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EFFECT OF NITROGEN FERTILIZATION AND ASCORBIC ACID ON GROWTH, ESSENTIAL OIL AND CHEMICAL COMPOSITION OF ROSEMARY PLANT

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ABSTRACT: A pot experiment was carried out during 2014 and 2015 seasons to study the effect of nitrogen fertilization (0, 1, 2 and 3 g N pot^{-1}) in the form of calcium nitrate Ca $(NO_3)_2$ (15.5% N) and four spray levels with ascorbic acid (0, 50 and 100 ppm) on growth, essential oil content and chemical composition of rosemary plant. Plastic pots of 25 cm diameter were used. Experimental treatments were laid out in a split plot design. Tow cuts were taken each season. Application of nitrogen up to 3 g N pot^{-1} enhanced all studied growth parameters in terms of plant height, number of branches, fresh and dry weight of leaves as well as total chlorophyll, total carbohydrates, N, P, K contents. Essential oil content was enhanced by adding of nitrogen up to 2 g N pot^{-1}, thereafter more increase in nitrogen, significantly decreased essential oil content. Spraying rosemary plants with ascorbic acid up to 100 ppm, significantly enhanced plant height, branch number, fresh and dry weight of leaves and essential oil content as well as total chlorophyll, total carbohydrates, N, P and K contents. Rosemary plants fertilized with 3 g N pot^{-1} and sprayed with 100 ppm ascorbic acid gave the highest value of each of plant height, number of branches, fresh and dry weight of leaves, total chlorophyll and total carbohydrates, while the highest essential oil percentage was obtained from plants fertilized with 2 g N pot^{-1} and sprayed with 100 ppm of ascorbic acid. Alpha-pinene, Eucalyptol, Linalool, Camphor, Endo-borneol and Cis-pinane were the major constituents of the essential oil profile, over all combinations of nitrogen and ascorbic acid levels in both cuts.

Key words: Rosmary, *Rosmarinus officinalis* L., nitrogen fertilization, ascorbic acid, growth, volatile oil.

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) of the family Lamiaceae is one of the most valuable spices and medicinal plants that widely used around the world. Rosemary is native to the Mediterranean regions. Therefore, it well grows under Egyptian conditions and available all throughout the year. Implementation of naturally occurring antioxidants rather than synthetic antioxidants is a promising technology to increase the shelf life of foods. Rosemary organic residues after the distillation of leaves after manufacturing of essential oils was used to prevent lipid oxidation and color changes in meat products (**Nieto *et al*., 2010 ; Banon *et al*., 2012**). Rosemary extract, phenolics and essential oil, stimulates blood circulation and used as an antibacterial, antifungal, antiviral and anticancer agents (**Oluwatuyi *et al*., 2004; Moreno *et al*., 2006; Sharabani *et al*., 2006; Cheung and Tai, 2007; Genena *et al*., 2008; Martínez *et al*., 2009; Yi and Wetzstein, 2010; Jiang *et al*., 2011; Moghtader *et al*., 2011; Tsai *et al*., 2011**).

Herbage yield and essential oils production of aromatic and medicinal plants are influenced by many agricultural practices, including, fertilization and harvesting time (**Min *et al.*, 2005; Stutte, 2006; Badawy *et al.*, 2009; Said-Al Ahl *et al*., 2009**). Plant nutrition is one of the most important factors that increase plant production. Nitrogen is most recognized in plants for its presence in the structure of the

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protein molecule. In addition, nitrogen is found in molecules of purines and pyrimidines which represent the basic construction units for the nucleic acids. Nitrogen fertilization has been reported to increase herbage yield and essential oil production in *Thymus vulgaris* (**Baranauskienne *et al.,* 2003**). Increasing N rates up to 1.2 g/pot (30cm diameter) significantly increased herb fresh yield and essential oil content of *Origanum vulgare* (**Said-Al Ahl *et al*., 2009). Puttanna *et al*. (2010)** evaluated the yield of herb and essential oil content of rosemary under 5 cuttings and 3 nitrogen rates (0, 150 and 300 kg N/ha). The yield of herb reached the highest value in the second cutting; thereafter it decreased in the next three cuttings. Herb yield and essential oil content were increased significantly by the application of 150 kg N/ha. Application of N increased the concentration of N, P and K in the herb. **Miguel *et al*. (2007)** determined essential oil and its compositions in the aerial parts of rosemary collected at different harvesting periods. Essential oil content varied significantly among different harvesting periods (0.5 – 1.4%). Analysis of essential oil at each harvesting time revealed nineteenth compounds with myrcene as the main component (23.2 -30.0%) followed by α-pinene (11.8 – 16.5%) and 1,8-cineole (8.6 – 12.2%). Application of nitrogen to rosemary plants enhanced leaf area, fresh and dry yield of herbs (**Singh and Wasnik, 2013**).

