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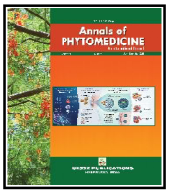

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Impact of elicitors on oil yield and essential oil profiling of sacred basil (*Ocimum sanctum* L.)

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Abstract

The experiment entitled impact of elicitors on oil yield and essential oil profiling of sacred basil (*Ocimum sanctum* L.) was done during 2021-22 and 2022-23 at College of Horticulture, SKLTGHU, Rajendranagar. Methyl jasmonate and salicylic acid, as signalling molecules have shown to promote the functioning of genes related to the synthesis of secondary metabolites. Salicylic acid and methyl jasmonate are important messengers in activating plant defense responses. They also reduce raw material production, resulting in the synthesis and buildup of numerous plant secondary compounds. Plant cells when exposed to the elicitors, a signal will be transmitted from the surface to the plasma membrane resulting in the production of reactive oxygen species, which triggers the plant defense mechanism and enhances the activity of key enzymes that catalyze chemical reactions and the synthesis of target secondary metabolites. In this study, an investigation on elicitors to examine the combined effect of varieties and different concentrations of elicitors on oil yield and composition of sacred basil was studied. The highest oil yield (113.96 kg/ha) was noted in CIM-Ayu when treated with salicylic acid @ 0.5 mM. The higher levels of eugenol (36.78), linalool (0.95), methyl chavicol (1.07), methyl eugenol (16.22), β-elemene (12.13), limonene (1.67), caryophyllene (11.64) and chlorophyll (41.53) content was recorded by the usage of salicylic acid @ 0.5 mM. From this study, it can be concluded that elicitors and varieties influence oil yield and essential oil profiling in sacred basil.

1. Introduction

O. sanctum is a biennial or triennial herbaceous, erect, branched, softly hairy plant growing upto 30-75 cm. The species is worshiped by hindus and commonly grown in temples and courtyards. The plant comprises phenols, tannins, aldehydes, fats and saponins. The essential oil consists of eugenol (71%), eugenol methyl ether (20%), carvacrol (3%) and trace amounts of nerol, α-pinene, caryophyllene, selinene, cineole, β-pinene, camphor and linalool, *etc.* (Lal *et al.*, 2003). The leaves are used as a condiment in salads as well as various dishes while the plant is used as a pot herb.

Salicylic acid (SA) which is a phenolic phytohormone governing physiological processes, including growth and development (Jakhar and Sheokand, 2015), photosynthesis, seed germination, transport and absorption of ions, glycolysis (Vicente and Plasencia, 2011). Salicylic acid, which is known for its systemic acquired resistance, triggers the response of plants to many pathogens and may also stimulate secondary metabolite production (Ankita Singh and Padmanabh Dwivedi, 2018).

Methyl jasmonate (MeJA), a volatile ester of jasmonic acid, represents another key signalling molecule involved in plant defence and stress response. It serves as an important regulator of tolerance against both biotic and abiotic stress factors (Cheong and Choi, 2003). MeJA-mediated responses include modification of growth and developmental processes through long-distance signalling pathways (Motallebi *et al.*, 2015), as well as stimulation of the secondary metabolic network. This compound also promotes the formation of defensive metabolites against herbivores and elevates reactive oxygen species (ROS) levels to improve resistance under stress (Meldau *et al.,* 2012).

Although, the role of SA and MeJA in defence and stress physiology is well established, limited information is available regarding their impact on oil yield and essential oil composition in sacred basil (*O. sanctum*) under field conditions. In view of this gap, the present investigation was undertaken to examine the effect of these elicitors on growth performance, yield and essential oil profile of two basil varieties.

2. Materials and Methods

The current study was done during 2021-22 and 2022-23 at SKLTGHU, Rajendranagar. Factorial randomized block design of 14 treatments and 3 replications was used. The treatments include 2 varieties CIM-Ayu (V_1) and CIM-Angana (V_2) and 7 elicitors E_1 : MeJA (0.25 mM), E_2 : MeJA (0.5 mM), E_3 : MeJA (1.0 mM), E_4 : SA (0.1 mM), E_5 : SA (0.5 mM), E_6 : SA (1 mM), E_7 : water spray (control).

CIM-Ayu (V_1) : High-yielding, eugenol-rich improved variety of sacred basil developed and released by CSIR-CIMAP, Lucknow in 2003 from the strain KRT-2 through open pollination to meet the demand for high eugenol content and superior essential oil yield for medicinal and aromatic industries.

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CIM-Angana (V_2) : High-herbage-yielding variety of sacred basil developed and released by CSIR-CIMAP, Lucknow in 2001 through systematic phenotypic selection from diverse germplasm.

2.1 Oil yield (kg/ha)

The hydrodistillation of freshly picked herbage and inflorescence yielded the essential oil content on a fresh weight basis using Clevenger type apparatus according to procedure from A.O.A.C (Anon, 1975). 500 g of the chopped plant material was taken in one litre volumetric flask and 1000 ml water was added. This was heated for 3½ h and oil was collected and expressed in percentage. The oil yield per hectare was computed by multiplying yield of fresh herbage per hectare with the percentage recovery of oil.

2.2 Essential oil profiling

By using gas chromatography, eugenol, linalool, methyl chavicol, limonene, methyl eugenol, β-elemene and caryophyllene content in the sacred basil was calculated and converted into percentage.

