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HERBAGE YIELD, ESSENTIAL OIL CONTENT AND COMPOSITION OF SUMMER SAVORY (*SATUREJA HORTENSIS* L.) AS AFFECTED BY SOWING DATE AND FOLIAR NUTRITION

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Summary: *Satureja hortensis* L., (summer savory) is a well-known herbaceous aromatic medicinal plant. It is traditionally used as carminative, digestive as well as an anti-spasmodic and antitussive drug. This study was conducted at the experimental farm of the National Research Center, Dokki, Cairo, Egypt during two successive seasons to evaluate the influence of the sundfloura fertilizer as a source of NPK and AT-amino as a source of amino acids in plants sown on two sowing dates (20 November and 20 February). The highest values for all parameters tested (fresh and dry herb, essential oil content and its constituents) were obtained in plants sown on the second sowing date. NPK and amino acids had a pronounced effect on these characters. The main constituents of the essential oil were γ-terpinene (31.5 – 46.08%), carvacrol (23.71 – 51.22%), and p-cymene (2.81 – 14.92%). All treatments affected significantly the essential oil constituents.

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Keywords: *Satureja hortensis* L.; amino acids; NPK; essential oil; carvacrol; γ-Terpinene.

Abbreviations: NPK – nitrogen, phosphorus, potassium; A3 Sandfloura – fertilizer, source of NPK; AT-amino – fertilizer, source of amino acids; DW – dry weight, FW – fresh weight.

INTRODUCTION

Summer savory (*Satureja hortensis* L., family Lamiaceae) is a well-known medicinal and spice plant cultivated in several areas of the world (Hadian et al., 2008). The aerial parts of this plant are frequently used as tea or additive in commercial spice mixtures for many foods (Sahin et al., 2003) and also have been used as a folk remedy to treat various ailments such as cramps, muscle

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pains, nausea, indigestion, diarrhea and infectious diseases (Sahin et al., 2003). It is a common understanding that secondary metabolites production of medicinal plants is influenced by the cultivation conditions such as climate, plant density and use of fertilizers (Baranauskiene et al., 2003).

Amino acids have traditionally been considered as precursors and constituents of proteins, act as precursors of other nitrogen containing compounds, e.g., nucleic acids, acting as regulatory and signaling molecules and also affect synthesis and activity of some enzymes, gene expression, and redox-homeostasis (Rai, 2002). Amino acids and enzymes play a key role in the biosynthesis of essential oil constituents (Koeduka et al., 2006). Nitrogen, one of the essential mineral elements, is used by plants to build many organic compounds, such as amino acids, proteins, enzymes and nucleic acids. A second important nutrient for plants is K, which is usually present in plants at quite a high concentration, in particular in meristematic tissues and phloem. In addition to the above-mentioned macronutrients, one should also notice the role of other minerals: P, Ca, Mg and S in the development of essential oil-producing plants and in essential oil biosynthesis (Dzida, 2010). The effect of sowing date on growth, yield and active ingredients of medicinal and aromatic plants has been studied by many investigators (Asghar and Manijeh, 2012; Omer et al., 2013).

The objective of the present study was to examine the effects of sowing date and foliar application of two sources of fertilizers on growth characters as well as quality and quantity of essential oil in summer savory plant.

MATERIALS AND METHODS

The present investigation was carried out at the Experimental Farm, National Research Center (NRC), Dokki, Cairo, Egypt during two successive seasons (2010/2011 and 2011/2012) by split plot design with three replicates. Some physical and chemical properties of the soil are shown in Table 1. The experimental layout was split plot design with three replications. The main plots were devoted to the two planting dates (20 November and 20 February), subplots were assigned for foliar application with A3 Sandfloura (0, 5.0, 7.5 and 10.0 g L^{-1}) which contains: N 18%, P_2O_5 2%, K_2O 6%, Zn 1000 ppm, Fe 3000 ppm, Mn 1000 ppm and Mg 1% and for complete nutrient fertilizer - Amino AT (0, 1.5 and 3 mlL^{-1}) which contains: Total Organic Acids + Amino Acids > 20%, Fe 2%, Zn 1.5%, Mn 0.7% and Mg 500 ppm. Both foliar fertilizers are manufactured by Misr El Dawlya for Agricultural & Industrial Development Co.