Vitamins could be considered as bio-regulators compounds in which a little concentrations have a great effect on plant growth. Ascorbic acid (Vitamin C) is considered as a regulator of growth and development of plants due to its effect on cell division and differentiation as well as it is involved in a wide range of important functions such as antioxidant defense, cofactor for several enzymes activity, regulation of photosynthesis processes and many physiological processes in plants **(Smirnoff and Wheeler, 2000; Blokhina *et al.,* 2003; Farooq *et al*., 2013; Zhang, 2013)**. Ascorbic acid is product of D-glucose metabolism which affects some nutritional cycles activity in higher plants and plays an important role in the electron transport system (**El-Kobisy *et al*., 2005**). Foliar application of ascorbic acid at different concentrations that ranges between 0.0 ppm and 100 ppm to many medicinal and aromatic plants showed considerable increases in lemongrass *Cymbopogon citratus* leaves contents of total carbohydrates and nitrogen (**Tarraf *et al.,* 1999);** sweet pepper leaves contents of N, P, and K (**Talaat, 2003);** vegetative growth traits, fresh and dry weights of *Hibiscus rosa sineses* plant organs and leaves contents of chlorophyll, carotenoids, soluble sugars, N, P and K (**El-Quesni *et al*., 2009).** Plant height, branch number, fresh and dry weights of plant, chlorophyll content and oil percentage of basil plant (**Khalil *et al*., 2010**). Plant height, number of branches and flower heads of fennel plant **(Hendawy and Ezz El-Din, 2010)**. Fresh and dry weights of roots, leaves and shoots as well as chlorophyll content of summer savory plants (**Nikee *et al*., 2014**).

This study aimed to evaluate the effect of nitrogen fertilization and foliar spray ascorbic acid on plant biomass and essential oil content and their main constituents of *Rosmarinus officinalis* L.

MATERIALS AND METHODS

Experimental Procedures and Design

During 2014 and 2015 seasons, a pot experiment was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza (30° 01' 39.36"N latitude and 31° 12' 36.50" E Longitude) Egypt, to study the effect of individual and combined effects of four nitrogen levels (0, 1, 2 and 3 g N pot^{-1}) and foliar spray with three levels of ascorbic acid (0, 50 and 100 ppm) on growth, essential oil content and chemical composition of rosemary (*Rosmarinus officinalis* L.) plant. Analysis of the experimental soil in which the pots was filled during the two seasons showed that the soil textural class is loamy clay with pH (7.85 and 7.12), EC (1.36 and 1.54 ds/m), organic matter (1.62 and 1.40%), available N (18.24 and 22.50 ppm), available P (13.32 and 15.45 ppm) and available K (135 and 150 ppm) in the first and second seasons, respectively. Rooted cuttings seedlings (10-12cm long) were obtained from a nursery of Al-Qanater Al-Kheireya, Kalyoubeya Governorate and transplanted into plastic pots of 25 cm diameter filled with a loamy clay soil on the first of March in the first and second seasons, respectively. Nitrogen was added in the form of calcium nitrate, Ca $(NO_3)_2$ (15.5% N) at different four splits (20%, 30%, 20% and 30%). The first dose was added to pots at seedlings transplanting, the second dose was added one month after transplanting, the third dose was added four months after transplanting (immediately after the first cut) and the fourth dose was added one month after the first cut. Nitrogen was applied with irrigation water (fertigation) by dissolving each dose in 0.75 L of water. Rosemary plants were sprayed with different concentrations of ascorbic acid (0, 50 and 100 ppm) four times; the first and the second were sprayed one month and two months after transplanting, respectively while the third and the fourth were sprayed one month and two months after the first cut, respectively. Spraying with tape water (0 ppm) represents control treatment. Water supply to plants was done as recommended. Experimental treatments were laid out in split plot arrangement in a randomized complete block design with three replicates. Nitrogen levels were allocated to the main plots, while ascorbic acid levels were assigned as sub plot. Each level of sub plot was represented by six pots for each replicate.

Experimental Measurements

Two cuts were taken each season, the first cut was taken on the 1st of July (4 months after transplanting), while the second cut was taken on 15th of November (4.5 months after the first one) by cutting the herb of the plants at 10 cm above the soil surface. Vegetative growth traits in terms of plant height (cm), number of branches per plant and fresh leaves weight (g) per plant were determined at harvest of each cut. The fresh leaves were dried at 70^{o}C for 48 hr., and the dry weight (g) of leaves per plant was recorded. Total chlorophylls content was measured one month before every cutting using chlorophyll meter, model SPAD-502, which SPAD unit = 10mg/100g fresh weight of leaves (**Netto *et al*., 2005**). Essential oil content was determined according to **British Pharmacopoeia (1963)** by hydro distillation of 100 g air dried leaves for three hours. The essential oil percentage was estimated as follows: Essential oil (%) = (reading measured pipette/sample weight) × 100. The essential oil samples obtained from different treatments were dried over anhydrous sodium sulfate and were subjected to GC/MS analysis according to **Adams (1995)** to determine their main constituents at the Central Laboratory of Faculty of Agriculture, Cairo University. Total carbohydrates content was determined in homogenized samples (0.2g) from dried leaves according to **Dubois *et al*. (1956).** Total nitrogen content was determined by the modified micro-Kjeldahl method as described by **AOAC (1990)**. Total phosphorus content was determined according to the method of **Jackson (1967)**. Potassium was determined using Model SP 1900 atomic absorption spectrophotometer with a boiling air-acetylene burner and recorded read out (**Issac and Kerber, 1971**). Essential oil constituents and chemical composition of rosemary plant were determined at the first and second cuts in samples collected across the two seasons.