2.3 GC operating conditions

Essential oil analysis was performed using a Gas Chromatograph ANALYTE 2900A fitted with a flame ionization detector (FID). A 1 µl aliquot of each sample was introduced in split mode, with the injector temperature set at 250°C to facilitate efficient vaporization. Compound separation was achieved using a non-polar fused silica capillary column. Helium was used as the carrier gas, maintained at a constant flow rate of 1.0 ml min^{-1}. The oven temperature was initially held at 60°C for 2 min, gradually increased to 180°C at a rate of 3°C min^{-1}, and then raised to 240°C at 5°C min^{-1} with a final hold of 10 min. The FID was operated at 280°C under standard gas supply conditions for hydrogen and air. Compound identification was based on comparison of retention times with authenticated standards and where applicable, further confirmed using spectral characteristics.

2.4 Chlorophyll content

Soil plant analysis development (SPAD) value was recorded from randomly selected plants with the help of SPAD meter (Kariya *et al.*, 1982) at 45, 60 and 75 DAT. The averages of top, middle and base value were expressed as SPAD value.

2.5 Statistical analysis and interpretation

Fisher method of analysis of variance was applied for data analysis and interpretation. The level of significance used in 'F' was at $p \leq 0.05$ and the critical difference (CD) values ware worked out wherever 'F' test was significant (Panse and Sukhatme, 1967).

3. Results

3.1 Oil yield per plant (g/plant)

The data on oil yield per plant (g/plant) as influenced by varieties, elicitors and their interactions during both the years and pooled is presented in Table 1. Among the varieties the highest oil yield per plant (g/plant) was observed in CIM-Ayu (V_1) (1.14, 1.14 and 1.14) than CIM-Angana (V_2) (1.06, 1.07 and 1.07) during 2021, 2022 and pooled, respectively. The use of elicitors also demonstrated a significant impact on the annual and pooled oil yield per plant, in which the maximum (1.73, 1.75 and 1.74) oil yield per plant (g/plant) was observed in E_5-SA @ 0.5 mM, followed by E_4-SA @ 0.1 mM (1.58, 1.57 and 1.57) and E_3-MeJA @ 1.0 mM (1.23, 1.26 and 1.24) during 2021, 2022 and pooled, respectively. In the same way, E_7-control (0.54, 0.50 and 0.52) had the lowest oil yield per plant (g/plant) in 2021, 2022 and pooled, respectively. In interaction the highest oil yield per plant (g/plant) (1.80, 1.84 and 1.82) was registered in T_5 (V_1E_5), followed by T_{12} (V_2E_5) (1.65, 1.66 and 1.65) and was at par with T_4 (V_1E_4) (1.64, 1.63 and 1.63) during 2021, 2022 and pooled, respectively. Whereas, the lowest oil yield per plant (g/plant) (0.53, 0.47 and 0.50) was registered in T_{14} (V_2E_7) and was on par with T_7 (V_1E_7) (0.54, 0.52 and 0.53) during 2021, 2022 and pooled.

3.2 Oil yield per plot (g/plot)

The results pertaining to oil yield per plot as influenced by varieties, elicitors and their combinations in the years 2021, 2022 and pooled are presented in the Table 1. The varieties, elicitors and their interaction differed significantly with respect to oil yield per plot. The highest oil yield per plot (g/plot) was noted in CIM-Ayu (V_1) (42.89, 42.72 and 42.81) than CIM-Angana (V_2) (39.83, 40.15 and 39.99) in 2021, 2022 and pooled, respectively. Among the elicitors maximum (64.69, 65.60 and 65.14) oil yield per plot (g/plot) was observed in E_5-SA @ 0.5 mM, followed by E_4-SA @ 0.1 mM (59.14, 58.93, and 59.03) and E_3-MeJA @ 1.0 mM (46.06, 47.24 and 46.65). Whereas, the lowest oil yield per plot (g/plot) was noted in E_7-control (20.08, 18.69 and 19.38) during 2021, 2022 and Pooled. Among the interactions maximum oil yield per plot (g/plot) (67.65, 69.10 and 68.38) was registered in T_5 (V_1E_5), followed by T_{12} (V_2E_5) (61.73, 62.10 and 61.91) and was at par with T_4 (V_1E_4) (61.48, 61.13 and 61.30). Whereas, the lowest oil yield per plot (g/plot) (19.83, 17.75 and 18.79) was registered in T_{14} (V_2E_7) and was on par with T_7 (V_1E_7) (20.33, 19.63 and 19.98) during 2021, 2022 and pooled.

3.3 Oil yield per hectare (kg/ha)

Table 1 displays information on the oil produced per hectare for the years 2021, 2022 and pooled, as influenced by varieties, elicitors and their interactions. The highest oil yield per hectare (kg/ha) was recorded in CIM-Ayu (V_1) (71.49, 71.20 and 71.42) than CIM-Angana (V_2) (66.39, 66.91 and 66.65) in 2021, 2022 and pooled, respectively. The maximum (107.81, 109.33 and 108.57) oil yield per hectare (kg/ha) was observed in E_5-SA @ 0.5 mM, followed by E_4-SA @ 0.1 mM (98.56, 98.21 and 98.65) and E_3-MeJA @ 1.0 mM (76.77, 78.73 and 77.75). While, the lowest oil yield per hectare (kg/ha) was recorded in E_7-control (33.46, 31.15 and 32.30) during 2021, 2022 and pooled. Among the interactions highest oil yield per hectare (kg/ha) (112.75, 115.17 and 113.96) was registered in T_5 (V_1E_5) CIM-Ayu + SA @ 0.5 mM, followed by T_{12} (V_2E_5) CIM-Angana + SA @ 0.5 mM (102.88, 103.50 and 103.19) and was at par with T_4 (V_1E_4) (102.46, 101.87 and 102.69) during 2021, 2022 and pooled. Whereas, the lowest oil yield per hectare (kg/ha) (33.04, 29.58 and 31.31) was registered in T_{14} (V_2E_7) CIM-Angana + control and was on par with T_7 (V_1E_7) (33.88, 32.71 and 33.29) during 2021, 2022 and pooled, respectively.