Seeds were sown in trays of 3.2x3.2x4.5 cm cell size in a medium of peat and sand (1:1) on either 20 November or 20 February in a screen house. After four weeks from sowing, the uniform seedlings were transplanted into pots, 30 cm^2 diameter. The different levels of A3 Sandfloura were applied after 2 weeks from planting into pots and AT-amino one week later. Eight plants were sampled from each replicate at full bloom (about 70-80 days after transplanting). The following parameters were recorded for each sample: plant height, branches number per plant, fresh and dry weight. In addition, the areal parts of harvested plants which were air-dried in the shad, flower head and leaves

Table 1. Main characteristics of soil.

1-Mechanical Analysis	Value		Value
Sand %	80.7	Clay %	12.5
Silt %	6.8	Texture	Sandy Clay Loam
2-Chemical Analysis			
pH	7.48	Total Nitrogen %	0.121
Electrical Conductivity	0.61	Total Phosphorus %	0.0072
Organic Carbon %	0.89	Total Potassium %	0.012
Organic Matter %	1.55	$CaCO_3$ %	2.23
Soluble cations (meq/L)		Soluble anions (meq/ L)	
Na^+	4.25	CO_3^{-2}	0.00
K^+	0.10	HCO_3^{-1}	2.68
Ca^{++}	1.21	Cl^-	1.64
Mg^{++}	0.49	SO_4^{-2}	2.55
CEC meq/100g	13.26		

were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The obtained oils were dried over anhydrous sodium sulphate and stored at 4°C prior to analysis.

The GC-MS analysis of the essential oil samples was carried out in the second season using a gas chromatography-mass spectrometry instrument: a Trace GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer).

RESULTS AND DISCUSSION

The growth characters of savory plants were significantly affected by the sowing date. The highest values for plant height (35.17 cm), number of branches plant^{-1} (11.46), herb FW (35.64 g plant^{-1}) and herb DW (23.91 g plant^{-1}) were obtained for the 2nd sowing date (Table 2). The late sowing date was more suitable for *Sideritis montana* (Abdel-Razek 2007) and savory (Jadczak 2007). The combination between sowing dates and A3 Sandfloura or amino AT application had a significant effect on the growth characters tested (Table 2). The combination between the second sowing date and A3 Sandfloura applied at a concentration of 10 g L^{-1} gave the maximum mean values of plant height (36.23cm), number of branches plant^{-1} (12.26), FW (43.21g plant^{-1}) and DW (20.89g plant^{-1}) (Table 2). Data presented in Table 2 on the combination treatments of AT-amino application with sowing dates showed clearly that the interaction between the second sowing date with AT-amino applied at a concentration of 3 ml l^{-1} gave the maximum mean values of plant height (36.40 cm), number of branches plant^{-1} (12.07), FW (39.45 g plant^{-1}) and DW (20.90 g plant^{-1}). It was evident that the highest concentrations of both mineral and organic fertilizers had a beneficial effect on plant biomass accumulation and essential oil content.

When plants were sown in February,

Table 2. Comparison of traits in summer savory (mean values for both seasons).