Statistical Analysis

Data recorded on vegetative growth traits, essential oil content and total chlorophylls were analyzed using analysis of variance based on a split-plot arrangement in a randomized complete block design according to procedures outlined by **Gomez and Gomez (1984)** using MSTAT-C computer package **(Freed *et al*., 1989)**. Treatment mean comparisons were performed using least significant difference (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

Vegetative Growth Traits

Nitrogen fertilization exhibited significant effect on growth traits in terms of plant height, number of branches, fresh and dry weights of leaves in the first and second cuts and over cuts during the two seasons (Tables 1, 2, 3 and 4). Nitrogen fertilization enhanced all studied growth traits up to 3 g N p^{-1} at both cuts during the two seasons. The increasing in plant height is mainly due to the role of N in stimulating the

Table 1. Effect of nitrogen fertilization, ascorbic acid and their interaction on plant height of rosemary plant at the first and second cuts during 2014 and 2015 seasons

Nitrogen (g /pot) (N)	Ascorbic acid (ppm) (A)	2014 season			2015 season		
		1st cut	2nd cut	Mean	1st cut	2nd cut	Mean
	0	53.44	42.22	50.33	53.50	43.50	51.00
0	50	59.00	50.45	57.22	63.34	53.67	61.01
	100	70.66	58.33	67.00	71.33	61.17	68.75
Mean		61.03	50.33	58.18	62.72	52.78	60.25
	0	61.22	50.67	58.45	62.00	52.00	59.50
1	50	66.22	57.50	64.36	69.83	60.25	67.54
	100	73.00	60.67	69.33	74.00	64.00	71.50
Mean		66.81	56.28	64.05	68.61	58.75	66.18
	0	76.78	62.33	72.06	77.17	63.50	72.84
2	50	81.00	66.33	76.17	82.50	69.00	78.25
	100	86.22	70.11	80.67	87.34	71.33	81.84
Mean		81.33	66.26	76.30	82.34	67.94	77.64
	0	87.78	69.33	81.06	85.83	72.00	81.42
3	50	89.22	71.00	82.61	88.67	71.84	82.75
	100	87.89	73.33	83.11	87.50	74.00	83.25
Mean		88.30	71.22	82.26	87.33	72.61	82.47
Grand mean		74.37	61.02	70.20	75.25	63.02	71.64
	0	69.81	56.14	65.48	69.63	57.75	66.19
Mean of ascorbic	50	73.86	61.32	70.09	76.09	63.69	72.39
	100	79.44	65.61	75.03	80.04	67.63	76.34
LSD at 5%							
Nitrogen (N)		2.77	2.17	1.56	2.34	1.90	1.34
Ascorbic acid (A)		2.55	2.19	1.62	1.48	1.38	0.97
N × A		4.09	3.39	2.23	2.96	2.75	1.94

Table 2. Effect of nitrogen fertilization ascorbic acid and their interaction on branch number/ plant of rosemary plant at the first and second cuts during 2014 and 2015 seasons

Nitrogen (g /pot) (N)	Ascorbic acid (ppm) (A)	2014 season			2015 season		
		1st cut	2nd cut	Mean	1st cut	2nd cut	Mean
	0	5.89	9.33	7.61	6.84	10.17	8.51
0	50	7.89	11.00	9.44	9.17	11.84	10.50
	100	8.78	12.72	10.75	9.84	12.67	11.25
Mean		7.52	11.02	9.27	8.62	11.56	10.09
	0	6.11	10.11	8.11	7.17	11.67	9.42
1	50	8.44	11.83	10.14	9.67	12.00	10.84
	100	9.47	13.00	11.23	10.50	12.75	11.63
Mean		8.01	11.65	9.83	9.11	12.14	10.63
	0	8.07	11.67	9.87	9.10	12.00	10.55
2	50	9.33	12.00	10.67	10.00	13.00	11.50
	100	10.50	13.17	11.83	12.09	13.75	12.92
Mean		9.30	12.28	10.79	10.40	12.92	11.66
	0	8.53	11.89	10.21	9.80	12.83	11.32
3	50	10.00	13.11	11.56	10.50	13.50	12.00
	100	11.18	14.00	12.59	12.27	14.00	13.14
Mean		9.90	13.00	11.45	10.86	13.44	12.15
Grand mean		8.68	11.99	10.33	9.75	12.51	11.13
	0	7.15	10.75	8.95	8.23	11.67	9.95
Mean of ascorbic	50	8.92	11.99	10.45	9.84	12.58	11.21
	100	9.98	13.22	11.60	11.18	13.29	12.23
LSD at 5%							
Nitrogen (N)		0.30	0.85	0.40	0.15	0.63	0.29
Ascorbic acid (A)		0.79	0.53	0.46	0.62	0.39	0.52
N × A		NS	NS	NS	0.85	0.77	1.04

Table 3. Effect of nitrogen fertilization, ascorbic acid and their interaction on fresh leaves weight/plant of rosemary plant at the first and second cuts during 2014 and 2015 seasons

