

Effects of indole-3-butyric acid and water stress on the fatty acid profile of *Ocimum basilicum L.* and *Ocimum kilimandscharicum Gürke*

Shahad Khalil Ibrahim, Alaa Hussain Ali

Department of Biology, College of Science, University of Mosul, Mosul, Iraq

Abstract

This study demonstrated that treating sweet basil (*Ocimum basilicum L.*) and camphor basil (*Ocimum kilimandscharicum Guerke*) with the growth regulator indole-3-butyric acid (IBA) at a concentration of 0.075 ppm, under varying levels of water stress, significantly influenced the fatty acid composition, particularly enhancing unsaturated fatty acids. GC-FID analysis revealed linoleic acid (C18:2n6) as the dominant fatty acid in all samples, followed by oleic and palmitic acids. The application of IBA promoted the activity of desaturase enzymes, which introduced additional double bonds into fatty acid chains, thereby improving membrane fluidity and stability under stress. Camphor basil exhibited a stronger response to IBA, accumulating linoleic acid concentrations exceeding 93% under 0.075 ppm IBA, which reduced overall fatty acid diversity. In contrast, sweet basil maintained a more balanced fatty acid profile. These findings highlight the role of IBA in enhancing the nutritional and therapeutic quality of basil essential oils and emphasize the importance of optimizing both hormonal treatments and irrigation practices to boost productivity and stress tolerance in basil cultivated in arid and semi-arid environments.

Keywords: Basil, IBA, water stress, fatty acids, linoleic acid, GC-FID

Introduction

The genus *Ocimum* belongs to the Lamiaceae family, which includes approximately 150 species of aromatic and medicinal plants (Güez *et al.*, 2017) [6, 7]. This genus is among the most diverse plant groups, comprising over 60 species of herbs and shrubs native to tropical and subtropical regions of Asia, Africa, and Central and South America. Due to its adaptability, *Ocimum* species are widely cultivated across different climatic zones worldwide, which enhances their potential in sustainable agriculture and various industrial applications (Padalia *et al.*, 2017; Zare *et al.*, 2021) [17, 23]. Basil species are grown for multiple purposes, including culinary use, traditional medicine, perfume production, and cosmetics. The most common species is *Ocimum basilicum L.*, also known as sweet basil, which is mainly classified into two types: green and purple basil. These types are highly valued in the food and pharmaceutical industries because of their rich essential oil and phenolic compound content (Zare *et al.*, 2021; Copolovici *et al.*, 2021; Ebrahimi *et al.*, 2022) [4, 5, 23]. The genus shows significant genetic and morphological diversity, attributed to variations in chromosome numbers, chromosomal abnormalities, and hybridization between and within species. This genetic variability explains the wide differences observed in chemical composition and therapeutic properties among basil varieties (Zare *et al.*, 2021) [23]. Notably, some varieties exhibit a purple coloration caused by the accumulation of anthocyanin pigments, mainly cyanidin derivatives, in leaf cells. This pigmentation enhances their antioxidant capacity beyond that found in many medicinal plants and colored fruits (Nazir *et al.*, 2020; Prinsi *et al.*, 2020) [16, 20]. Chemical and molecular analyses demonstrate considerable variation in essential oil yield among basil species, ranging from 0.2% to 5.22%, influenced by species, phenological stage, and environmental conditions (Mulugeta *et al.*, 2024) [14]. These essential oils contain diverse compounds such as camphor, limonene, thymol, citral, geraniol, and linalool. Some

species also have high levels of phenolic compounds like eugenol, methyl chavicol (estragole), and methyl cinnamate, along with varying amounts of linalool (Gurav *et al.*, 2022; Padalia *et al.*, 2017) [8, 17]. Beyond essential oils, basil contains various phenolic and polyphenolic compounds, including rosmarinic acid, caffeic acid, and cichoric acid, as well as complex flavonoids and anthocyanins. These compounds contribute to basil's potent antioxidant and anti-inflammatory effects (Bajomo *et al.*, 2022; Beltrán-Noboa *et al.*, 2023) [2, 3]. Among its bioactive constituents, fatty acids are particularly significant, especially in basil seeds. Gas chromatography analyses have shown that basil seed oil is rich in unsaturated fatty acids, mainly alpha-linolenic acid (omega-3), followed by linoleic acid (omega-6), as well as saturated fatty acids such as palmitic and stearic acids. This composition gives basil seed oil valuable nutritional and health-promoting properties, positioning it as a promising source of essential fatty acids for food and pharmaceutical applications (Mostafavi *et al.*, 2019) [12, 13], key compounds like eugenol and linalool contribute to the therapeutic effects of basil essential oils, including antioxidant, anti-inflammatory, antimicrobial, and chronic disease resistance properties (Mulugeta *et al.*, 2024) [14]. Recent research has also highlighted some basil chemical constituents as natural insecticides, offering an eco-friendly alternative to synthetic pesticides in agriculture (Padalia *et al.*, 2017) [17].

