



Impact of Foliar Application of Some Growth stimulants on the Vegetative Growth and the Essential Oil Characters of *Ocimum basilicum* var. *thyrsoflorum* (L.)



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Abstract

This study aimed to investigate the effect of foliar application by synthetic and natural growth stimulants (salicylic acid (SA), selenium (SE) and orange peel extract (OPE)), on vegetative growth, oil production and the total phenolic content of *Ocimum basilicum* thyrsoflorum L. essential oil. A pot experiment was carried out and the treatments of foliar application ((SA) at (0.5, 1 and 1.5 mm), (SE) at (6, 8, and 10 mg /L), and OPE at (600, 800, 1000 mg/L) and the control treatment (spray with distilled water)) started 60 days after sowing date. Plant shoot was cut each 45 days intervals, then plant growth and biochemical traits were estimated. It was noticed that plant treated with SA, SE at high concentration and OPE at medium concentration showed the highest vegetative growth values, and essential oil production. The total phenolic content and photosynthetic pigments exhibited greater development due to increase in vegetative growth characters and increase in volatile oil chemical characters of *Ocimum basilicum*. In respect to chlorophyll a, b and carotenoids in plant green leaves recorded the highest values with the high level of synthetic promoters and medium concentration of natural one (OPE) at the third cut.

Key words: Salicylic acid; Selenium; Orange peel extract; Growth stimulants; *Ocimum basilicum* L; essential oil

1. Introduction

Natural products are now staffed as a therapeutic option in the control and diagnosis of a wide range of diseases for their low toxicity and significant pharmacological capability. Any plant species are regarded as a significant source for the extraction of essential oils, which have a variety of vital biological functions and may be employed to treat a wide range of diseases [1]. The essential oils of the Lamiaceae family are notable for their therapeutic benefits [2,3]. Basil (*Ocimum basilicum* L.), member of the Lamiaceae family, is one of the most broadly utilized fragrant herbs in the world and commonly grown in Mediterranean areas as well as semitropical and tropical parts of America, Asia, and Africa [4]. Basil essential oil has been broadly applied in perfumery, cooking, food industry, cosmetics and medications due to its various biological activities as: antioxidant, anti-microbial, anti-fungal, antiviral, analgesic, and anti-inflammatory

properties [5, 6]. The major biological effects of basil essential oil are based on the chemical components, such as estragole, linalool, 1,8-cineol, eugenol, eucalyptol, and bergamotene, which can affect in isolation or synergism [6]. There are several varieties of *Ocimum basilicum* which differ in their morphological structure, this consequently influences the content of essential oil, as well as its chemical composition [7].

Salicylic acid (SA) as a plant growth regulator, responsible for some metabolic and physiological reactions in plants, influencing plant growth and development. It is a phenolic compound that had a vital role in plant resistance against harmful pathogens [8]. It plays a dynamic role in the production and stimulation of volatile compounds, phenolics antioxidants, anthocyanins, and other important components in aromatic plants [9].

Selenium is a trace element which is necessary for animals, humans, and plants [10]. Selenium had a

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role in hormone balance, anti-oxidative reactions, and several physiological functions in plant cells. It can enhance glutathione peroxidase GPX functions, which increase tolerance to substandard biotic variables influencing crops [11].

Fruit peels waste is one of several kinds of waste that accumulate daily, it is a critical problem that must be solved to reduce the environmental pollution. Fruit peels contain high macro and micronutrients that promote plant development. We may reduce waste load and gain more advantages by applying fruit peel as fertilizers [12, 13]. Orange peel contains vital compounds such as sugars and acids, which have a function in soil acidity and have an impact on plant development and photosynthesis [14].

El- Bassiouny et al [15] illustrated that orange peels possess natural antioxidants such as flavonoids and vitamin C, which increase antioxidant enzyme activity, promote photosynthesis, and stimulate protein synthesis and prevent ageing.

The aim of this study is to investigate the effect of foliar application by salicylic acid (SA), selenium (SE) and orange peel extract (OPE), on number of cuts treatment with the previous stimulants and the interaction between both of them on vegetative growth, oil production, pigment in fresh leaves and the total phenolic content of essential oil of *Ocimum basilicum* thyriflorum 'Siam Queen'.

2. Material and Methods

2.1. Generals

Sodium selenate (Na_2SeO_4), salicylic acid ($\text{C}_7\text{H}_6\text{O}_3$), Folin-Ciocalteu reagent, Gallic acid, N, N-Dimethyl formamide, were purchased from Sigma-Aldrich. Orange peel extract, the seed of Basil " *Ocimum basilicum* thyriflorum 'Siam Queen' Family: Lamiaceae were obtained from Haraz market. The absorbance of the total phenolic and photosynthetic pigments contents assay was measured using UV spectrophotometer (Jasco, serial No. C317961148, Japan). Rotary evaporator: Heidolph G2, Clevenger-type apparatus for oil extraction by hydro-distillation method. Ethanol and Methanol were applied from Algomhuria Company Egypt.

2.2. Plant Identification

This study was carried out at the Department of Botany Faculty of Agriculture, Ain shams University, Shubra Elkhema, Egypt and at the National Research Centre, Dokki, Giza, Egypt during 2020. The seed of Basil " *Ocimum basilicum* thyriflorum 'Siam Queen' Family: Lamiaceae were obtained from the Haraz market and the scientifically identified and deposited in CAIRC.

2.3. Plant cultivation

The seeds of *Ocimum basilicum* thyriflorum 'Siam Queen' were manually sown in trays on first of March 2020 in a mixture soil of sand and compost (1:1) and located in nursery inside greenhouse. The uniform healthy *Ocimum* seedling (60 days old and 12-14 cm height) were transplanted on the first of May 2020 to the previously prepared pots (30 cm), each pot filled 6 kg with sandy soil, washed three times with distilled water. The pots were kept outdoor under natural environmental conditions (open field). The seedlings were thinned leaving one plant per pot⁻¹. The plants were irrigated with Hoagland's solution half-strength according to [16] for two weeks after transplanting and they were irrigated with full-strength Hoagland's solution until harvest. All pots were irrigated at 65% of the water-holding capacity of the soil.

