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EFFECT OF GUANINE, ALANINE AND THYMINE ON THE GROWTH, CHEMICAL COMPOSITION AND OIL QUALITY OF *Rosmarinus officinalis,* L. PLANT

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ABSTRACT

Two field experiments were carried out to study the effect of the nitrogenous compounds (guanine, alanine and thymine) as foliar application on growth, chemical composition and oil quality of *Rosmaninus officinalis*, L. plant. All these compounds increased plant height, number of branches/plant, fresh and dry weights of leaves and stems. This promotive effect reached maximum in response to alanine combined with guanine each at 200 ppm. Similar responses for oil percentage, carbohydrates, nitrogen, phosphorus and potassium contents were attained due to foliar application of nitrogenous substances.

Oil analysis showed promotive effects for major component of essential oil i.e. 1,8 cineole, camphor, borneol and bornyl acetate.

The highest 1, 8 cineole percentage was obtained by 200 ppm guanine combined with alanine at 100 ppm, foliar application of thymine at 150 ppm alone or combined with guanine at 100 ppm resulted in increasing camphor percentage, while alanine at 100 ppm was effective an increasing bornyl acetate percentage. Borneol percentage was decreased due to all nitrogenous substances used.

Keywords: Rosemary (*Rosmarinus officinalis*, L.), Guanine, Alanine, Thymine, Growth, Chemical composition, Essential oil, GLC.

INTRODUCTION

Rosmary (*Rosmarinus officinalis*, L.) is an aromatic and medicinal plant of Fam. Lamiaceae, native to the Mediterranean region. It is an evergreen woody shrub, well known for its highly aromatic leaves used medicinally either fresh or dried. Rosemary leaves contain phenolic acids, phenolic diterpenoid, triterpenoid acids, flavonoids plus tannins and at least 1.2% (v/w) essential oil in the dried leaves, of which 1,8 cineole, camphor and borneol are the main compounds (Budavari, 1996 and ESCOP, 1997). These substances give rosemary pronounced rubefacient action. Rosemary has been used internally for improvement of hepatic, biliary of function and dyspeptic complaints (ESCOP, 1997), while the aqueous infusion and essential oil are used externally as adjuvant therapy in rheumatic condition, promotion of wound healing, and as a mild antiseptic (Leung and Foster, 1996 and ESCOP, 1997) it has been used as antibacterial and spasmolytic action (Newall *et al.*, 1996). According to Mabey *et al.* (1988) rosemary oil has antibacterial and antifungal properties, also it improves the circulation and strengthens fragile blood vesels. However, rosemary leaves are components of dietary supplement products, pharmaceutical and cosmetic industries.

Little information are available about the role of growth substances in regulating the biosynthesis of essential oil in plant. The beneficial effect of amino acids on new cells production through restoring the specific enzymes for protein synthesis has been stated by Levitt (1980). Amino acids as organic nitrogenous compounds are the building blocks in thynthesis of proteins which probably occurs by a process in which ribosomes catalyze the polymerization of amino acids (Davies, 1982). Moursy *et al.* (1988) on *Datura stramonium* L. stated that phenylalanine or ornithine at 500 ppm showed the highest values of fresh and dry weights of callus explant. Gamal El-Din (1997) on *Cymbopogon citratus* found that foliar application of ornithine and phenylalanine at 50 and 100 mg/L led to significant increase in vegetative growth, number of leaves and tillers as well as fresh and dry weights of the herb. Refaat and Naguib (1998) found a favorable effects of alanine, guanine, cytosine, L-tyrosine and thymine on the yield and oil quality of peppermint. Several other authors indicated the promotive effect of amino acids on some ornamental and medicinal plants including; Mohamed and Khalil (1992) on *Antirrhinum majus*, *Mathiola incana* and *Callistephus chinensis*, Hussein *et al.* (1992) on *Datura metel*, Mohamed and Whaba (1993) on *Rosmarinus officinalis* and Hendawy (2000) on *Echinacea purporea*, L. who reported that phenylalanine was the most effective amino acid on improving plant growth.

The regulation of plant growth and biosynsethesis of important economic chemical constituents could be achieved through many precursor substances. However, recent trends prefer the use of naturally occurring compounds as amino acids.

This work aims to study the effect of amino compounds on rosemary growth and its chemical contents of total carbohydrates, nitrogen, phosphorus, potassium, percentage and quality of oil.

MATERIALS AND METHODS

This study was carried out in two successive seasons of 2003/2004 and 2004/2005 at the Experimental Farm of Faculty of Agriculture, Moshtohor, Benha University, to study the response of rosemary plants (*Rosmarinus officinalis,* L.) to foliar application of some nitrogenous compounds (e.g. guanine, alanine and thymine).

Vegetative uniform cuttings (15-20 cm length) were taken from rosemary plants grown at the same experimental Farm at November 15th and planted in pots till rooted.

The experimental area was arranged in a complete randomized block design with three replicates. Plot area was one m^2 containing 3 rows. During soil preparation 30 m^3/fed compost were applied. Seedlings were planted on rows at 30x25 cm (12 plant/plot), planting date was 15th of March for both seasons.

Mineral fertilizers as 100 kg ammonium sulphate (20.5% N), 75 kg calcium super phosphate (15.5% P_2O_5) and 50 kg potassium sulphate (48.5% K_2O) were added at two portions, the first one was a month after planting, the second one was 15 days after the first cut.

