Egypt. J. Chem. **Vol. 60,** No.5, pp. 805 - 816 (2017)

Effect of Nitrogen Fertilization Rates, Plant Spacing and Their Interaction on Essential Oil Percentage and Total Flavonoid Content of Summer Savory (*Satureja hortensis* L.) Plant

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SUMMER savory (*Satureja hortensis* L.) plant is one of the most promising plants suitable for cultivation in the conditions of Egypt. A field experiment was carried out at Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, with Department of Medicinal and Aromatic Plants Research, National Research Center, Dokki, Giza, Egypt. during two successive seasons 2014 and 2015. The experimental design was a factorial in split plot with three replicates. The aim of the work was to study the effect of nitrogen fertilization at the rates of N0, N1, N2 and N3 (0, 40, 80 and 120 kg N/ feddan) as equivalents 0.0, 200, 400 and 600 kg/feddan of ammonium sulfate fertilizer 20.5% N, respectively as the main plots. Plant spacing was defined as S1, S2 and S3 (45×50, 30×50 and 15×50 cm, respectively) between the plants, as sub-plots. The effect of these treatments on essential oil percentage, essential oil constituents and total flavonoid content of *S. hortensis* L. was recorded. The results showed that the highest level of N fertilizer to combined with the least plant spacing had a significant effect on increasing the essential oil percentages in both seasons. The major essential oil constituents were carvacrol (47.26 and 42.90%) and γ-terpinene (38.30 and 39.10%) at the first and second cuts, respectively. Generally, carvacrol content was not affected by N fertilizer, but the γ-terpinene content increased slightly. The other lower percentage components arranged in descending order including ρ-cymene, terpinolene, α-terpinene, α-pinene and α-thujene contents were not significantly affected by such treatments. Total flavonoid content decreased with all N fertilizer levels at the first cut (June), the opposite trend was noticed at the second cut (August) in both seasons. Plant spacing at intermediate density was associated with the production of high contents of total flavonoids at both cuts during both seasons.

Keywords: Summer savory, *Satureja hortensis* L., N fertilization, Plant spacing, Carvacrol, γ-terpinene, Total flavonoids.

Introduction

Summer savory (*Satureja hortensis* L.) plant which belongs to Lamiaceae family is an annual herb up to 45 cm high with slender, erect, slightly hairy stems, linear leaves and small, pale lilac flowers. It is native to Europe, naturalized in North America. It is extensively cultivated in Spain, France, Yugoslavia and the USA and recently in Egypt for the production of essential oil. It is popular culinary herb, with a peppery flavour. It has been used therapeutically mainly as a tea for various ailments including digestive complaints (cramp, nausea, indigestion, intestinal parasites), menstrual disorders and respiratory conditions (asthma, catarrh, sore throat). Applied externally, the fresh leaves bring instant relief from insect bites, bee and wasp stings [1].

Plant nutrition is one of the most important factors that increase plant production. Nitrogen has the most recognized role in plant nutrition as it is in corporate in the structure of the protein molecule. Nitrogen is found in such important

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DOI :10.21608/ejchem.2017.1296.1074

molecules as purines, pyrimidines, porphyrines and coenzymes. purines and pyrimidines are found in the nucleic acids RNA and DNA essential for protein synthesis. Accordingly, nitrogen plays an important role in synthesis of the plant constituents through the action of different enzymes [2-5].

Plant spacing is an important factor in determining the microenvironment for the *Satureja hortensis* L. and other medicinal and aromatic plants. The optimization of this factor can lead to a higher essential oil yield and a better chemical composition [6-10].

Essential oil of summer savory (*Satureja hortensis* L.) was extracted by hydro distillation from the whole fresh and dried herb. An oleoresin is also produced by solvent extraction. The principal constituents are carvacrol, γ-terpinene,pinene, cymene, camphene, limonene, phellandrene and borneol. This essential oil and oleoresin are used in perfumery work and in most major food categories especially meat products and canned food [1,11,12].

Total flavonoids are the largest group of phytonutrients, with more than 6.000 types. Some of the best – known flavonoids are quercetin and kaempferol. Flavonoids are powerful antioxidant with anti-inflammatory and immune system benefits. Diets rich in flavonoid containing foods are sometimes associated with prevention of cancer, neurodegenerative and cardiovascular disease. [13-15].

The objective of this study was to investigate the effect of nitrogen fertilizer, plant spacing and their interaction on the essential oil percentage and constituents as well as total flavonoids of *Satureja hortensis* L. plant in loamy soil conditions.

Materials and Methods

This study was carried out at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University. The experiments investigations were carried out at Department of Medicinal and Aromatic Plants Research, National Research Center Dokki, Giza, Egypt, in the two successive seasons of 2014 and 2015.

