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IMPACT OF CHITOSAN ON GROWTH, CHEMICAL COMPONENTS AND ESSENTIAL OIL YIELD OF *LAVANDULA OFFICINALIS* PLANTS.

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ABSTRACT

This study was carried out during two successive seasons of 2019 and 2020 in an open field condition at a private farm in EL-Behira governorate, Egypt. This work aim to study the effect of spraying chitosan with different concentrations (0.0, 1, 2 and 3 g L^{-1}) on plant growth, chemical components, essential oil yield, photosynthetic pigments, total flavonoids, total amino acids, total phenols and total carbohydrates of lavender plants. The Experimental layout was randomized complete blocks design (RCBD) with 3 replicates. The obtained results showed that using chitosan concentration of 3g L^{-1} significantly increased plant height, number of branches $plant^{-1}$, fresh and dry weights of plants, volatile oil percentage and volatile oil yield per plant as well as chemical constituents such as Linalool and 1, 8cineol. It also increased chlorophyll content, total amino acids and total carbohydrates. While chitosan treatment at 2 g L^{-1} gave the maximum mean values of total carotenoids, total flavonoids and total phenols compared to control and the other chitosan levels under this study conditions.

Keywords: *Lavandula officinalis;* chitosan; growth; yield; essential oil; Linalool; 1,8 cineol.
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INTRODUCTION

Lavender (*Lavandula officinalis* L.) belongs to the Lamiaceae family, which is characterized by its ornamental, medicinal, and economic value. Lavender plants characterize by its high-quality essential oil which is considered raw material for many industries such as perfumes, cosmetics, and pharmaceutical industries (**Chrysargyris *et al*., 2020; Silva *et al*., 2020; González-Coloma *et al*., 2011; Touati *et al*., 2011**). Lavender herbage is rich in its oil content which has a high content of linalool, 1, 8 cineole, camphor, borneol, fenchol, pinene, and trans-pinocarveol (**Mambrí *et al*., 2018**). According to (**Woronuk *et al*., 2011** and **Meftahizade *et al*., 2011**) the antiseptic activity of lavender plants and lavender essential oils enabled them to be traditionally used as an agent in swabbing of wounds, burns, insect bites, and in veterinary practice to kill parasites. Lavender leaves and flowers have a high number of essential oil constituents, which are effective in biological therapeutics, aromatherapy as a relaxant, carminative and sedative agents (**El-Ghadban *et al*., 2008**; **Hritcu *et al*., 2012***).*

Ecological toxicity is at a critical point due to the high-level production and usage of inorganic fertilizers (**Gabr *et al*., 2022**). Different planning has been investigated to find the eco-friendly solutions for enhancing medicinal and aromatic plants growth and productivity among which chitosan. Therefore, biodegradable biofertilizers, like chitosan, are attracting the research community to avoid the hazards of using inorganic fertilizers.

Chitosan is a suitable candidate, taking into account sustainable agriculture (**Malerba** and **Cerana, 2018**; **Maluin** and **Hussein, 2020**). Moreover, chitosan may act as an exogenous elicitor to enhance plant protection (**Pirbalouti *et al.*, 2017**). Chitosan gets degraded enzymatically without affecting the soil-borne beneficial rhizosphere biota at low concentrations, and also induces the symbiotic exchange between plant and microbes (**Escudero *et al.*, 2017**). In addition, chitosan is a polysaccharide-based biopolymer, which stimulates the activity of plant symbiotic microbes, resulting in the alteration of

rhizosphere microbial equilibrium, thus disadvantaging the plant pathogens (**Bell *et al*., 1998**; **Cruz-Romero *et al*., 2013**). Chitosan is considered as a low toxic, biodegradable and assess efficient substance created by deacetylation process of chitin (**Iriti *et al*., 2009**), that is utilized in several agricultural production and medicine industries (**Pichyangkura** and **Chadchawan**, **2015**). Chitosan showed significant increasing of yield components (**El-Gamal and Ahmed, 2016**). In addition, (**Al-Tawaha *et al*., 2020**) indicate that chitosan may be useful in the cultivation of barley, due to its positive influence on growth and yielding of this plant. (**Malekpoor *et al*., 2016**) on basil, (**El-Khateeb *et al*., 2017**) on marjoram, (**Byczyńska, 2018**) on pineapple (**lily, Abdul-Qader** and **Rabie, 2019**) on stevia plants have reported that chitosan treatments increased volatile oil yield per plant , total chlorophyll content and total carbohydrates percentage compared with control. Moreover, lavender volatile oil production and chemical constituents were gradually increased by the increasing in chitosan concentration. On fennel plants, (**El-Bassiony *et al*., 2014**) indicated that foliar spray of chitosan gave the highest leaves number, dry weight of leaves and total yield. (**Massoud *et al*., 2016**) on *Coriandrum sativum* showed that chitosan significantly affected growth characters, fruit yield and essential oil productivity. Application of chitosan significantly increased total carbohydrates and N, P and K % in plant organs (**El- Attar, 2017**) on Snapdragon plants. The aim of the present study was to investigate the effect of foliar application of chitosan on the growth, productivity and essential oil constituents of lavender plants.