Gas Chromatography (GC) is an analytical technique used to separate and analyze chemical compounds that can be vaporized or converted into the gaseous state without decomposition. GC is an essential tool in analytical chemistry for accurately determining the quantity and identity of components in complex mixtures (Al-Bukhaiti *et al.*, 2020).

Keywords Basil, Fatty acids, IBA, Water Stress Materials and Methods

The fatty acid composition of samples from two basil cultivars, *Ocimum basilicum L.* (sweet basil) and *Ocimum*

kilimandscharicum Guerke (camphor basil), was analyzed using gas chromatography coupled with a flame ionization detector (GC-FID). The objective was to evaluate the effects of varying concentrations of the growth regulator indole-3-butyric acid (IBA) at 0, 0.025, and 0.075 ppm, under two water stress levels (80 g/L and 20 g/L). Seeds were germinated and seedlings were treated with the designated IBA concentrations and subjected to the specified water stress conditions. Fatty acids were then extracted from the leaf samples following standard lipid extraction protocols and subsequently analyzed by GC-FID. The experiment was designed with nine replicates per treatment to ensure statistical robustness. The obtained chromatograms were used to quantify and compare the fatty acid profiles between treatments and cultivars, thereby assessing the physiological responses of *O. basilicum* and *O. kilimandscharicum* to combined effects of IBA application and water stress

Esterification of Fats

The sample was prepared according to the method adopted by AOAC (1995), which is based on the esterification of fats by reacting them with methanolic potassium hydroxide. This reagent was prepared by dissolving 11.2 g of potassium hydroxide in 100 ml of methanol. Then, 1 g of fat was taken and mixed with 8 ml of methanolic potassium hydroxide and 5 ml of hexane. The mixture was shaken rapidly for 30 seconds and then left to separate into two layers. The upper layer (hexane layer), containing the fat esters, was collected and injected into the device.

Chromatographic Analysis of the Sample

Fatty acid compounds were analyzed using a gas chromatography device (GC-2010), model Shimadzu, Japanese origin. A flame ionization detector (FID) was used, along with a capillary separation column (SE-30) with

dimensions of 30 m length × 0.25 mm diameter, under the following conditions: As shown in the table (1)

Table 1: GC conditions

No.	Parameter	Temperature/Value
1	Injection Port Temperature	280 °C
2	Detector Temperature	310 °C
3	Column Oven Temperature	120 – 290 °C (10 °C/min)
4	Carrier Gas Flow Rate	100 kPa

Fatty acid composition in basil plants was analyzed using Gas Chromatography equipped with a Flame Ionization Detector (GC-FID). Four replicate samples (R1, R3, R6, R9) were collected from both *Ocimum basilicum* L and *Ocimum kilimandscharicum* Gürke cultivars.

Five standard fatty acids were used for calibration and identification

- α -Linolenic acid (0.02 g/mL) Figure (1)
- Linoleic acid (0.005 g/mL) Figure (2)
- Oleic acid (0.005 g/mL) Figure (3)
- Palmitic acid (0.005 g/mL) Figure (4)
- Stearic acid (0.02 g/mL) Figure (5)

Each standard was injected in a volume of 1 μ L under specific GC conditions (injector and detector temperatures ranging from 250°C to 340°C, and programmed column oven temperatures appropriate for each compound). Retention times and peak areas were recorded for standard identification. For sample analysis, fatty acids were extracted from basil leaves, then methylated (if applicable) and analyzed using the same GC method. The chromatographic profiles were used to identify and quantify fatty acids based on comparison with standards. Table (2)

Table 2: Information of Samples

Sample Name	Concentration (g/mL)	Injection Volume	Tem Injector	Tem Detector (FID)	Column Oven (ZB-1)	Pressure
a-Lenolinic	0.02 g/mL	1 μ L	280°C	330°C	100–300°C (10°C/min)	100 kPa
Linoleic	0.005 g/mL	1 μ L	280°C	340°C	60–90°C (5°C/min)	100 kPa
Oleic	0.005 g/mL	1 μ L	280°C	330°C	100–200°C	100 kPa
Palmitic	0.005 g/mL	1 μ L	250°C	300°C	220°C	100 kPa
stearic acid	0.02 g/mL	1 μ L	280°C	330°C	100–300°C (10°C/min)	100 kPa