The seeds of summer savory (*Satureja hortensis* L.) were imported from Jellitto Staudensamen GmbH, Schwarmstedt, Germany by Sekem Company, Egypt. The seeds were sown on 15th February 2014 and 2015 (in the two seasons) at the seeds were sown directly into a "nursery" seed bed inside green house in peat moss medium. After 45 days from sowing the seeds on 1st April 2014 and 2015, when the seedlings were 12-15 cm. height, they were transplanted to prepared plots in the experimental field.

The soil was prepared on 15th March 2014 for the first season and 20th March 2015 for the second season: Compost $5m^3$/fed and super-phosphate 15.50% P_2O_5 (200kg/fed) were added during soil preparation, while Potassium sulfate (48% K_2O) as a mineral fertilizer (100 kg /fed) was added in two doses, the first dose added during the soil preparation, while the second one was added two weeks after the first cut.

The experiment included 12 treatments, in three replicates, each was comprised of randomly distributed plots. The experiment was designed as split plots, with the main plots were assigned to different nitrogen levels (N0, N1, N2 and N3 as 0N, 40 N, 80 N and 120 N kg/feddan, respectively), while subplots were assigned to plant spacing S1, S2 and S3 (45, 30 and 15 cm, respectively) between the plants .Each experimental unit (plot) which was 2×1.6 m (3.2 m^2) divided into 4 rows with 50 cm apart and 45, 30 and 15 cm between the plants, i.e. the plots contained 16, 24 and 36 plants, respectively. Then, the treatments replicated three times (36 plots).

Nitrogen fertilizer at different levels 0, 40, 80 and 120 kg N equivalents 0.0, 200, 400 and 600 kg/feddan as Ammonium sulphate (20.5% N) in two doses, the first dose was added 21 days after transplanting, while the second one was added after the first cut during both seasons.

Recorded data:

Summer savory (*Satureja hortensis* L.) plant were harvested two times at the early bloom stage on 2nd June and 5th August 2014 for the first and the second cuts, respectively, in the first season and on 10th June and 13th August 2015 for the first and the second cuts, respectively, in the second season. The plants were harvested by cutting vegetative parts at 10 cm above the soil surface.

Data were recorded in the two seasons as the following:

1.Essential oil percentage in fresh herb.

2.Essential oil components by GC (Gas Chromatography).

3.Total of flavonoids mg / g. Dry Weight.

1. Essential oil percentage in the fresh herb

The oil percentage was determined in fresh herb in both seasons using the hydro-distillation method by Clevenger apparatus according to Guenther[16]. A known weight of fresh herb (100 g) was placed in a flask of 1 L capacity for distillation, and an adequate amount of water was added. A proper essential oil trap and condenser were attached to the flask and enough water was added to fill the trap. The distillation continued for 3.0 hours until no further increase in the oil was observed. After finishing the distillation process the apparatus was left to be cooled. The volume of the extracted essential oil was determined and recorded.

The obtained oil samples were then dehydrated over anhydrous sodium sulfate and stored in glass vials with Teflon-sealed at 2°C in the absence of light for oil analysis the essential oil percentage was estimated as follows:

$$\text{Essential oil \%} = \frac{\text{Essential oil vol. (Measuring pipette reading)}}{\text{Weight of sample}} \times 100$$

2.Essential oil components

Samples taken from the essential oil obtained in the first season were analyzed using GC analysis, to determine their main constituents. The use of GC in the quantitative determinations was performed using the methods described by Tatjana [17].

The GC analysis of the essential oil samples was carried out in the first season using gas chromatography instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Center with the following specifications.

Instrument: Instrument capillary GC-2010 plus Gas Chromatographs (Shimadzu Corp., Japan), coupled with a Shimadzu FID 2010 Plus detector (Flame Ionization Detector). The GC system was equipped with a Stabilwax column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 40ºC for 1 min; rising at 4.0º C/min to 150º C and held for 6 min; rising at 4º C/min to 210º C and held for 1min. The injector and detector were held at 210º and 250º C, respectively. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Most of the compounds were identified using GC standards (Sigma Aldrich GC standards). The obtained chromatogram and analysis report for each sample were analyzed to calculate the percentage of the main volatile oil components. The area of each peak was first calculated by an automatic integrator. The areas were then summed, and the total area of the peaks represented the whole sample. The percentage of each component was the ratio between its peak area to the total peak areas, multiplied by 100.

3.Total flavonoid content Quercetin equivalent / g Dry Weight.