The experimental plots included 21 treatments, which were two concentrations of guanine (100 and 200 ppm) as main plot, in addition to the control (distilled water), also three concentrations of either alanine or thymine each at 100, 150 and 200 ppm as the sub plot in addition to 12 treatments of guanine combined with alanine or thymine. The plants were sprayed with guanine, one month from planting two times at one month interval (at first week of each April and May in the first cut and during first week of August and September for the second cut). Alanine and thymine were also sprayed one week later for both cuttings.

Plants were cut two dates, in the first one on July 15th and the second one on November 15th in which they were cut at 15 cm over the ground.
Data recorded were: Plant height (cm), number of branches/plant, fresh and dry weight (g) of leaves and stems/plant. The determination of the chemical compounds were performed as follows: essential oil percentage in fresh leaves. The essential oil of each treatment was extracted by hydrodistillation. The dehydrated oil of all treatments were subjected to GLC analysis using apparatus Shimadzu GC-17A, column carbwax. 50 m length, 0.25 mm diameter. Detector flame ionization. Column flow 2.3 ml/min at 280°C. Injection temperature 250°C, temperature program: initial temperature 50°C, time 3 min. Ramp rate 10°C/min. Final temperature 220°C, final time 10 min.

Total carbohydrates content was determined according to Herbert *et al.* (1971) in dry leaves. N, P and K percentages in the dried leaves were determined according to Wilde *et al.* (1985).

Results were statistically analyzed according to Snedecor and Cochran (1989). The L.S.D. test was used to compare the mean values. The physical properties and chemical analysis of soil are shown in Tables (a and b), physical analysis was estimated according to Jackson (1973), whereas, chemical analysis was estimated according to Blake *et al.* (1982).

Table (a): Physical properties of the experimental soil.

Analysis	First season	Second season
Soil texture	Clay loamy	Clay loamy
Clay %	39.71	40.14
Silt %	34.78	33.96
Sand %	25.51	25.90

Table (b): Chemical analysis of the experimental soil.

Analysis	Unit	1st season (2003/2004)	2nd season (2004/2005)
Organic matter	(%)	1.54	1.57
Total nitrogen	(mg/100 g soil)	1061.2	1061.9
Phosphorous	(mg/100 g soil)	239.0	239.3
Potassium	(mg/100 g soil)	611.4	611.6
Cl^-	(mg/100 g soil)	52.8	52.6
HCO_3	(mg/100 g soil)	138.1	138.5
SO_4	(mg/100 g soil)	34.5	34.6
Ca^{++}	(mg/100 g soil)	30.7	30.9
Mg^{++}	(mg/100 g soil)	14.6	14.8
Na^+	(mg/100 g soil)	38.8	39.1
Zn	(mg/100 g soil)	1.10	1.11
Fe	(mg/100 g soil)	0.89	0.91
E.C.	mmhos/cm	1.81	1.79
pH		7.79	7.81

RESULTS AND DISCUSSION

1. Effect of guanine, alanine and thymine on plant growth:

1.1. Plant height:

Data shown in Table (1) indicate that all treatments significantly increased *Rosmarinus officinalis* plant height. This was observed in both seasons for both cuts. Spraying alanine combined with guanine at the rate of 200 ppm was the best treatment in this concern, since it increased the plant height for the first season by 45.9 and 52.5% over control (not sprayed plant) in the first and second cuts, respectively, followed by spraying thymine at 200 ppm combined with guanine at the same rate, as it increased the main plant height by 40.9 and 45.0% over the control in the first and second cuts, respectively. Alanine or thymine each alone was better than guanine for increasing plant height, especially at the 200 ppm concentration. The increase was parallel to the increase in the applied concentration of nitrogenous compounds. These results are in agreement with those reported by Talaat and Youssef (2002) on basil and Refaat and Naguib (1998) on peppermint who found that amino acids led to taller plants compared to control.

1.2. Number of branches/plant:

Data in Table (1) revealed that the treatment of 200 ppm alanine combined with guanine was superior than all other treatments, while the control plants had the least number of branches/plant. The mean number of branches per plant in the second cut was more than that in the first one. The superiority of the high level of alanine (200 ppm) combined with guanine at the same level reached more than twice that of control plants for the two cuts of both seasons. This was followed by guanine at 200 ppm combined with thymine at the same concentration. These increase of the branches number for the first season reached 126.3 and 116.7% over the control (untreated plant) in the first and second cuts, respectively. Several investigators on different plants reported similar results as Refaat and Naguib (1998) on peppermint, Hendawy (2000) on *Echinacea purpurea* and Talaat and Youssef (2002) on basil.

1.3. Fresh and dry weights of leaves/plant:

Data in Table (2) indicate that both the fresh and dry weights of leaves per plant of the two cuts were considerably and gradually increased by foliar application of guanine, alanine or thymine. These results were noticed in the two cuts. Generally, there was a gradual increase in the fresh weight/plant by applying alanine, thymine or guanine at the different concentrations. The high level of alanine resulted in the heaviest plants [53.2% over the control in the first cut], followed by thymine at 200 ppm concentration of (48.8%) then the treatment of 200 ppm of guanine (41.9%). The fresh weight of leaves/plant in the second cut was more than that in the first one.

Table (1): Effect of guanine, alanine and thymine on plant height and number of branches of *Rosmarinus officinalis*, L. plant in the two cuts during 2003/2004 and 2004/2005 seasons.