MATERIALS AND METHODS

This study was conducted during the two successive seasons of 2019 and 2020 at a private farm in EL-Behira governorate, Egypt. The aim of the present study was to investigate the effect of foliar application of chitosan concentrations (0.0, 1, 2 and 3 g L^{-1}) on plant growth, chemical components, essential oil content, photosynthetic pigments, total flavonoids, total free amino acids, total phenols and total carbohydrates of lavender (*Lavandula officinalis*). This experiment was designed using a randomized complete blocks design (RCBD) with 3

replicates. Physical and chemical analyses of the experimental soil were performed according to (**Jackson 1973**) and (**Cottenie *et al.*, 1982**) methods and the results are present in Table (1).

Table (1) Physical and chemical analysis of the experimental soil (average of the two seasons).

Soil properties		**Available nutrients (mm/100gm)**	
Texture	Clay loam	**N**	10.4
pH	8.0	**P**	0.5
EC (ds m^{-1})	1.2	**K**	3.3

Plant material and cultivation procedures

Lavender seedlings

Lavender seedlings were obtained from the experimental station at El-Qanater El-Khairia. Seedlings were transplanted at the age of 60 days on 15thOctober in plastic pots (30 cm diameter and 50 cm height)

All the recommended agricultural practices of planted lavender were done when ever needed. All treatments were fertilized with single calcium superphosphate (15.5 % P2O5) at 200 kg, potassium sulphate (48 % K2O) at 100 kg and ammonium nitrate (33 % N) at 150 kg per feddan. Phosphorus and potassium fertilizers were applied during soil preparation, while, nitrogen fertilizer was divided into three equal doses and were added to the soil at 35, 60 and 85 days after planting date.

Chitosan treatments

Chitosan (CAS no. 9012-76-4; degree of deacetylation degree of 85%) was purchased from Sigma-Aldrich (Shanghai, China).

Chitosan dosages were administered using a solution was prepared according to (**Dzung *et al*., 2011**) by dissolving 1 g of chitosan powder in 100 mL of 0.5% acetic acid for 12 hours. Then the corresponding concentrations (0, 1, 2 and 3g L^{-1}) were obtained by

diluting this solution with distilled water. The pH of chitosan solutions was adjusted to 6.5with 2mol L NaOH. The chitosan treatments were sprayed using a hand sprayer on the aerial parts until complete wetness and the pot surface was covered with polyethylene prior to the addition of chitosan to prevent spray droplets from dripping into the growth media.

Foliar application of chitosan solutions on plants was performed three times throughout the growing season; after 5 weeks from transplanting and repeated twice every 3 weeks after the first application. Untreated control plants were sprayed with an equivalent volume of distilled water.

Recorded Data

The plants were harvested two times (on 10th Feb) and after 3 months from the 1st cut. The plants were harvested by cutting vegetative parts at 20 cm above the soil surface. Data were recorded as the following:

Growth and yield

- Plant height
- Number of branches
- Fresh and dry weights (g plant^{-1})

Essential oil

The oil percentage was determined in fresh herb in both cuts using the hydro-distillation method by Clevenger apparatus according to Guenther(1961)then the oil was dried by sodium sulphate anhydrous.

- **Essential oil percentage was estimated as follows:**

Essential oil % = {Essential oil vol. (Measuring pipette reading) / Weight of sample} ×100

Essential oil yield/ plant (ml)

- **Essential oil yield per plant was estimated as follows:**

Essential oil yield per plant = oil percentage × herb fresh weight/plant.

- **Identification of Essential oil (EO) compounds**:

The EO was analyzed using DsChrom 6200 Gas Chromatograph equipped with a flam ionization detector for separation of volatile oil constituents were as follows: The chromatograph apparatus was fitted with capillary column DB-WAX 122-7032 Polysillphenylene- siloxane 30mx0.25 mm IDXO.25 µm film. Temperature program ramp increase with a rate of 13℃/min from 60° C to 220° C. Flow rates of gases were

nitrogen at 1ml/min, hydrogen at 30 ml/min and 330ml/min for air. Detector and injector temperatures were 280̊C and 250̊C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of EO. The identification of the compounds of the essential oil was achieved by matching their retention times with those of authentic samples injected under the same conditions.