Nitrogen (g /pot) (N)	Ascorbic acid (ppm) (A)	2014 season			2015 season		
		1st cut	2nd cut	Mean	1st cut	2nd cut	Mean
	0	45.52	56.08	50.8	53.7	61.85	57.78
0	50	56.63	73.62	65.12	64.47	80.18	72.32
	100	75.68	80.82	78.25	76.19	83.91	80.05
Mean		59.28	70.17	64.73	64.79	75.31	70.05
	0	52.11	66.88	59.5	59.17	69.54	64.35
1	50	61.49	75.49	68.49	67.46	76.73	72.09
	100	72.71	83.27	77.99	75.06	88.02	81.54
Mean		62.10	75.21	68.66	67.23	78.10	72.66
	0	68.11	80.52	74.31	73.83	84.28	79.06
2	50	74.8	86.83	80.82	83.39	90.63	87.01
	100	88.69	108.56	98.63	95.25	110.7	102.97
Mean		77.20	91.97	84.59	84.16	95.20	89.68
	0	77.85	85.89	81.87	84.69	88.51	86.6
3	50	85.87	102.32	94.09	94.2	106.32	100.26
	100	92.94	114.9	103.92	98.45	118.53	108.49
Mean		85.55	101.04	93.3	92.44	104.45	98.45
Grand mean		71.03	84.60	77.82	77.15	88.27	82.71
	0	60.9	72.34	66.62	67.85	76.04	71.95
Mean of ascorbic	50	69.7	84.57	77.13	77.38	88.46	82.92
	100	82.51	96.89	89.7	86.24	100.29	93.26
LSD at 5%							
Nitrogen (N)		6.54	5.28	4.47	5.34	3.98	2.96
Ascorbic acid (A)		4.62	3.67	3.22	3.92	3.01	2.37
N × A		7.24	6.34	5.45	6.83	5.02	4.75

Table 4. Effect of nitrogen fertilization and ascorbic acid on dry leaves weight/plant of rosemary plant at two cuts during 2014 and 2015 seasons

Nitrogen (g /pot) (N)	Ascorbic acid (ppm) (A)	2014 season			2015 season		
		1st cut	2nd cut	Mean	1st cut	2nd cut	Mean
	0	12.59	15.78	14.19	15.12	17.33	16.23
0	50	17.19	21.62	19.41	19.58	23.55	21.57
	100	22.92	24.46	23.69	23.94	25.25	24.6
Mean		17.57	20.62	19.09	19.55	22.05	20.8
	0	15.52	19.84	17.68	17.59	20.56	19.08
1	50	18.78	22.81	20.8	20.86	23.25	22.06
	100	22.04	25.39	23.72	23.87	26.69	25.28
Mean		18.78	22.68	20.73	20.77	23.5	22.14
	0	21.97	25.45	23.71	23.71	26.69	25.2
2	50	23.18	26.62	24.9	25.87	27.35	26.61
	100	30.18	35.24	32.71	32.34	35.47	33.91
Mean		25.11	29.1	27.11	27.31	29.84	28.57
	0	26.22	26.33	26.27	28.48	27.25	27.86
3	50	27.89	31.36	29.63	30.61	32.53	31.57
	100	29.64	35.92	32.78	31.3	37.15	34.23
Mean		27.92	31.2	29.56	30.13	32.31	31.22
Grand mean		22.34[a]	25.90[a+]	24.12	24.44[a]	26.92[a]	25.68
	0	19.08	21.85	20.46	21.23	22.96	22.09
Mean of ascorbic	50	21.76	25.6	23.68	24.23	26.67	25.45
	100	26.2	30.25	28.23	27.86	31.14	29.5
LSD at 5%							
Nitrogen (N)		3.18	1.58	1.58	1.95	1.09	0.99
Ascorbic acid (A)		2.02	1.1	1.1	1.44	0.86	0.8
N × A		4.03	2.2	2.21	2.87	1.72	1.61

meristematic growth activity which contributes to the increase in number of cells in additions to cell enlargement. The increases in the number of branches/plant led to an increase in the plant biomass represented here in the yield of plant leaves. In the first season, raising N level from 0 to 1, 2 and 3 g N pot^{-1} increased fresh yield of leaves (Table 3) by about 4.76%, 30.23% and 44.32%, respectively in the first cut, corresponding to 7.18%, 31.07% and 43.99%, respectively in the second cut. In the second season, the increasing in fresh yield of leaves amounted to 3.77%, 29.90% and 42.68%, respectively in the first cut, corresponding to 3.70%, 26.41% and 38.69%, respectively in the second cut. Dry weight of leaves in relation to raising N level followed the same trend as fresh weight of leaves at each cut during the two seasons. Similar findings were reported by **Baranauskienne *et al.* (2003)** on thyme plant and **Singh and Wasnik (2013)** on rosemary plant.

Foliar application of ascorbic acid either at 50 ppm or 100 ppm significantly increased (P<0.05) all studied growth traits as compared to control treatment (spraying with tap water) at each cut and across cuts during the two seasons (Tables 1, 2, 3 and 4). Spraying rosemary plants with ascorbic acid significantly enhanced plant height, branch number, fresh and dry weight of leaves up to 100 ppm at both cuts during the two seasons. In the first season, the yield of fresh leaves plant^{-1} (Table 3) significantly increased by about 14.45% and 35.48% at the first cut when ascorbic acid concentrations increased from 0 to 50 and 100 ppm, respectively, corresponding to 16.91% and 33.94%, respectively at the second cut. In the second season, the increasing in fresh yield of leaves amounted to 14.05% and 27.10%, respectively at the first cut, corresponding to 16.33% and 31.89%, respectively at the second cut. The yield of dry leaves plant^{-1} in relation to ascorbic acid followed the same trend as fresh yield of leaves.