Guanine levels(ppm)	Plant height (cm)								Branches number /plant							
	1st cut				2nd cut				1st cut				2nd cut			
B / A	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean
	First season															
Control	42.5	46.5	49.5	46.17	40.0	44.5	48.0	44.17	9.5	12.5	15.5	12.50	12.0	15.5	16.0	14.50
Alanine at: 100 ppm	47.0	50.0	53.5	50.17	45.5	49.0	52.0	48.83	11.5	15.0	19.0	15.17	16.5	19.5	19.5	18.50
150 ppm	53.5	57.5	59.0	56.67	49.5	53.5	57.0	53.33	14.0	17.5	19.5	17.00	19.0	22.0	23.0	21.30
200 ppm	56.5	60.5	62.0	59.67	51.0	60.0	61.0	57.33	18.0	19.5	21.5	19.67	21.5	24.5	26.0	24.00
Thymine at: 100 ppm	45.5	47.5	51.5	48.17	44.0	46.5	51.5	47.33	11.0	14.0	17.5	14.17	15.5	19.0	20.0	18.17
150 ppm	52.0	55.5	56.0	54.5	49.0	52.5	55.5	52.33	15.5	17.0	20.0	17.50	18.0	21.0	22.0	20.33
200 ppm	55.5	58.5	59.9	57.83	50.5	56.5	58.0	55.00	17.5	18.5	21.5	19.17	20.0	23.5	24.5	22.67
Means	50.36	53.71	55.86		47.07	51.79	54.71		13.86	16.29	19.21		17.50	20.71	21.57	
L.S.D. at:	5%		1%		5%		1%		5%		1%		5%		1%	
Main plots (A)	0.38		0.54		0.43		0.57		0.63		0.81		0.42		0.53	
Sub plots (B)	0.51		0.65		0.58		0.71		0.67		0.88		0.54		0.71	
Interaction (AxB)	0.67		0.82		0.73		0.82		0.76		0.97		0.72		0.96	
	Second season															
Control	44.5	49.5	51.5	48.50	43.0	46.5	49.5	46.33	10.0	12.0	15.5	12.50	13.5	16.0	18.0	15.83
Alanine at: 100 ppm	50.5	53.0	55.5	53.00	48.5	50.5	52.5	50.50	12.0	15.5	17.0	14.83	15.0	18.5	19.5	17.67
150 ppm	54.0	58.5	59.5	57.33	53.0	54.5	56.5	54.67	15.5	16.0	19.5	17.00	18.0	20.0	21.5	19.83
200 ppm	59.5	61.5	63.5	61.50	57.5	58.0	61.0	58.67	17.5	18.0	21.0	18.83	20.0	22.0	23.0	21.67
Thymine at: 100 ppm	46.5	51.0	54.0	50.50	45.5	48.0	51.0	48.17	11.5	13.5	16.0	13.67	15.5	17.0	18.5	17.00
150 ppm	51.5	54.5	57.5	54.17	50.0	53.0	55.0	52.67	14.0	15.0	18.5	15.83	17.5	19.5	20.5	19.17
200 ppm	57.5	59.0	60.5	59.00	54.0	57.0	59.0	56.67	15.5	17.0	19.5	17.33	20.5	22.5	22.5	21.83
Means	52.00	55.29	57.43		50.21	52.50	54.93		13.71	15.29	18.14		17.14	19.36	20.50	
L.S.D. at:	5%		1%		5%		1%		5%		1%		5%		1%	
Main plots (A)	0.45		0.68		0.54		0.65		0.47		0.61		0.38		0.49	
Sub plots (B)	0.55		0.72		0.63		0.78		0.52		0.79		0.42		0.56	
Interaction (AxB)	0.68		0.89		0.80		0.93		0.73		0.92		0.61		0.74	

A = Guanine levels as main plot.
B = Alanine and thymine levels as sub plot.
A x B = Interaction.

The applied substances improved the herb production of the two cuts of rosemary plant. This increment might be due to the increased plant height and the number of branches/plant, which is consequently reflected on the fresh and dry weights of the plant. These results are in harmony with those reported by Gamal El-Din *et al.* (1997) on lemongrass, Rafaat and Naguib (1998) on peppermint and Talaat and Youssef (2002) on basil.

Table (2): Effect of guanine, alanine and thymine on leaves fresh and dry weights of *Rosmarinus officinalis*, L. plant in the first and second cuts during 2003/2004 and 2004/2005 seasons.