Photosynthetic pigments

According to **Lichtenthaler (1987)** samples of lavender fresh leaves were kept in solution of 80% acetone and 95% ethanol in the refrigerator. The chlorophylls concentrations were measured in extract at 663, 645 and 470 nm using a spectrophotometer

Flavonoids compounds (TFC)

The aluminium chloride colorimetric method was used to determine TFC in herb according to **Pourmorad *et al.*** (**2006**).

Total amino acids

Total amino acids were determined according to (**Cocking, 1954)**

Total phenolic compounds (TPC)

The total phenolic content was determined according to the Folin-Ciocalteu procedure **(Žilić *et al.*, 2012**).

Total carbohydrates (TC)

The TC contents were determined in herb collected at the end of vegetative growth stage for each treatment with the method of (**Dubois *et al.*, 1956).**

Statistical Analysis

The experimental design was a randomized complete block design (RCBD) in three replications. The analysis of variance (ANOVA) was conducted by using SAS software. The Duncan test was used to compare the means within treatments at a 5% level of probability.

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RESULTS AND DISCUSSION

The effect of chitosan on plant growth

The research showed that treated lavender plants with chitosan had a stimulating effect on the plant height, the number of branches as well as herb fresh and dry weights in both seasons (Table 2, 3). The recorded mean values in most cases were increased steadily as a result of raising the concentration of CHT compared to the control with some exceptions. The highest mean values of plant height (57 and 77 cm in the first season and 59 and 80 cm in the second season) in both cuts were obtained from the plants treated with chitosan 3g L^{-1}. Concerning, the effect of chitosan on number of branches per plant, it is clear that, chitosan at 3gL^{-1} gave the maximum mean value of branches number per plant (21, 24 branch $plant^{-1}$) in the 1st season and (32, 35 branch $plant^{-1}$) in the 2nd season in both cuts.

Spraying chitosan on lavender plants improved herb fresh and dry weights significantly in both studied seasons (Table 2, 3). Fresh weight of the herb reached to their maximum mean values (137.4 and 285.0 g $plant^{-1}$ in the first season and 139.4 and 288.0 g $plant^{-1}$ in the second season) as a result of chitosan application at 3 g L^{-1} followed by 2g L^{-1} during both cuts.

Dry weight of the herb reached to their maximum mean values as a result of chitosan application at 3 g L^{-1} (82.4 and 104.8 g $plant^{-1}$ in the first season and 87.2 and 110.3 g $plant^{-1}$ in the second season) followed by 2g L^{-1} during both cuts.

Effect of chitosan on essential oil percentage and essential oil yield

It is obvious from Table (3) that all chitosan levels increased essential oil percentage compared with control in both seasons. In the both cuts chitosan at 3gL^{-1} gave the highest mean value of essential oil (0.55 and 0.57%) during the 1st cut and (0.46 and 0.48 %) in the 2nd cut of both seasons, respectively.

As a result of increasing the plant weight and essential oil percentage the yield of oil yield per plant increased with increasing chitosan levels compared with the control in both seasons. Chitosan treatment of 3 g L^{-1} gave the highest mean values of essential oil yield

in the 1st cut (0.419 and 0.423 mL plant^{-1}) and during the 2nd cut (0.461 and 0.473 mL plant^{-1}) of the first and second seasons, respectively.

Table (2): Effect of chitosan treatments on plant height (cm), branches number and fresh weight (g plant^{-1}) of lavender plants

Treat. Chitosan	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
Plant height (cm)				
Control	43±1.29^{c}	58±1.74^{c}	42±0.2^{c}	59±4.72^{c}
1g L^{-1}	54±1.62^{b}	61±1.83^{b}	56±3.36^{b}	64±3.82^{b}
2g L^{-1}	55± 1.65^{b}	62±1.86^{b}	57±2.28^{ab}	65±2.6^{b}
3g L^{-1}	57±1.71^{a}	77±2.31^{a}	59±1.77^{a}	80±2.4^{a}
$LSD_{0.05}$	1.683	2.160	2.626	2.18
Branches number per plant				
Control	18±0.54^{d}	25±0.75^{c}	16±1.28^{d}	26±2.08^{d}
1g L^{-1}	19±0.57^{c}	28±0.84^{cb}	19±1.14^{c}	29±1.74^{c}
2g L^{-1}	20±0.6^{b}	29±0.87^{ab}	21±0.63^{b}	31±1.24^{b}
3g L^{-1}	21±0.63^{a}	32±0.96^{a}	24±0.96^{a}	35±1.05^{a}
$LSD_{0.05}$	3.438	3.782	0.561	0.938
Fresh weight (g plant^{-1})				
Control	95.8±2.874^{d}	134.5±4.035^{d}	97.6±7.81^{d}	137.3±10.98^{d}
1g L^{-1}	105.6±3.168^{c}	190.8±5.724^{c}	108.5±6.51^{c}	194.6±11.68^{c}
2g L^{-1}	133.6±4.008^{b}	230.4±6.912^{b}	135.8±5.43^{b}	234.4±9.38^{b}
3g L^{-1}	137.4±4.122^{a}	285.0±8.55^{a}	139.4±4.18^{a}	288.0±8.64^{a}
$LSD_{0.05}$	2.869	4.119	3.089	2.8