Sample Name : a-Lenolinic

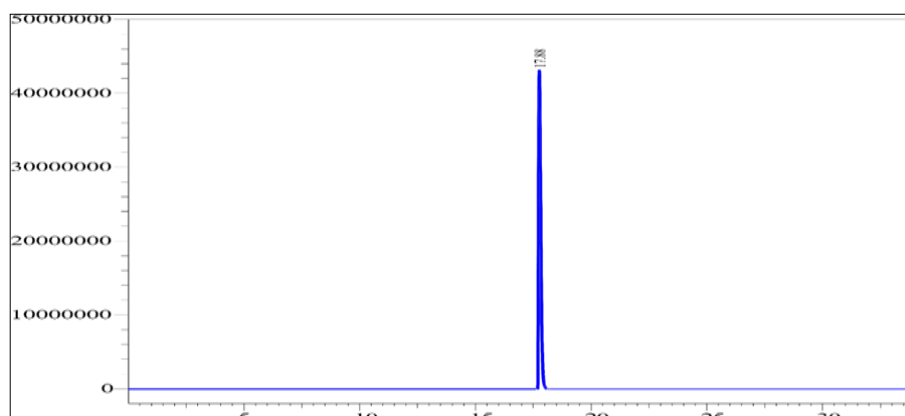


Fig 1: Chromatogram D:\data1\Data1L3286.gcd - Channel 1

Peak Table - Channel 1 min

Peak#	Ret.Time	Area	Area%	Height	Name
1	17.88	2541455	100.0000	39453929	
Total		2541455	100.0000	39453929	

Sample Name = Linoleic

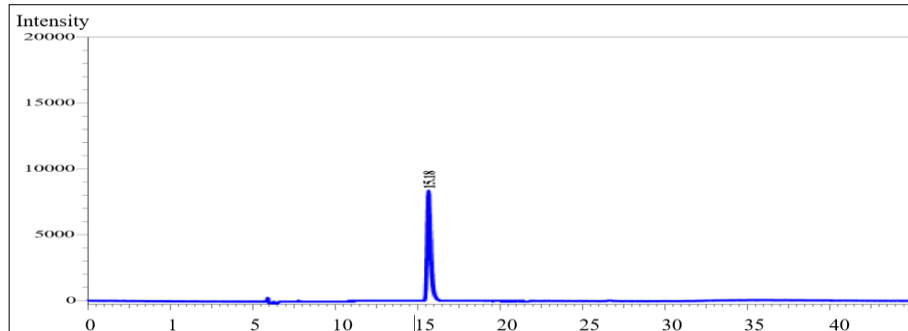


Fig 2: Chromatogram C:\G Csolution Data Project1 Data 1L193. gcd - Channel 1

Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	15.18	854798	100.0000	42718	
Total		854798	100.0000	42718	

Sample Name = oleic

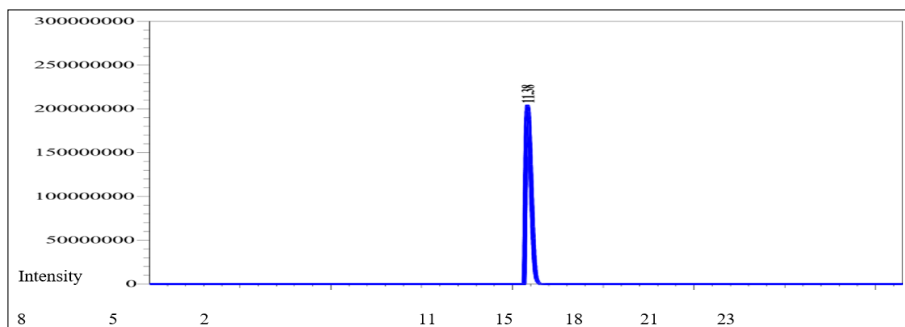


Fig 3: Chromatogram C: G Csolution Sample Data 1L4828 .gcd - Channel 1

min PeakTable - Channel

Peak#	Ret.Time	Area	Area%	Height	Name
1	11.38	1258741	100.0000	54925408	
Total		1258741	100.0000	54925408	

Sample Name = palmitic

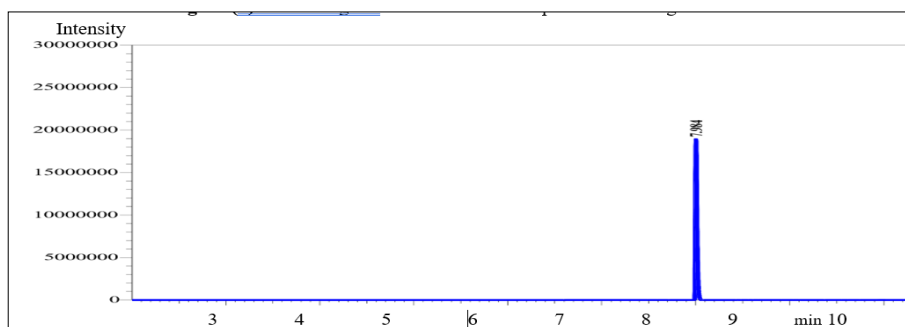


Fig 4: Chromatogram C: G C solution Sample Data 1L891. gcd - Channel 1

Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	7.984	8547858	100.0000	18352655	
Total		8547858	100.0000	18352655	

