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### **Research Article**

## Comparison Between Salicylic Acid and Selenium Effect on Growth and Biochemical Composition of Celery

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#### Abstract

**Background and Objective:** Salicylic acid (SALA) and Selenium (Se) play important roles in physiological process in celery (*Apium graveolens* L.). Celery has different medical and biological properties. In this study, the Influence of SALA or Se on growth and chemical composition of celery plants were investigated at vegetative, flowering and fruiting stages. **Materials and Methods:** Celery plants treated with SALA or Se was applied to foliage at 10 or 20 mg L<sup>-1</sup> compared with an untreated control. Plant height, vegetative fresh and dry weights and contents of essential oils composition, photosynthetic pigments, total carbohydrates, soluble sugars and antioxidant enzymes (SOD, CAT, POX) were measured. The averages of data were analyzed using 2-ways analysis of variance. **Results:** The SALA and Se significantly affected the growth and yield of celery crop. Treatment of 20 mg L<sup>-1</sup> SALA produced the best growth and the highest chemical contents during the flowering stage. **Conclusion:** The SALA and Se resulted in significant changes on growth, yield and chemical constituents of celery crop.

Key words: Celery, salicylic acid, selenium, growth, essential oil, chemical contents

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**Competing Interest:** The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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#### **INTRODUCTION**

Celery (*Apium graveolens* L.) contains essential oil used in food and pharmaceutical industries<sup>1</sup>. The SALA and Se are chemicals that could be used as elicitors to modify plant growth, yield, secondary products and bioactivities processes of medicinal and aromatic plants<sup>2,3</sup>.

Salicylic acid (SALA), a plant phenol, was recognized as a regulator of plant physiological processes when applied exogenously to plants, the most investigated roles of SALA are associated with its interference in plant resistance response to pathogen attacks and less than optimal biotic conditions<sup>4</sup>. Improvement in growth, yield and essential oil yield occurred due to SALA treatment of lemongrass (Cymbopogon citratus)<sup>5</sup>. Biomass production and seed yield of coriander (Coriandrum sativum) were increased due to treatment with SALA6. Treatment with SALA increased fresh and dry weights and sugar content of Salvia officianlis L. and Plectranthus tenuiflorus<sup>7,8</sup>. Application of SALA on Calendula officinalis indicated that SALA enhanced shoot and root dry weights and inflorescences number<sup>9,10</sup>. Abdou and Mohamed<sup>11</sup> reported that SALA improved mint (Mentha piperita L.) production, rates of photosynthetic pigments (chlorophyll a and b and total carotenoid), essential oil amounts and major constituents of essential oil. Khodary<sup>12</sup> reported that SALA accelerated growth, photosynthesis and carbohydrate metabolism of maize (Zea mays L). Pirbalouti et al. 13 reported that monoterpene hydrocarbons and major constituents of summer savory (Satureja hortensis) essential oils (carvacrol,  $\gamma$ -terpinene (Z)-β-ocimene,  $\alpha$ -pinene and  $\alpha$ -terpinene) were increased by foliar application of SALA. Peppermint (Mentha piperita L.) essential oil was improved due to application of SALA but essential oil constituents were not changed 14. The antioxidant enzymes catalase, CAT; peroxidase, POX; superoxide dismutase, SOD of Brassica juncea, wheat (Triticum aestivum) and sunflower (Helianthus annuus) were enhanced in response to SALA<sup>15-17</sup>.

Selenium (Se) is required in various crops at low doses, it has an important role in hormone balance, antioxidative reactions and many physiological processes in plant cell. It can promote glutathione peroxidase (GPX) activities which increase a resistance to substandard biotic factors affecting crops<sup>18-21</sup>. Application of Se enhanced growth, yield and accumulation of photosynthetic pigments in cucumber, alfalfa, peanut and chives<sup>22-26</sup>. Selenium application was associated with increased *Brassica rapa* L. seed production<sup>27</sup>. Essential oil productions in aromatic plants were affected by Se application<sup>28</sup>. Basil and lemon balm plants treated with Se