Means in columns followed by the same letters are not statistically different at the 0.05 significance level. Data are mean values ± SE (n= 3).

Table (3) Effect of chitosan treatments on dry weight (g plant^{-1}), essential oil percentage (%), and essential oil yield (mL plant^{-1}) of lavender plants

Treat. Chitosan	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
Dry weight (g plant^{-1})				
Control	61.3±1.839^{c}	57.0±1.71^{d}	62.6±5.01^{d}	59.2±4.74^{d}
1g L^{-1}	71.8±2.154^{b}	68.1±2.043^{c}	73.8±4.43^{c}	70.1±4.21^{c}
2g L^{-1}	80.2±2.406^{a}	83.8±2.514^{b}	84.5±3.38^{b}	86.5±3.46^{b}
3g L^{-1}	82.4±2.472^{a}	104.8±3.144^{a}	87.2±2.62^{a}	110.3±3.31^{a}
$LSD_{0.05}$	3.106	1.890	2.132	1.335
Essential oil percentage				
Control	0.46±0.014^{c}	0.34±0.01^{c}	0.48±0.038^{d}	0.36±0.029^{c}
1g L^{-1}	0.50±0.015^{b}	0.41±0.012^{b}	0.51±0.031^{c}	0.45±0.027^{b}
2g L^{-1}	0.52±0.016^{a}	0.44±0.013^{a}	0.55±0.022^{b}	0.47±0.019^{a}
3g L^{-1}	0.55±0.017^{a}	0.46±0.014^{a}	0.57±0.017^{a}	0.48±0.014^{a}
$LSD_{0.05}$	0.041	0.059	0.019	0.014
Essential oil yield (mL plant^{-1})				
Control	0.304±0.009^{c}	0.194±0.006^{d}	0.302±0.024^{c}	0.196±0.016^{d}
1g L^{-1}	0.373±0.011^{b}	0.293±0.009^{c}	0.375±0.022^{b}	0.325±0.019^{c}
2g L^{-1}	0.417±0.012^{a}	0.369±0.011^{b}	0.420±0.017^{a}	0.357±0.014^{b}
3g L^{-1}	0.419±0.013^{a}	0.461±0.014^{a}	0.423±0.013^{a}	0.473±0.018^{a}
$LSD_{0.05}$	0.006	0.056	0.01	0.005

Means in columns followed by the same letters are not statistically different at the 0.05 significance level. Data are mean values ± SE (n = 3).

Effect of chitosan on essential oil constituents

In GC-MS chromatographic analyzes all 13 main constituents were identified representing 87.76 - 98.28 % of the total essential oil in plant leaves (Table 4). The superiority of linalool varied from one cut to another. The highest value was attained by chitosan at 2 g L^{-1} which

recorded 51.43 % at 1st cut while untreated plants gave the highest value of linalool (54.15 %) during the 2nd cut. 1, 8 cineol was the second major constituent in all treatments which ranged from 13.88 to 17.05% and showed variable fluctuations due to chitosan application.

Table (4): Effect of chitosan treatments on the relative percentages of essential oil constituents of Lavender plants (average of the two seasons).

Constituents	Chitosan treatments g/l							
	0		1gL^{-1}		2g L^{-1}		3g L^{-1}	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
α-tujone	0.07	0.11	0.06	0.12	0.08	0.09	0.07	0.09
α- pinene	3.92	3.99	5.92	6.99	6.39	7.71	6.36	7.69
Camphene	3.26	3.94	5.23	4.10	4.03	3.12	4.48	3.69
Myrcene	0.87	0.98	0.85	0.76	0.90	0.77	1.97	1.02
β- pinene	2.93	2.12	2.51	2.34	2.51	1.89	3.73	3.42
1,8 cineol	16.93	15.33	15.24	14.64	14.94	13.88	15.68	17.05
Limonene	2.66	2.30	3.66	2.13	2.90	3.91	2.21	1.38
Linalool	47.09	54.15	51.15	48.77	51.43	52.76	50.72	49.67
Camphor	2.26	2.80	3.03	2.39	2.03	2.68	2.88	3.28
Broneol	2.08	4.09	2.08	8.80	5.12	3.02	2.62	3.11
Terpine-4-01	2.78	2.95	3.88	3.44	3.07	3.70	3.15	3.94
linalyl acetate	2.00	2.95	2.90	2.06	3.25	2.87	2.36	2.08
Eugenol	0.91	1.06	1.11	1.19	1.63	1.38	1.51	1.00
Total identified	87.76	96.77	97.62	97.73	98.28	97.78	97.74	97.42