3.4 Essential oil profiling (%)

Among the varieties, CIM-Ayu (V_1) had higher values of eugenol, methyl eugenol and caryophyllene (35.73, 22.25 and 10.84, respectively) than CIM-Angana (V_2) (26.14, 5.10 and 10.09) (Figure 1). Methyl chavicol, limonene, linalool and β- elemene, the maximum content was noted in CIM-Angana (V_2) (1.29, 1.68, 1.45 and 14.20, respectively) compared to CIM-Ayu (V_1) (0.56, 1.35, 0.26 and 7.87)

(Table 2). Among the elicitors highest linalool, methyl chavicol, limonene, methyl eugenol, eugenol, caryophyllene and β-elemene, respectively (0.92, 1.05, 1.66, 16.16, 35.71, 11.60 and 12.06) was recorded by the application of E_5-SA @ 0.5 mM. The lowest content of linalool, limonene, methyl chavicol, eugenol, β-elemene, methyl eugenol and caryophyllene was recorded in E_7-control (0.75, 1.40, 0.80, 24.11, 9.81, 11.16 and 9.09, respectively). Among the interactions, treatment T_5 (V_1E_5) recorded significantly maximum content of (26.92, 41.96 and 12.29) methyl eugenol, eugenol and caryophyllene, respectively, while the minimum (4.70, 20.52 and 8.94, respectively) was recorded in T_{14} (V_2E_7). Methyl chavicol, limonene, linalool and β-elemene maximum (1.45, 1.82, 1.54 and 15.78, respectively) was noticed in T_{12} (V_2E_5), while, the minimum (0.48, 1.23, 0.20 and 7.16, respectively) was recorded in T_7 (V_1E_7).

Table 1: Influence of elicitors on oil yield of sacred basil (*O. sanctum*)

Treatments	Oil yield per plant (g/plant)			Oil yield per plot (g/plot)			Oil yield per hectare (kg/ha)		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Varieties									
V_1	1.14	1.14	1.14	42.89	42.72	42.81	71.49	71.20	71.42
V_2	1.06	1.07	1.07	39.83	40.15	39.99	66.39	66.91	66.65
S.Em ±	0.01	0.01	0.007	0.27	0.32	0.26	0.45	0.53	0.43
CD at 5%	0.02	0.02	0.021	0.78	0.92	0.75	1.30	1.54	1.25
Elicitors									
E_1	0.68	0.66	0.67	25.53	24.86	25.19	42.54	41.44	41.99
E_2	0.93	0.96	0.94	34.79	35.81	35.30	57.99	59.69	58.84
E_3	1.23	1.26	1.24	46.06	47.24	46.65	76.77	78.73	77.75
E_4	1.58	1.57	1.57	59.14	58.93	59.03	98.56	98.21	98.65
E_5	1.73	1.75	1.74	64.69	65.60	65.14	107.81	109.33	108.57
E_6	1.05	1.04	1.04	39.25	38.91	39.08	65.42	64.85	65.14
E_7	0.54	0.50	0.52	20.08	18.69	19.38	33.46	31.15	32.30
S.Em ±	0.01	0.02	0.013	0.50	0.60	0.48	0.84	0.99	0.80
CD at 5%	0.04	0.05	0.038	1.46	1.73	1.41	2.43	2.88	2.33
Interactions (Varieties and Elicitors)									
T_1 (V_1E_1)	0.73	0.72	0.72	27.23	26.85	27.04	45.38	44.75	45.06
T_2 (V_1E_2)	0.94	0.96	0.95	35.29	36.08	35.68	58.81	60.13	59.47
T_3 (V_1E_3)	1.26	1.25	1.26	47.23	47.03	47.13	78.71	78.38	78.54
T_4 (V_1E_4)	1.64	1.63	1.63	61.48	61.13	61.30	102.46	101.87	102.69
T_5 (V_1E_5)	1.80	1.84	1.82	67.65	69.10	68.38	112.75	115.17	113.96
T_6 (V_1E_6)	1.09	1.05	1.07	41.05	39.25	40.15	68.42	65.42	66.92
T_7 (V_1E_7)	0.54	0.52	0.53	20.33	19.63	19.98	33.88	32.71	33.29
T_8 (V_2E_1)	0.64	0.61	0.62	23.83	22.88	23.35	39.71	38.13	38.92
T_9 (V_2E_2)	0.91	0.95	0.93	34.30	35.55	34.93	57.17	59.25	58.21
T_{10} (V_2E_3)	1.20	1.27	1.23	44.90	47.45	46.18	74.83	79.08	76.96
T_{11} (V_2E_4)	1.51	1.51	1.51	56.80	56.73	56.76	94.67	94.54	94.60
T_{12} (V_2E_5)	1.65	1.66	1.65	61.73	62.10	61.91	102.88	103.50	103.19
T_{13} (V_2E_6)	1.00	1.03	1.01	37.45	38.58	38.01	62.42	64.29	63.35
T_{14} (V_2E_7)	0.53	0.47	0.50	19.83	17.75	18.79	33.04	29.58	31.31
S.Em ±	0.02	0.02	0.018	0.71	0.84	0.68	1.18	1.40	1.13
CD at 5%	0.05	0.07	0.053	2.06	2.45	1.99	3.44	4.08	3.30