Guanine levels(ppm)	Fresh weight of leaves (g/plant)								Dry weight of leaves (g/plant)							
	1st cut				2nd cut				1st cut				2nd cut			
B A	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean
	First season															
Control	125.0	143.5	155.0	141.2	159.0	171.5	190.5	173.7	21.5	25.5	29.0	25.3	30.0	32.0	36.0	32.7
Alanine at: 100 ppm	152.5	160.5	172.5	161.8	182.5	193.5	199.0	191.7	27.0	29.5	31.5	29.3	35.0	36.5	37.5	36.3
150 ppm	170.0	181.0	183.0	178.0	190.0	200.0	208.0	199.3	31.5	32.5	33.5	32.5	36.0	37.5	39.5	37.7
200 ppm	185.0	192.0	197.5	191.5	201.0	208.0	223.0	210.7	33.5	35.0	37.0	35.2	38.0	38.5	41.5	39.3
Thymine at:100 ppm	147.5	155.0	161.0	154.5	176.0	187.5	195.5	186.3	25.5	28.5	30.5	28.2	33.5	34.0	36.5	34.7
150 ppm	168.0	174.0	180.0	174.0	181.0	192.5	206.0	193.2	30.5	32.0	33.0	31.8	34.0	36.0	38.0	36.0
200 ppm	179.5	186.0	192.5	186.0	188.5	203.0	219.0	203.5	32.0	35.0	36.5	34.5	36.0	38.5	40.5	38.3
Means	161.1	170.3	177.4		182.6	193.7	205.9		28.8	31.1	33.0		34.6	36.1	38.4	
L.S.D. at:	5%		1%		5%		1%		5%		1%		5%		1%	
Main plots (A)	0.76		0.92		0.82		0.96		0.22		0.29		0.18		0.27	
Sub plots (B)	0.88		1.11		0.95		1.13		0.28		0.37		0.21		0.35	
Interaction (AxB)	1.32		1.57		1.37		1.66		0.33		0.41		0.29		0.38	
	Second season															
Control	132.0	164.5	170.0	155.5	163.5	175.0	182.0	173.5	23.0	29.0	30.5	27.5	29.5	31.5	33.0	31.3
Alanine at: 100 ppm	163.0	178.0	185.0	175.3	176.0	184.0	195.0	185.0	29.0	32.0	34.5	31.8	30.5	33.5	35.5	33.2
150 ppm	172.5	186.0	193.0	183.8	181.0	195.0	211.0	195.7	31.5	35.0	36.0	34.2	32.0	36.0	38.5	35.5
200 ppm	185.5	197.0	201.0	194.5	194.0	204.0	221.0	206.3	35.0	36.5	37.5	36.3	35.5	38.5	39.5	37.8
Thymine at:100 ppm	161.0	172.5	183.0	172.2	171.0	182.0	188.0	180.3	28.0	30.5	34.0	30.8	30.0	33.0	34.5	32.5
150 ppm	169.5	185.0	190.5	181.7	183.0	197.0	198.0	192.7	30.0	35.0	35.5	33.5	33.0	35.0	36.0	34.7
200 ppm	182.0	196.0	199.0	192.3	192.5	201.0	207.0	200.2	33.5	36.5	37.0	35.7	35.0	36.5	38.5	36.7
Means	166.5	182.7	188.8		180.2	191.1	200.3		30.0	33.5	35.0		32.2	34.9	36.5	
L.S.D. at:	5%		1%		5%		1%		5%		1%		5%		1%	
Main plots (A)	0.83		1.03		0.78		0.93		0.24		0.33		0.21		0.30	
Sub plots (B)	0.96		1.15		0.94		1.08		0.28		0.39		0.25		0.42	
Interaction (AxB)	1.22		1.48		1.13		1.38		0.36		0.47		0.33		0.46	

A = Guanine levels as main plot.
B = Alanine and thymine levels as sub plot.
A x B = Interaction.

With respect to the response of the fresh and consequently the dry weights of leaves in the rosemary cuts to foliar application with the different nitrogenous compounds was shown in (Table, 2). A clear trend was observed in the interaction treatment. Application of alanine combined with guanine at the concentration of 200 ppm to rosemary recorded the highest values compared with control and the other treatments. The increase in the fresh and dry weights of leaves reached 58.0 and 72.1% in the first cut over control, respectively and 40.3 and 38.3% in the second one over control, respectively, followed by thymine combined with guanine at the high level (200 ppm). The increases were 54.0 and 69.8% over the same control, respectively in the first cut, while reached 37.7 and 35.0% in the second one over the same control, respectively. The positive effect on yield may be due to the vital effect of growth substances on stimulating of the growth of plant cells. Stimulative effects of nitrogenous compounds on growth were stated by Goss (1973) who indicated that the amino acids can serve as a source of carbon and energy when carbohydrates become deficient in the plant, amino acids are determinate releasing the ammonia and organic acid from which the amino acid was originally formed. The organic acid then enter the Krebs' cycle, to be broken down to release energy through respiration. In addition, Thom *et al.* (1981) pointed out that amino acids provide plant cells with an immediately available source of nitrogen which generally can be taken by cells more rapidly than inorganic nitrogen.

1.4. Fresh and dry weights of stems/plant:

The data in Table (3) showed the effect of spraying alanine, thymine or guanine when applied individually and in combination on the fresh and the dry weights of stems/rosemary plant. The fresh and consequently the dry weight of stems/plant shows significant differences due to nitrogenous compounds application. The highest significantly weights of stems resulted from spraying alanine at the high and medium concentration combined with guanine at 200 ppm or thymine combined with guanine at the high concentrations (200 ppm) compared with control plants. The highest rates of increase resulted from the foliar application of alanine combined with guanine at 200 ppm for each in the two cuts. The increase reached 54.5 and 32.6% over control in first cut, respectively, while in the second cut, the increase reached 55.8 and 27.1% over control, respectively, followed by thymine combined with guanine at 200 ppm concentration (the increase of fresh and dry weights of stems reached 49.1 and 30.4% over non treated plant) in the first cut, and 52.5 and 25.0% over control in the second cut, respectively. These results were in agreement with those obtained by El-Saeid *et al.* (1996) on *Tagetes patula* and Refaat and Naguib (1998) on peppermint.