Extraction of plant materials

One gram of each dry sample was extracted with 25 ml of methanol using an Ultrasonic bath Bandelin Sonorex (Germany) at 35 kHz and 200 W for 60 min at room temperature. The extracted samples were filtered through Whatman filter paper No. 4 and the filtrate was evaporated to dryness under vacuum and the yield of the extract from each sample was calculated. Each extract was dissolved in 10 ml methanol, 0.1 ml of sample solution, 1.5 ml of methanol, 2.8 ml of water, 0.1 ml of potassium acetate (1M) and 0.1 ml 2% $AlCl3$ solution dissolved in methanol were added and mixed. After incubation at room temperature for 30 min, the absorbance was determined using spectrophotometer at $\lambda max = 415$ nm. , the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of Quercetin equivalent (mg of Quercetin /g of extract).was expressed as milligrams of Quercetin equivalent per 100 g of dried plant (mg Quercetin/100 g) [18].

Statistical analysis of data:

Data recorded on essential oil percentage was statistically analyzed, and separation of means was performed using the Least Significant Difference (L.S.D.) test at the 5% level [19].

Results and Discussion

I. Effect of nitrogen fertilization rates, plant spacing and their interaction on essential oil percentage of Summer savory (Satureja hortensis L.) plant.

Table 1 shows the effect of different

concentrations of nitrogen fertilizer on the essential oil percentage of summer savory in the first season. Data indicated that increasing the nitrogen fertilizer application rate caused gradual steady increases significantly the essential oil percentage at the first and second cuts in the first season. The favorable effect of nitrogen fertilizer on essential oil percentage was particularly evident at the 80 and 120 kg N / feddan, which gave significantly higher essential oil percentages with mean values 0.596 and 0.612% in the first cut and 1.017 and 1.129% in the second cut, respectively than any other N fertilization treatments.

TABLE 1. Effect of nitrogen fertilization, plant spacing and their interaction on essential oil percentage of *Satureja hortensis* L. plant in the first season, 2014.

Plant spacing (S)	Nitrogen fertilization (N)				Mean
	N0	N1	N2	N3	
		First cut			
S1	0.540	0.543	0.550	0.553	**0.547**
S2	0.583	0.590	0.593	0.583	**0.588**
S3	0.607	0.597	0.643	0.700	**0.637**
Mean	**0.577**	**0.577**	**0.596**	**0.612**	**0.590**
L.S.D at 0.05 for N			**0.031**		
S			**0.036**		
N x S			**0.054**		
		Second cut			
S1	0.800	0.873	0.950	1.013	**0.909**
S2	0.803	0.950	0.973	1.203	**0.983**
S3	0.837	1.127	1.127	1.170	**1.065**
Mean	**0.813**	**0.983**	**1.017**	**1.129**	**0.986**
L.S.D at 0.05 for N			**0.044**		
S			**0.011**		
N x S			**0.077**		

N. fertilization: N0= 0.0, N1= 40, N2= 80 and N3= 120 Kg N/feddan
Plant spacing: S1= 45cm, S2= 30cm and S3= 15cm

Data presented in the second season in Table 2 indicated that increasing the nitrogen fertilizer levels (from 0 to 120 kg N/feddan) caused gradual increases with insignificant different between N treatments in the first cut. Whereas in the second cut the essential oil percentages of summer savory plant were gradual steady increased significantly, i.e. all nitrogen fertilizer levels (N1, N2 and N3) significantly increased the essential oil percentage with mean values 0.946, 0.948 and 0.992%, respectively compared with 0.858% for untreated plants (without nitrogen fertilizer). Similar results were recorded in both cuts of the first season Many investigators agreed with these results [11,5,3].

TABLE 2. Effect of nitrogen fertilization, plant spacing and their interaction on essential oil percentage of *Satureja hortensis* L. plant in the second season, 2015.

Plant spacing(S)	Nitrogen fertilization(N)				Mean
	N0	N1	N2	N3	
		First cut			
S1	0.660	0.693	0.680	0.667	**0.675**
S2	0.713	0.713	0.773	0.773	**0.743**
S3	0.733	0.750	0.780	0.787	**0.762**
Mean	**0.702**	**0.719**	**0.744**	**0.742**	**0.727**
L.S.D at 0.05 for N			**NS**		
S			**0.036**		
N x S			**0.094**		
		Second cut			
S1	0.840	0.913	0.923	0.913	**0.898**
S2	0.853	0.930	0.940	0.933	**0.914**
S3	0.880	0.993	0.980	1.130	**0.996**
Mean	**0.858**	**0.946**	**0.948**	**0.992**	**0.936**
L.S.D at 0.05 for N			**0.044**		
S			**0.051**		
N x S			**0.077**		

N. fertilization: N0= 0.0, N1= 40, N2= 80 and N3= 120 Kg N/feddan
Plant spacing: S1= 45cm, S2= 30cm and S3= 15cm

Values for essential oil percentage in the fresh herb of summer savory (*Satureja hortensis* L.) plants during the two seasons (Tables 1 and 2) show variation in different plant spacing in both seasons. The narrowest spacing S3 (15×50 cm) gave the highest significant volatile oil percentage in comparison with the medium S2 (30×50 cm) and widest S1 (45×50 cm) spacing at the two cuts in both seasons, which recorded 0.637, 0.588 and 0.547% in the first cut, and 1.065, 0.983 and 0.909% in the second cut during the first season for narrowest, medium and widest spacing, respectively Also, the mean values showed the same trend at the first and second cuts of the second season.