2.4. Plant treatments

The experiment included 40 treatments arranged in a completely randomized block design, growth stimulants of both salicylic acid at (0.5, 1.0, and 1.5 mm), selenium at (6, 8, and 10 mg /L) as sodium selenate (Na_2SeO_4), and orange peel extract at 600, 800, 1000 and control treatment (spray with distilled water) and number of cuts treatments (four cuts). Both growth stimulants were foliar sprayed 8 times during the growing season which started from 15/6/ 2020 up to 1/11/2020. The first foliar application was after two weeks from transplanting date, second spraying was after two weeks from the first one while third up to eight were sprayed twice before two weeks for each cut and the time between the first and second sprays for each cut was two weeks. The herb of the plants was cut four times during the growing season starting after second spraying foliar application by two weeks, where the first cut was harvested after 45 days from transplanting and the reaming cuts carried out every 45 days intervals till the fourth cut. The plants were harvested by cutting the herb for each plant 12-15cm above the soil surface leaving 2-3 branches with some leaves for regrowth.

2.5. Extraction method of orange peel.

Orange peel extraction estimated according to [17] 500gm of fresh fruits of orange peel was extracted with distilled water with the aid of mixer the extract filtered into dark-colored flasks, the extraction was repeated till exhaustion, the combined filtered extract was evaporated to dryness under vacuum at 50°C in a rotary evaporator to give total water extract. The evaporated water extract was subjected to lyophilization, dried extract was kept in dark bottle stored under -4°C until use.

2.6.1. Vegetative growth characters

The following traits were investigated: plant height (cm) plant-1, number of branches plant-1, fresh and dry weight gplant-1

2.6.2. Essential oil production

Extraction of essential oils - The essential oil was extracted from fresh herb (100 g) of *Ocimum basilicum* plants and was subjected to hydro-distillation using Clevenger-type apparatus for three hours as mentioned in Egyptian pharmacopeia [18]. The resulted essential oil from each treatment was separately dehydrated with anhydrous sodium sulphate and kept in the deep freezer.

2.6.3. Oil percentage and oil yield plant-1

The amount of obtained oil from plants was measured and oil percentage was calculated as follows; Essential oil % = Essential oil volume (Measuring pipette reading) divided on weight of sample x 100.

Essential oil yield (ml plant⁻¹) was estimated in proportion to the herb fresh weight.

3. Chemical investigation

3.1. Determination of the photosynthetic pigments

The photosynthetic pigments (chlorophyll-a, b and total carotenoids) were determined in representative fresh leaves samples according to [19].

3.2. Total phenolic determination

The total phenolic contents for essential oil of *Ocimum basilicum* were determined using Folin-Ciocalteu reagent following the method of [20]. The absorbance was measured at 750 nm. The total phenolic content (TPC) was expressed as mg gallic acid/g essential oil and calculated as follows: TPC= Conc. (gallic) × V × m M⁻¹, where conc. (gallic) is the concentration of the standard (gallic acid) established from the calibration curve, V is dilution factor, m is total volatile oil wt. (g) and M is the concentration of dry plant volatile oil.

3.3. Statistical analysis

The combined analysis of variance for the data of the treatments was performed after testing the error homogeneity and Fisher's Least Significant Difference (LSD) test at 0.05 level obtained data were subjected to the proper statistical analysis of variance of significance was used for the comparison between means according to [21].

4. Results

4.1. Vegetative growth parameters

This study carried out to investigate the effect of both salicylic acid (SA), selenium (SE) and orange peel extract (OPE), as a synthetic and natural growth stimulant as well as number of cuts and the interaction between each growth stimulant and number of cuts

treatment on vegetative growth, oil production, and chemical constituents of *Ocimum basilicum* var. thyrsoflorum (L.). Results in Tables from 1 to 6 were arranged in descending order according to their activities.

4.2. Plant height (cm/plant) and Number of branches /plant.

Data tabulated in Table (1) show clearly that all growth stimulants sources, salicylic acid (SA), selenium (SE), and (OPE) at different concentrations have significant effect on plant height and number of branches of *Ocimum basilicum* var. thyrsoflorum (L.), as compared to untreated plants. The maximum mean values of plant height and number of branches / plant produced from SA treatment (55.15 cm and 25.65 branches plant-1), respectively followed by orange peel extract (51.11cm and 23.03 branches plant-1) then SE treatment (47.54 cm and 20.05 branches plant-1), respectively. The effect of different growth promoters concentrations.results indicated that the high concentrations (1.5 and 1.0 mm) of SA were the best treatments which produced the tallest plants and the highest number of branches plant-1 (57.73, 55.14 cm and 27.10, 25.55 branches plant-1), respectively. The differences between these two doses of SA were insignificant. On the other side SE at three doses produced the minimum values in this concern comparing to SA and OPE treatments.

Also the results in Table (1) cleared that plant height and number of branches plant-1 of *Ocimum basilicum* var. thyrsoflorum (L.) was affected by the number of cuts, it was noted a reduction of plant height and the number of plant-1branches with the first cut (40.54 cm and 10.78 branchplant-1), respectively and gradual increase up to the third cut which produced the maximum plant height (cm/plant) and number of branches plant-1 (56.22 cm and 30.5 branch plant-1), respectively while the fourth cut gave the values (53.99 cm and 26.70 branch plant⁻¹) higher than plants that harvested with first and second cut (40.54, 50.55 cm and 10.78 and 20.96 branch plant⁻¹) for first and second cut, respectively. The differences between all cuts were high significantly.