2. Effect of guanine, alanine and thymine on chemical composition:

2.1. Effect on essential oil percentage:

As for the effect on oil content, data obtained in Table (4) indicated clearly that all amino compounds treatments increased the oil percentage in leaves of rosemary plants to both seasons. The highest recorded values were obtained in plants treated with alanine at 200 ppm combined with guanine at the same concentration as the increase reached 54.2% over control, followed by alanine alone at 200 ppm or combined with guanine at 100 ppm which were more effective for increasing oisl percentage in the leaves (the increase reached 50 and 40% over control in the two cuts, respectively). On the other hand, the lowest concentration of guanine and alanine gave slight increase in oil percentage of the leaves. This finding may be due to the enhancement ffect of the nitrogenous compounds on the volatile oil biosynthesis through playing direct or indirect role in this concern. A similar finding was obtained by El-Saeid *et al.* (1996) on *Tagetes patula* and Rafaat and Naguib on peppermint (1998). Hess (1975) pointed out that the secondary plant substances such as terpenoids, phenols and alkaloids are derived biosynthetically from the metabolism of carbohydrates, fats and amino acids.

Table (3): Effect of guanine, alanine and thymine on stems fresh and dry weights of *Rosmarinus officinalis*, L. plant in the first and second cuts during 2003/2004 and 2004/2005 seasons.

Guanine levels (ppm) B A	Fresh weight of stems (g/plant)								Dry weight of stems (g/plant)							
	1st cut				2nd cut				1st cut				2nd cut			
	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean
	First season															
Control	83.5	98.5	103.5	95.2	90.5	103.5	115.5	103.2	23.0	26.5	27.5	25.5	24.0	25.5	27.5	25.5
Alanine at: 100 ppm	94.5	106.0	109.5	103.3	113.0	112.0	125.0	119.0	25.5	27.5	28.0	27.0	26.0	26.5	28.5	27.0
150 ppm	101.5	117.0	121.0	113.2	119.5	125.0	132.0	125.5	26.0	28.5	29.0	27.8	27.5	28.0	29.5	28.3
200 ppm	108.0	124.0	129.0	120.7	123.5	130.0	141.0	131.5	27.5	29.5	30.5	29.2	28.0	29.0	30.5	29.2
Thymine at: 100 ppm	85.5	101.5	105.0	97.5	108.0	114.0	126.0	116.0	24.0	25.0	28.0	25.7	26.0	27.0	28.0	27.0
150 ppm	93.5	112.0	117.0	107.5	117.0	123.5	130.0	123.5	25.5	27.0	29.5	27.3	27.0	29.0	29.0	28.3
200 ppm	105.0	121.0	124.5	116.8	124.0	136.0	138.0	132.7	26.5	28.0	30.0	28.2	27.5	29.0	30.0	29.0
Means	95.9	110.6	115.6		113.6	120.6	129.6		25.4	27.4	28.9		25.1	27.7	29.0	
L.S.D. at:	5%		1%		5%		1%		5%		1%		5%		1%	
Main plots (A)	0.76		0.85		0.73		0.84		0.12		0.19		0.14		0.22	
Sub plots (B)	0.92		1.07		0.96		1.15		0.13		0.22		0.13		0.25	
Interaction (AxB)	0.98		1.21		1.02		1.28		0.18		0.27		0.15		0.26	
	Second season															
Control	87.5	102.0	115.0	101.5	93.5	108.5	119.5	107.2	24.0	26.5	28.5	26.3	25.0	27.0	28.5	26.8
Alanine at: 100 ppm	96.5	117.5	127.0	113.7	106.0	119.0	128.5	117.8	25.0	28.0	29.5	27.5	27.0	27.5	29.0	27.8
150 ppm	111.0	120.0	132.0	121.0	117.0	123.5	136.0	125.5	27.5	28.5	30.0	28.7	28.0	29.0	29.5	28.8
200 ppm	122.0	130.0	139.0	130.3	123.0	130.0	143.5	132.2	28.5	29.5	30.5	29.5	29.0	23.5	31.0	29.8
Thymine at: 100 ppm	92.5	100.0	121.0	104.5	102.5	109.5	121.0	108.3	24.5	26.5	29.0	26.7	27.0	27.0	29.0	27.7
150 ppm	103.5	109.0	130.3	114.2	111.5	112.5	133.5	119.2	25.5	27.5	30.0	27.7	28.5	29.0	30.0	29.2
200 ppm	118.0	125.5	130.0	126.8	119.5	127.0	141.5	129.3	27.0	28.0	30.5	28.5	29.0	30.5	31.0	30.2
Means	104.4	114.9	128.7		110.4	118.4	131.9		26.0	27.8	29.7		27.6	28.5	29.7	
L.S.D. at:	5%		1%		5%		1%		5%		1%		5%		1%	
Main plots (A)	0.78		0.91		0.77		0.91		0.14		0.23		0.09		0.18	
Sub plots (B)	0.97		1.16		0.98		1.17		0.17		0.30		0.12		0.22	
Interaction (AxB)	1.05		1.30		1.08		1.35		0.21		0.33		0.16		0.26	

A = Guanine levels as main plot.
B = Alanine and thymine levels as sub plot.
A x B = Interaction.

2.2. Effect on total carbohydrates percentage:

Regarding the effect of nitrogenous compounds on total carbohydrates percentage, it is clear from Table (4) that application of all nitrogenous compounds increased the total carbohydrates percentage in rosemary leaves and the highest record was obtained with the application of 200 ppm alanine combined with guanine. The promotive effect of these nitrogenous compounds on the total carbohydrates content may be due to their important role on the biosynthesis of chlorophyll molecules which in turn affected carbohydrates content. A similar finding was obtained by Mohamed and Whaba (1993) on *Rosmarinous officinalis* and Refaat and Naguib (1998) on peppermint. In this concern, Devlin (1972) stated that there is a general agreement that succinyl COA (Kreb's cycle intermediate) and the amino acid glycine, initiate the biosynthetic pathway leading to chlorophyll formation.