The increase in rosemary plant biomass due to spraying with ascorbic acid confirms the role of vitamin C in regulation of photosynthesis processes and growth activation (**Zhang, 2013; Farooq *et al.*, 2013).** The obtained results are in agreement with those reported by **El-Quesni *et al.* (2009); Hendawy and Ezz El-Din (2010); Khalil *et al.* (2010) and Nikee *et al.* (2014)**. The highest values of each plant height and branch number was measured at the first and second cuts, respectively in both seasons. Although, the yield of fresh leaves plant^{-1} (Table 3) obtained from the second cut was significantly higher than that obtained from the first one in both seasons, the difference between the two cuts in the dry leaves yield (Table 4) was insignificant due to the difference in moisture content of plants harvested at the two cuts. The interaction between nitrogen and ascorbic acid levels was significant for all studied growth traits at both cuts and across two cuts during the two seasons, except for branch number plant^{-1} that was affected with interaction only in the second season. Rosemary plants fertilized with 3 g N pot^{-1} and spray it with 100 ppm ascorbic acid gave the highest values of plant height, number of branches, fresh and dry weight of leaves (Tables 1, 2, 3 and 4) across the two cuts in both seasons.

Essential Oil (%) and its Compositions

Essential oil percentage

Essential oil percentage in the dried rosemary leaves (Table 5) was enhanced by adding of nitrogen up to 2 g N pot^{-1} in both cuts and over cuts in both seasons, thereafter more increase in nitrogen, significantly decreased (P<0.05) essential oil (%). The adverse effect of excessive nitrogen, fertilization on essential oil (%) was reported by **Puttanna *et al.* (2010).** The essential oil (%) was significantly increased by foliar application of ascorbic acid at the levels of 50 and 100 ppm compared with control treatment at the two cuts and over cuts in both seasons. The highest and significant essential oil percentage was obtained from leaves of plants sprayed with 100 ppm. Essential oil obtained from the second cut was significantly higher than that obtained from the first cut in both seasons. The positive effect of ascorbic acid on essential oil content was reported by **Khalil *et al.* (2010).** The interaction between nitrogen and

Table 5. Effect of nitrogen fertilization, ascorbic acid and their interaction on essential oil percentage of the dried rosemary leaves at the first and second cuts during 2014 and 2015 seasons

Nitrogen (g /pot) (N)	Ascorbic acid (ppm) (A)	2014 season			2015 season		
		1st cut	2nd cut	Mean	1st cut	2nd cut	Mean
	0	0.25	0.31	0.28	0.27	0.33	0.30
0	50	0.31	0.42	0.36	0.32	0.45	0.39
	100	0.38	0.58	0.48	0.40	0.62	0.51
Mean		0.31	0.44	0.37	0.33	0.47	0.40
	0	0.29	0.36	0.33	0.30	0.39	0.35
1	50	0.33	0.47	0.40	0.37	0.47	0.42
	100	0.36	0.59	0.47	0.39	0.63	0.51
Mean		0.33	0.47	0.40	0.35	0.50	0.43
	0	0.35	0.43	0.39	0.38	0.44	0.41
2	50	0.37	0.48	0.43	0.40	0.50	0.45
	100	0.42	0.68	0.55	0.42	0.72	0.57
Mean		0.38	0.53	0.46	0.40	0.55	0.48
	0	0.29	0.35	0.32	0.32	0.36	0.34
3	50	0.31	0.38	0.35	0.33	0.41	0.37
	100	0.34	0.42	0.38	0.36	0.42	0.39
Mean		0.32	0.38	0.35	0.34	0.40	0.37
Grand mean		0.33	0.46	0.40	0.35	0.48	0.42
	0	0.30	0.36	0.33	0.32	0.38	0.35
Mean of ascorbic	50	0.33	0.44	0.38	0.36	0.46	0.41
	100	0.38	0.57	0.47	0.39	0.60	0.49
LSD at 5%							
Nitrogen (N)		0.04	0.05	0.02	0.02	0.05	0.03
Ascorbic acid (A)		0.03	0.03	0.02	0.01	0.03	0.02
N × A		0.05	0.05	0.04	0.02	0.05	0.04

ascorbic acid levels was significant for leaves essential oil (%) in both cuts and across two cuts during the two seasons. The highest essential oil percentage was obtained from leaves of plants fertilized with 2 g N pot^{-1} and sprayed with 100 ppm of ascorbic acid.

Essential oil composition

The analysis of rosemary essential oil samples of different treatments of this experiment showed variability in both qualitative and quantitative of essential oil composition. Essential oil contained more than fifty components and the majority of these components found in the form of traces (< 0.5%). Twenty compounds, which account for more than 80% of essential oil constituents, are listed in Table 6. Nitrogen fertilization and ascorbic acid affected the chemical profile of essential oil samples from the two cuts. Six compounds (Alpha-pinene, Eucalyptol, Linalool, Camphor, Endo-borneol and Cis-pinane) accounted 59.72%, 59.95%, 55.77% and 59.41% under nitrogen levels of 0, 1, 2 and 3g pot^{-1}, respectively at the first cut, corresponding to 55.30%, 55.02%, 53.75 and 58.51%, respectively at the second one. The previous six compounds accounted 57.42%, 59.34% and 59.38% of the essential oil components under ascorbic acid levels of 0, 50 and 100 ppm, respectively in the first cut, corresponding to 54.54%, 56.36% and 56.04%, respectively in the second one. The aforementioned compounds were the major constituents of the essential oil profile, over all combinations of nitrogen and ascorbic acid levels at both cuts.