The interaction between the foliar spraying of SA, SE, and OPE at all concentrations with number of cuts increased plant height and number branches plant-1 comparing to the control plants (without spray). This trend was true with all cuts. The growth stimulants (SA and SE) and natural extract OPE at all concentrations gave the maximum values of plant height and number of branches plant-1 with the third cut comparing to the first, second and fourth cuts, but the foliar application of SA treatment with third cut gave the maximum values of plant height (cm) and branches number plant⁻¹ (57.34, 59.10 and 63.20 cm and 34.6,35.8, and 36.0 branch plant-1) with different concentrations (0.5,1.0, and 1.5 mm), respectively. The differences between these treatments were insignificant.

Table (1): Effect of foliar application treatments for each SA, OPE, SE, number of cuts and their interaction on plant height and number of branches plant⁻¹ of *Ocimum basilicum* var. *thyrsoflorum* (L.)

Treat.	Plant height (cm)					Number of branches plant ⁻¹					
	Cut1	Cut2	Cut3	Cut4	Mean	Cut1	Cut2	Cut3	Cut4	Mean	
Control (D.W)	32.24	42.64	47.28	45.32	41.87	8.60	15.40	20.40	20.20	16.15	
SA	0.5 mm	44.40	53.50	57.34	55.06	10.60	23.20	34.60	28.80	24.30	
	1.0 mm	47.20	56.20	59.10	58.04	12.80	23.60	35.80	30.00	25.55	
	1.5 mm	49.20	57.32	63.20	61.20	57.73	13.60	25.80	36.00	27.10	
	Mean	46.93	55.67	57.23	58.10	55.15	12.33	24.20	35.47	30.60	25.65
OPE	600 mg/L	38.30	50.50	55.30	51.30	48.85	10.60	19.60	31.80	24.60	21.65
	800 mg/L	43.30	53.20	58.40	54.40	52.33	11.00	22.40	33.80	30.60	24.45
	1000 mg/L	41.30	51.20	58.00	58.10	52.15	10.60	22.00	32.80	26.60	23.00
	Mean	40.97	51.63	57.23	54.60	51.11	10.73	21.33	32.80	27.27	23.03
SE	6.0 mg/L	34.26	45.70	52.30	51.20	45.87	9.40	18.60	22.40	24.20	18.65
	8.0 mg/L	36.20	47.20	55.30	52.30	47.75	10.20	19.00	26.20	25.00	20.10
	10 mg/L	39.00	48.00	56.00	53.00	49.00	10.40	20.00	31.20	24.00	21.40
	Mean	36.49	46.97	54.53	52.17	47.54	10.00	19.20	26.60	24.40	20.05
Mean of cuts	40.54	50.55	56.22	53.99		10.78	20.96	30.50	26.70		
LSD (0.05)	Tr.		3.10				1.63				
	Cuts		1.96				1.03				
	Tr*Cuts		6.19				3.26				

* SA Salicylic acid *OPE Orange peel extract *SE selenium *Tr. Treatments * D.W Distilled water

4.3. Herb fresh and dry weight (g plant⁻¹).

Data presented in Table (2) recorded that foliar spray for each (SA), (SE) and (OPE), as a synthetic and natural growth stimulant on basil plants at all studied concentrations significantly increased the herb fresh and dry weight (gplant⁻¹) compared to un-treated plants. These increments raised gradually with increasing the concentrations of all studied growth promoters. Salicylic acid (SA) treatments produced the maximum mean values fresh and dry weight (113.55 and 16.57g plant⁻¹), respectively followed by orange peel extract (102.33 and 14.96 g plant⁻¹) then selenium (91.91 and 13.8 gplant⁻¹) compared to control treatment which recorded (76.89 and 10.52 gplant⁻¹) for both fresh and dry weight, respectively. On the other side, the maximum values in this concern were obtained from salicylic acid at high level 1.5mm (131.37 and 19.18 gplant⁻¹) for fresh and dry weight, respectively versus (113.80 and 16.65 and 103.34 and 15.63 g plant⁻¹) for fresh and dry weight plant⁻¹, respectively for each OPE and SE with high concentrations 1000 mgL⁻¹ and 10 mL⁻¹, respectively. In general, increasing the concentration of this growth stimulants increased herb fresh and dry weight per plant. These increments were gradually raising concentration up to 1.5 mm SA, 800 mgL⁻¹ OPE and 10 mgL⁻¹ of SE, but the high level of SA was the superior treatment enhanced herb fresh and dry weight plant⁻¹ comparing to other concentrations of all growth promoters, the differences between them were significant.

Jiang et al. [22] investigated the probability that selenium's benefit for herb dry weight results from its support of plant development through improving photosynthetic capacity.

In respect to the effect of cuts, data in the same table (2) cleared that fresh and dry weight of herb was significantly influenced by the number of cuts. The values of the weight of herb were increased gradually up to the fourth cut. Therefore, the maximum values of the fresh and dry weight of herb were in the fourth cut (130.67 and 18.5 g/plant) compared to 73.7, 90.57, 105.16 and 10.89, 13.51, 15.68 g /plant) for the first cut, the second cut, and the third cut for both fresh and dry weight, respectively. The differences between four cuts were high significant.

As for cuts, the data in the same table (2), show that significantly influenced by the number of herb cuts. The highest values of fresh and dry weight of the herb were obtained from taking fourth cut when compared to first or second and third cuts. The fresh and dry weight of herb for four cuts increased by (77.29%, 44.27%, 24.26% and 69.88%, 36.93% and 17.98%) when compared to the plants that were harvested first or second and third times, for fresh and dry weight, respectively. So, the minimum values of fresh and dry weight of herb were obtained from plants were harvested on first cut only.