Sample Name: stearic acid

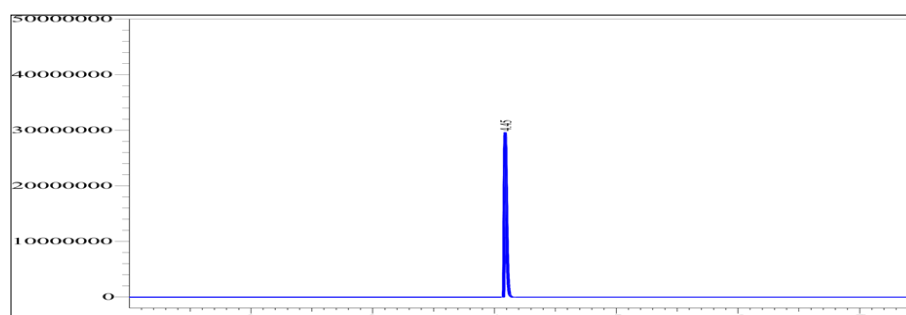


Fig 5: Chromatogram D:\data1\Data1L3285.gcd - Channel 1

Peak Table - Channel 1 min

Peak#	Ret.Time	Area	Area%	Height	Name
1	4.45	199100	100.0000	25761265	
Total		199100	100.0000	25761265	

Results

Fatty acid analysis was conducted on leaf samples from two basil cultivars (*Ocimum basilicum* L.) and (*Ocimum kilimandscharicum* Gürke)—using gas chromatography with flame ionization detection (GC-FID). The objective was to evaluate the effects of different concentrations of the growth regulator indole-3-butyric acid (IBA) (0, 0.025, and 0.075 ppm) and two levels of water stress (80 and 20 g/L). The results revealed clear differences in fatty acid composition among the samples, indicating distinct physiological responses by each cultivar to the applied treatments.

Retention times observed in the samples matched those of the standards

- α -Linolenic acid \approx 17.88 min
- Linoleic acid \approx 15.18 min
- Oleic acid \approx 11.38 min
- Palmitic acid \approx 7.98 min
- Stearic acid \approx 4.45 min

The fatty acid composition was analyzed in samples of *Ocimum basilicum* L. (samples 1 to 4) and *Ocimum kilimandscharicum* Gürke (samples 5 to 8) under the influence of two main factors: the concentration of the growth regulator indole butyric acid (IBA) and the level of water stress. The results showed that the first sample of sweet basil, which did not receive any IBA treatment and was subjected to high water stress (80 g/L), recorded the highest percentage of linoleic acid. Linoleic acid is an essential fatty acid associated with plant stress tolerance mechanisms, indicating an adaptive response by the plant to enhance linoleic acid production as a defense mechanism under severe water stress conditions (Figure 6). With the reduction of water stress in the second sample of the same cultivar, without IBA application, a decrease in linoleic acid concentration was observed alongside an increase in other fatty acids such as oleic acid and palmitic acid. This reflects

an improvement in the plant's metabolic balance and a reduced impact of water stress on fatty acid composition (Figure 7). In the third sample, treated with a low concentration of IBA (0.025 ppm) under continued mild water stress, a more balanced distribution of fatty acids was observed with relative stability in linoleic acid levels. This suggests that the growth regulator contributed to enhancing the plant's metabolic flexibility and improving its response to stress without causing significant metabolic disruption (Figure 8). In the fourth sample, treated with a higher IBA concentration (0.075 ppm) under mild water stress, greater stability in fatty acid proportions was noted, reflecting the positive role of higher IBA concentrations in stabilizing metabolic processes and improving the plant's adaptability (Figure 9). Regarding camphor basil, the fifth sample, exposed to high water stress without IBA treatment, showed a marked increase in linoleic acid percentage, indicating an adaptive response similar to that observed in sweet basil under water stress conditions (Figure 10). With the reduction of water stress intensity in the sixth sample without IBA application, an increase in fatty acid diversity was noted, suggesting relative stability of the plant's metabolic processes (Figure 11). In the seventh sample, treated with 0.025 ppm IBA under low water stress, broader activation of fatty acid biosynthesis pathways was observed, along with a relative increase in secondary fatty acids such as oleic and palmitic acids. This indicates the positive effect of the growth regulator in enhancing metabolic flexibility. The eighth sample, treated with the highest IBA concentration (0.075 ppm) under low water stress, exhibited a significant increase in linoleic acid percentage at the expense of other fatty acid diversity, which may reflect an excessive adaptive response or an imbalance in metabolic homeostasis caused by the high growth regulator concentration. Based on these findings, it can be concluded that water stress induces an increase in linoleic acid production as a common defense mechanism in both basil types. Conversely, the growth regulator Indole-3-butyric

acid (IBA) significantly modulates this response. Low to moderate concentrations of IBA improve the balance of fatty acids and enhance the metabolic flexibility of the plants. However, high concentrations of IBA lead to a more specialized response, focusing on linoleic acid production,

with a clear variation in its effect between the two basil types. These results reflect distinct differences in the metabolic adaptation mechanisms of sweet basil and camphor basil under water stress conditions and the influence of the growth regulator.