The results on the essential oil profiling is showed in Table 3. Among the varieties, CIM-Ayu (V_1) recorded maximum (22.38, 37.88 and 10.93) methyl eugenol, eugenol and caryophyllene, respectively than CIM-Angana (V_2) (5.23, 28.38, and 10.18). Whereas, β-elemene, linalool, methyl chavicol and limonene, was recorded maximum in CIM-Angana (V_2) (14.33, 1.50, 1.33 and 1.72, respectively) compared to CIM-Ayu (V_1) (8.00, 0.31, 0.60 and 1.38) (Figure 2). The maximum content of limonene, linalool, eugenol, methyl chavicol, β-elemene, methyl eugenol and caryophyllene, respectively was observed by the use of E_5-SA @ 0.5 mM (1.69, 0.97, 37.86, 1.09, 12.19, 16.29 and 11.68, respectively) while, the lowest methyl eugenol, linalool, limonene, methyl chavicol, eugenol, â-elemene and caryophyllene was observed in E_7-control (11.28, 0.80, 1.44, 0.82, 26.56, 9.95 and 9.17, respectively). Among the interactions, T_5 (V_1E_5) CIM-Ayu + SA @ 0.5 mM recorded significantly maximum content of (44.11, 27.05 and 12.38, respectively) eugenol, methyl eugenol and caryophyllene, respectively, followed by T_4 (V_1E_4) (42.23, 25.76 and 12.01), while the minimum (23.28, 4.82 and 9.01, respectively) was recorded in T_{14} (V_2E_7) CIM-Angana and control. In case of linalool, methyl chavicol, limonene and β-elemene highest content (1.59, 1.49, 1.85 and 15.91, respectively) was noticed in T_{12}-CIM-Angana and SA @ 0.5 mM (V_2E_5) while, the minimum (0.25, 0.52, 1.26 and 7.29, respectively) was recorded in T_7 (V_1E_7) CIM-Ayu and control.

Table 2: Influence of elicitors on essential oil profiling of sacred basil (*O. sanctum*) during the year 2021

Treatments	Essential oil profiling						
	Limonene	Linalool	Methyl chavicol	Eugenol	Methyl eugenol	âelemene	Caryophyllene
	Varieties						
V_1	1.35	0.26	0.56	35.73	22.25	7.87	10.84
V_2	1.68	1.45	1.29	26.14	5.10	14.20	10.09
S.Em ±	0.01	0.00	0.003	0.30	0.01	0.02	0.01
CD at 5%	0.03	0.01	0.009	0.86	0.04	0.05	0.03
Elicitors							
E_1	1.47	0.82	0.86	27.71	12.03	10.33	9.73
E_2	1.50	0.85	0.91	29.88	12.59	10.80	10.19
E_3	1.57	0.89	0.96	33.46	14.69	11.47	10.89
E_4	1.50	0.90	1.00	34.21	15.46	11.74	11.27
E_5	1.66	0.92	1.05	35.71	16.16	12.06	11.60
E_6	1.53	0.86	0.93	31.49	13.65	11.04	10.49
E_7	1.40	0.75	0.80	24.11	11.16	9.81	9.09
S.Em ±	0.02	0.01	0.006	0.55	0.03	0.03	0.02
CD at 5%	0.06	0.02	0.018	1.61	0.08	0.10	0.06
Interactions (Varieties and Elicitors)							
$T_1(V_1E_1)$	1.27	0.23	0.52	31.00	19.16	7.60	9.84
$T_2(V_1E_2)$	1.29	0.25	0.55	34.46	20.15	7.70	10.52
$T_3(V_1E_3)$	1.39	0.28	0.58	38.52	24.13	8.13	11.23
$T_4(V_1E_4)$	1.43	0.28	0.61	40.08	25.63	8.25	11.92
$T_5(V_1E_5)$	1.49	0.30	0.64	41.96	26.92	8.34	12.29
$T_6(V_1E_6)$	1.34	0.26	0.56	36.44	22.14	7.92	10.82
$T_7(V_1E_7)$	1.23	0.20	0.48	27.69	17.61	7.16	9.25
$T_8(V_2E_1)$	1.67	1.41	1.20	24.42	4.90	13.06	9.62
$T_9(V_2E_2)$	1.70	1.45	1.27	25.31	5.02	13.89	9.85
$T_{10}(V_2E_3)$	1.75	1.49	1.34	28.39	5.26	14.81	10.56
$T_{11}(V_2E_4)$	1.56	1.51	1.39	28.34	5.28	15.23	10.62

$T_{12}(V_2E_5)$	1.82	1.54	1.45	29.45	5.39	15.78	10.90
$T_{13}(V_2E_6)$	1.73	1.46	1.29	26.54	5.15	14.16	10.15
$T_{14}(V_2E_7)$	1.57	1.30	1.11	20.52	4.70	12.46	8.94
S.Em ±	0.03	0.01	0.009	0.78	0.04	0.05	0.03
CD at 5%	0.09	0.03	0.025	2.27	0.11	0.14	0.08