Total chlorophyll content

Results presented in Table 7 show that application of nitrogen to rosemary plants significantly increased total chlorophyll readings taken at the two cuts in both seasons. Total chlorophyll increased gradually with increasing nitrogen level up to 3 g N pot^{-1}. Spraying plants with ascorbic acid either at 50 ppm or 100 ppm significantly increased total chlorophyll content compared to control treatment in both cuts during the two seasons. The highest chlorophyll readings resulted from spraying plants with 100 ppm of ascorbic acid in both seasons. The role of ascorbic acid in enhancing chlorophyll content in leaves of different medicinal and aromatic plants was reported by **El-Quesni *et al.* (2009), Khalil *et al.* (2010)** and **Nikee *et al.* (2014).** Total chlorophyll content was significantly affected by the interaction between nitrogen and ascorbic acid levels at the two cuts in both seasons. The highest total chlorophyll was recorded when plants sprayed with 100 ppm of ascorbic acid and fertilized with 2 and /or 3 g N pot^{-1} at both seasons.

Chemical constituents

Results in Table 8 reveal that application of nitrogen fertilization up to 3 g N pot^{-1} increased total carbohydrates, N, P and K contents in herb of rosemary plants at the two cuts. Similar result was reported by **Puttanna *et al.* (2010).** Spraying plants with ascorbic acid either at 50 ppm or 100 ppm increased total carbohydrates, N, P and K contents compared to control treatment at both cuts. The highest values of total carbohydrates (26.50 and 23.40%), N (2.00 and 2.09%), P (0.26 and 0.31%) and K (1.72 and 2.10%) contents resulted from fertilized plants with 3 g N pot^{-1} and sprayed them with100 ppm of ascorbic acid at the first and second cuts, respectively. These results agreed with findings obtained by **Tarraf *et al.* (1999)** on lemongrass and **Talaat (2003)** on sweet pepper plants who found that total carbohydrates, N, P, and K in herb of lemongrass and sweet pepper were increased by application of ascorbic acid.

Conclusion

According to the results of this study on rosemary plant; it may be recommended that to obtain high herbage yield, the plants should be fertilized with 3 g N/pot and sprayed with 100 ppm of ascorbic acid. For obtaining high essential oil content, application of 2 g N/pot with 100 ppm of ascorbic acid is recommended.

Table 6. Chemical composition of rosemary essential oil as affected by nitrogen fertilization, ascorbic acid and their interaction

Compound	N (g/pot)	Relative concentration of compound (%)					
		Ascorbic acid (ppm)					
		1st cut			2nd cut		
		0	50	100	0	50	100
Alpha- pinene	0	8.44	6.37	9.61	5.99	9.10	8.80
Eucalyptol		10.20	10.95	9.84	9.01	9.51	9.75
Linalool		6.00	7.31	5.86	6.88	5.68	5.77
Camphor		11.99	15.67	13.42	12.55	11.86	12.44
Endo-borneol		10.99	11.69	10.15	11.42	10.54	10.90
Alpha- terpineol		3.03	3.46	2.56	2.53	2.30	3.04
Cis-pinane		9.49	11.18	9.99	7.56	9.21	8.93
Trans-caryophyllene		3.20	2.27	2.18	1.98	1.71	1.49
Camphene		2.45	1.64	3.14	1.47	3.59	3.39
P-Cymene		1.75	1.32	4.83	2.31	2.78	2.56
beta-Pinene		0.76	1.60	1.46	0.67	1.47	1.39
beta-myrcene		0.85	0.63	1.47	1.14	1.43	1.23
Alpha-terpinene		0.26	0.28	0.21	0.16	0.43	0.40
3-Carene		0.93	0.74	1.71	0.75	1.13	1.00
Palatinol IC		4.54	5.72	2.05	3.65	2.86	3.04
Alpha-terpineolene		1.08	1.00	0.56	1.34	1.67	1.46
Terpinene-4-ol		1.19	1.78	1.45	1.29	1.27	1.41
Alpha-campholenol		2.28	2.60	1.65	3.32	1.68	2.69
Grandlure II		3.89	3.03	2.75	4.52	2.96	2.07
Bornyl acetate		1.91	2.85	3.02	3.74	3.45	3.38
Alpha- pinene	1	8.16	7.91	6.25	4.85	10.22	8.96
Eucalyptol		9.02	11.09	6.23	9.01	10.55	9.51
Linalool		6.37	6.47	7.76	6.88	5.73	6.31
Camphor		12.78	13.56	16.00	12.55	12.79	12.23
Endo-borneol		10.37	10.45	14.39	11.42	10.27	10.31
Alpha- terpineol		2.99	2.82	3.88	2.53	2.61	2.46
Cis-pinane		10.92	9.32	12.79	7.56	7.71	8.20
Trans-caryophyllene		2.56	2.77	3.73	1.98	1.90	3.06
Camphene		2.46	2.11	1.35	1.47	3.47	3.14
P-Cymene		2.50	1.84	0.87	2.31	2.06	1.78
beta-Pinene		0.94	0.78	0.85	0.67	1.51	1.40
beta-myrcene		1.17	1.07	0.22	1.14	1.38	1.27
Alpha-terpinene		0.36	0.35	0.24	0.26	0.52	0.49
3-Carene		1.30	1.11	0.21	0.75	1.20	1.14
Palatinol IC		3.74	5.45	5.82	3.65	4.38	3.58
Alpha-terpineolene		1.37	1.26	0.83	1.34	1.73	1.85
Terpinene-4-ol		1.41	1.33	1.73	1.29	1.32	1.22
Alpha-campholenol		1.76	2.19	2.88	3.32	2.22	2.98
Grandlure II		3.36	3.08	4.28	4.52	3.27	3.44
Bornyl acetate		2.59	2.34	2.20	3.74	3.68	4.02

Table 6. Cont.