The highest relative percentage of 1,8 cineol were resulted from untreated plants (16.93 %) for the 1st cut while the application of chitosan at 3 g L^{-1} gave the highest values of 1,8 cineol (17.05) for the second cut. The third main component was α- pinene which ranged from 3.92 to 7.71%. In the 1st cut the highest relative percentage of α-pinene (6.39 %) which obtained from plants treated by chitosan at 2 g L^{-1} while chitosan at 2g L^{-1} gave the highest relative percentage of α-pinene (7.71%) in the 2nd cut.

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Table (5): Effect of chitosan treatments on chlorophyll a, chlorophyll b, and total carotenoids of lavender plants

Treat. Chitosan	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
Chlorophyll a (mg g^{-1} FW)				
Control	0.179±0.0053^{c}	0.157±0.004^{c}	0.177±0.014^{c}	0.152±0.012^{c}
1g L^{-1}	0.182±0.0054^{b}	0.174±0.005^{b}	0.183±0.011^{bc}	0.175±0.01^{b}
2g L^{-1}	0.185±0.0055^{ab}	0.176±0.0053^{ab}	0.188±0.007^{ab}	0.179±0.009^{ab}
3g L^{-1}	0.188±0.0056^{a}	0.179±0.0054^{a}	0.193±0.006^{a}	0.184±0.005^{a}
$LSD_{0.05}$	0.006	0.004	0.007	0.006
Chlorophyll b (mg g^{-1} FW)				
Control	0.088±0.0026^{d}	0.057±0.0017^{d}	0.091±0.003^{d}	0.055±0.002^{d}
1g L^{-1}	0.101±0.003^{c}	0.06±0.0018^{c}	0.105±0.006^{c}	0.064±0.005^{c}
2g L^{-1}	0.110±0.0033^{b}	0.064±0.0019^{b}	0.114±0.004^{b}	0.073±0.004^{b}
3g L^{-1}	0.130±0.004^{a}	0.078±0.002^{a}	0.132±0.011^{a}	0.081±0.003^{a}
$LSD_{0.05}$	0.004	0.001	0.007	0.003
Total carotenoids (mg g^{-1} FW)				
Control	0.084±0.0025^{d}	0.063±0.0021^{d}	0.085±0.007^{c}	0.066±0.006^{d}
1g L^{-1}	0.087±0.0026^{c}	0.069±0.002^{c}	0.086±0.005^{c}	0.068±0.002^{c}
2g L^{-1}	0.101±0.003^{a}	0.081±0.0024^{a}	0.104±0.004^{a}	0.087±0.003^{a}
3g L^{-1}	0.091±0.0027^{b}	0.075±0.0022^{b}	0.093±0.003^{b}	0.074±0.004^{b}
$LSD_{0.05}$	0.001	0.004	0.003	0.003

Means in columns followed by the same letters are not statistically different at the 0.05 significance level. Data are mean values ± SE (n=3).

Effect of chitosan on photosynthetic pigments

Data presented in Table (5) shows that most chitosan treatments had a positive effect on photosynthetic pigments content (chlorophyll a

and b as well as total carotenoids) in both seasons. The highest content of chlorophyll a was obtained from plants treated with chitosan at 3 g L^{-1} which recorded 0.188 and 0.179 mg g^{-1} for the first season and 0.193 and 0.184 mg g^{-1} for the second season during the 1st and 2nd cuts, respectively. Concerning, the effect of chitosan on chlorophyll b, it is clear that treated plants with 3 g L^{-1} chitosan gave the highest value of chlorophyll b (130 and 0.078 mg g^{-1} Fw for the first season and 0.132 and 0.081 mg g^{-1} Fw for the second season) in both cuts, respectively. Moreover, Chitosan at 2 g L^{-1} gave the maximum mean values of total carotenoids (0.101 and 0.081 mg g^{-1} FW for the first season and 0.104 and 0.087 mg g^{-1} FW for the second season) for both cuts in both seasons.