Table (4): Effect of guanine, alanine and thymine on essential oil and total carbohydrates percentages of *Rosmarinus officinalis*, L. plant in the first and second cuts during 2003/2004 and 2004/2005 seasons.

Guanine levels(ppm)	Essential oil (%)								Total carbohydrates (%)							
	1st cut				2nd cut				1st cut				2nd cut			
B / A	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean
	First season															
Control	0.24	0.28	0.31	0.28	0.24	0.29	0.32	0.28	12.5	14.0	14.5	13.7	13.5	14.5	15.0	14.33
Alanine at: 100 ppm	0.29	0330	0.32	0.30	0.28	0.31	0.33	0.31	15.0	15.5	16.0	15.5	15.0	16.0	16.5	14.83
150 ppm	0.32	0.33	0.35	0.33	0.32	0.33	0.33	0.33	16.0	16.5	17.0	16.5	16.0	16.5	17.5	16.67
200 ppm	0.34	0.36	0.37	0.36	0.35	0.36	0.37	0.36	16.5	17.0	17.0	16.8	16.5	17.0	18.5	17.33
Thymine at: 100 ppm	0.28	0.29	0.31	0.29	0.27	0.30	0.31	0.29	13.5	14.5	15.0	14.3	13.5	15.0	15.5	14.67
150 ppm	0.30	0.32	0.33	0.32	0.31	0.31	0.32	0.31	15.0	16.0	16.5	15.8	14.5	15.5	16.5	15.50
200 ppm	0.31	0.32	0.35	0.33	0.32	0.33	0.35	0.33	15.5	16.0	16.5	16.0	15.5	16.0	17.0	16.17
Means	0.30	0.31	0.33		0.30	0.32	0.33		14.86	15.64	16.07		14.93	15.79	16.64	
	Second season															
Control	0.25	0.27	0.30	0.27	0.26	0.28	0.31	0.28	13.0	14.5	15.0	14.17	14.0	15.5	16.0	15.17
Alanine at: 100 ppm	0.28	0.31	0.32	0.30	0.27	0.30	0.32	0.30	15.5	16.0	16.5	16.00	16.0	17.0	17.5	16.83
150 ppm	0.30	0.32	0.34	0.32	0.30	0.32	0.34	0.32	16.0	16.5	17.0	16.50	17.0	18.0	18.0	17.67
200 ppm	0.34	0.35	0.35	0.35	0.32	0.35	0.35	0.34	16.5	17.0	18.0	17.16	17.5	18.5	19.0	18.33
Thymine at: 100 ppm	0.28	0.30	0.30	0.31	0.26	0.29	0.32	0.29	14.5	15.0	15.5	15.00	14.5	15.5	16.5	15.50
150 ppm	0.30	0.33	0.34	0.32	0.29	0.30	0.33	0.31	15.5	16.0	17.0	16.17	15.0	16.0	17.5	16.17
200 ppm	0.32	0.33	0.34	0.33	0.31	0.32	0.33	0.32	16.0	16.0	17.5	16.50	16.0	17.0	18.0	17.00
Means	0.30	0.32	0.33		0.29	0.31	0.33		15.29	15.86	16.64		15.71	16.79	17.5	

A = Guanine levels as main plot.
B = Alanine and thymine levels as sub plot.

2.3. Effect on essential oil constituents:

Data presented in Table (5) and illustrated in Figs. (1-8) obviously reveal that oil composition responded greatly to foliar spray with the nitrogenous compounds guanine, alanine and thymine at different rates of application. The GLC analysis of essential oil extracted from *Rosmarinus officinalis*, L. plants treated with these compounds revealed the presence of each of α-pinene, 1, 8 cineole, camphor, borneol and bornyl acetate constituents in the leaves oil. This finding is in accordance with that obtained by Soliman *et al.* (1994) and Youssef and Talaat (2003) on rosemary. 1, 8 cineole (6.09 to 10.22%), camphor (21.21 to 25.97%), borneol (8.62-10.8%) and bornyl acetate (16.07 to 24.7%) are the major constituents in all treatments. The maximum percentage of cineol was observed with guanine combined with alanine at the levels of 200 and 100 ppm, respectively, followed by guanine at 100 ppm level combined with thymine at the same level. These treatments were higher than control plants by 50.52 and 50.07%, respectively.

Table (5): Effect of guanine, alanine and thymine on essential oil composition of *Rosmarinus officinalis,* L. plant.