Compound	N (g/pot)	Relative concentration of compound (%)					
		Ascorbic acid (ppm)					
		1st cut			2nd cut		
		0	50	100	0	50	100
Alpha- pinene	2	8.52	11.03	5.61	9.10	10.43	8.90
Eucalyptol		10.31	9.71	9.38	9.51	3.26	8.32
Linalool		5.34	6.84	6.69	5.68	4.07	5.91
Camphor		12.35	4.97	13.31	11.86	11.29	12.23
Endo-borneol		10.50	9.60	10.33	10.54	10.76	10.80
Alpha- terpineol		2.57	2.47	3.05	2.30	2.98	2.65
Cis-pinane		7.88	13.06	11.89	9.21	8.91	10.47
Trans-caryophyllene		3.35	2.07	2.97	1.71	2.74	2.41
Camphene		2.75	2.49	1.78	3.59	3.38	2.96
P-Cymene		1.78	1.76	2.63	2.78	2.03	2.14
beta-Pinene		0.75	0.75	0.86	1.47	1.31	1.42
beta-myrcene		0.89	0.97	1.17	1.43	1.23	1.31
Alpha-terpinene		0.34	0.29	0.40	0.43	0.43	0.57
3-Carene		0.96	1.00	1.10	1.13	1.10	1.19
Palatinol IC		4.14	6.34	2.61	2.86	6.06	2.57
Alpha-terpineolene		1.24	1.11	1.70	1.67	1.54	1.85
Terpinene-4-ol		1.22	1.14	1.45	1.27	1.18	1.47
Alpha-campholenol		1.67	1.39	1.88	1.68	2.36	1.86
Grandlure II		2.43	2.54	3.28	2.96	3.95	1.83
Bornyl acetate		2.07	1.91	2.58	3.45	4.00	4.00
Alpha- pinene	3	8.47	4.37	6.30	8.80	10.95	9.15
Eucalyptol		10.43	7.19	2.44	9.75	12.70	8.61
Linalool		5.56	6.65	8.93	5.77	5.81	6.17
Camphor		13.67	15.88	14.77	12.44	14.73	11.33
Endo-borneol		10.46	13.03	14.80	10.90	10.90	10.17
Alpha- terpineol		2.87	3.84	4.09	3.04	2.25	2.49
Cis-pinane		11.44	13.05	10.79	8.93	8.45	9.98
Trans-caryophyllene		2.03	3.32	3.40	1.49	1.40	2.36
Camphene		2.60	2.25	2.14	3.39	3.31	3.34
P-Cymene		3.27	0.69	0.38	2.56	1.80	2.48
beta-Pinene		1.01	0.98	0.85	1.39	1.23	1.70
beta-myrcene		1.33	1.21	1.16	1.23	1.05	1.45
Alpha-terpinene		0.38	0.36	0.28	0.40	0.37	0.60
3-Carene		1.49	1.21	0.95	1.00	0.90	1.24
Palatinol IC		2.96	5.57	3.61	3.04	6.36	2.30
Alpha-terpineolene		1.46	0.76	0.60	1.46	1.32	1.88
Terpinene-4-ol		1.45	1.64	2.07	1.41	1.17	1.36
Alpha-campholenol		1.76	2.85	1.34	2.69	1.43	1.89
Grandlure II		2.92	3.95	4.89	2.07	2.74	3.82
Bornyl acetate		2.29	3.56	2.69	3.38	3.64	3.88

Table 7. Effect of nitrogen fertilization and ascorbic acid and their interaction on total chlorophyll (SPAD-502) of rosemary plant at two cuts during 2014 and 2015 seasons

Nitrogen (g/pot) (N)	Ascorbic acid (ppm) (A)	2014 season			2015 season		
		1st cut	2nd cut	Mean	1st cut	2nd cut	Mean
	0	29.23	28.47	28.85	27.60	29.25	28.43
0	50	34.03	36.13	35.08	33.35	39.00	36.18
	100	37.80	37.23	37.52	38.00	40.75	39.38
Mean		33.69	33.94	33.82	32.98	36.33	34.66
	0	35.12	33.83	34.48	32.11	33.05	32.58
1	50	36.72	36.10	36.41	35.00	40.45	37.73
	100	39.13	37.63	38.38	38.50	41.50	40.00
Mean		36.99	35.86	36.42	35.20	38.33	36.77
	0	38.73	36.17	37.45	40.40	35.85	38.13
2	50	39.10	37.83	38.47	39.45	42.40	40.93
	100	41.40	39.50	40.45	42.80	42.55	42.68
Mean		39.74	37.83	38.79	40.88	40.27	40.58
	0	40.23	38.93	39.58	40.30	40.50	40.40
3	50	42.33	42.13	42.23	42.30	43.00	42.65
	100	42.42	43.00	42.71	42.14	43.20	42.67
Mean		41.66	41.36	41.51	41.58	42.23	41.91
Grand mean		38.02	37.25	37.63	37.66	39.29	38.48
	0	35.83	34.35	35.09	35.10	34.66	34.88
Mean of ascorbic	50	38.05	38.05	38.05	37.53	41.21	39.37
	100	40.19	39.34	39.77	40.36	42.00	41.18
LSD at 5%							
Nitrogen (N)		1.90	2.99	2.80	0.91	2.37	2.42
Ascorbic acid (A)		1.43	2.74	1.49	0.80	1.89	0.99
N × A		2.85	3.49	2.23	1.60	2.78	1.97