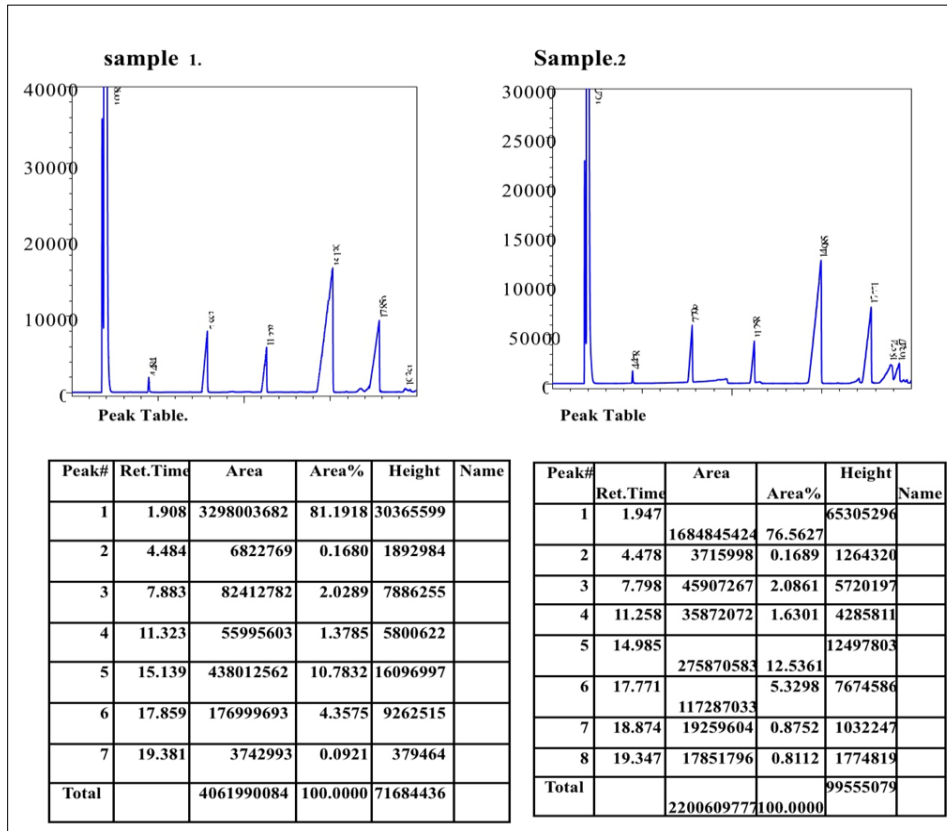


Fig 6

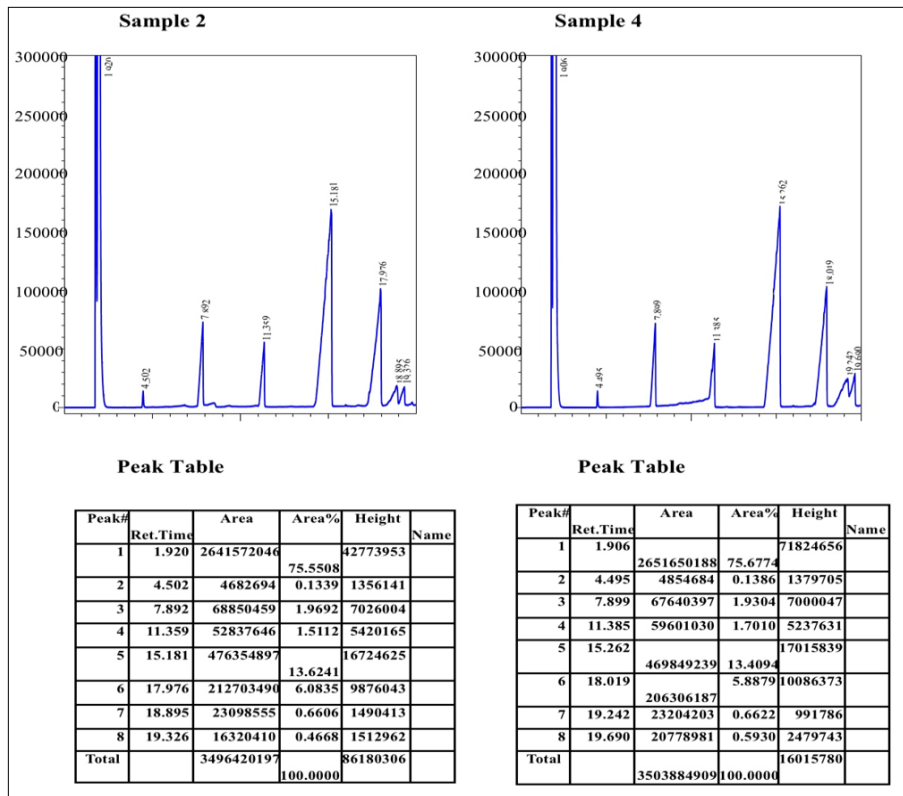


Fig 7

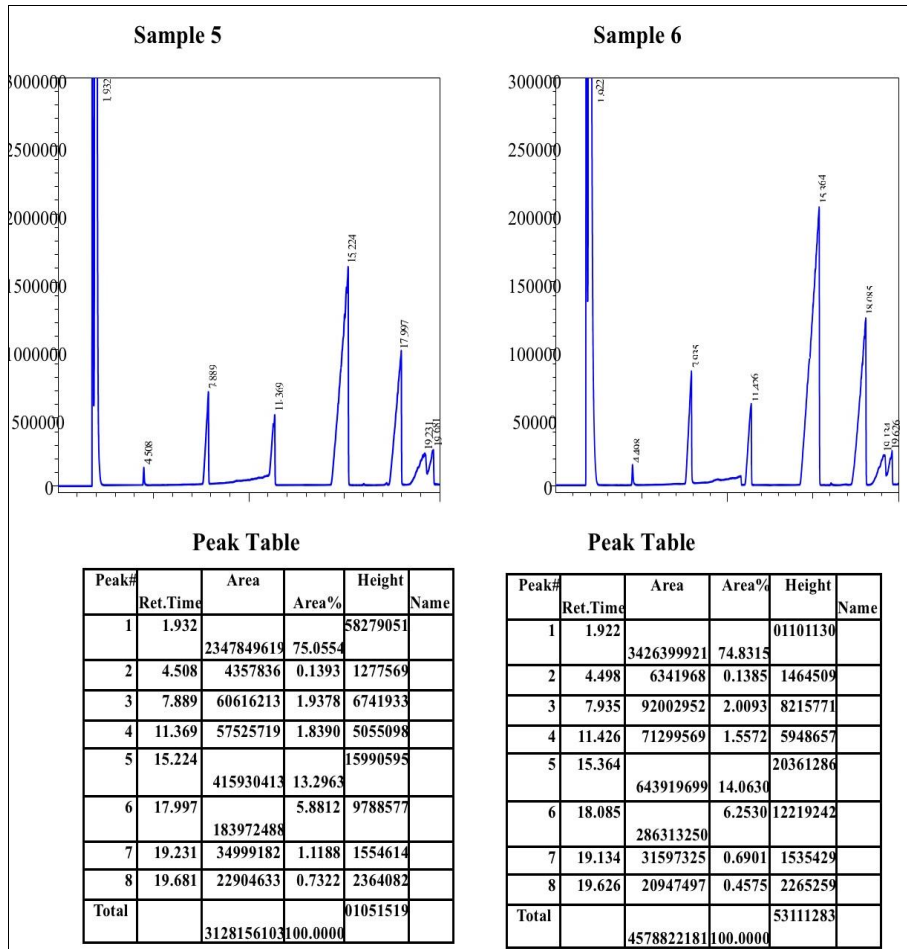


Fig 8, 9

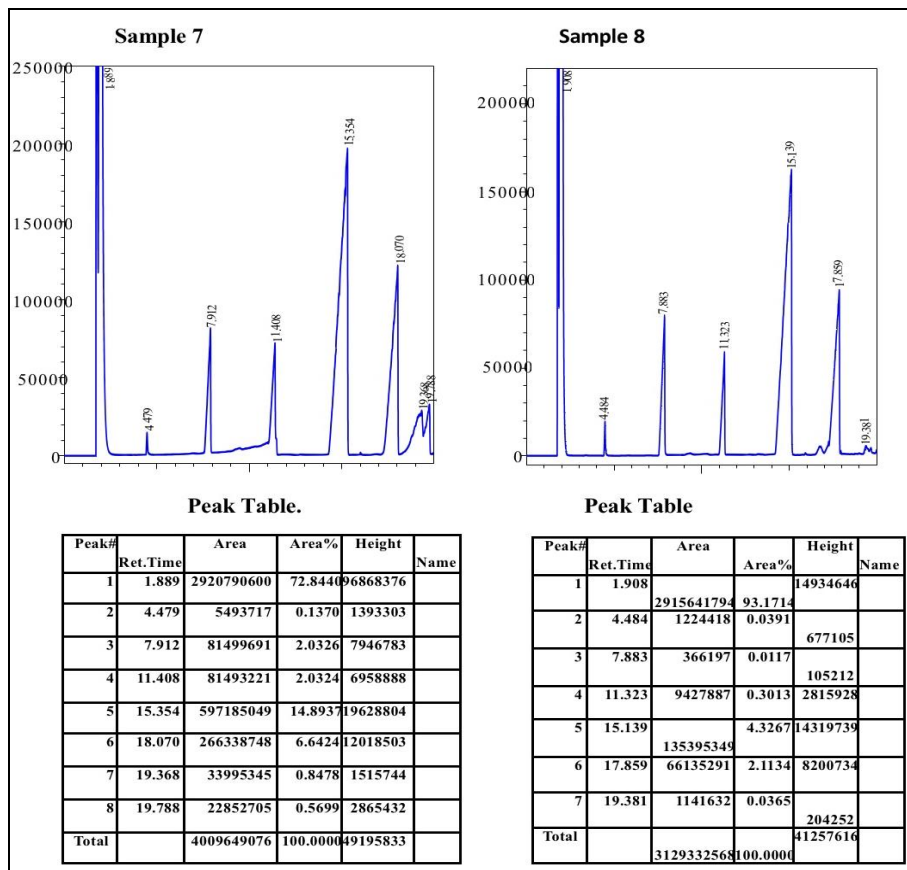


Fig 10, 11

Table 3: Fatty acid concentrations

Name %	Oleic	Palmitic	Stearic	Linoleic	α -Linolenic
1	7.58	5.22	3.25	44.98	2.58
2	8.45	6.52	4.15	46.85	3.00
3	9.22	7.99	5.99	47.82	3.58
4	11.65	9.80	7.89	49.80	4.89
5	7.98	6.11	4.00	45.08	2.98
6	9.88	7.58	5.58	47.00	3.88
7	10.58	8.98	6.98	48.98	4.78
8	12.66	11.06	8.99	50.33	6.00

Table (3) The results revealed clear differences in fatty acid profiles among the tested samples. A progressive increase in fatty acid concentrations was observed with increasing IBA levels and decreasing water stress. Linoleic acid was found to be the most abundant fatty acid across all samples, followed by oleic acid and palmitic acid. Stearic and α -linolenic acids were present in lower amounts. These findings indicate that IBA application positively influenced fatty acid biosynthesis pathways, especially under low water stress conditions. Samples treated with high IBA concentrations showed the highest levels of unsaturated fatty acids, reflecting enhanced oil quality in basil. Conversely, untreated samples under severe water stress exhibited the lowest fatty acid contents, highlighting the crucial role of growth regulators in mitigating stress and enhancing lipid metabolism. Overall, the interaction between IBA and water stress significantly modified the fatty acid composition in basil, increasing the content of unsaturated fatty acids, which are known for their high nutritional and therapeutic value. The following formula was used to calculate the concentration of each fatty acid in the sample