resulted in improved essential oil contents<sup>29</sup>. Selenium increased essential oil and monoterpenes of geranium<sup>28</sup>. Salvia officinalis had improved levels of  $\alpha$ -thujone,  $\beta$ -thujone, camphor and ketones while mono and sesquiterpenes were reduced due to Se treatment<sup>30</sup>. Selenium affected essential oils in chives<sup>26</sup>. Application of Se increased soluble sugars and total carbohydrate in potato, alfalfa and maize<sup>31-34</sup>. Effects of Se concentrations  $(0, 10, 20, 40, 80, 150, 175, 200, 250 \text{ mg L}^{-1})$  on activities of glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT) and Gua-dep peroxidases (POD) of Spirulina platensis were investigated<sup>35</sup>, application with >175 mg L<sup>-1</sup> producing increased GPX, SOD, CAT and POD activities. Antioxidant enzymes of lettuce were enhanced due to treatment with low doses of Se<sup>36</sup>. Treated tomato plants with Se resulted in higher activities of CAT than control<sup>37</sup>.

Scientific research had different techniques to increase the medicinal plants productivity which must increase as demand for food and natural pharmaceutical row materials production increases. The applications of SALA or Se are two ways of research that have the potential to increase the productivity of medicinal plants. Therefore, the effects of SALA and Se on growth, yield and chemical composition of celery plants were evaluated.

#### **MATERIALS AND METHODS**

Experimental: Two pot experiments were conducted in a greenhouse of the National Research Centre, Dokki, Cairo, Egypt, during 2 seasons of 2015/2016 and 2016/2017. Celery seeds were obtained from the Department of Medicinal and Aromatic Plants (MAP), Ministry of Agriculture, Giza, Egypt. Ten seeds were sown in each clay pot (30 cm diameter) in the 3rd week of October during both seasons. Each pot was filled with 10 kg of air-dried clay:sand (1:1, V:V) mix. Eight weeks after sowing, seedlings were thinned to 3 plants per pot. Pots were divided into 3 groups. The first group was exposed to SALA at 10 or 20 mg  $L^{-1}$ . The second group was subjected to Se at 10 or 20 mg  $L^{-1}$ . The third group was subjected to distilled water (as control). The SALA and Se were applied to run-off to foliage at 10 weeks after sowing. All agricultural practices were conducted according to the recommendations by the Egyptian Ministry of Agriculture.

**Growth characters:** Plant height and vegetative fresh and dry weights were recorded during the vegetative stage, 120 days after sowing (120 DAS), flowering stage, 210 days after sowing (210 DAS) and fruiting stage, 225 days after sowing (225 DAS).

**Essential oil isolation:** Fresh above ground tissue was collected from each treatment during vegetative, flowering, fruiting stages and fruit yield, air dried and weighed to extract the essential oil, then 100 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus<sup>38</sup>. The essential oil content was calculated as a relative percentage (v/w). Total essential oil per 100 plants was calculated. The essential oil extracted from celery fruit were collected from each treatment and dried over anhydrous sodium sulfate to identify the chemical constituents.

**Gas chromatography-mass spectrometry (GC-MS):** The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. DB-5 column (60 m×0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL min<sup>-1</sup>). The GC oven temperature was kept at 60°C for 10 min and programmed to reach 220°C at a rate of 4°C min<sup>-1</sup> and then kept constant at 220°C for 10 min followed by elevating the temperature to 240°C at a rate of 1°C min<sup>-1</sup>. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was m/z 35-450.

**GC analysis:** The GC analysis was carried out using an Agilent 6890N GC system using FID detector temperature of 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column at the same operational conditions. Relative (%) amounts of separated compounds were calculated from FID chromatograms.

**Identification of components:** Identification of essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their Retention Index (RI) to series of n-alkanes. Computer matching was against commercial (Wiley GC/MS Library, Mass Finder 3 Library)<sup>39,40</sup> and in-house Başer Library of essential oil constituents built up by genuine compounds and components of known oils. Additionally, MS literature data were used for identification<sup>41,42</sup>.

**Determination of photosynthetic pigments:** Chlorophyll (Ch a, Ch b) and total carotenoids (TC) in fresh leaves which collected at vegetative and flowering stages of each treatment were determined using methods described by the Anonymous<sup>41</sup>.

#### Determination of total carbohydrate and soluble sugars:

Total carbohydrate and soluble sugars contents were determined from leaves collected at the vegetative and flowering stages of each treatment. Their contents were determined with the method of Dubois *et al.*<sup>43</sup>.