Effect of chitosan on total flavonoids, amino acids, phenols and carbohydrates contents

The data shown in Table (6) exhibited that different levels of chitosan increased total flavonoids compared with control during both cuts in both seasons. Total flavonoids reached to its maximum values as the result of 2 g L^{-1} which recorded 17.04 and 14.85 mg 100 g-1 FW in the first season and 19.07 and 16.95 mg 100 g-1 FW in the second season during the two cuts, respectively.

Concerning the effect of chitosan on total amino acids, results in Table (6) indicate that total amino acids increased gradually with increment doses of chitosan during both cuts in both seasons. So, the highest values of amino acids were obtained from plants treated with 3 g L^{-1} which recorded 12.24 and 10.74 mg 100 g^{-1} FW for 1st and 2nd cuts, respectively in the first season. While, the second season recorded 14.24 and 12.94 mg/100g for 1st and 2nd cuts, respectively.

As shown in Table (6) the data revealed that application of chitosan had a noticeable effect on total phenols in both seasons. Where total phenols reached to its maximum values as a result of chitosan application at 2g gL^{-1} which reached 3.53 and 3.11 mg 100 g^{-1} FW for 1st and 2nd cuts in the first season, respectively. In the second season at 2 g L^{-1} recorded 4.22 and 4.01 mg 100 g^{-1} FW for 1st and 2nd cuts, respectively.

Moreover data in Table (6) exhibited that total carbohydrate (%) was increased progressively with raising chitosan levels from 1-3 g L^{-1} compared to control during both cuts in both seasons. So, chitosan

at 3 g L^{-1} caused the highest accumulation of carbohydrate which recorded 29.99 and 28.81% for 1st and 2nd cuts in the first season, respectively. While, second season recorded 32.57 and 29.92 % for 1st and 2nd cuts, respectively.

Table (6): Effect of chitosan treatments on total flavonoids, total amino acids, total phenols and total carbohydrates of lavender plants

Treat. Chitosan	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
Total flavonoids (mg 100 g^{-1} FW)				
Control	14.09±0.423^{d}	12.01±0.36^{d}	14.36±1.15^{d}	12.13±0.97^{d}
1g L^{-1}	16.33±0.489^{c}	13.85±0.415^{c}	16.62±1^{c}	13.74±0.41^{c}
2g L^{-1}	17.04±0.511^{a}	14.85±0.445^{a}	19.07±0.76^{a}	16.95±0.68^{a}
3g L^{-1}	16.93±0.508^{b}	13.95±0.418^{b}	17.81±0.53^{b}	14.98±0.9^{b}
$LSD_{0.05}$	0.044	0.043	0.540	0.504
Total amino acids (mg 100 g^{-1} FW)				
Control	9.08±0.27^{d}	8.13±0.24^{d}	9.87±0.79^{d}	8.53±0.68^{d}
1g L^{-1}	10.02±0.3^{c}	8.91±0.26^{c}	11.12±0.67^{c}	9.91±0.59^{c}
2g L^{-1}	10.54±0.316^{b}	9.32±0.27^{b}	12.50±0.5^{b}	10.30±0.41^{b}
3g L^{-1}	12.24±0.367^{a}	10.74±0.32^{a}	14.24±0.43^{a}	12.94±0.39^{a}
$LSD_{0.05}$	0.044	0.036	0.326	0.281
Total phenols (mg 100 g^{-1} FW)				
Control	2.29±0.07^{d}	2.20±0.06^{d}	2.43±0.19^{d}	2.28±0.18^{d}
1g L^{-1}	3.15±0.09^{c}	2.42±0.07^{c}	3.75±0.22^{c}	3.41±0.2^{c}
2g L^{-1}	3.53±0.105^{a}	3.11±0.09^{a}	4.22±0.17^{a}	4.01±0.16^{a}
3g L^{-1}	3.50±0.106^{b}	3.09±0.09^{b}	3.99±0.12^{b}	3.75±0.11^{b}
$LSD_{0.05}$	0.248	0.158	0.088	0.079
Total carbohydrates %				
Control	24.03±0.72^{d}	24.12±0.72^{d}	24.24±1.94^{d}	25.02±2^{d}
1g L^{-1}	27.44±0.82^{c}	26.33±0.79^{c}	28.09±1.69^{c}	26.83±1.61^{c}
2g L^{-1}	29.51±0.88^{b}	28.57±0.85^{b}	29.91±1.2^{b}	29.14±1.17^{b}
3g L^{-1}	29.99±0.89^{a}	28.81±0.86^{a}	32.57±0.98^{a}	29.92±0.9^{a}
$LSD_{0.05}$	0.098	0.109	0.879	0.969

Means in columns followed by the same letters are not statistically different at the 0.05 significance level. Data are mean values ± SE (n = 3).