Compound concentration (ppm) = (Standard concentration \times Sample peak area \div Standard peak area) \times (Dilution factor \div Sample weight)

Variation Between Species

Fatty acid profiling revealed distinct metabolic responses between the two studied species. Sweet basil exhibited metabolic flexibility, maintaining a stable and balanced fatty acid profile under low water stress combined with elevated IBA concentration, showing a harmonious increase in both primary and secondary fatty acids. This suggests a regulated activation of lipid biosynthesis pathways, which is physiologically advantageous. In contrast, camphor basil showed pronounced sensitivity to the high IBA concentration (0.075 ppm), resulting in an excessive accumulation of linoleic acid (over 93%) and a marked decrease in other fatty acids. This indicates a metabolic imbalance and possibly a channeling of biosynthetic pathways toward the production of a single dominant fatty acid, reducing overall biochemical diversity.

Effect of IBA and Water Stress on Fatty Acid Biosynthesis

Application of IBA at moderate concentrations (0.025 ppm) enhanced the production of unsaturated fatty acids, particularly under moderate water availability. This observation is consistent with the findings of Bettaieb *et al.* (2011), who reported that water deficit significantly affects fatty acid content and essential oil composition in cumin (*Cuminum cyminum* L.), highlighting the critical role of water stress and growth regulators in modulating fatty acid

biosynthesis. Furthermore, the presence of IBA coupled with reduced water stress likely stimulates enzymes such as $\Delta 9$ -desaturase, which convert saturated fatty acids into unsaturated forms, thereby improving the nutritional quality of plant oils (Bettaieb *et al.*, 2011).