Table 3: **Influence of elicitors on essential oil profiling of sacred basil (*O. sanctum*) during the year 2022**

Treatments	Essential oil profiling						
	Limonene	**Linalool**	**Methyl chavicol**	**Eugenol**	**Methyl eugenol**	**β elemene**	**Caryophyllene**
Varieties							
V_1	1.38	0.31	0.60	37.88	22.38	8.00	10.93
V_2	1.72	1.50	1.33	28.38	5.23	14.33	10.18
S.Em ±	0.01	0.004	0.01	0.18	0.02	0.02	0.01
CD at 5%	0.03	0.01	0.02	0.54	0.05	0.05	0.03
Elicitors							
E_1	1.50	0.87	0.90	29.86	12.16	10.46	9.82
E_2	1.53	0.90	0.95	32.03	12.72	10.93	10.27
E_3	1.60	0.94	1.00	35.61	14.82	11.60	10.98
E_4	1.53	0.95	1.04	36.36	15.59	11.87	11.36
E_5	1.69	0.97	1.09	37.86	16.29	12.19	11.68
E_6	1.56	0.91	0.97	33.64	13.78	11.17	10.57
E_7	1.44	0.80	0.82	26.56	11.28	9.95	9.17
S.Em ±	0.02	0.007	0.01	0.35	0.03	0.03	0.02
CD at 5%	0.06	0.02	0.03	1.01	0.09	0.10	0.06
Interactions (Varieties and Elicitors)							
$T_1 (V_1E_1)$	1.30	0.28	0.56	33.15	19.29	7.73	9.93
$T_2 (V_1E_2)$	1.32	0.30	0.59	36.61	20.28	7.83	10.61
$T_3 (V_1E_3)$	1.42	0.33	0.62	40.67	24.26	8.26	11.31
$T_4 (V_1E_4)$	1.46	0.33	0.65	42.23	25.76	8.38	12.01
$T_5 (V_1E_5)$	1.52	0.35	0.68	44.11	27.05	8.47	12.38
$T_6 (V_1E_6)$	1.37	0.31	0.60	38.59	22.27	8.05	10.91
$T_7 (V_1E_7)$	1.26	0.25	0.52	29.84	17.74	7.29	9.33
$T_8 (V_2E_1)$	1.70	1.46	1.24	26.57	5.03	13.19	9.71
$T_9 (V_2E_2)$	1.73	1.50	1.31	27.46	5.15	14.02	9.94
$T_{10} (V_2E_3)$	1.78	1.54	1.38	30.54	5.39	14.94	10.65
$T_{11} (V_2E_4)$	1.59	1.56	1.43	30.49	5.41	15.36	10.71
$T_{12} (V_2E_5)$	1.85	1.59	1.49	31.60	5.52	15.91	10.99
$T_{13} (V_2E_6)$	1.76	1.51	1.33	28.69	5.28	14.29	10.24
$T_{14} (V_2E_7)$	1.61	1.35	1.13	23.28	4.82	12.60	9.01
S.Em ±	0.03	0.010	0.01	0.49	0.04	0.05	0.03
CD at 5%	0.09	0.03	0.04	1.42	0.12	0.14	0.08

Table 4: Impact of elicitors on essential oil profiling (pooled) of sacred basil (*O. sanctum*)

Treatments	Essential oil profiling						
	Limonene	Linalool	Methyl chavicol	Eugenol	Methyl eugenol	âelemene	Caryophyllene
Varieties							
V_1	1.37	0.28	0.58	36.81	22.31	7.94	10.88
V_2	1.70	1.48	1.31	27.26	5.17	14.27	10.13
S.Em ±	0.01	0.004	0.004	0.24	0.02	0.02	0.01
CD at 5%	0.03	0.011	0.013	0.69	0.04	0.05	0.02
Elicitors							
E_1	1.49	0.84	0.88	28.78	12.10	10.40	9.77
E_2	1.51	0.88	0.93	30.96	12.65	10.86	10.23
E_3	1.59	0.91	0.98	34.53	14.76	11.53	10.94
E_4	1.51	0.92	1.02	35.29	15.52	11.81	11.32
E_5	1.67	0.95	1.07	36.78	16.22	12.13	11.64
E_6	1.55	0.89	0.95	32.57	13.71	11.11	10.53
E_7	1.42	0.77	0.81	25.33	11.22	9.88	9.13
S.Em ±	0.02	0.007	0.008	0.45	0.03	0.03	0.01
CD at 5%	0.06	0.020	0.024	1.30	0.08	0.10	0.03
Interactions (Varieties and Elicitors)							
T_1 (V_1E_1)	1.29	0.26	0.54	32.07	19.23	7.67	9.88
T_2 (V_1E_2)	1.31	0.28	0.57	35.53	20.22	7.77	10.56
T_3 (V_1E_3)	1.41	0.31	0.60	39.60	24.20	8.20	11.27
T_4 (V_1E_4)	1.45	0.31	0.63	41.15	25.69	8.32	11.97
T_5 (V_1E_5)	1.51	0.33	0.66	43.04	26.99	8.41	12.33
T_6 (V_1E_6)	1.36	0.29	0.58	37.52	22.20	7.99	10.87
T_7 (V_1E_7)	1.25	0.23	0.50	28.76	17.67	7.23	9.29
T_8 (V_2E_1)	1.69	1.43	1.22	25.49	4.97	13.13	9.66
T_9 (V_2E_2)	1.71	1.48	1.29	26.39	5.09	13.96	9.89
T_{10} (V_2E_3)	1.76	1.52	1.36	29.47	5.32	14.87	10.60
T_{11} (V_2E_4)	1.58	1.54	1.41	29.42	5.35	15.30	10.66
T_{12} (V_2E_5)	1.84	1.57	1.47	30.53	5.46	15.85	10.94
T_{13} (V_2E_6)	1.74	1.49	1.31	27.62	5.22	14.23	10.19
T_{14} (V_2E_7)	1.59	1.32	1.12	21.90	4.76	12.53	8.97
S.Em ±	0.03	0.01	0.012	0.63	0.04	0.05	0.02
CD at 5%	0.09	0.03	0.034	1.84	0.12	0.14	0.04