#### Extraction and assaying antioxidant enzymes activities:

Enzyme extraction was with the method described by Mukherjee and Choudhuri<sup>44</sup>. Catalase activity (CAT) EC 1.11.1.6 assayed according to the method of Kar and Mishra<sup>45</sup>. Superoxide dismutase activity (SOD) EC 1.15.1.1 was determined by measuring inhibition of auto-oxidation of pyrogallol with the method of Marklund and Marklund<sup>46</sup>. Peroxidase activity (POX) EC 1.11.1.7 assayed with the method of Kar and Mishra<sup>45</sup> with slight modifications.

**Statistical analysis:** The experiment was arranged as a  $2\times2\times3$  factorial (SALA, SE, growth stages) with 4 replicates using a randomized complete block design using STAT-ITCF program (Statistica, ver. 7.1, Statsoft Inc., Tulsa, OK)<sup>47</sup>. According to De Smith<sup>48</sup> averages of data of both seasons were analyzed using 2-ways analysis of variance.

#### **RESULTS**

#### Effect of SALA and Se on growth characters and fruit yield:

Growth characters [plant height (cm) and vegetative fresh and dry weights (g/plant)] were affected by SALA and Se treatments during vegetative, flowering and fruiting stages (Table 1). All SALA or Se, except the Se at 20 mg L $^{-1}$  caused increases in all growth characters compared with the control at the various stages. All growth characters were increased toward the fruiting stage. The greatest growth characters were obtained from treatment of 20 mg L $^{-1}$  SALA with the values of 27.1, 102.5 and 119.8 cm; 4.8, 49.7 and 55.3 g/plant; 2.7, 17.0 and 19.9 g/plant at vegetative, flowering and fruiting stages respectively. Changes in growth characters were significant for SALA or Se treatments, growth stages and treatment  $\times$  growth stages. Plants treated with doses of SALA or Se produced high fruit yield compared with the control (Table 2). The greatest fruit yield was due to treatment with 20 mg L $^{-1}$  SALA.

**Effect of SALA and Se on essential oil content:** The contents of essential oil isolated from celery were affected by SALA and Se levels during vegetative, flowering and fruiting stages (Table 1). Plants treated with levels of SALA and Se had higher essential oil contents than the control at different growth

Table 1: Effect of SALA or Se levels and growth stages on growth characters and essential oil in vegetative tissues

			Growth character		-	Essential oil in v	egetative tissue
Growth stages	Treatment (mg L <sup>-1</sup> )		Plant height (cm)	Fresh weight (g/plant)	Dry weight (g/plant)	Percentage	Yield (mL/100 plants)
Vegetative	Control	0	17.6±0.1	2.2±0.0	1.5±0.0	0.9±0.2	1.4±0.2
	SALA	10	$23.0\pm0.1$	$3.4\pm0.0$	$2.4 \pm 0.1$	1.5±0.1	$3.6 \pm 0.2$
		20	$27.1 \pm 0.2$	4.8±0.1	$2.7 \pm 0.0$	1.9±0.1	$5.1 \pm 0.1$
	Se	10	$21.3 \pm 0.1$	$2.9 \pm 0.1$	$2.1 \pm 0.1$	$1.3 \pm 0.2$	2.7±0.2
		20	15.9±0.1	$2.1 \pm 0.1$	$1.3 \pm 0.0$	1.5±0.1	$2.0\pm0.1$
Overall vegetative			21.0±0.2	3.1±0.1	2.0±0.2	1.4±0.1	3.0±0.1
Flowering	Control	0	$61.1 \pm 0.2$	$10.8 \pm 0.2$	5.7±0.1	$1.1 \pm 0.1$	$6.3 \pm 0.2$
	SALA	10	69.7±0.1	$30.9\pm0.1$	11.1±0.2	$1.6 \pm 0.2$	$17.8 \pm 0.1$
		20	$102.5 \pm 0.1$	49.7±0.1	$17.0\pm0.1$	$2.1 \pm 0.1$	35.7±0.3
	Se	10	65.3±0.1	13.7±0.1	6.6±0.1	1.5±0.1	9.9±0.2
		20	58.0±0.1	8.6±0.1	4.9±0.1	1.7±0.1	$8.3 \pm 0.3$
Overall flowering			71.3±1.6	22.7±1.6	9.1±0.5	1.6±0.2	15.6±0.3
Fruiting	Control	0	$70.6 \pm 0.1$	12.7±0.1	6.6±0.0	$0.8 \pm 0.1$	5.3±0.3
	SALA	10	95.8±0.0	34.2±0.2	13.6±0.0	$1.1 \pm 0.1$	$15.0\pm0.2$
		20	119.8±0.1	55.3±0.1	19.9±0.1	$1.3 \pm 0.1$	25.9 0.3
	Se	10	$79.8 \pm 0.0$	15.7±0.1	8.0±0.1	$0.9 \pm 0.1$	$7.2 \pm 0.2$
		20	65.7±0.1	10.4±0.1	5.4±0.1	$1.0 \pm 0.1$	5.4±0.1
Overall fruiting			86.4±2.3	25.7±1.7	10.7±0.4	1.0±0.1	11.8±0.3
Overall treatments	Control	0	49.8±2.4	8.6±0.3	4.6±0.1	$0.9 \pm 0.1$	4.3±0.2
	SALA	10	62.8±3.2	22.8±0.5	9.0±0.2	$1.4 \pm 0.1$	12.1±0.3
		20	83.1±4.2	36.6±2.4	13.2±0.7	$1.8 \pm 0.1$	$22.2 \pm 0.3$
	Se	10	55.5±2.2	10.8±0.6	5.6±0.3	1.2±0.1	6.6±0.1
		20	46.5±2.3	$7.0 \pm 0.3$	3.9±0.2	$1.4 \pm 0.1$	5.2±0.1
F-ratio							
Treatments			170710.7***	165369.7***	18473.3***	8.5***	2416.6***
Growth stages			1571192.6***	265534.6***	44051.4***	13.8***	3067.6***
Treatments×growtl	n stages		25227.9***	34706.1***	3510.2***	0.4ns	440.7***