Experimental Treatments		Plant height [cm]	Branches [number plant^{-1}]	Herb FW [g plant^{-1}]	Herb DW [g plant^{-1}]	Essential oil [%]	Essential oil yield [ml plant^{-1}]
Planting Dates							
20 November		20.9^{a}	7.71^{a}	21.28^{a}	11.59^{a}	0.400	0.0846
20 February		35.17^{b}	11.46^{b}	35.64^{b}	23.91^{b}	1.011	0.3631
LSD		0.75	0.21	0.90	0.55	0.004	0.0001
Sowing date	Sandfloura [g l^{-1}]						
1st sowing date	0	19.36^{a}	7.46^{a}	18.95^{a}	9.06^{a}	0.3867	0.0733
	5	20.48^{ab}	7.46^{a}	20.35^{ab}	10.38^{ab}	0.4033	0.0823
	7.5	21.47^{ab}	7.96^{a}	21.88^{bc}	10.62^{b}	0.4000	0.0877
	10	22.26^{b}	8.10^{a}	23.92^{c}	11.56^{b}	0.3967	0.0950
2nd sowing date	0	34.00^{c}	10.33^{b}	28.55^{d}	17.73^{c}	0.9633	0.2757
	5	35.00^{c}	11.30^{c}	31.63^{e}	17.32^{c}	1.0200	0.3230
	7.5	35.43^{c}	11.94^{cd}	39.15^{f}	19.61^{d}	1.0267	0.4033
	10	36.23^{c}	12.26^{d}	43.21^{g}	20.89^{d}	1.0367	0.4503
LSD		2.45	0.85	2.56	1.32	0.078	0.0003
Sowing date	AT-amino [ml l^{-1}]						
1st sowing date	0	19.74^{a}	7.11^{a}	19.42^{a}	9.14^{a}	0.3775	0.0735
	1.50	20.74^{a}	7.77^{ab}	21.68^{ab}	10.34^{a}	0.3950	0.0855
	3.00	22.21^{a}	8.26^{b}	22.73^{b}	11.74^{b}	0.4175	0.0948
2nd sowing date	0	34.00^{b}	10.84^{c}	31.41^{c}	16.71^{c}	0.9750	0.3088
	1.50	35.10^{b}	11.47^{cd}	36.05^{d}	18.74^{d}	1.0175	0.3673
	3.00	36.40^{b}	12.07^{d}	39.45^{e}	20.90^{e}	1.0425	0.4133
LSD		2.59	0.87	2.62	1.36	0.0060	0.0003

increased plant biomass accumulation and essential oil yield per plant independent of the kind of fertilization (Sandflora or AT-amino) were observed. This effect could be due to the different mean temperatures during the two growing seasons.

Moreover, it was found that supplying plants with different levels of A3 Sandfloura and AT-amino on both sowing dates led to a significant effect on different growth characters (Table 3). The combination between A3 Sandfloura (10 g L^{-1}) and AT-amino (3 ml l^{-1}) under the 2nd sowing date resulted in the highest mean values of plant height (38.20 cm), number of branches plant^{-1} (12.81), FW (48.09 g plant^{-1}) and DW (48.09 g plant^{-1}) compared with the other interaction treatments.

Our data indicated that delaying the sowing date from November to February resulted in the highest promotional effect on essential oil content (Table 2). The yield of essential oil in the herb was significantly affected by the sowing date. The second

sowing date gave the maximum yield of essential oil and this increment reached 329.20%. Si and Walton (2004) reported that a later sowing date resulted in the reduction of oil concentration in canola and Indian mustard by 1.1 percentage points for every two-week-delay of sowing. Soheir et al. (2008) concluded that sowing rue plants at the end of November was more favorable for herb production and in addition, for stronger accumulation of essential oil than in October. Concerning the combination between sowing dates and A3 Sandfloura fertilizer or amino AT (Table 2), it can be noticed that these treatments had a significant effect on essential oil content and yield (ml plant^{-1}) compared with non-fertilized plants. The

Table 3. Interaction effects between Sandfloura and AT amino under different sowing dates on growth characters and essential oil content of summer savory (mean values for both seasons).