Concerning the effect of interaction between the foliar spraying of synthetic and natural growth stimulant (SA, SE, and OPE) and number of cuts on fresh and dry herb of *Ocimum basilicum* var. *thyrsoflorum* (L.), data in table (2) show that under the same cut all concentrations of growth stimulants application increased the fresh and dry herb g/plant comparing to without growth stimulants sprayed plants.

The combination of treatments at high dose of each SA, OPE, and SE under all cuts gave the

maximum values compared to low and medium concentrations, but the heaviest herb fresh and dry weight (155.06 and 21.4 g/plant) were obtained from the plants treated at high dose of SA (1.5 mm) and fourth cut, while the medium dose (1.0 mm) with fourth cut gave the second value (148.74 and 20.6 g/plant), followed by (141.22 and 20.11 g/plant) produced from the medium dose (800 mg/L) OPE with fourth cut, respectively. The differences between SA at 1.0 mm and OPE at 800 mg/L were insignificant.

4.4. Essential oil (EO) percentage and yield (EOY).

The results in Table (3) show the effect of different concentrations of SA and SE as growth stimulants as well as OPE as a natural extract, number of cuts and interaction between growth promoters with number of cuts on the percentage and yield (ml/plant) of essential oil in fresh herb of *Ocimum basilicum* var. *thyriflorum* (L.).

Data in Table (3) exhibit the effect of three concentrations of both SA, OPE, and SE of the essential oil percentage (EOP) and essential oil yield (EOY) in fresh weight of *Ocimum basilicum* var. *thyriflorum* (L.), generally spraying plants with all these treatments at all concentrations lead to positive increase in EOP and EOY (ml/plant) compared to unsprayed plants. These increments increased gradually with increasing the concentrations for these treatments. The maximum value of the EOP and EOY were obtained by application of SA (0.24 % and 0.28 ml/plant), while OPE treatments come in the second rank (0.23 % and 0.24 ml/plant) followed by SE treatments which produced (0.18 % and 0.16 ml/plant)

versus (0.13% and 0.10 ml/ plant), for each EOP and EOY, respectively with un treated plants. On the other hand SA at high concentration (1.5 mm) was the most effective in EOP and EOY which recorded (0.27 % and 0.36 ml/plant) followed by (0.25% and 0.27 ml/plant) with OPE at medium concentration (800 ml/L), The differences between all concentrations between three treatments were significant.

These results are in parallel with those who investigated the effect of SA on *Ocimum basilicum* and *Mentha piperita* L., [28, 34]. Additionally a study were made on the effect on *Ocimum basilicum* as well as *Stachys byzantine* plants, showed that treating plants with SE enhanced oil percentage of EO [35,36]. El-Bassiouny et al [15] illustrated that treating quinoa plants with OPE caused a remarkable effect on EOP.

Concerning the effects of the number of cuts on EOP, data in Table (3), show that significantly influenced on EOP parameter in this investigation by the number of cuts. Also, the EOP was raised gradually with increasing number of cuts up to third cut. However, the maximum value of EOP was obtained with the third cut (0.22%) comparing the second and fourth cut (0.21 %, 0.21%), respectively, while the least value in this regard (0.19 %) recorded in the essential oil percentage at the first cut.

In respect to essential oil yield (EOY), the results in the same table cleared that EOY ml plant-1 was significantly influenced by the number of cuts, and it was increased gradually with increasing number of cuts up to the fourth cut.

Table (2): Effect of foliar application treatments for each SA, OPE, SE, number of cuts and their interaction on herb fresh and dry weight of *Ocimum basilicum* var. *thyriflorum* (L.).

Treat.	Herb fresh weight (g/plant)					Herb dry weight (g/plant)					
	Cut1	Cut2	Cut3	Cut4	Mean	Cut1	Cut2	Cut3	Cut4	Mean	
Control (D.W)	45.00	74.44	80.10	108.00	76.89	6.40	10.51	10.92	14.26	10.52	
SA	0.5 mm	69.72	80.42	100.42	127.74	94.58	10.52	11.30	16.28	17.6	13.93
	1.0 mm	81.52	101.04	127.50	148.74	114.70	12.08	14.82	18.74	20.6	16.56
	1.5 mm	115.30	122.00	133.12	155.06	131.37	16.72	18.41	20.20	21.4	19.18
Mean	88.85	101.15	120.35	143.85	113.55	13.11	14.84	18.41	19.87	16.57	
OPE	600 mg/L	57.00	77.58	92.02	121.00	86.90	8.38	11.59	13.60	16.93	12.63
	800 mg/L	71.42	95.46	117.02	141.22	106.28	10.70	14.23	17.32	20.11	15.59
	1000 mg/L	97.42	105.02	114.02	138.72	113.80	14.50	15.51	16.60	19.98	16.65
Mean	75.28	92.69	107.69	133.65	102.33	11.19	13.78	15.84	19.01	14.96	
SE	6.0 mg/L	51.62	74.10	88.02	116.98	82.68	7.48	11.50	13.52	16.13	12.16
	8.0 mg/L	65.00	81.50	92.34	120.04	89.72	9.64	12.61	13.80	18.40	13.61
	10 mg/L	83.00	94.14	107.00	129.20	103.34	12.50	14.60	15.80	19.60	15.63
Mean	66.54	83.25	95.79	122.07	91.91	9.87	12.90	14.37	18.04	13.8	
Mean of cuts	73.7	90.57	105.156	130.67		10.89	13.51	15.68	18.50		
LSD (0.05)	Tr.		4.52				0.45				
	Cuts		2.86				0.29				
	Tr*Cuts		9.04				0.91				

* SA Salicylic acid *OPE Orange peel extract *SE Selenium *Tr. Treatments* D.W Distilled water

The highest value of EOY was recorded from the plants were harvested at the fourth cut (0.28 ml plant⁻¹) than the first, second and third cuts which recorded (0.15, 0.19 and 0.23 ml plant⁻¹), respectively. These increments at the fourth cut were estimated by (87.11%, 53 % and 27 %) for the same respective treatments, respectively.