<sup>\*\*\*</sup>Significant at p<0.001, ANOVA, SALA: Salicylic acid, S: Selenium

Table 2: Effect of SALA or Se levels and on fruit yield and essential oil of fruit

			Essential oil (fru	Essential oil (fruits)		
Treatme	nt (mg L <sup>-1</sup> )	Fruit yield (g Plant <sup>-1</sup> )	Percentage	Yield (mL) (100 Plant) <sup>-1</sup>		
Control	0	7.9±0.1	1.2±0.2	13.4±0.4		
SALA						
	10	$17.8 \pm 0.2$	1.9±0.1	33.8±0.1		
	20	23.8±0.2	$2.3 \pm 0.2$	$54.7 \pm 0.3$		
Se						
	10	18.7±0.3	$1.6\pm0.1$	$29.9 \pm 0.3$		
	20	13.8±0.2	$1.4\pm0.1$	$19.3 \pm 0.3$		
F-value		2403.7***	6.8***	9753.3***		

<sup>\*\*\*</sup>Significant at p<0.001, ANOVA, SALA: Salicylic acid, Se: Selenium

stages. The greatest essential oil content was due to treatments with 20 mg L $^{-1}$  SALA at flowering stage. The SALA or Se treatments increased contents of essential oil extracted from celery fruit (Table 2). About 20 mg L $^{-1}$  SALA treatment produced the highest values of essential oil in fruit.

**Effect of SALA and Se on essential oil components:** Analysis with GC-MS indicated the presence of 19 compounds of essential oil from celery fruit (Table 3). Limonene,  $\beta$ -selinene, sedanolide and sedanenolide were identified as major constituents that produced the highest amounts of essential

oil due to treatment with SALA or Se. Increases occurred in the major essential oil constituents with all doses of SALA or Se. The 20 mg L $^{-1}$  SALA produced the highest amounts of limonene, β-selinene, sedanolide and sedanenolide (Table 3). All identified components were classified into 4 fractions. Monoterpene hydrocarbons (MCH), sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO) were the main fractions. Oxygenated monoterpenes (MCHO) formed a minor fraction. Treatment with 20 mg L $^{-1}$  SALA produced the highest amounts of MCH, SCH and SCHO; treatment with 20 mg L $^{-1}$  of Se produced the greatest amount of MCHO. There were highly significant variations in β-pinene, myrcene, p-cymene, limonene, carvone, β-selinene, sedanolide, sedanenolide and MCHO due to treatment.