Sowing date	Sandfloura [g plant^{-1}]	Amino [ml L^{-1}]	Plant height [cm]	Branches [number plant^{-1}]	FW [g plant^{-1}]	DW [g plant^{-1}]	Essential oil [%]	Essential oil yield [ml plant^{-1}]
1st sowing date	0	0	18.30^{a}	6.63^{a}	17.32^{a}	8.12^{a}	0.380	0.066
		1.5	18.39^{a}	7.83^{bcde}	19.90^{b}	9.50^{b}	0.380	0.076
		3	21.40^{bcd}	7.92^{bcde}	19.62^{ab}	9.55^{b}	0.400	0.078
	5	0	19.55^{ab}	7.29^{abcd}	19.09^{ab}	9.22^{ab}	0.370	0.071
		1.5	20.40^{ab}	7.15 ^{ab}	21.02^{b}	10.12^{b}	0.410	0.086
		3	21.50^{bcd}	7.50^{bcd}	20.95^{b}	11.81^{c}	0.430	0.090
	7.5	0	20.50^{ab}	7.30^{abcd}	19.95^{b}	9.21^{ab}	0.380	0.076
		1.5	21.00^{bcd}	8.09 ^{de}	21.05^{b}	10.25^{b}	0.400	0.084
		3	22.92^{cd}	8.50^{ef}	24.64^{c}	12.40^{cd}	0.420	0.103
	10	0	20.62^{abc}	7.20^{abc}	21.33^{b}	9.99^{b}	0.380	0.081
		1.5	23.17^{d}	8.00^{cde}	24.73^{c}	11.50^{c}	0.390	0.096
		3	23.00^{cd}	9.11^{f}	25.70^{c}	13.20^{d}	0.420	0.108
2nd sowing date	0	0	32.00^{e}	10.50^{gh}	26.66^{cd}	15.20^{e}	0.890	0.237
		1.5	34.50^{fg}	10.00^{g}	28.69d^{e}	18.16^{hi}	1.000	0.287
		3	35.50^{fg}	10.50^{gh}	30.29^{ef}	19.82^{k}	1.000	0.303
	5	0	33.50^{ef}	10.95^{h}	28.30^{de}	16.61^{fg}	1.000	0.283
		1.5	35.00^{fg}	11.03^{h}	30.36^{ef}	15.55^{ef}	1.030	0.313
		3	36.50^{gh}	11.93^{i}	36.23^{g}	18.55^{ij}	1.030	0.373
	7.5	0	35.00^{fg}	10.85^{h}	31.45^{f}	17.00^{gh}	1.000	0.315
		1.5	35.90^{gh}	11.93^{i}	42.82^{i}	19.72^{jk}	1.020	0.437
		3	35.40^{fg}	13.03^{j}	43.19^{i}	22.11^{lm}	1.060	0.458
	10	0	35.50^{fg}	11.05^{h}	39.24^{h}	18.04 ^{hi}	1.010	0.400
		1.5	35.00^{fg}	12.92^{j}	42.31^{i}	21.51^{l}	1.020	0.432
		3	38.20^{h}	12.81^{j}	48.09^{j}	23.11^{m}	1.080	0.519
LSD			2.38	0.81	2.45	1.26	0.013	0.001

maximum mean values of essential oil content (1.037 %) and essential oil yield (0.450 ml plant^{-1}) were obtained as a result of the combination between the 2nd sowing date and 10 g L^{-1} A3 Sandfloura (Table 2). Moreover, the combination between the 2nd sowing date and 3 ml L^{-1}AT-amino gave the highest values of essential oil content (1.043 %) and essential oil yield (0.413 ml plant^{-1}) compared with other combination treatments between sowing dates and amino AT fertilizer as well as control.

There were significant differences in the interaction between A3 Sandfloura, AT-amino under different sowing dates for essential oil content and essential oil yield (Table 3). The 2nd sowing date with A3 Sandfloura applied at 10 g l^{-1} + AT-amino applied at 3 g l^{-1} was the best combination affecting essential oil content (1.020%) and essential oil yield (0.519 ml l^{-1}).

The effects of A3 Sandfloura and amino AT treatments under different sowing dates on essential oil constituents of *S. hortensis L.* are presented in Figs. 1 and 2. The essential oil composition varied between treatments and was characterized by a high percentage of non-oxygenated compounds ranging from 42.87 to 61.85 % and from 56.05 to 74.26 % for the 1st and 2nd sowing dates, respectively. The relative percentage of monoterpenes ranged from 44.01 to 67.98% and from 96.42 to 98.49 % while sesquiterpenes ranged from 31.94