Concerning the interaction treatments, there was a significant effect on the oil percentage of summer savory plant at both cuts in both seasons. During both seasons, the highest mean values of essential oil percentage were obtained as a result of the combination treatment between the highest level of nitrogen fertilizer (120 Kg / Fed.) with the widest spacing (45 cm x 50) which recorded 0.700 and 1.170 % for the first season as well as 0.787 and 1.130% for the second season during 1^{st} and 2^{nd} cuts, respectively. On the other hand, the combination between unfertilized plants with the narrowest spacing (15 x 50 cm) gave the lowest values of essential oil percentage where the values were 0.540 and 0.800% at the two cuts in the first season and 0.660 and 0.840 % at both cuts in the second seasons, respectively.

In general, data in Tables 1 and 2 show that the essential oil percentage in all treatments in the first cut (June cut) were lower than those in the second one (August cut) in both seasons. Also, the general means of the essential oil percentages were 0.590 and 0.727% in the first cut and 0.986 and 0.936% in the second cut in the first and second seasons, respectively, this effect presumably due to longer light duration, higher temperature and higher light intensity previously dominated during summer growing season. This would have been the most suitable condition for oil synthesis and accumulation in the leaves [20-23].

II. Effect of nitrogen fertilization rates, plant spacing and their interaction with essential oil constituents by GC

Data presented in Tables 3 and 4 and illustrated in Fig. 1 show the main constituents of the essential oil of summer savory, *Satureja hortensis* L. of all treatments at both cuts in the first season as identified by GC. Seven components had been identified in the essential oil. The mean of major components were carvacrol (47.26 and 42.90%) and γ- terpinene (38.30 and 39.10%) in the first and second cuts, respectively, followed by five components arranged in descending order were ρ-cymene (3.91 and 4.02%), terpinolene (2.48 and 4.40%), α-terpinene (2.65 and 2.82%), α-pinene (1.57 and 1.86%) and α-thujene (0.84 and 1.18%) in the first and second cuts, respectively. The total identified components in the essential oil 97.01% and 96.28% at the first and second cuts, respectively [11,12,3].

Carvacrol content (the first major component) is a phenolic compound

Recorded data in Tables 3 and 4 and Fig. 1 show the effect of nitrogen fertilizer treatments on the carvacrol content in both cuts in the essential oil extracted from summer savory plants. The highest mean contents 48.51 and 44.34% were obtained in the oil of plants unfertilized by N0 at both cuts, respectively compared to fertilized plants by different nitrogen levels (N1, N2 and N3) had slightly decreased between them at the first and second cuts when compared to control N0 plants. This effect may be due to carcavrol synthesis and accumulation in essential oil of summer savory plants not need to more nitrogen uptake from the soil medium.

The plants grown at widest spacing S1 (45×50 cm) contained the slightly higher carvacrol contents in the oil, which gave 48.80 and 43.43% in both cuts, respectively compared to other plant spacings medium and narrow (30×50 cm and 15×50 cm), the means were 45.83, 47.16% and 41.97, 43.31% at both cuts, respectively.

Regarding the interaction between the effect of nitrogen levels and plant spacing on the carvacrol percentage in the essential oil, data presented in Tables 3 and 4 and Fig. 1 show that the highest carvacrol percentages were recorded in oil extracted from plants fertilized by nitrogen at N1 and planted at a spacing of S1 (45×50 cm) in the first cut (52.50%) and the plants unfertilized by nitrogen when transplanting at a spacing of S1 (45×50 cm) in the second cut (47.04%).

γ-terpinene content (the second major component) is a mono-terpenes hydrocarbon compound

γ-terpinene percentage was the lowest mean

TABLE 3. Effect of nitrogen fertilization and plant spacing and their interactions on the essential oil components (%) of *Satureja hortensis* L. plant at the first cut in the first season, 2014.