Table 8. Chemical composition of rosemary plant as affected by nitrogen fertilization and ascorbic acid treatments

Chemical constituent (%)	N (g/pot)	Ascorbic acid (ppm)							
		1st cut			Mean	2nd cut			Mean
		0	50	100		0	50	100	
Total carbohydrates	0	14.20	16.40	18.10	16.23	11.60	13.30	14.40	13.10
	1	15.00	17.60	19.30	17.30	14.40	17.70	19.60	17.23
	2	16.60	18.70	22.50	19.27	17.20	19.10	21.40	19.23
	3	17.70	20.10	26.50	21.43	18.50	21.50	23.40	21.13
Mean		15.88	18.20	21.60	18.56	15.43	17.90	19.70	17.68
Total N	0	1.07	1.16	1.45	1.23	1.27	1.41	1.67	1.45
	1	1.16	1.42	1.58	1.39	1.44	1.67	1.93	1.68
	2	1.35	1.61	1.71	1.56	1.74	1.87	2.03	1.88
	3	1.61	1.65	2.00	1.75	1.87	1.96	2.09	1.97
Mean		1.30	1.46	1.69	1.48	1.58	1.73	1.93	1.75
Total P	0	0.12	0.15	0.22	0.16	0.14	0.17	0.24	0.18
	1	0.14	0.21	0.23	0.19	0.15	0.18	0.25	0.19
	2	0.17	0.21	0.24	0.21	0.15	0.19	0.25	0.20
	3	0.23	0.24	0.26	0.24	0.21	0.23	0.31	0.25
Mean		0.17	0.20	0.24	0.20	0.16	0.19	0.26	0.21
K	0	0.94	1.05	1.34	1.11	1.02	1.10	1.15	1.09
	1	1.16	1.26	1.42	1.28	1.18	1.26	1.26	1.23
	2	1.18	1.49	1.57	1.41	1.26	1.34	1.42	1.34
	3	1.34	1.49	1.72	1.52	1.34	1.42	2.10	1.62
Mean		1.16	1.32	1.51	1.33	1.20	1.28	1.48	1.32

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تأثير التسميد النيتروجينى وحامض الأسكوربيك على النمو ومحتوى الزيت الطيار والمحتوى الكيميائي لنبات الحصالبان

آمال عبد الخالق محمد هيكل – سامية سامي حلمي

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يعتبر الحصالبان أحد أهم النباتات الطبية ونباتات التوابل التابعة للعائلة الشفوية حول العالم، تم تنفيذ تجربة أصص خلال موسمي ٢٠١٤، ٢٠١٥ لدراسة تأثير التسميد النيتروجينى فى صورة نترات الكالسيوم (صفر، ١ ، ٢ ، ٣ جم وحدة أزوت/أصيص) والرش بحامض الأسكوربيك (صفر، ٥٠ ، ١٠٠ جزء فى المليون) على النمو ومحتوى الزيت الطيار والتركيب الكيميائي لنبات الحصالبان، تم استخدام أصص بلاستيك بقطر ٢٥ سم، تم وضع المعاملات التجريبية فى تصميم القطع المنشقة مرة واحدة، تم أخذ حشتين لكل موسم، أظهرت نتائج الدراسة أن إضافة السماد النيتروجينى بمعدل ٣ جم للأصيص أدى إلى تحسين جميع صفات النمو تحت الدراسة والمتمثلة فى ارتفاع النبات وعدد الأفرع والوزن الطازج والجاف للأوراق بالإضافة إلى صفات الكلوروفيل الكلى والكربوهيدرات الكلية ومحتوى النبات من عناصر النيتروجين والفوسفور والبوتاسيوم، أدى إضافة السماد النيتروجينى بمعدل ٢ جم للأصيص إلى زيادة نسبة الزيت الطيار، بينما أدت زيادة التسميد النيتروجينى بمعدل ٣ جم للأصيص إلى انخفاض معنوي فى نسبة الزيت الطيار. أدى رش النباتات بحامض الأسكوربيك بمعدل ١٠٠ جزء فى المليون إلى زيادة معنوية فى صفات ارتفاع النبات وعدد الأفرع والوزن الطازج والجاف للأوراق ونسبة الزيت بالإضافة إلى صفات الكلوروفيل الكلى والكربوهيدرات الكلية ومحتوى النبات من عناصر النيتروجين والفوسفور والبوتاسيوم، أعطى تسميد النباتات بمعدل ٣ جم وحدة أزوت للأصيص مع رشها بمعدل ١٠٠ جزء في المليون من حامض الأسكوربيك أعلى القيم لصفات ارتفاع النبات وعدد الأفرع والوزن الطازج والجاف للأوراق والكلوروفيل الكلى والكربوهيدرات الكلية، بينما أدى التسميد النيتروجينى بمعدل ٢ جم للأصيص مع الرش بمعدل ١٠٠ جزء في المليون إلى الحصول على أعلى نسبة من الزيت الطيار، أظهر تحليل الزيت أن Alpha-pinene، Eucalyptol، Linalool ، Camphor ، Endo-borneol ، Cis-pinane هي المكونات الأساسية للزيت تحت جميع معاملات هذه الدراسة للحشتين الأولى والثانية.

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