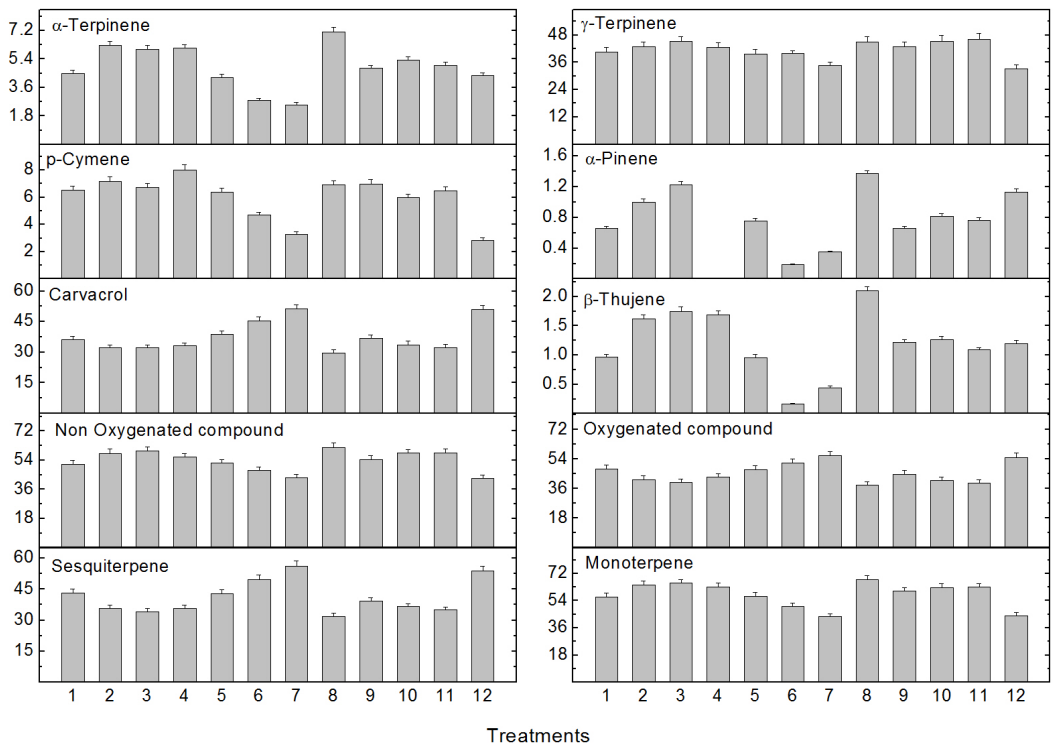

Figure 1. Essential oil composition (%) of summer savory as affected by foliar application of A3 Sandfloura and AT-amino at different concentrations, under first sowing date (20 November). 1 - (0^{a}:0^{b}), 2 - (0:1.5), 3 - (0:3), 4 - (5:0), 5 - (5:1.5), 6- (5:3), 7 - (7.5:0), 8 - (7.5:1.5), 9 - (10:3), 10 - (10:0), 11 - (10:1.5), 12 - (10:3). Values are means ± SE, n=3; t: compounds less than 0.05. ^{a}Sandfloura [g/plant], ^{b}Amino AT [ml/L].