	The components (%) of the essential oil				
	Nitrogen fertilization (N)				
Plant spacing (S)	**N0**	**N1**	**N2**	**N3**	**Mean**
			α-Thujene		
S1	1.01	0.42	0.45	0.86	**0.69**
S2	0.89	0.97	1.01	1.01	**0.97**
S3	0.90	0.99	0.59	0.97	**0.86**
Mean	**0.93**	**0.79**	**0.68**	**0.95**	**0.84**
			α-Pinene		
S1	1.78	0.82	0.85	1.66	**1.28**
S2	1.64	1.83	1.88	1.79	**1.79**
S3	1.70	1.93	1.10	1.77	**1.63**
Mean	**1.71**	**1.53**	**1.28**	**1.74**	**1.57**
			α-Terpinene		
S1	2.75	2.16	2.23	2.71	**2.46**
S2	2.64	2.89	2.90	2.77	**2.80**
S3	2.73	2.91	2.35	2.79	**2.70**
Mean	**2.71**	**2.65**	**2.49**	**2.76**	**2.65**
			ρ-Cymene		
S1	3.99	3.29	3.30	4.07	**3.66**
S2	3.84	4.37	4.25	4.10	**4.14**
S3	3.95	4.15	3.55	4.08	**3.93**
Mean	**3.93**	**3.94**	**3.70**	**4.08**	**3.91**
			γ-Terpinene		
S1	36.77	35.69	38.63	39.53	**37.66**
S2	35.96	41.77	39.95	38.43	**39.03**
S3	37.17	40.55	36.55	38.51	**38.20**
Mean	**36.63**	**39.34**	**38.38**	**38.82**	**38.30**
			Terpinolene		
S1	2.48	2.31	2.52	2.59	**2.48**
S2	2.50	2.38	2.18	2.47	**2.38**
S3	2.28	2.97	2.49	2.63	**2.59**
Mean	**2.42**	**2.55**	**2.40**	**2.56**	**2.48**
			Carvacrol		
S1	48.01	52.50	49.33	45.34	**48.80**
S2	49.27	42.87	44.91	46.27	**45.83**
S3	48.26	43.35	50.21	46.82	**47.16**
Mean	**48.51**	**46.24**	**48.15**	**46.14**	**47.26**
Seven components representing = (identified)					**97.01%**
Other components representing = (unidentified)					**2.99%**

N. fertilization: N0= 0.0, N1= 40, N2= 80 and N3= 120 Kg N/feddan
Plant spacing: S1= 45cm, S2= 30cm and S3= 15cm

content in the oil obtained from plants unfertilized by (N0) nitrogen, the values were 36.63 and 37.85% in both cuts, respectively compared to the plants fertilized by different nitrogen levels of N1, N2 and N3 (40, 80 and 120 kg N/feddan,) recorded the highest values of γ-terpinene content, the means were 39.34, 38.38 and 38.82% at the first cut and 39.94, 39.88 and 38.55% at the second cut, respectively.

The highest mean content of γ-terpinene was recorded when the plants cultivated at medium spacing (39.03 and 39.17%) in both cuts compared to widest and narrowest plant spacing slightly decreases, the values were 37.66, 38.20% and 39.13, 38.87% at both cuts, respectively.

Concerning the interaction effect between nitrogen levels and plant spacing on γ-terpinene percentage in volatile oil of summer savory, data presented in Table (3 and 4) and Fig. (1) reveal that the highest content (41.77%) was recorded in the oil of the plants fertilized by N1 (40 kg N/fed.) and planted at S2 (30×50cm) in the first cut. Also, the plants fertilized by N1 and planted at wide spacing S1 (45×50 cm) resulted the highest content of γ-terpinene (43.01%) compared to other interaction treatments in the second cut.

Other components in essential oil α-Thujene, α-Pinene, α-Terpinene, ρ-Cymene and Terpinolene contents

Generally, these components mentioned before in the essential oil of summer savory, *Satureja hortensis* L., the percentage had no detectable trend as affected by different N levels fertilizer and plant density as well as interaction between them at the first and second cuts in the

TABLE 4. Effect of nitrogen fertilization and plant spacing and their interactions on the essential oil components (%) of *Satureja hortensis* L. plant at the second cut in the first season, 2014.