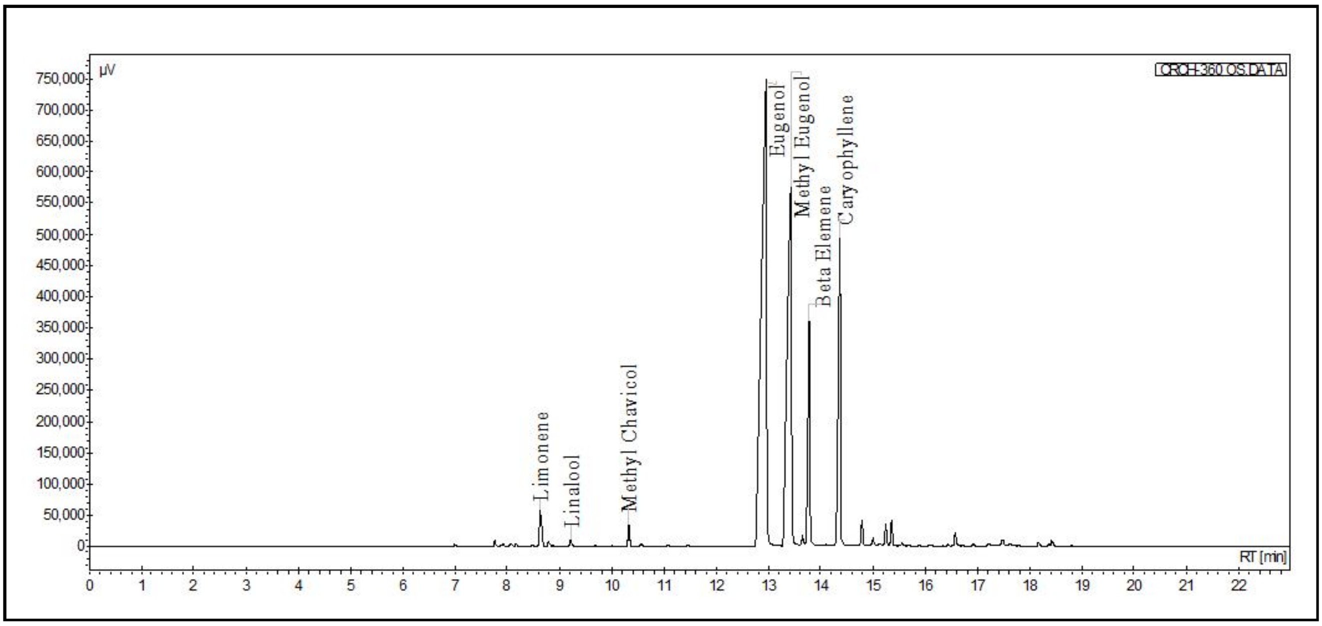

Figure 1: Gas chromatogram of the CIM-Ayu sample for essential oil profiling.