**Effect of SALA and Se on photosynthetic pigments:** Most levels of SALA or Se caused increases in photosynthetic pigments (chlorophyll a, b and total carotenoids) during vegetative and flowering stages, except for 20 mg  $L^{-1}$  (Se) which caused a decrease (Table 4). Higher values were found in the photosynthetic pigments at the flowering stage than at the vegetative stage. The greatest amounts of chlorophyll a, b and total carotenoids were due to treatment with 20 mg  $L^{-1}$  SALA (Table 4).

Table 3: Effect of SALA or Se levels e on fruit essential oil constituents

			Treatments (mg L <sup>-1</sup> )					
				SALA		Se		
Number	Components (%)	RI	Control 0	10	20	10	20	F-value
1	α-Pinene	939	0.6±0.1	0.5±0.1	0.3±0.1	0.6±0.1	0.5±0.1	4.5*
2	Sabinene	976	$0.9 \pm 0.1$	$0.9 \pm 0.1$	$0.8 \pm 0.2$	$0.7 \pm 0.2$	$0.7 \pm 0.2$	1.1ns
3	β-Pinene	980	$0.3 \pm 0.1$	$0.3 \pm 0.1$	$0.9 \pm 0.1$	$0.3 \pm 0.1$	$0.6 \pm 0.2$	13.7***
4	Myrcene	991	$0.3 \pm 0.1$	$0.1 \pm 0.0$	$0.5 \pm 0.1$	$0.4 \pm 0.1$	$0.2 \pm 0.1$	9.4***
5	p-Cymene	1026	$0.2 \pm 0.1$	$0.4 \pm 0.1$	$0.6 \pm 0.2$	$0.9 \pm 0.1$	$0.8 \pm 0.2$	11.2***
6	Limonene	1031	32.3±0.3	$32.8\pm0.2$	$33.5 \pm 0.5$	32.4±0.4	32.9±0.1	6.2***
7	Limonene Oxide	1138	$0.4 \pm 0.1$	$0.5 \pm 0.1$	$0.3 \pm 0.1$	$0.7 \pm 0.2$	$0.6 \pm 0.1$	4.7*
8	Menthol	1173	$1.3 \pm 0.3$	$1.1 \pm 0.1$	$1.2 \pm 0.2$	$1.6 \pm 0.1$	$1.6 \pm 0.1$	1.7ns
9	Citronellol	1228	$1.4\pm0.1$	$1.3 \pm 0.3$	$1.2 \pm 0.1$	$1.1 \pm 0.1$	$1.5 \pm 0.1$	0.7ns
10	Carveol	1229	$1.5 \pm 0.1$	$1.7 \pm 0.2$	$1.6\pm0.2$	$1.7 \pm 0.1$	$1.4 \pm 0.1$	0.4ns
11	Citral	1240	$1.3 \pm 0.1$	$1.2 \pm 0.2$	$1.4\pm0.2$	$1.1 \pm 0.1$	$1.6 \pm 0.1$	1.6ns
12	Carvone	1242	$0.2 \pm 0.1$	$0.5 \pm 0.1$	$0.6 \pm 0.1$	$0.4 \pm 0.1$	$0.7 \pm 0.1$	11.1***
13	Phellandral	1249	$0.4 \pm 0.1$	$0.9 \pm 0.1$	$0.8 \pm 0.2$	$0.5 \pm 0.1$	$0.6 \pm 0.2$	5.8ns
14	β-Elemene	1375	$0.5 \pm 0.1$	$0.6 \pm 0.1$	$0.4 \pm 0.1$	$0.3 \pm 0.1$	$0.6 \pm 0.2$	3.2ns
15	Tetradecane	1399	$0.7 \pm 0.2$	$0.6 \pm 0.2$	$0.5 \pm 0.1$	$0.6 \pm 0.2$	$0.5 \pm 0.1$	0.8ns
16	β-Humulene	1440	$0.5 \pm 0.2$	$0.8 \pm 0.2$	$0.9 \pm 0.1$	$0.7 \pm 0.1$	$0.8 \pm 0.2$	2.5ns
17	β-Selinene	1485	$26.8 \pm 0.2$	$27.3 \pm 0.3$	$28.9 \pm 0.1$	$26.9 \pm 0.1$	$27.1 \pm 0.1$	69.4***
18	Sedanolide	1601	9.8±0.2	$10.9 \pm 0.1$	$11.1 \pm 0.1$	$9.9 \pm 0.1$	$10.4 \pm 0.4$	22.0***
19	Sedanenolide	-	$10.2 \pm 0.2$	$10.9 \pm 0.1$	11.8±0.2	$10.5 \pm 0.1$	$10.6 \pm 0.4$	11.3***
MCH			$34.6 \pm 0.4$	$35.0 \pm 0.1$	$36.6 \pm 0.4$	$35.3 \pm 0.3$	35.7±0.2	6.0*
MCHO			6.5±0.1	$7.2 \pm 0.2$	$7.1 \pm 0.1$	$7.1 \pm 0.1$	$8.0 \pm 0.1$	7.7***
SCH			$28.5 \pm 0.4$	29.3±0.3	$30.7 \pm 0.7$	$28.5 \pm 0.5$	29.0±0.5	2.4ns
SCHO			20.0±0.2	$21.8 \pm 0.4$	22.9±0.1	$20.4 \pm 0.4$	21.0±0.3	3.5*
Total iden	tified		89.6	93.3	97.3	91.3	93.7	