	The components (%) of the essential oil				
	Nitrogen fertilization (N)				
Plant spacing (S)	**N0**	**N1**	**N2**	**N3**	**Mean**
			α-Thujene		
S1	0.83	0.80	1.30	1.20	**1.03**
S2	1.27	1.77	1.10	1.27	**1.35**
S3	1.32	1.12	1.07	1.16	**1.17**
Mean	**1.14**	**1.23**	**1.16**	**1.21**	**1.18**
			α-Pinene		
S1	1.43	1.29	2.18	2.09	**1.75**
S2	1.94	2.24	1.45	2.03	**1.92**
S3	1.86	1.68	1.93	2.11	**1.90**
Mean	**1.74**	**1.74**	**1.85**	**2.08**	**1.86**
			α-Terpinene		
S1	2.60	2.63	3.01	2.89	**2.78**
S2	2.83	3.02	2.65	2.89	**2.85**
S3	2.82	2.71	2.84	2.94	**2.83**
Mean	**2.75**	**2.79**	**2.83**	**2.91**	**2.82**
			ρ-Cymene		
S1	3.76	3.88	4.43	3.94	**4.00**
S2	3.95	3.76	3.92	4.38	**4.00**
S3	4.07	3.95	4.06	4.20	**4.07**
Mean	**3.93**	**3.86**	**4.14**	**4.17**	**4.02**
			γ-Terpinene		
S1	36.79	43.01	42.10	34.60	**39.13**
S2	37.25	37.62	39.74	42.07	**39.17**
S3	39.52	39.20	37.79	38.97	**38.87**
Mean	**37.85**	**39.94**	**39.88**	**38.55**	**39.10**
			Terpinolene		
S1	4.15	3.97	3.83	4.28	**4.10**
S2	5.22	7.76	3.21	3.66	**4.96**
S3	4.93	4.02	3.97	3.68	**4.15**
Mean	**4.77**	**5.25**	**3.67**	**3.87**	**4.40**
			Carvacrol		
S1	47.04	40.72	39.65	46.32	**43.43**
S2	44.13	39.38	44.35	40.00	**41.97**
S3	41.85	43.56	44.94	42.90	**43.31**
Mean	**44.34**	**41.22**	**42.98**	**43.07**	**42.90**
Seven components representing = (identified)					**96.28%**
Other components representing = (unidentified)					**3.72%**

N. fertilization: N0= 0.0, N1= 40, N2= 80 and N3= 120 Kg N/feddan

Plant spacing: S1= 45cm, S2= 30cm and S3= 15cm

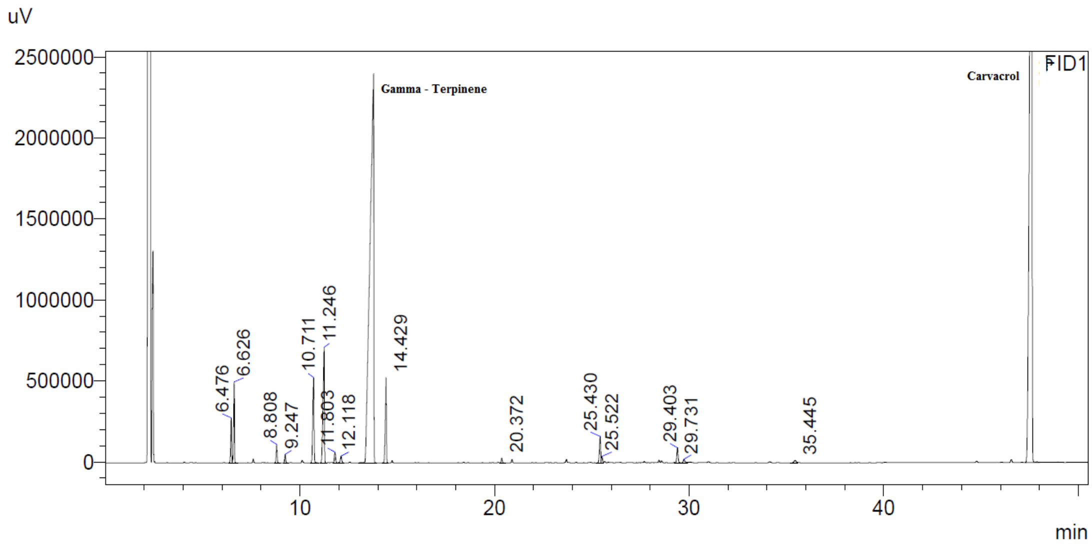

Fig.1. GC chromatogram of *Satureja hortensis* L. essential oil distilled from treated plants in the first season, 2014.

first season [24-26].

III. Effect of nitrogen fertilization rates, plant spacing and their interaction on total flavonoid content (mg / g D.W.)

Data presented in Tables 5 and 6 show that supplying *Satureja hortensis* L. plants with nitrogen fertilization treatments resulted in a marked decrease in the total flavonoids content compared to the control (unfertilized plants). The plants fertilized by N1 (40 kg N), N2 (80 kg N) and N3 (120 kg N / feddan) caused a steady reduction in the total flavonoid content with mean values of 0.555, 0.505 and 0.410 mg/g DW respectively, while the control plants N0 (unfertilized) resulted 0.711 mg/g D.W., the reduction percent compared to control were 21.94, 28.97 and 42.33% in nitrogen fertilizer at N1, N2 and N3, respectively at the first cut in the first season.