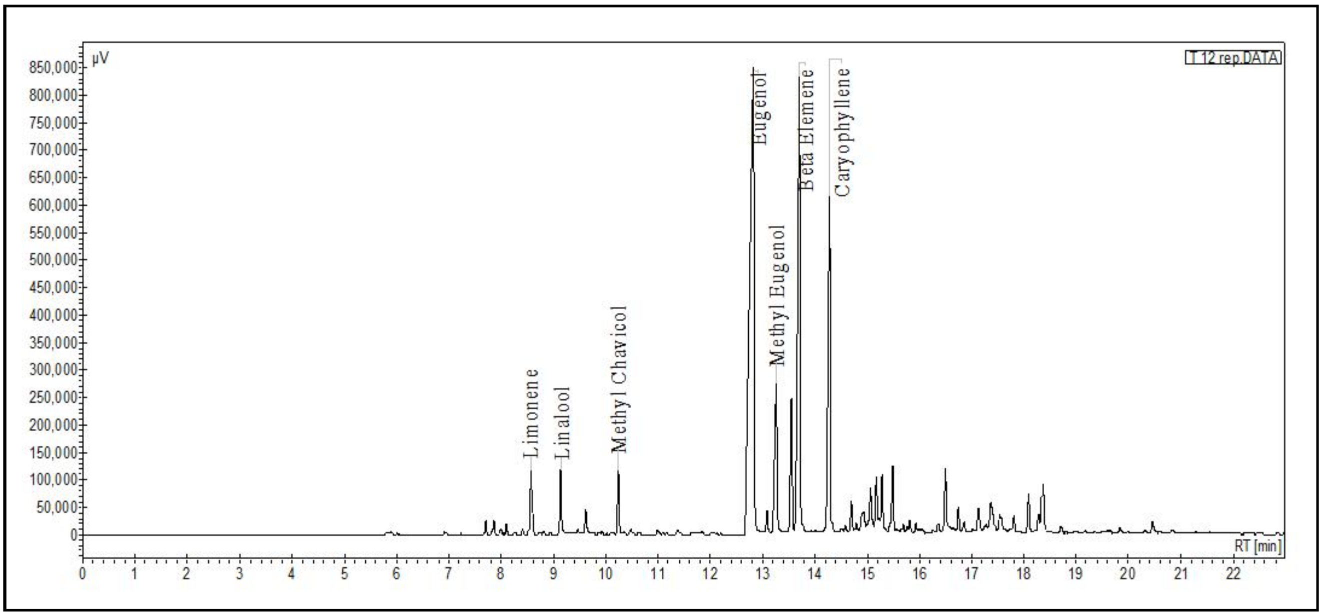

Figure 2: Gas chromatogram of the CIM-Angana sample for essential oil profiling.

In pooled data (Table 4), among the varieties, CIM-Ayu (V_1) recorded maximum (22.31, 36.81 and 10.88, respectively) content of methyl eugenol, eugenol and caryophyllene, respectively, than CIM-Angana (V_2) (5.17, 27.26 and 10.13). In case of linalool, limonene, methyl chavicol and β-elemene, the maximum content was recorded in CIM-Angana (V_2) (1.48, 1.70, 1.31 and 14.27, respectively) compared to CIM-Ayu (V_1) (0.28, 1.37, 0.58 and 7.94). The use of elicitors was found to be significant on the essential oil profiling. The maximum content of methyl chavicol, limonene, linalool, eugenol, β-elemene, methyl eugenol and caryophyllene, respectively was observed by the use of E_5-SA @ 0.5 mM (1.07, 1.67, 0.95, 36.78, 12.13, 16.22 and 11.64, respectively), while, the minimum content of caryophyllene, eugenol, linalool, methyl chavicol, methyl eugenol, β-elemene and limonene was observed in E_7-control (9.13, 25.33, 0.77, 0.81, 11.22, 9.88 and 1.42, respectively). In the present study among the interactions, T_5 (V_1E_5) CIM-Ayu + SA @ 0.5 mM recorded significantly maximum content of (26.99, 43.04 and 12.33, respectively) methyl eugenol, eugenol and caryophyllene, respectively, followed by T_4 (V_1E_4) (25.69, 41.15 and 11.97), while the minimum (4.76, 21.90 and 8.97) was recorded in T_{14} (V_2E_7) CIM-Angana and control. In case of linalool, limonene, methyl chavicol and β-elemene maximum content (1.57, 1.84, 1.47 and 15.85, respectively) was noticed in T_{12} CIM-Angana and SA @ 0.5 mM (V_2E_5) while, the minimum (0.23, 1.25, 0.50 and 7.23, respectively) was recorded in T_7 (V_1E_7) CIM-Ayu and control.

3.5 Chlorophyll content (SPAD value)

The results pertaining to the chlorophyll content as influenced by varieties, elicitors and their combinations during the years 2021, 2022 and pooled are presented in the Table 5. The varieties differed significantly with respect to chlorophyll content during 2021, 2022 and pooled. The highest chlorophyll content was recorded in CIM-Ayu (V_1) (34.22, 36.51 and 35.36) than CIM-Angana (V_2) (32.77, 34.75 and 33.76) during the years 2021, 2022 and pooled, respectively.

The application of elicitors also showed a significant effect on the chlorophyll content during both the years and pooled, in which the maximum (40.50, 42.56 and 41.53) chlorophyll content was recorded in E_5-SA @ 0.5 mM, followed by E_4-SA @ 0.1 mM (39.12, 41.27 and 40.19) and E_3-MeJA @ 1.0 mM (38.39, 40.54 and 39.46) during 2021, 2022 and pooled, respectively. Likewise, the minimum chlorophyll content was recorded in E_7-control (25.32, 27.27 and 26.29) during 2021, 2022 and pooled, respectively.

The interaction effect between varieties and elicitors was observed to be significant during the years 2021, 2022 and pooled. The highest chlorophyll content (40.98, 43.54 and 42.26) was registered in T_5 (V_1E_5) CIM-Ayu + SA @ 0.5 mM, and was at par with T_4 (V_1E_4) CIM-Ayu + SA @ 0.1 mM (40.54, 42.69 and 41.62), during 2021, 2022 and pooled, respectively. Whereas, the lowest chlorophyll content (25.01, 26.86 and 25.94) was registered in T_{14} (V_2E_7) CIM-Angana + control and was on par with T_7 (V_1E_7) (25.63, 27.67 and 26.65) during 2021, 2022 and pooled, respectively.