Our study correlate with those of [26, 37] who reported that *Ocimum basilicum* L., herb collected in the third cut had a higher proportion of oil%. As well as [27] who revealed that the maximum content of basil oil was obtained from the last cut whereas the lowest content was found in the first cut, in four cuts experiment.

The interaction between growth stimulants SA, OPE, and SE and number of cuts, the results indicated that SA was the most effective treatment on the accumulation of essential oil percentage (EOP) and EOY compared to OPE and SE treatments with all cuts, but the maximum values in this concern were third cut while in EOY in the fourth one. In the other words, SA treatment at high concentration (1.5 mm) gave the maximum values of EOP and EOY (0.30 % and 0.42 ml plant⁻¹) comparing (0.26 % and 0.35 ml/plant) for the medium concentration SA (1.0 mm) and OPE (800 mgL⁻¹) with third cut for EOP and the fourth one treatment.

This is agreeing with [30] who stated that foliar spray of basil plants with salicylic acid enhanced essential oil yield in three cuts, and [15] on quinoa plants treated with OPE.

4.5. Plant photosynthetic pigments.

Data presented in Tables (4 & 5) cleared the effect of foliar application of salicylic acid (SA), natural extract of orange peel (OPE) and selenium (SE) treatments and number of cuts and the interaction between growth stimulants natural and synthetic with number of cuts on content of pigments, chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids in leaves of *Ocimum basilicum* var. *thyrsoiflorum* (L.).

Table (4) shows that content of Chl a and Chl b increased with all studied growth stimulants (SA, OPE, and SE) compared to untreated plants. The highest values of chl a and b (1.74 and 0.41 mg g⁻¹ fresh weight) were obtained by supplying plants with SA treatment followed by both OPE and SE treatments which produced (1.49, 1.13 and 0.30, 0.23 mg g⁻¹ fresh weight), while the least value in this respect was (1.00 and 0.19 mg g⁻¹ fresh weight) with control plants for Chl a and b respectively. Also, the results cleared those increasing concentrations of all studied growth stimulants gradually increased content of Chl a and b in leaves of *Ocimum basilicum* var. *thyrsoiflorum* (L.) plant. Plants of *Ocimum* were sprayed with SA at high or medium concentration (1.5 and 1.0 mm) and OPE at medium dose (800 mgL⁻¹) produced the maximum values (2.02, 1.71, and 1.58 Chl a mg g⁻¹ fresh weight) and (0.51, 0.41 and 0.33 Chl b mg g⁻¹ fresh weight), respectively.

Also, resulting data in Table (4) showed that the content of Chl a and b in leaves of *Ocimum basilicum* var. *thyrsoiflorum* (L.) plant were gradually increased with increasing the number of cuts up to third cut, while the content of Chl a and b in leaves markedly decreased of the fourth cut.

Table (3): Effect of foliar application treatments for each SA, OPE, SE, number of cuts and their interaction on essential oil percentage and yield in herb of *Ocimum basilicum* var. *thyrsoiflorum* (L.).

Treat.	Cut	Essential oil%				Essential oil yield (ml plant ⁻¹)					
		Cut1	Cut2	Cut3	Cut4	Mean	Cut1	Cut2	Cut3	Cut4	Mean
Control (D.W)		0.12	0.14	0.13	0.13	0.13	0.05	0.10	0.10	0.14	0.10
SA	0.5 mm	0.20	0.22	0.21	0.23	0.22	0.14	0.18	0.21	0.29	0.21
	1.0 mm	0.22	0.23	0.26	0.24	0.24	0.18	0.23	0.32	0.36	0.27
	1.5 mm	0.25	0.26	0.30	0.27	0.27	0.29	0.32	0.40	0.42	0.36
Mean		0.22	0.24	0.26	0.25	0.24	0.20	0.24	0.31	0.36	0.28
OPE	600 mg/L	0.19	0.21	0.22	0.22	0.21	0.11	0.16	0.2	0.27	0.19
	800 mg/L	0.22	0.26	0.26	0.25	0.25	0.16	0.25	0.30	0.35	0.27
	1000 mg/L	0.2	0.22	0.24	0.23	0.22	0.20	0.23	0.27	0.32	0.26
Mean		0.20	0.23	0.24	0.23	0.23	0.16	0.21	0.26	0.31	0.24
SE	6.0 mg/L	0.16	0.17	0.15	0.16	0.16	0.08	0.13	0.13	0.19	0.13
	8.0 mg/L	0.17	0.18	0.19	0.17	0.18	0.11	0.15	0.18	0.20	0.16
	10 mg/L	0.17	0.19	0.20	0.21	0.19	0.14	0.18	0.21	0.27	0.20
Mean		0.17	0.18	0.18	0.18	0.18	0.11	0.15	0.17	0.22	0.16
Mean of cuts		0.19	0.21	0.22	0.21		0.15	0.19	0.23	0.28	
LSD (0.05)	Tr.			0.008				0.003			
	Cuts			0.005				0.002			
	Tr*Cuts			0.016				0.005			

* SA Salicylic acid *OPE Orange peel extract *SE Selenium *Tr. Treatments *D. W Distilled water

However, the content of Chl a and b at the second cut increased by (15.55 and 15.4%) compared to the first cut, while this increment was (14.10 and 40%) with the third cut compared to second cut. Generally, the variations between all number of cuts were significant.

Regarding the interaction between various concentrations of growth promoters (SA, OPE, and SE) and number of cuts, the data reported in Table (4), cleared that under the same cut, application of studied growth promoters gradually increased the content of Chl a and b. except, , these increase were observed

with increasing the concentration of growth promoters except the OPE treatment with medium concentration (800 mgL⁻¹) which gave the maximum value in this concern compared to two other concentrations. The optimum values of Chl a and b were estimated with plants sprayed by SA at high concentration (1.5 mm) with raising the number of cut up to the third cut.