Similar results were obtained in the first cut in the second season with some modification, i.e the total flavonoid contents were decreased by 47.61, 31.20 and 41.79% in N1, N2 and N3 treatments, respectively compared to unfertilized control plants [27].

The results recorded in the first cut of the two seasons in Tables 5 and 6 show that similar trend was noticed, the plants cultivated at medium spacing S2 (30×50 cm) resulted an increase in the total flavonoid content compared to widest S1 (45×50 cm) and narrowest S3 (15×50 cm), in the first season, the means were 0.557, 0.542 and 0.537 mg / g D.W., whereas, in the second season were 0.495, 0.474 and 0.435 mg / g D.W., respectively.

Regarding the interaction between the effect of plant spacing and nitrogen fertilizer rates on the total flavonoid content, data in Tables 5 and 6 show that the highest content 0.771 mg / g DW at the first cut of the first season was produced by plant grown at the narrowest spacing (15×50 cm) and unfertilized with nitrogen, while the lowest content 0.406 mg / g DW was recorded from plants grown at the medium spacing (30×50 cm) and receiving the high level of nitrogen fertilizer N3 (120 kg N / feddan).

In the second season, at the second cut, the highest content of total flavonoids 0.702 mg / g DW was produced by plants cultivated at the widest spacing (45×50 cm) and unfertilized with nitrogen, whereas the lowest 0.291 mg / g DW was determined from plants grown at widest spacing and fertilized by low level of nitrogen N1 (40 kg N / feddan).

In both seasons, at the second cut, the opposite trend was noticed in Tables 5 and 6 show that all nitrogen levels (N1, N2 and N3) increased the total flavonoids content compared to control (unfertilized plants) in both seasons.

In both seasons, at the second cut, planting at the medium spacing (30×50 cm) gave the

TABLE 5. Effect of nitrogen fertilization, plant spacing and their interaction on total flavonoids (mg/g D.W) of *Satureja hortensis* L. leaves in the first season, 2014.

Plant Spacing(S)	Nitrogen fertilization(N)				Mean
	N0	N1	N2	N3	
			First cut		
S1	0.675	0.590	0.486	0.416	**0.542**
S2	0.687	0.578	0.556	0.406	**0.557**
S3	0.771	0.496	0.472	0.408	**0.537**
Mean	**0.711**	**0.555**	**0.505**	**0.410**	
			Second cut		
S1	0.372	0.265	0.612	0.712	**0.490**
S2	0.645	0.726	0.800	0.777	**0.737**
S3	0.472	0.582	0.733	0.689	**0.619**
Mean	**0.496**	**0.524**	**0.715**	**0.726**	

N. fertilization: N0= 0.0, N1= 20, N2= 40 and N3= 80 Kg N/feddan
Plant spacing: S1= 45cm, S2= 30cm and S3= 15cm

TABLE 6. Effect of nitrogen fertilization, plant spacing and their interaction on total flavonoids (mg/g D.W) of *Satureja hortensis* L. leaves in the second season, 2015.

Plant Spacing (S)	Nitrogen fertilization(N)				Mean
	N0	N1	N2	N3	
			First cut		
S1	0.702	0.291	0.420	0.483	**0.474**
S2	0.651	0.412	0.555	0.360	**0.495**
S3	0.656	0.351	0.407	0.327	**0.435**
Mean	**0.670**	**0.351**	**0.461**	**0.390**	
			Second cut		
S1	0.299	0.202	0.310	0.489	**0.325**
S2	0.324	0.324	0.304	0.486	**0.360**
S3	0.321	0.366	0.319	0.277	**0.321**
Mean	**0.315**	**0.297**	**0.311**	**0.417**	

N. fertilization: N0= 0.0, N1= 20, N2= 40 and N3= 80 Kg N/feddan
Plant spacing: S1= 45cm, S2= 30cm and S3= 15cm

highest content, with mean values of 0.737 and 0.360 mg /g D.W. in the first and second seasons, respectively, whereas the widest and narrowest spacings gave the lowest content, similar results recorded at the first cut in both seasons.

Concerning the interaction between the effect of nitrogen levels and plant spacings on the total flavonoid contents, data in Tables 5 and 6 show that the highest total flavonoid content 0.800 mg / g D.W. produced by plants receiving the N2 level (80 kg N / feddan) and planted at S2 (30×50 cm) spacing. On the other hand, the lowest content 0.265 mg / g D.W. resulted from plants fertilized by low level of nitrogen N1 (40 kg N / feddan) and transplanted at the widest spacing S1 (45×50 cm) at the second cut in the first season. Moreover, the highest content of total flavonoids 0.489 mg /g DW was produced from plants receiving the high level of nitrogen N3 (120 kg N / feddan) and planted at the widest spacing (45×50 cm), while the lowest content 0.202 mg /g DW was recorded from plants fertilized with N1 level and cultivated at the widest spacing S1 (45×50 cm) in the second season.