Importance of Hormonal and Water Balance

This study underscores the importance of finely tuning the levels of IBA and water stress to maintain fatty acid compositional balance. Sweet basil maintained metabolic homeostasis under these treatments, while camphor basil experienced compositional disruption at high IBA levels. This highlights the necessity of regulating IBA concentrations carefully to avoid excessive accumulation of a single fatty acid and maintain metabolic diversity.

The findings suggest that agricultural practices incorporating growth regulators such as IBA, alongside controlled water management, can be used to improve the quality of basil oils by:

1. Increasing the production of valuable unsaturated fatty acids like linoleic and α -linolenic acids, known for their health-promoting properties.
2. Preserving fatty acid balance, especially in sweet basil, thereby enhancing metabolic stability under fluctuating environmental conditions.
3. Preventing the overaccumulation of a single fatty acid, as observed in camphor basil, through careful regulation of IBA concentration

Conclusions

The current study demonstrated a clear effect of the interaction between the growth regulator indole-3-butyric acid (IBA) and water stress levels on the fatty acid composition of two basil species: *Ocimum basilicum* L. (sweet basil) and *Ocimum kilimandscharicum* Gürke (camphor basil). Linoleic acid was the most abundant fatty acid in all samples, followed by oleic acid and palmitic acid, while stearic acid and α -linolenic acid were present at lower concentrations. These findings align with previous studies reporting the predominance of unsaturated fatty acids in plant oils under varying environmental conditions (Ghasemi Pirbalouti *et al.*, 2017).

