnas.0914428107

**Valero, D., & Serrano, M. (2013).** Growth and ripening stage at harvest modulates postharvest quality and bioactive compounds with antioxidant activity. Stewart Postharvest Review, 3(3), <https://doi.org/10.2212/spr.2013.3.7>

**Wang, M., Zheng, Q., Shen, Q., & Guo, S. (2013).** The critical role of potassium in plant stress response. International Journal of Molecular Sciences, 14(4), 7370–7390.

Wasternack, C., & Hause, B. (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Annals of Botany*, 111(6), 1021-1058.

Wasternack, C., & Song, S. (2017). Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *Journal of Experimental Botany*, 68(6), 1303–1321.

**Zhang, Y., Butelli, E., De Stefano, R., Schoonbeek, H.-J., Magusin, A., Pagliarani, C., Wellner, N., Hill, L., Orzaez, D., Granell, A., Jones, J. D. G., & Martin, C. (2013).** Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. Current Biology, 23(12), 1094–1100.

Zlotek, U., Michalak-Majewska, M., & Szymanowska, U. (2016). Effect of jasmonic acid elicitation on the yield, chemical composition, and antioxidant and anti-inflammatory properties of essential oil of lettuce leaf basil (*Ocimum basilicum* L.). *Food Chemistry*, 213, 1-7.