In general, the plants fertilized by the different nitrogen levels (N1, N2 and N3) resulted gradual steady increase in the total flavonoid content at both cuts of the first and second seasons. The favorable effect of nitrogen fertilization on the total flavonoid content in summer savory plant may be explained by increase in the N uptake by the plants of the nutrients involved in the synthesis and accumulate of the different secondary products such as total flavonoids [22,26,28,29].

Conclusion

Improvement of essential oil production of summer savory, *Satureja hortensis* L. plant was achieved by increasing the amount of nitrogen fertilizer to the rates of 400 and 600 kg / feddan of ammonium sulfate fertilizer (20.5%N) under Egyptian weather conditions. The maximum production of flavonoids was that of plants grown without application of nitrogen fertilization in June harvesting. On the other hand, the plant of August harvesting had improved synthesis and accumulation of flavonoids with higher N fertilization values. The optimum production of essential oil and flavonoid content was recorded with the heavy planting (30 or 15 cm spacing). The results of study may help the Egyptian growers to increase essential oil.

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(*Received* 5/7/2017;
accepted 13/8/2017)

تأثير التسميد النيتروجينى و مسافات الزراعة على محتوى الزيت العطرى و الفلافونيدات الكليه فى نبات الندغ البستانى *Satureja hortensis* L

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نبات الندغ البستانى summer savory من النباتات الواعدة و التى يناسبها الظروف السائدة فى مصر. إجريت التجربة فى مشتل كلية الزراعة – جامعة القاهرة ومعامل المركز القومى للبحوث بالدقى خلال موسمى ٢٠١٤ و ٢٠١٥. تهدف التجربة إلى دراسة تاثير التسميد النيتروجينى بسماد سلفات الامونيوم ٢٠,٦٪ نيتروجين بتركيزات N٣, N٢, N١, N٠ (صفر, ٤٠, ٨٠, ١٢٠ كجم نيتروجين / الفدان و التى تكافىء صفر, ٢٠٠, ٤٠٠, ٦٠٠ كجم سماد للفدان) على التوالى و زرعت النباتات على مسافات ٤٥, ٣٠, ١٥ سم فى خطوط و كان عرض الخط ٥٠سم فى تجربة عاملية بنظام القطع المنشقة و كان العامل الرئيسى هو التسميد و العامل تحت الرئيسى هو مسافات الزراعة و التداخل بينهما على النسبة المئوية للزيت العطرى و مكوناته و محتوى الفلافونيدات الكلية لنبات الندغ البستانى Summer savory.

كانت اهم النتائج هى:

- المعدلات العالية من التسيمد النيتروجينى (٨٠, ١٢٠ كجم نيروجين / فدان) و الزراعة فى المسافات الضيقة ١٥سم أعطت زيادة معنوية فى النسبة المئوية للزيت العطرى عند المقارنة بالنباتات التى لم تسمد و التى زرعت على مسافات واسعة ٤٥سم.
- تقدير مكونات الزيت العطرى بواسطة GC و GC-MS أثبت أن الزيت يحتوى على مكونين رئيسيين هما الكارفاكرول carvacrol و وصلت نسبته إلى ٤٧,٢٦ و ٤٢,٩٠٪ و جاما تربينين γ-Terpinene و وصلت نسبته إلى ٣٨,٣٠ و ٣٩,١٠ ٪ فى الحشة الاولى و الحشة الثانية فى الموسم الاول على التوالى. و بصفة عامة لم يتأثر محتوي carvacrol بمستويات التسميد النيتروجينى اما مركب γ-Terpinene زادت نسبته زيادة بسيطة مع مستويات التسيمد النيتروجينى بالمقارنة بالكنترول. و إحتوى الزيت على نسبة صغيرة من المركبات الاتية فى ترتيب تنازلى و هى: α-Pinene, α-Terpinene, ρ-Cymene, Terpinolene α-Thujene, و لم يكن للتسميد النيتروجينى او مسافات الزراعة تأثير محدد الاتجاه لهذه المركبات.
- الفلافونيدات الكلية حدث لها نقص مع مستويات التسميد النيتروجينى المختلفة فى الحشة الاولى (حشة يونيو)- و حدث العكس فى الحشة الثانية (حشة اغسطس) حيث زاد محتواها مع التسميد النيتروجينى بالمستويات المختلفة عند المقارنه بالكنترول فى الموسمين بينما مسافات الزراعة المتوسطة ٣٠سم أعطت أحسن محتوى من الفلافونيدات الكلية فى الحشتين فى الموسمين.