Table 5: Impact of elicitors on chlorophyll content (SPAD value) of sacred basil (*O. sanctum*)

Treatments	Chlorophyll		
	2021	2022	Pooled
Varieties			
V_1	34.22	36.51	35.36
V_2	32.77	34.75	33.76
S.Em ±	0.12	0.11	0.10
CD at 5%	0.35	0.32	0.29
Elicitors			
E_1	26.42	29.02	27.72
E_2	30.46	32.35	31.40
E_3	38.39	40.54	39.46
E_4	39.12	41.27	40.19
E_5	40.50	42.56	41.53
E_6	34.28	36.43	35.35
E_7	25.32	27.27	26.29
S.Em ±	0.22	0.20	0.19
CD at 5%	0.65	0.59	0.54
Interactions (Varieties and Elicitors)			
T_1 (V_1E_1)	26.91	29.67	28.29
T_2 (V_1E_2)	31.38	33.53	32.45
T_3 (V_1E_3)	39.02	41.17	40.10
T_4 (V_1E_4)	40.54	42.69	41.62
T_5 (V_1E_5)	40.98	43.54	42.26
T_6 (V_1E_6)	35.11	37.26	36.19
T_7 (V_1E_7)	25.63	27.67	26.65
T_8 (V_2E_1)	25.93	28.36	27.15
T_9 (V_2E_2)	29.55	31.16	30.36
T_{10} (V_2E_3)	37.75	39.90	38.82
T_{11} (V_2E_4)	37.69	39.84	38.77
T_{12} (V_2E_5)	40.03	41.57	40.80
T_{13} (V_2E_6)	33.44	35.59	34.52
T_{14} (V_2E_7)	25.01	26.86	25.94
S.Em ±	0.31	0.29	0.26
CD at 5%	0.91	0.84	0.77

4. Discussion

SA serves as a stress transmitter, activating the plants defense system. Plants build up low molecular weight defense compounds like essential oils. Primarily in response to stress or stimulus molecules. Gharib (2006) indicated the impact of SA on basil and marjoram at a dose of 10^{-4} mM has increased the quantity and quality of essential oils. Rowshan and Bahmanzadegan (2013). It has been stated that external application of SA at 200 and 400 mgl^{-1} may change secondary metabolites and their pathways by influencing plastids and chlorophyll content.

The higher oil yield per ha was obtained with SA @ 0.5 mM concentration. Similar enhancement in the essential oil content and yield per plant in response to the exogenous application of SA was reported in *Ocimum basilicum* and *Origanum majorana* (Gharib, 2006). According to Idrees *et al.* (2010), the increase in vegetative growth, nutrient uptake, increase in leaf oil gland population and synthesis of monoterpene has enhanced oil yield in lemon grass. In contrast to this, Ram *et al.* (1997) reported the SA application (100 ppm) restricts herbage and essential oil yields in *Pelargonium graveolens, Mentha arvensis* and *Cymbopogon martini*. The above results are in harmony with the findings of Rodrigues *et al.* (2009) where the SA based stimulant paste enhanced the production of oleoresin in *Pinus elliotti*.

SA is reported to improve phenolic content in chilli (Zunun *et al.*, 2017), fennel (Gorni *et al.*, 2017) and cotton (El Beltagi *et al.*, 2017). Plant cells use various processes to maintain redox homeostasis. The crucial one is production of phenolic compounds, which are potent inhibitors of oxidative stress (Kovacik *et al.*, 2009). Even at minute concentrations, exogenous SA reacts with stress-signaling mechanisms (Gorni *et al.*, 2017). Thus, a spike in SA may activate cell signaling, which governs the expression of genes that encode enzymes involved in phenylpropanoid pathway. As a result, enzymes engaged with synthesis of secondary plant defense metabolites like phytoalexins and lignin may become more active. (La Camera *et al.*, 2004).

In the present study, it is observed that the chlorophyll pigments were significantly enhanced by the application of SA. The best results were obtained with SA 0.5 mM concentration in both the years of observation. Similar findings were obtained by Yildirim *et al.* (2008) in *Cucumis sativus*, Li *et al.* (2014) in *Torreya grandis*, Manaa *et al.* (2014) in *Solanum lycopersicum* and Al-Rubaye and Atia (2016) in *Cucurbita pepo*. Leaf chlorophyll is a critical component of the photosynthetic system that regulates dry matter accumulation. When a plant is stressed, it acts as one of the antioxidant substances concentrated in the chloroplast and protects the photosynthetic apparatus by scavenging the excess reactive oxygen species known as free radicals (Khodary, 2004). In *Artemisia annua*, foliar spray with SA 1.0 mM enhanced the chlorophyll content (Aftab *et al.,* 2010).

In summary, SA at 0.5 mM emerged as the most promising elicitor for improving oil concentration, metabolic quality and physiological performance of *O. sanctum*. The response, however, appears to be influenced by both elicitor strength and varietal behaviour, suggesting the need for further optimization under diverse environmental conditions.

5. Conclusion

The findings of this investigation clearly establish that foliar application of SA, particularly at 0.5 mM, substantially improved essential oil yield, oil profile and chlorophyll concentration in *O. sanctum*. Among the varieties evaluated, CIM-Ayu exhibited superior performance, indicating a strong genotype × elicitor interaction. The overall enhancement in oil productivity and chemical composition suggests that SA based elicitation promotes metabolic activity linked to secondary metabolite formation and strengthens physiological performance.

Given the positive outcomes, SA at 0.5 mM can be considered a promising and cost-effective option for improving essential oil quality and yield in commercial basil cultivation. Further work is required to elucidate the biochemical pathways involved in elicitation and to validate results across wider agro-ecological conditions. Future studies may also benefit from testing synergistic combinations of elicitors, refining application schedules and integrating biostimulants within sustainable or organic cultivation practices to maximize production efficiency.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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