SALA: Salicylic acid, Se: Selenium, RI: Retention index, \*\*\*p<0.001, \*p<0.01, \*p<0.05, MCH: Monoterpenes hydrocarbons, MCHO: Oxygenated monoterpenes, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpenes

Table 4: Effect of SALA or Se levels on photosynthetic pigments, total carbohydrates and total soluble sugars during various growth stages

			Photosynthetic pigments (mg $g^{-1}$ )				
						Total carbohydrates	Total soluble
Growth stages	Treatments (mg $L^{-1}$ )		Ch a	Ch b	TC	(mg g <sup>-1</sup> )	sugars (mg g <sup>-1</sup> )
Vegetative	Control	0	3.1±0.0	2.9±0.0	1.4±0.0	56.9±0.1	36.6±0.1
	SALA	10	$3.4 \pm 0.0$	$3.3 \pm 0.0$	$1.5 \pm 0.0$	$72.0\pm0.1$	46.8±0.2
		20	$3.5 \pm 0.0$	$3.4\pm0.0$	$1.5 \pm 0.0$	85.3±0.1	48.2±0.1
	Se	10	$3.2 \pm 0.0$	$3.1 \pm 0.0$	1.5±0.0	69.6±0.2	44.9±0.1
		20	$3.0\pm0.0$	$1.8 \pm 0.0$	1.4±0.0	52.2±0.2	29.9±0.2
Overall vegetative			3.2±0.2	2.9±0.2	1.5±0.0	67.2±0.1	41.3±0.2
Flowering	Control	0	$3.3 \pm 0.0$	$3.2 \pm 0.0$	1.6±0.0	67.7±0.1	38.2±0.1
	SALA	10	$3.7 \pm 0.0$	$3.5 \pm 0.0$	1.7±0.1	78.6±0.3	$47.1 \pm 0.1$
		20	$4.0 \pm 0.0$	$3.7 \pm 0.0$	1.8±0.1	$102.0\pm0.1$	$72.0 \pm 0.1$
	Se	10	$3.5 \pm 0.0$	$3.3 \pm 0.0$	1.6±0.0	68.0±0.1	40.6±0.1
		20	$3.1 \pm 0.1$	$3.0\pm0.0$	1.5±0.0	65.5±0.4	35.9±0.2
Overall flowering			3.5±0.3	3.3±0.2	1.6±0.1	76.4±0.1	46.7±0.1
Overall treatments	Control	0	$3.2 \pm 0.1$	$3.0\pm0.1$	1.5±0.1	62.3±0.2	37.4±0.2
	SALA	10	$3.6\pm0.2$	$3.4\pm0.1$	1.6±0.1	75.3±0.3	46.9±0.2
		20	$3.7 \pm 0.3$	$3.5 \pm 0.2$	1.6±0.2	93.6±0.1	$60.1 \pm 0.1$
	Se	10	$3.3 \pm 0.2$	$3.2 \pm 0.1$	1.6±0.1	68.8±0.3	$42.8\pm0.2$
		20	$3.1 \pm 0.1$	$2.4\pm0.2$	1.4±0.0	58.9±0.3	$32.9\pm0.3$
F-ratio							
Treatments			1434.6***	6632.7***	24.4***	30939.0***	54614.5***
Growth stages			2275.7***	7751.5***	173.1***	17227.0***	18722.3***
Treatments x growth stages			135.6***	1648.8***	9.1***	2029.4***	14852.1***