References

1. Alwattar AM. Role of fatty acids in cellular signaling and inflammation regulation. *J Lipid Res*,2021;62(4):100045. doi: 10.1016/j.jlr.2021.100045.
2. Bajomo A, Smith J, Adeyemi O. Phenolic compounds and antioxidant activities in medicinal plants: A review. *J Herbal Sci*,2022;14(3):215-230.
3. Beltrán-Noboa MJ, Torres D, Ramos F. Polyphenols and flavonoids in *Ocimum* species and their health effects. *Phytochem Rev*,2023;22(1):45-60.

4. Copolovici L, Farkas Á, Niinemets Ü. Variation in essential oil yield and composition among basil (*Ocimum basilicum*) varieties. *Ind Crops Prod*,2021;164:113384.
5. Ebrahimi S, Hashemi S, Rahimi H. Chemical composition and biological activity of *Ocimum basilicum* L.: A comprehensive review. *Nat Prod Commun*,2022;17(2):1-12.
6. Güez A, Ben Jannet H, Kchouk ME. Diversity and uses of *Ocimum* species: A review. *Med Plants-Int J Phytomedicines Related Ind*,2017;9(1):1-9.
7. Güez A, El Kahoui S, Benkirane R. Diversity of aromatic and medicinal plants of the Lamiaceae family in Morocco. *Moroc J Bot*,2017;5(1):12-28.
8. Gurav S, Patil P, Deshmukh P. Chemical constituents and bioactivities of basil essential oils: A systematic review. *J Essent Oil Res*,2022;34(1):1-15.
9. Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M. Plant response and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In: *Crop Stress Management*. Elsevier, 2017, 261-315. doi: 10.1016/B978-0-12-805268-1.00010-9.
10. Khan MIR, Fatma M, Per TS, Anjum NA, Khan NA. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front Plant Sci*,2014;5:529. doi: 10.3389/fpls.2014.00529.
11. Los DA, Murata N. Membrane fluidity and its roles in the perception of environmental signals. *Biochim Biophys Acta Biomembr*,2004;1666(1-2):142-157. doi: 10.1016/j.bbamem.2004.06.013.
12. Mostafavi H, Jafari M, Zare S. Fatty acid profile and nutritional properties of *Ocimum basilicum* seed oil. *Food Chem*,2019;274:70-76.
13. Mostafavi SA, Saei-Dehkordi SS, Hosseini SMH. Fatty acid composition and health benefits of *Ocimum* seed oils: A review. *J Food Sci Technol*,2019;56(5):2331-2340. doi: 10.1007/s13197-019-03722-3.
14. Mulugeta D, Bekele T, Tesfaye K. Variation in essential oil composition and antioxidant activity of *Ocimum* species grown in Ethiopia. *J Med Plants Res*,2024;18(2):85-98.
15. Muñoz-Mayor A, Pérez-Pérez JM, Sánchez-Blanco MJ, Flores FB. The role of fatty acid unsaturation in drought tolerance in plants. *Plant Sci*,2012;196:12-21. doi: 10.1016/j.plantsci.2012.08.003.
16. Nazir M, Sultana S, Qureshi R. Anthocyanin accumulation and antioxidant potential in purple basil (*Ocimum basilicum*). *Plant Physiol Rep*,2020;25(3):194-202.
17. Padalia H, Verma RS, Chauhan A. Chemical composition, biological activities, and potential uses of basil (*Ocimum* spp.) essential oils. *Med Aromat Plants*,2017;6(3):1-12.
18. Padalia H, Verma RS, Chauhan A. Essential oils of *Ocimum* species: Their chemical diversity and biological activity. *Ind Crops Prod*,2017;95:117-131. doi: 10.1016/j.indcrop.2016.09.010.
19. Pandey A, Singh N, Singh A. Genetic variation in lipid metabolism pathways of basil cultivars under stress. *Plant Physiol Rep*,2017;22(3):321-330. doi: 10.1007/s40502-017-0313-4.
20. Prinsi B, Negri AS, Failla O. Anthocyanin-related compounds and antioxidant activity in basil leaves: A comparative study. *Front Plant Sci*,2020;11:123.
21. Sharma S, Kumar V, Singh R. Role of fatty acid desaturase enzymes in plant stress tolerance. *Plant Mol Biol*,2015;87(4-5):431-446. doi: 10.1007/s11103-015-0313-9.
22. Upchurch RG. Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnol Lett*,2008;30(6):967-977. doi: 10.1007/s10529-008-9652-5.
23. Zare H, Azizi M, Abolghasemi M. Genetic diversity and essential oil composition of *Ocimum* species: A review. *Biotechnol Rep*,2021;30:e00661.
24. Zhang Y, Liu Y, Liu J, Wang Z. Physiological and metabolic responses of plants under drought stress: An overview. *J Am Soc Hortic Sci*,2018;143(3):131-142. doi: 10.21273/JASHS04213-18.