Regarding the effect of SA, the following studies agree with these results, [46] on *Carthamus tinctorius* L. and [34] on *Ocimum basilicum* L. as well as [47] on *Ocimum basilicum* L. treated with SE, and [48] on chickpea plants treated with OPE.

Table (4): Effect of foliar application treatments by SA, OPE, SE and number of cuts and their interaction on Chlorophyll a and b (mg g⁻¹ fresh weight) of *Ocimum basilicum* var. *thyriflorum* (L.)

Treat.	Cut1	Chlorophyll a				Chlorophyll b				
		Cut2	Cut3	Cut4	Mean	Cut1	Cut2	Cut3	Cut4	Mean
Control (D.W)	0.94	1.06	1.13	0.63	1.00	0.17	0.17	0.24	0.16	0.19
SA	0.5 mm	1.47	1.65	1.86	1.02	1.50	0.26	0.34	0.44	0.23
	1.0 mm	1.66	1.82	2.22	1.14	1.71	0.33	0.37	0.62	0.31
	1.5 mm	1.73	2.21	2.71	1.44	2.02	0.39	0.48	0.83	0.34
Mean	1.62	1.89	2.26	1.20	1.74	0.33	0.40	0.63	0.29	0.41
OPE	600 mg/L	1.35	1.59	1.72	0.90	1.39	0.26	0.29	0.41	0.25
	800 mg/L	1.61	1.77	1.98	0.96	1.58	0.28	0.32	0.41	0.29
	1000 mg/L	1.55	1.76	1.83	0.92	1.52	0.26	0.27	0.37	0.22
Mean	1.50	1.70	1.84	0.93	1.49	0.27	0.29	0.40	0.25	0.30
SE	6.0 mg/L	1.03	1.11	1.34	0.76	1.03	0.20	0.22	0.25	0.18
	8.0 mg/L	1.04	1.28	1.44	0.82	1.13	0.23	0.25	0.29	0.20
	10 mg/L	1.10	1.38	1.61	0.85	1.23	0.21	0.26	0.32	0.20
Mean	1.06	1.26	1.46	0.81	1.13	0.21	0.24	0.29	0.19	0.23
Mean of cuts	1.35	1.56	1.78	0.94		0.26	0.30	0.42	0.24	
LSD (0.05)	Tr.		0.024					0.017		
	Cuts			0.015				0.011		
	Tr*Cuts			0.051				0.032		

* SA Salicylic acid *OPE Orange peel extract *SE Selenium *Tr. Treatments* D.W Distilled water

Table (5): Effect of foliar application treatments by SA, OPE, SE, number of cuts and their interaction on carotenoids content (mg g⁻¹ fresh leaves) of *Ocimum basilicum* var. *thyriflorum* (L.)

Cuts	Treatments	First cut	Second cut	Third cut	Fourth cut	Mean
		Carotenoids				
Control	D.W.	0.36	0.38	0.41	0.32	0.37
	0.5 mm	0.43	0.46	0.62	0.4	0.48
	1.0 mm	0.49	0.56	0.82	0.44	0.58
	1.5 mm	0.61	0.62	1.04	0.47	0.69
Mean		0.51	0.55	0.83	0.44	0.58
OPE	600 mg/L	0.41	0.42	0.53	0.39	0.44
	800 mg/L	0.45	0.47	0.6	0.42	0.49
	1000 mg/L	0.43	0.44	0.53	0.4	0.45
Mean		0.43	0.44	0.55	0.40	0.46
SE	6 mg/L	0.38	0.4	0.45	0.34	0.39
	8 mg/L	0.40	0.42	0.46	0.36	0.41
	10 mg/L	0.40	0.43	0.46	0.38	0.42
Mean		0.39	0.42	0.46	0.36	0.41
Mean of cuts		0.44	0.46	0.59	0.39	
L.S.D(0.05)	Tr.			0.017		
	Cuts			0.01		
	Tr*Cuts			0.032		

* SA Salicylic acid *OPE Orange peel extract *SE Selenium *Tr. Treatments* (D.W) Distilled water

4.5.2. Carotenoids Content.

Data in Table (5) showed that plants of *Ocimum* plants treated with growth promoters of SA, OPE, and SE treatments considerably higher carotenoid content than non-sprayed plants. The highest content of carotenoid was resulted by treating plants with SA (0.58 mg g⁻¹ fresh weight) compared to OPE and SE which recorded (0.46 and 0.41 mg g⁻¹ fresh weight), respectively. Also, in this regard increasing the concentration of SA treatment up to high dose (1.5 mm) led to obtaining the maximum content of carotenoids (0.69 mg g⁻¹ fresh weight) in leaves of *Ocimum basilicum* L. plant while the second dose for each SA (1.0 mm) and OPE (800 mgL⁻¹) gave (0.58 and 0.49 mg g⁻¹ fresh weight) content of carotenoids. The variation between these treatments were significant.

As for the effect of number of cuts on carotenoids content in leaves of *Ocimum basilicum* L. plants, data tabulated in Table (5) show that the third cut had the superiority carotenoid content (0.59 mg g⁻¹ fresh weight) when compared to the plants with first, second and fourth cuts which produced (0.44, 0.46, and 0.39 mg g⁻¹ fresh weight), respectively. Generally, the differences between third cut with both first, second and fourth were significant, but differences between first and second cut were insignificant. It was observed from the results of this study carotenoid contents in leaves of *Ocimum basilicum* L. plants had a significant response to interaction between sources and concentration of growth stimulants (SA, OPE, and SE) with number of cuts compared to the untreated plants.