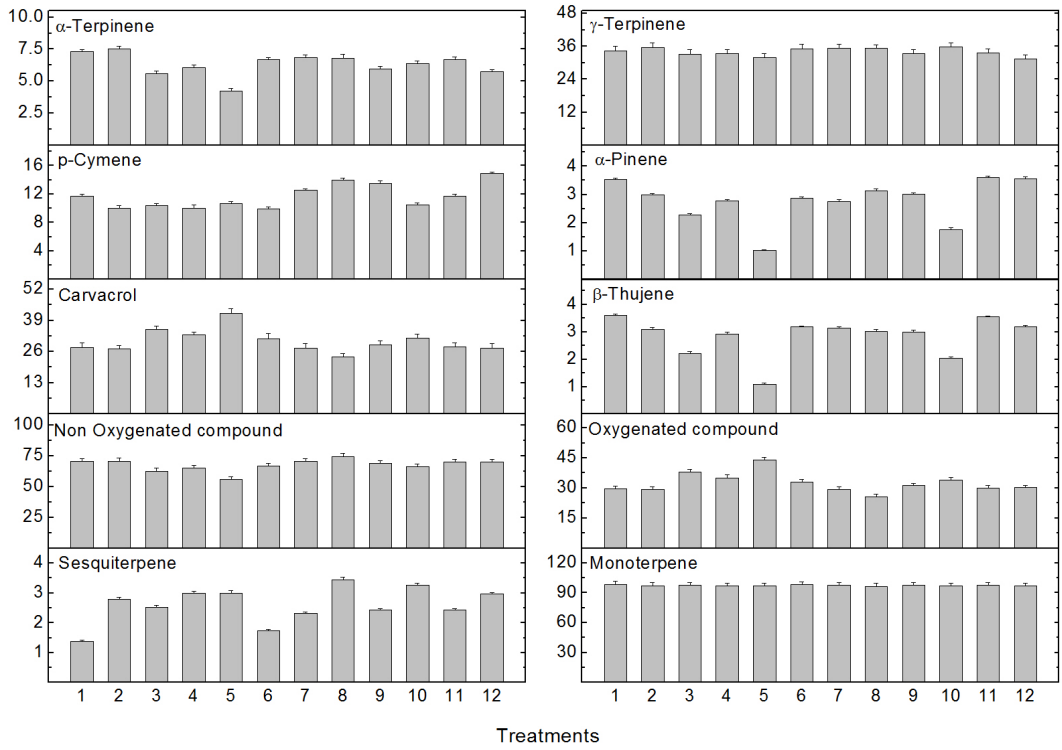

Figure 2. Essential oil composition (%) of summer savory as affected by foliar application of A3 Sandfloura and AT-amino at different concentrations, under second sowing date (20 February). 1 - (0^{a}:0^{b}), 2 - (0:1.5), 3 - (0:3), 4 - (5:0), 5 - (5:1.5), 6- (5:3), 7 - (7.5:0), 8 - (7.5:1.5), 9 - (10:3), 10 - (10:0), 11 - (10:1.5), 12 - (10:3). Values are means ± SE, n=3; t: compounds less than 0.05. [a]Sandfloura [g/plant], [b]Amino AT [ml/L].

to 56.18% and from 1.37 to 3.44% for the 1st and 2nd sowing dates, respectively. The increment of monoterpenes as a result of the 2nd sowing date was accompanied with a decrease in sesquiterpines content. The major components of the essential oil were found to be γ-terpinene (ranging from 33.11 to 46.08 % and from 31.50 to 35.81% for the 1st and 2nd sowing dates, respectively), carvacrol (ranging from 32.07 to 51.22 % and from 23.71 to 41.98% for the 1st and 2nd sowing dates, respectively) and P-cymene (ranging from 2.81 to 7.17 % and from 9.95 to 14.92% for the 1st and 2nd sowing dates, respectively), which were similar to those reported earlier (Zawislak, 2008; Hasan et al., 2011). It can be noticed that the highest percentage of carvacrol (51.22%) was obtained as a result of the application of A3 Sandfloura at 7.5 g l^{-1} followed by A3 Sandfloura applied at 10 g l^{-1} + AT-amino at 3 ml l^{-1} which produced 50.90 % under the 1st sowing date (Figs. 1 and 2). Also, the combination between A3 Sandfloura at 10 g l^{-1} and AT-amino at 1.5 ml l^{-1} under the 1st sowing date produced the maximum relative percentage of γ–terpinene (46.08%). On the other hand, the highest relative percentage of p-cymene (14.92%) was produced as a result of the combined treatment with A3 Sandfloura at 10 g l^{-1} and AT-amino at 3 ml l^{-1} under the 2nd sowing date. From the results presented in Figs. 1 and 2, it can be concluded that

there was a negative correlation between carvacrol and both γ-terpinene (-0.035) and p-cymene (-0.767). Since the biosynthesis of carvacrol is strongly dependent on its precursors (ã-terpinene, *p*-cymene) (Nhu-Trang et al., 2006), the amount of these compounds changed in parallel with each other.

The other identified essential oil components were detected in values less than 1% from the total amount: camphene, β-pinene, sabinene, 3-carene, α-phellandrene, β-myrcene, dl-limonene, β-phellandrene, 1,8-cineol, 2-hexenal, cis-β-ocimene, 1-octen-3-ol, isomenthone, cis-β-terpineol, l-menthone, trans-2-caren-4-Ol, cis-sabinene hydrate, trans-caryophyllene, carvacrol methyl ether, terpinene-4-ol, α-caryophyllene, α-terpineol, β-bisabolene, bicyclogermacrene, trans-α-bisabolene, anethole, carvacrol acetate.

CONCLUSION

In conclusion, the results of the current experiment showed that A3 Sandfloura applied at a concentration of 10 g L^{-1} and AT-amino applied at a concentration of 3 ml l^{-1} in combination with the 2nd sowing date (20th February) resulted in the highest mean values of vegetative growth parameters, growth yield, essential oil content (%) and essential oil yield of *Satureja hortensis* compared with the other interaction treatments.

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