SALA: Salicylic acid, Se: Selenium, \*\*\*p<0.001

Table 5: Effect of SALA or Se levels on antioxidant enzymes activities during various growth stages

			Antioxidant enzymes (unit gFW/ min)			
Growth stages	Treatments (mg $L^{-1}$ )		SOD	CAT	POX	
Vegetative	Control	0	1.3±0.0	25.9±0.0	0.6±0.0	
	SALA	10	$1.5 \pm 0.0$	26.1±0.0	1.0±0.0	
		20	1.6±0.0	28.3±0.0	$1.1 \pm 0.0$	
	Se	10	1.4±0.0	26.2±0.2	$0.9 \pm 0.0$	
		20	1.2±0.0	25.7±0.0	$0.5 \pm 0.0$	
Overall vegetative			1.4±0.1	26.4±0.1	0.8±0.2	
Flowering	Control	0	2.1±0.1	26.2±0.1	0.8±0.0	
	SALA	10	2.2±0.0	27.9±0.1	$1.1 \pm 0.0$	
		20	2.5±0.0	31.5±0.5	1.2±0.0	
	Se	10	$2.1 \pm 0.0$	26.7±0.3	0.9±0.0	
		20	1.8±0.0	26.0±0.0	$0.6 \pm 0.0$	
Overall flowering			2.1±0.2	27.7±0.2	0.9±0.2	
Overall treatments	Control	0	1.7±0.4	26.1±0.2	$0.7 \pm 0.1$	
	SALA	10	1.9±0.4	27.0±0.9	$1.0\pm0.1$	
		20	2.0±0.5	29.9±0.8	1.2±0.0	
	Se	10	1.7±0.4	26.5±0.3	0.9±0.0	
		20	1.5±0.3	25.9±0.2	$0.5 \pm 0.1$	
F-ratio						
Treatments			803.4***	448.4***	1119.4***	
Growth stages			16073.3***	310.9***	243.2***	
Treatments x growth stage	?S		59.0***	67.1***	13.1***	

SALA: Salicylic acid, Se: Selenium, \*\*\*p<0.001, CAT: Catalase, SOD: Superoxide dismutase, POX: peroxidase

**Effect of SALA and Se on total carbohydrate and total soluble sugars:** Foliar application of SALA, Se or the interaction affected total carbohydrate and soluble sugars during various growth stages compared with control (Table 4). Celery plants had lower amounts of total carbohydrates and soluble sugars at the vegetative stage than at the flowering stage. The highest amounts of total carbohydrate and soluble sugars were due to treatment with 20 mg L<sup>-1</sup> SALA at flowering stage.

**Effect of SALA and Se on antioxidant enzymes:** Treatments, growth stages and the interaction affected antioxidant enzymes activities (SOD, CTA and POX) (Table 5). Activities of antioxidant enzymes due to treatment with SALA or Se, except 20 mg L<sup>-1</sup> Se which resulted in a decrease compared with control, varied at the various growth stages. During flowering stage, the 20 mg L<sup>-1</sup> SALA produced higher values in activities of antioxidant enzymes than other treatments or the control.

#### **DISCUSSION**

The stimulating effects of SALA on plant growth characters at the growth stages could be attributed to SALA effects on ion uptake, cell elongation, cell division, cell differentiation, sink/source regulation, changes in the hormonal status, improvement of photosynthesis, transpiration and stomatal conductance<sup>49-54</sup>. The SALA