The health-promoting and pharmaceutical properties of plants depend on the content of biologically active compounds. Plant species belonging to the Lamiaceae family are a rich source of essential oil, polyphenolic compounds, in particular phenolic acids with high antioxidant activity (**Zgorka** and **Głowniak, 2001**). The obtained increases in vegetative growth parameters due to chitosan treatments are is in agreement with reports of several researches (**Ohta*, et al*., 1999; Salachna *et al*., 2015; Salachna *et al*., 2017; Pirbalouti *et al*., 2017; Byczyńska, 2018; El-Khateeb *et al*., 2018**).

In this study, we used chitosan as biological elicitor, with the aim of increase the amount of essential oil, phenolic and flavonoid content or in other words and secondary metabolites of lavender plants. That it was closer to our study, we have seen, the growth of lavender plants increased with chitosan foliar spray. It may be due to the role of chitosan as a natural biopolymer stimulates and increases nutrient uptake, chlorophyll content, photosynthetic and chloroplast enlargement, oil yield and its composition, and the synthesis of secondary metabolites, such as polyphenols, lignin and flavonoids (**Hadwiger, 2013; Malekpoor *et al*., 2016 and Salachna *et al*., 2017**).

Indeed, it has been found that signal molecules like chitosan are very potential elicitors for induction of active substances (**Zhao *et al*., 2005**). This results are in agreement with the previous reports about Greek oregano (**Yin *et al.*, 2012**), basil (**Kim *et al*., 2005; Pirbalouti *et al*., 2017**), shoot cultures of lemon balm (**Shabani** and **Razavizadeh, 2019**), and marjoram (**Amer** and **Shoala, 2020**). Foliar application of a chitosan was shown to have a positive effect on the net photosynthetic rate in soybean and corn leaves several days after application. This was correlated with an increase in the stomatal conductance and transpiration rate; however, the use of chitosan and chitin oligomers did not affect plant growth attributes (**Khan *et al*., 2002**).

On *Ruta graveolens* it has been concluded that, chitosan significantly increased growth rate and induced a significant increase in the oil concentrations (**Orlita *et al.*, 2008**). In *Origanum vulgare*, chitosan significantly enhanced the biomass and essential oil yield, but there was no effect on essential oil composition (**Yin *et al*., 2012**). On fennel plants, (**El-Bassiony *et al*., 2014**) concluded that foliar spray of chitosan gave the highest leaves number, dry weight of leaves and total

yield. (**Massoud *et al.*, 2016**) on *Coriandrum sativum* indicated that chitosan significantly affected growth characters, fruit yield and essential oil productivity.

Chitosan elicitation has also been reported to cause two-fold higher bioaccumulation of curcumin in turmeric (**Sathiyabama *et al.*, 2016**). According to (**Bistgani *et al.*, 2017**), chitosan treatments raised the essential oil content of *T. daenensis*. On napdragon plants application of chitosan significantly increased total carbohydrates and N, P and K % in plant organs (**El-Attar, 2017**). **El-Khateeb *et al.*, 2017** showed that humic acid, chitosan and brassinolide are recommended for improving plant growth, oil yield and its ingredients of *Majorana hortensis* plants.

Adding chitosan enhanced basil essential oil yield (**Pirbalouti *et al.*, 2017**). (**Vosoughi et al., 2018**) reported that the highest essential oil content was obtained from *Salvia officinalis* L. grown under water-stress condition by spraying with 0.50 g L^{-1} chitosan. **Amer and Shoala (2020)** concluded that using of H_2O_2 and nano chitosan at the growing stage are a practical strategy for enhancing the productivity attributes after harvest and reducing weight loss, respiratory with steadily decreasing of essential oil during storage period.

Foliar chitosan application associated with citric acid (400 mg L^{-1}) improved the yield quality and quantity in *Mentha piperita* grown under field and greenhouse conditions (**Pourhadi *et al.*, 2018**), and influenced the amount of volatile constituents in *Mentha arvensis in vitro* plants 100 mg L^{-1} (**de Oliveira *et al.*, 2020**).

The main characteristics such as flower number, weight and yield, essential oil yield, and the percentage of chamazulene in the volatile oil from chamomile plants improved using the foliar application of chitosan (**Abdul-Hafeez** and **Ibrahim, 2021**). The accumulation of phenolics and the activities of defense enzymes, i.e. polyphenol oxidase and β-1, 3-glucanases, were enhanced after chitosan elicitation in *Ocimum basilicum* L. and *Melissa officinalis* L. (**Hawrylak-Nowak *et al.*, 2021**). (**Kahromi** and **Khara, 2021**) indicated that the amounts of hydrogen peroxide and the activities of enzymatic and non-enzymatic and nutrient absorption increased after the foliar spray of chitosan.