On the other side, combination between treatments of growth stimulants gave the highest value of the carotenoids content with third cut compared to first, second as well as fourth cut which is the least value in this concern. In contrast, the optimum value was estimated from plants sprayed by raising the dose of SA up to (1.5 mm) with third cut which recorded (1.04 mg g⁻¹ fresh weight). Carotenoids act as free radical scavengers, increasing plants' capacity to minimize ROS damage, which in turn causes their chlorophyll content to enhance [45,49].

4.6. Total phenolic content in essential oil (TPC).

As results of vegetative growth parameters (fresh and dry weight, plant height, branches number, oil production percentage and oil yield) as well as plant pigments (chlorophyll a, b and carotenoids). According to these previous results we select the more potent concentration of different growth stimulants synthetic as SA and SE, and natural one (OPE). These results showed that the high concentration of both synthetic growth stimulants (SA and SE) had excellent improvements to all vegetative growth parameters compared to the low and medium concentration of the same promoters. In contrast OPE natural one the medium concentration (800 mg L⁻¹) had more effective comparing with the

high and Low concentrations. So, we determine the Total phenolic content (TPC) of *Ocimum* volatile oil sprayed with each growth stimulants of SA, SE and OPE with the four cuts.

Table (6 and Fig. 1) illustrated the effect of growth promoters (SA, SE and OPE) on content of total phenolic in essential oil of *Ocimum basilicum* L. at different four cuts compared with untreated plants. The results showed that plants of *Ocimum* plants sprayed with growth promoters of SA, OPE, and SE treatments higher TPC content than control plants. The highest value of TPC of *Ocimum* volatile oil was recorded by spraying plants with SA treatment (243.75 mg GAE/g oil) compared to OPE and SE which recorded (188.25 and 175.5 mg GAE/g oil), respectively. The variation between these treatments of TPC was significant.

As for the effect number of cuts on TPC in essential oil of *Ocimum basilicum* L. plants, data tabulated in the same Table (6) and Fig. (1) show that the third cut had the superiority TPC (215.75 mg GAE/g oil) when compared to the plants with first, second and fourth cuts which produced (127.75, 189.25, and 134.75 mg GAE/g oil), respectively. Generally, the differences between third cut with both first, second and fourth were significant, but differences between first and fourth cut were insignificant.

It was cleared from the results of this study TPC in essential oil of *Ocimum basilicum* L. plants had a significant response to interaction between sources and concentration of growth stimulants synthetic and natural with number of cuts compared to the untreated plants. On the other side, the combination between treatments of growth stimulants gave the highest value of the TPC with third cut compared to first, second as well as fourth cut which is the least value in this concern. In contrast, the optimum value was estimated from plants sprayed by high concentrations of SA up to (1.5 mm), SE (10 mg L⁻¹) and medium concentration of OPE which recorded (302, 221 and 280 mg GAE/g volatile oil), respectively with third cut.

5. Discussion

Salicylic acid is a plant growth regulator it had a role in the production of auxin in the plant's meristematic tissue which is responsible for increasing plant height. SA is thought to play a role in modifying the hormonal condition of the plant, which is important for plant height [23].

The current study also demonstrated the favorable influence of SA on herb development, which may be attributed to SA's role in the control of various physiological processes in plants like influencing plant growth and development. It is a phenolic characterization that had a vital role in plant resistance against harmful pathogens [8].

Table (6): Total phenolic content of *Ocimum basilicum L.* essential oil in mg GAE/g oil

Treat.	Cut1	Cut2	Cut3	Cut4	Mean
Control (D.W)	60 ± 0.0013	60 ± 0.0013	60 ± 0.0013	60 ± 0.0013	60 ^d
SA 1.5 mM	185 ± 0.009 ^b	290 ± 0.005 ^a	302 ± 0.007 ^a	198 ± 0.006 ^b	243.75 ^a
OPE 800 mg/L	123 ± 0.005 ^c	229 ± 0.01 ^b	280 ± 0.002 ^a	121 ± 0.002 ^c	188.25 ^b
SE 10 mg/L	143 ± 0.01 ^c	178 ± 0.012 ^b	221 ± 0.01 ^a	160 ± 0.009 ^{bc}	175.5 ^c
Mean of cuts	127.75 ^d	189.25 ^b	215.75 ^a	134.75 ^c	

* SA Salicylic acid *OPE Orange peel extract *SE Selenium *Tr. Treatments *(D.W) Distilled water

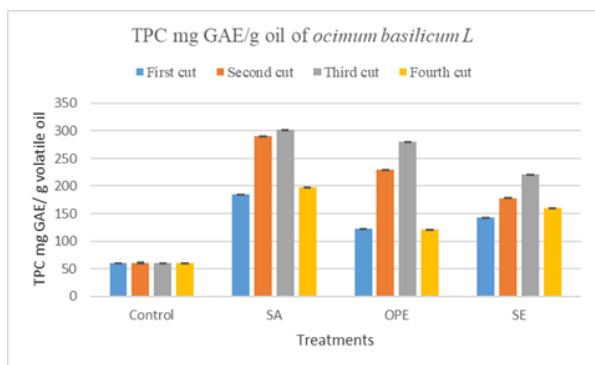


Fig.1 Total phenolic content of *Ocimum basilicum* essential oil mg GAE/g oil.

Pierpoint [24] argued that SA enhances the vegetative growth. It is affecting a different physiological response in plants, such as inhibition of ethylene production, and the prevention of auxin oxidation, control of indole-3-acetic acid (IAA), and gibberellin content. Which in turn required for cell division and cell elongation, and this may be the reason of increasing all vegetative growth characters as (plant height, branches number, fresh and dry weight).