increased rate of cell metabolism, prerequisite for synthesis of auxin and/or cytokinin55,56. The stimulating effects of SALA on growth characters were confirmed by Khodary<sup>12</sup> on maize, Hayat et al.57 on wheat, Abdel-Wahed et al.58 on yellow maize, El-Khallal et al.59 on maize, Delavari et al.60 on Ocimum basilicum and Dawood et al.61 on sunflower. The effect of SALA on the essential oil has been confirmed. Rowshan et al.<sup>62</sup> indicated that increased essential oil contents due to treatment with SALA may be due to increase in numbers of leaf oil glands and enzyme activities of mono and sesquiterpenes biosynthesis. The results agreed with Abdou and Mohamed<sup>11</sup> and Talaat et al.<sup>63</sup>, they reported that SALA caused a significant increase in *Mentha piperita* and Ammi visnaga essential oil and its major constituents. The increases in photosynthetic capacity due to treatment with SALA could be attributed to stimulatory effects on pigment composition, rubisco activities, CO2 assimilation, photosynthetic rate and nutrient uptake<sup>12,64</sup>. The SALA has beneficial effects on photosynthetic apparatus through increase of antioxidants and new protein and decreases its degradation 54,65. Salicylic acid inhibits synthesis of ACC enzyme that prevents formation of ethylene and chlorophyll loss<sup>63</sup>. Positive effects of SALA on photosynthetic pigments were confirmed by some previous investigators. Salicylic acid at low doses caused increases in photosynthetic pigments of wheat, Brassica juncea, Mytrus communis and Phaseolus vulgeris<sup>12,57,66-67</sup>. The increase in total carbohydrates

and soluble sugars under SALA treatments may be implicated in osmotic adjustment as it has been reported in tomato plants treated with SALA<sup>68</sup>. The results from this work agree with Tari et al.68, Talaat69, El-Din and Reda70 and El-Moursi et al.71, who reported that total carbohydrates and soluble sugars were increased due to SALA treatment of camellia, pelargonium, chamomile, plectranthus and sweet marjoram. Activities of SOD, CTA and POX were increased due to treatment with SALA doses due to enhanced capacity of tissues to scavenge excess ROS. Salicylic acid application influences a wide variety of plant inductions of antioxidant synthesis<sup>72,73</sup>. The effects of SALA on the growth and biochemical characters of Mentha suaveolens under salt stress were investigated 74, the results decided that application of SALA at 30 mM caused significant increases in growth parameters, chlorophyll pigments, total phenolic compounds, tannins, soluble sugars, proline and hydrogen peroxide.

Variation occurred in growth characters, photosynthetic pigments and essential oil due to treatment with Se which may be due to increased chlorophyll content and amount of respiration value and glutathione peroxidase (GSH-Px) activity in mitochondria and dry matter content<sup>75-79</sup>. That treatment with Se affects photosynthetic pigments has been reported80-82. Low doses of Se can affect chlorophyll by increasing uptake of magnesium (Mg) in leaves<sup>83</sup>. Treatment with Se caused an increase in essential oil contents and major constituents. This may be due to Se ability to increase essential oils<sup>28</sup>. Selenium affects CO<sub>2</sub> assimilation rates that increase photosynthetic pigments content and ultimately accumulation of essential oil composition<sup>28</sup>. Obtained results agree with Lee et al.29, Khalid 30 and Khalid et al.26 who reported that Se increased essential oil of basil and lemon balm and parsley compared with control. Selenium caused an increase in carbohydrate and soluble sugars, which may be due to higher CO<sub>2</sub> fixation as result of enhanced stomatal conductance or activation of enzymes involved in CO2 assimilation producing a more efficient photosynthetic process and producing more carbohydrates<sup>32,84</sup>. The activation of antioxidant enzymes by Se has been reported in dill<sup>85,86</sup>. On other hand, the effects of Se on enzymatic activities and productivity of dill under saline condition were investigated<sup>87</sup>; the results decided that Se caused various improvements in antioxidant enzymes activities and osmotic adjustment; therefore, adding Se under saline condition could be a better strategy for maintaining the dill productivity in arid regions. Application of Se with iodine resulted in an increase of carrot productivity88.

#### **CONCLUSION**

It may be summarized that SALA and Se caused significant effects on growth characters and chemical composition of celery plants. The treatment of 20 mg L<sup>-1</sup> (SALA) resulted in higher values in growth characters, essential oil yield and major constituents of essential oil, photosynthetic pigments, total carbohydrates, soluble sugars and some antioxidant enzymes activities than control and other treatments.

#### SIGNIFICANCE STATEMENT

This study discovered that production of celery crop under SALA treatments is required. The SALA application caused significant variations in the active principals (essential oil) isolated from celery; so this investigation help the producers, ministry of agriculture and pharmaceutical companies to increase the yield and active principal of celery as a natural source of pharmaceutical and drug industries.

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