In addition, they found that chitosan is very effective elicitor for improving rosmarinic acid, and quercetin apigenin (anticancer

flavonoid) contents. The results of previous investigations (**Bistgani *et al*., 2017a, 2017b; Pirbalouti *et al*., 2017; Goudarzian *et al*., 2020; Momeni *et al*., 2020**) showed that the quantity volatile oils from some medicinal and aromatic plants cultivated in Iran (*Ocimum ciliatum* L. and *O. basilicum* L., *Thymus daenensis* Celak., *Thymbra spicata* L. *Salvia officinalis* L., and *Mentha* × *piperita* L.) improved by the foliar spraying of chitosan.

The highest essential oil yield of Savory (*Satureja hortensis*) was obtained in the amino acid treatments in 0.5 mM salicylic acid and chitosan (**Poorghadir *et al*., 2020**). **Evandri *et al*. (2005**) reported that linalyl acetate (43.1%) and linalool (32.7%) were the major volatile oil components of *L. officinalis* plants from Italy (**Evandri *et al*., 2005**). In a previous study from the Isfahan province in middle parts of Iran, **Afsharypour** and **Azarbayejany** (**2006**) noted that the predominant constituents of *Lavandula officinalis* Chaix.

inflorescence volatile oil were linalool (34.1%), 1,8-cineole (18.5%), borneol (14.5%) and camphor (10.2%). Linalyl acetate (47.6%), linalool (28.1%) and lavandulyl acetate (4.4%) have been characterized as the main inflorescence volatile oil components of lavender (**Verma *et al*., 2010**).

Furthermore, linalool (44.6%), geraniol (11.1%) and lavandulyl acetate (10.8%) were the major chemical constituents of *L. angustifolia* plants cultivated in Xinjiang, China (**Cong *et al*., 2008**). Despite these reports on different wild and cultivated plants from diverse regions of the world, there is no documented research on the chemical composition of *L. officinalis* aerial part volatile oil and also the influence of chitosan on it. Therefore, owing to the multi-purpose usage of L. *officinalis* plants and as an ornamental plant for aromatic parks and garden design as well as the wide application of lavender essential oil in pharmaceutical and fragrance industries this work was done to study the effect of chitosan treatments on growth, chemical components and essential oil yield of *Lavandula officinalis* plants.

CONCLUSION

Chitosan may have essential role for the growth and productivity of lavender plants. The current study recommended that spraying *Lavandula officinalis* plants with chitosan at a rate of 3 gL^{-1} three times throughout the growing season may enhance chlorophyll content, plant growth parameter, chemical components, and volatile oil yield production, while chitosan treatment at 2 g L^{-1} gave the maximum mean values of total carotenoids, total flavonoids and total phenols.

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الملخص العربي

تأثيرالشيتوزان علي النمو والمكونات الكيميائية ومحصول الزيت العطري لنباتات اللافندر

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أجريت هذه الدراسة خلال الموسمين المتعاقبين لعامي 2019 و 2020 في حقل مفتوح بمزرعة خاصة بمحافظة البحيرة – جمهورية مصرالعربية. وذلك بهدف دراسة تأثير الرش بمركب الشيتوزان بتركيزات مختلفة (0.0، 1 ، 2 و 3 جم لكل لتر) على نمو النبات ، المحتوي الكيميائي ، محصول الزيت العطري, صبغات التمثيل الضوئي ، الفلافونيدات الكلية ، الأحماض الأمينية الكلية ، الفينولات الكلية والكربوهيدرات الكلية لنبات اللافندر. وقد صممت التجربة في صورة قطاعات كاملة العشوائية والمحتويه علي ثلاث مكررات. وقد أظهرت النتائج المتحصل عليها أن استخدام الشيتوزان بتركيز 3 جم لكل لتر أدى إلى زيادة معنوية في ارتفاع النبات وعدد الأفرع لكل نبات والوزن الطازج والجاف للنبات. كما ادي الي زيادة النسبه المئوية للزيت العطري ومحصول الزيت بالاضافة الي المكونات الكيميائية للزيت مثل اللينالول و1,8 سينيول كما ارتفع محتوي الكلوروفيل والاحماض الامينيه الكليه والكربوهيدرات الكليه في النبات. أعطي تركيز الشيتوزان2جم لكل لترأعلي قيمة لمتوسط نسبة الكاروتينودات الكلية والفينولات الكلية وأخيرا الفلافونيدات الكلية مقارنة بالكنترول ومستويات الشيتوزان الأخري تحت ظروف هذة الدراسة.

الكلمات الدالة: اللافندر, شيتوزان, النمو, المحصول, الزيت الطيار, اللينالول, 1,8 سينيول.