Treatments	α-Pinene (%)	1,8 Cineole (%)	Camphor (%)	Borneol (%)	Bornyl acetate (%)
Control	2.00	6.79	24.79	10.80	24.12
Alanine at: 100 ppm	2.35	6.99	24.03	9.78	24.70
150 ppm	2.28	9.38	24.13	9.85	23.29
200 ppm	2.12	9.06	21.99	8.62	22.40
Thymine at: 100 ppm	2.25	6.09	25.31	10.31	23.73
150 ppm	2.30	6.93	25.97	9.74	23.21
200 ppm	1.87	9.30	22.87	9.13	20.06
Guanine at level (100 ppm)	1.90	8.87	23.65	9.67	20.04
Guanine (100 ppm) + alanine (100 ppm)	2.02	9.02	21.54	8.84	19.50
Guanine (100 ppm) + alanine (150 ppm)	2.06	9.23	21.21	8.89	23.00
Guanine (100 ppm) + alanine (200 ppm)	2.05	8.50	24.99	10.06	19.38
Guanine (100 ppm) + thymine (100 ppm)	2.12	10.19	22.54	8.90	22.95
Guanine (100 ppm) + thymine (150 ppm)	2.49	10.09	25.34	10.60	23.47
Guanine (100 ppm) + thymine (200 ppm)	2.41	9.33	23.79	10.12	21.99
Guanine at level (200 ppm)	2.03	8.74	22.28	8.91	21.03
Guanine (200 ppm) + alanine (100 ppm)	2.46	10.22	21.52	8.80	17.55
Guanine (200 ppm) + alanine (150 ppm)	2.13	9.19	22.04	9.67	20.77
Guanine (200 ppm) + alanine (200 ppm)	2.27	8.80	24.30	9.84	22.41
Guanine (200 ppm) + thymine (100 ppm)	1.79	9.18	22.74	9.26	20.94
Guanine (200 ppm) + thymine (150 ppm)	2.60	9.99	21.89	9.30	16.07
Guanine (200 ppm) + thymine (200 ppm)	2.18	7.28	22.43	9.37	20.39
Retention time (RT. Min) of compound	7.13	10.35	16.45	18.28	19.35

These results may be attributed to the effect of nitrogen source consumed directly in purine group erection which consequently affect apparently the enzyme reaction system.

As for the camphor compound, data showed that both thymine at the levels of 150, 100 ppm alone and guanine (100 ppm) combined with thymine at level 150 ppm have a stimulatory effect on increasing the percentage of camphor compound in the oil of rosemary plants compared with other treatments and control plants.

The data showed that treating rosemary plants with alanine at the 100 ppm level enhanced the formation of bornyl acetate in the oil.

As for the borneol compound, data show that all treatments decreased the percentage of borneol compound in the oil of rosemary plants. A similar finding was obtained by Mohamed and Whaba (1993) on rosemary and Refaat and Naguib (1998) on peppermint.

2.4. Effect on the minerals content:

Data in Tables (6 and 7) show also the effect of the nitrogenous compounds on the minerals percentage of N, P and K of rosemary leaves in the two cuts for both seasons. Foliar application with all substances increased N, P and K comparing with untreated plants. The highest values were obtained in leaves of rosemary plants which received the high level (200 ppm) of alanine combined with guanine in the first cut of the first season. The increase N, P and K% 13.2, 24.1 and 14.6% over control, respectively. In the second cut, the increases reached 15.9, 28.6 and 14.9% over control, respectively. Davies (1982) stated that synthesis of all proteins probably occurred by a process in which ribosomes by the sequence of nucleotides in a nucleic acid template. A similar finding was obtained by Refaat and Naguib (1998) on peppermint.

Table (6): Effect of the alanine, thymine and guanine on the minerals perecentage in the leaves of *Rosamarinus officinalis* L. plant in the first cut during 2003/2004 and 2004/2005 seasons.

B \ Guanine levels (ppm) A		N (%)				P (%)				K (%)			
		0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean
		First season											
Control		1.36	1.41	1.46	1.41	0.29	0.30	0.32	0.30	1.21	1.28	1.31	1.27
Alanine at:	100 ppm	1.42	1.48	1.49	1.46	0.31	0.32	0.33	0.32	1.30	1.32	1.33	1.32
	150 ppm	1.47	1.50	1.52	1.50	0.32	0.34	0.35	0.34	1.34	1.36	1.37	1.36
	200 ppm	1.50	1.53	1.54	1.52	0.34	0.35	0.36	0.35	1.37	1.38	1.39	1.38
Thymine at:	100 ppm	1.40	1.43	1.46	1.43	0.29	0.31	0.33	0.31	1.28	1.31	1.32	1.30
	150 ppm	1.44	1.46	1.47	1.46	0.31	0.32	0.34	0.32	1.31	1.34	1.35	1.33
	200 ppm	1.49	1.49	1.48	1.49	0.33	0.33	0.34	0.33	1.34	1.35	1.37	1.35
Mean		1.44	1.47	1.49		0.31	0.32	0.34		1.31	1.33	1.35	
		Second season											
Control		1.38	1.40	1.45	1.41	0.30	0.32	0.33	0.32	1.24	1.27	1.31	1.27
Alanine at:	100 ppm	1.43	1.45	1.48	1.45	0.32	0.33	0.34	0.33	1.29	1.31	1.34	1.31
	150 ppm	1.46	1.48	1.52	1.49	0.34	0.34	0.35	0.34	1.33	1.35	1.38	1.35
	200 ppm	1.50	1.50	1.54	1.51	0.34	0.35	0.37	0.35	1.36	1.37	1.39	1.37
Thymine at:	100 ppm	1.40	1.44	1.45	1.43	0.31	0.32	0.33	0.32	1.30	1.31	1.32	1.31
	150 ppm	1.43	1.45	1.46	1.45	0.33	0.34	0.35	0.34	1.31	1.34	1.35	1.33
	200 ppm	1.47	1.49	1.49	1.48	0.34	0.34	0.36	0.35	1.34	1.35	1.36	1.35
Mean		1.44	1.46	1.48		0.33	0.33	0.35		1.31	1.33	1.35	

A = Guanine levels as main plot.
B = Alanine and thymine levels as sub plot.