Also, the role of selenium [25] found that the improvement of vegetative growth characters under study might be related to that selenium enhances the storage of starch in chloroplasts, which in turn promotes plant development and, ultimately, plant height

The above results of plant height, number of branches plant-1, fresh and dry weight g/ plant were in harmony with [26] admitted these results on *Ocimum basilicum L.* in three cuts experiment and revealed that third cut has the highest values of all vegetative growth parameters followed by first and second cut, respectively.

Baczek et al [27] described that the effect of the cuts and revealed the gradually increase up to fourth cut in the plant height and the main shoot number of *Ocimum basilicum L.* plants.

The improvement in plant growth, photosynthetic pigments, and general plant metabolism may be responsible for the increase in volatile oil production. Abdi, & Karami, 2020 and Hayat, & Ahmad, 2007, [28,29] discussed the effect of salicylic acid on volatile oil percentage and yield may be connected to the salicylic acid's enhancement in

growth parameters and metabolism, as well as salicylic acid is revealed to be involved in the signal transduction system, resulting in the balance and increased yield of secondary metabolites like essential oil.

Gharib [30] Mentioned that the improvement in essential oil yield could be attributed to increasing of growth parameters, food intake, or variations in the leaf oil glands and monoterpenes synthesis. Also, it was noticed that monoterpenes hydrocarbon the main constituent of *Satureja hortensis L.* increased as a response to SA application [31]. Khalid, 2011 [32] argued the benefit of selenium on oil production may be attributed to the fact that SE is a crucial element needed for numerous metabolic pathways and functions as an antioxidant in the various redox reactions of primary and secondary plant biomolecule production, including the production of essential oils.

The effectiveness in EOP may be returns to the positive effect of OPE in vegetative parameters especially that OPE possess a natural antioxidant such as flavonoids and vitamin C, which increase the activity of antioxidant enzyme promote photosynthesis, stimulate protein synthesis, and prevent aging, as well as this increase could be linked to total phenols' role in the control of plant metabolic functions. Furthermore, the phenolic compounds have an antioxidant property as a scavenger of free radicals due to their activity as an electron or hydrogen donor to stabilize and delocalize the chelator unpaired electron, and from their effect as transition metal ions [33, 15].

Chlorophyll a, b and carotenoid levels are signals of leaf photosynthesis, and the main function of the carotenoids are to serve as light receptors and safeguards for the photosynthetic machinery [38]. Salicylic acid influences the structure of leaves and chloroplasts, the closure of stomata, and the concentrations of chlorophyll and carotenoids, making it a crucial regulator of photosynthesis [39]. SA application to plants resulted in an enhancement in growth and photosynthetic rates. Possibly caused on by changes in the activity of the Rubisco enzyme and photosystem II [40], or facilitation of iron absorption by ATPase pump activation [41]. According to [42] SA enhances photosynthesis, chlorophyll production, and the concentration of alpha amino levulinic acid (a-ALA), a key step in the synthesis of chlorophyll.

According to Hartikainen et al [43] the beneficial effect of selenium on chlorophyll can be attributed to its ability to reduce lipid peroxidation, which may help preserve the integrity of several organelles' cellular and subcellular membranes, including the chloroplast. These membranes are thought to be involved in the production and localization of leaf pigments. Germ et al [44] investigated that the ideal Se concentration may be crucial in improving leaf pigments by raising antioxidant capability and delaying the aging of leaf tissues.

Treatment plants with orange peel extract enhanced the content of Chlorophyll a, Chlorophyll b, and total Chlorophyll which in turn boosted the photosynthetic process, improving plant productivity, as well enhanced carotenoids content which act as a free radical, increasing the chlorophyll in these plants [45]. According to El-Bassiouny et al [15] orange peels maintain enzyme function because they include natural antioxidants such flavonoids and vitamin C, which increase activities of antioxidant enzymes and induce photosynthesis. Fruit peels enhanced plant nutrient levels, particularly N and P, which are needed for chlorophyll production.

Regarding the effect of SA, the following studies agree with these results, on *Salvia coccinea*, [46] on *Carthamus tinctorius* L. and [34] on *Ocimum basilicum* L., as well as [47] on *Ocimum basilicum* L. treated with SE, and [48] on chickpea plants treated with OPE.

Phenolic compounds are a type of non-enzymatic antioxidant defense in plants, and they are generated in response to stress [50]. The increase in synthesis of several phenolic constituents in response to salicylic acid application, may be attributed by the activation of an oxidative stress state in plants. [51].

Treating basil with SA caused a positive effect on total phenol content it may returns to that SA promotes the phenylpropanoid pathway, resulting in the production of several secondary metabolites [52]. In this regard, [53]. argued that SA promoted the accumulation of phenylalanine mRNA, resulting in higher phenylalanine synthesis which in turn enhance accumulation of phenylpropanoids like phenolic acids. As well as [54] it was reported that SA activates the phenylpropanoid pathway, resulting in enhancement production of several secondary metabolites, including terpenoids and flavonoids with defense-related functions

Phenolics plays an important function as a free radical scavenger due to their reactivity as an electron or hydrogen donor, to stabilizing and delocalize the chelator unpaired electron, as well as from their purpose as transition metal ions [33]. The presence of natural antioxidants such as flavonoids, phenols, and vitamin C in orange peel extract may contribute to the positive effect on total phenolics content of basil essential oil [15].

Conclusion

Results showed that the treatments of foliar spray application with salicylic acid (SA), selenium as synthetic growth stimulants and orange extract as natural one, on number of cuts and the interaction between treatments and cuts were superior in terms of improving plant height, number of branches, fresh and dry weight, and volatile oil ratio, essential oil yield, and total phenolic content in essential oil with the third cut. Recommend the use of treatments promoters synthetic SA and SE at high concentrations (1.5mm & 10mg/l) and natural one orange peel extract at medium concentration (800mg/l) with the third cut.

Conflicts of interest

“There are no conflicts to declare”.

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Reference

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