Table (7): Effect of the alanine, thymine and guanine on the minerals percentage in the leaves of *Rosamarinus officinalis,* L. plant in the second cut during 2003/2004 and 2004/2005 seasons.

B \ Guanine levels (ppm) A		N (%)				P (%)				K (%)			
		0.0	100	200	Mean	0.0	100	200	Mean	0.0	100	200	Mean
		First season											
Control		1.32	1.43	1.45	1.40	0.28	0.31	0.34	0.31	1.23	1.31	1.36	1.30
Alanine at:	100 ppm	1.40	1.46	1.48	1.45	0.32	0.33	0.35	0.33	1.29	1.37	1.39	1.35
	150 ppm	1.45	1.49	1.50	1.48	0.33	0.35	0.36	0.35	1.32	1.38	1.40	1.37
	200 ppm	1.49	1.50	1.53	1.51	0.34	0.35	0.36	0.35	1.36	1.38	1.41	1.38
Thymine at:	100 ppm	1.36	1.44	1.47	1.42	0.30	0.32	0.34	0.32	1.25	1.33	1.38	1.32
	150 ppm	1.43	1.46	1.48	1.46	0.31	0.33	0.35	0.33	1.32	1.35	1.40	1.36
	200 ppm	1.46	1.48	1.49	1.48	0.33	0.34	0.36	0.34	1.35	1.37	1.40	1.37
Mean		1.42	1.47	1.49		0.32	0.33	0.35		1.30	1.40	1.39	
		Second season											
Control		1.35	1.39	1.43	1.39	0.31	0.34	0.35	0.33	1.26	1.30	1.37	1.31
Alanine at:	100 ppm	1.41	1.43	1.45	1.43	0.34	0.35	0.36	0.35	1.32	1.34	1.38	1.35
	150 ppm	1.46	1.47	1.50	1.48	0.35	0.37	0.37	0.36	1.36	1.36	1.38	1.37
	200 ppm	1.51	1.52	1.53	1.52	0.36	0.37	0.38	0.37	1.37	1.38	1.39	1.38
Thymine at:	100 ppm	1.39	1.42	1.44	1.42	0.32	0.34	0.36	0.34	1.30	1.32	1.35	1.35
	150 ppm	1.43	1.46	1.48	1.46	0.34	0.36	0.36	0.35	1.33	1.33	1.37	1.34
	200 ppm	1.44	1.47	1.49	1.47	0.35	0.36	0.37	0.36	1.35	1.36	1.37	1.36
Mean		**1.43**	**1.45**	**1.47**		**0.34**	**0.36**	**0.36**		**1.33**	**1.35**	**1.37**	

A = Guanine levels as main plot.
B = Alanine and thymine levels as sub plot.

From the present results it can be concluded that rosemary plant had a great response to nitrogenous compounds application. It can be recommended that alanine and guanine can be applied at 200 ppm to in yield of herb and oil with good quality.

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تأثير الجوانين والألانين والثيمين على النمو والمحصول والتركيب الكيميائى وجودة زيت نبات حصالبان

حسناء عبدالحسيب حسن جودة
الهيئة القومية للرقابة ولبحوث الدوائية

أجريت تجربتان حقليتان لدراسة تأثير إضافة ثلاث مركبات نيتروجينية هى الجوانين والألانين والثيمين على النمو والتركيب الكيميائى وجودة الزيت لنبات حصالبان وكانت أهم النتائج المتحصل عليها:

- زادت قياسات النمو (طول النبات – عدد الأفرع/نبات والوزن الطازج والجاف لكل من الأوراق والسيقان) نتيجة لاستخدام المواد الثلاثة بدرجة واحدة تقريبا.
- تم التحصل على أقصى زيادة لصفات النمو عند استخدام مادتى: الألانين والجوانين معاً بالتركيز العالى لكل منهما (٢٠٠ جزء فى المليون).
- كان لجميع المواد النيتروجينية المستخدمة أثرا واضحا على زيادة النسبة المئوية لكل من الزيت الطيار والكربوهيدرات الكلية فى العشب فى كلا الحشتين فى موسمى التجربة. كذلك زاد المحتوى الكيماوى لكل من النيتروجين والفوسفور والبوتاسيوم فى الأوراق فى كلا الحشتين لموسمى التجربة.
- زادت المركبات الرئيسية فى الزيت وهى السينيول والكامفور والبورنيول وخلات البورنيول كنتيجة لاستخدام المواد النيتروجينية الثلاث. وقد أظهرت المعاملة بالجوانين + الألانين بتركيز ٢٠٠، ١٠٠ جزء فى المليون لكل منها على التوالى إلى زيادة نسبة السينيول بدرجة واضحة. بينما أدت المعاملة بالثيمين على حدة بتركيز ١٥٠ جزء فى المليون أو مضافا إليه الجوانين بتركيز ١٠٠ جزء فى المليون إلى زيادة واضحة فى النسبة المئوية للكامفور فى الزيت الطيار. أما بالنسبة للألانين فكان لتركيز ١٠٠ جزء فى المليون أحسن الأثر فى الوصول إلى أعلى نسبة مئوية من خلات البورنيول. كما أظهرت جميع المعاملات نقصا واضحا فى النسبة المئوية للبورنيول.