

Effect of Riboflavin, Ascorbic Acid and Dry Yeast on Vegetative Growth, Essential Oil Pattern and Antioxidant Activity of Geranium (*Pelargonium graveolens* L.)

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Abstract: The response of vegetative growth, essential oil content and constituents, phenolic and flavonoids content and their antioxidant activity of Geranium plants (*Pelargonium graveolens* L.) was studied after foliarly treated with riboflavin, ascorbic acid and dry yeast (different concentrations) at two cuttings. Improved vegetative growth and photosynthetic pigments content were obtained, especially at treatments of dry yeast (4000 mg/l), ascorbic acid (150 mg/l) and riboflavin (90 mg/l). Pronounced increases were obtained in essential oil percent, total phenols and total flavonoids in treated plants, especially at cutting (I). Consequently, pronounced antioxidant activity (DPPH radical scavenging activity) was obtained in plants treated with the above-mentioned levels of ascorbic acid, riboflavin and dry yeast treatments.

Key words: *Pelargonium graveolens* L. • Antioxidant activity • Essential oil • Flavonoids • Ascorbic acid • Dry yeast • Riboflavin

INTRODUCTION

A great attention was recently focused for exogenous application of vitamins and yeast to improve plant growth and production, due to their advantages of being natural products. Ascorbic acid was synthesized in higher plants and affected plant growth and development, played an important role as co-enzymatic reaction in the electron transport system and metabolism [1]. Ascorbic acid is considered an important antioxidant which protects not only with H_2O_2 but also with O_2 , OH and lipid hydroperoxidase [2]. Bolikhina *et al.* [3] stated that ascorbic acid is the most abundant antioxidant which protected plant cells, ascorbic acid is currently considered to be a regulator on cell division and differentiation. They added that ascorbic acid is involved in a wide range of important functions as antioxidant defense, photoprotection and regulation of photosynthesis and growth. Farahat *et al.* [4] reported that pronounced increases in vegetative growth and chemical constituents of *Cupressus sempervirens* L. plants by foliar application of ascorbic acid. Abdel Aziz *et al.* [5] stated that ascorbic acid showed a stimulatory effect on all chemical constituents of *Gladiolus* plants. Eid *et al.* [6] reported that foliar application of ascorbic acid significantly increased Jasmine concrete, essential oil percent and oil yield of *Jasminum grandiflorum* L.

Riboflavin is one of vitamin B acted as coenzyme; enhance growth and metabolism of various plant species. Riboflavin is known to be the precursor of the coenzymes riboflavin monophosphate and flavin adenine dinucleotide, which serve indispensable redox cofactors in all organisms. In plants, these cofactors required for numerous critical cellular processes as diverse as the citric acid cycle, fatty acid oxidation and mitochondrial electron transport [7]. Dry yeast had stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis and chlorophyll formation and source of cytokinins [8, 9]. Active dry yeast enhanced growth, essential oil and photosynthetic pigments of some plants as *Nigella sativa* L. [10], *Ruta graveolens* [11] and *Lupinus termis* [12]. Geranium species are used in aromatherapy as antiasthmatic, antiallergic, antioxidant and other pharmaceutical and cosmetic purposes [13]. *Pelargonium graveolens* are commercially grown in Egypt, Morocco and China mainly for their essential oil [14]. In addition, Geranium plants also contain different phenolic and flavonoids compounds which have antioxidant capacity [13]. The role of ascorbic acid, riboflavin and dry yeast on *P. graveolens* could not be traced in the literature.

Therefore, the aim of the present work was to reveal the best efficient level of exogenously applied natural bioactive compounds, i.e. ascorbic acid, riboflavin and

dry yeast, which could increase and improve growth, photosynthesis and essential oil content. In addition, the contents of total phenols, total flavonoids and antioxidant activity were determined.

MATERIALS AND METHODS

A pot experiment was carried out at the greenhouse of National Research Centre, Dokki, Giza, Egypt during two seasons 2007/08 and 2008/09 to study the effect of foliar application of vitamin B₂ (riboflavin), vitamin-C (ascorbic acid) and active dry yeast on geranium (*Pelargonium graveolens* L.) plants. Uniform cuttings were planted at 17th June, 2007 and 18th June, 2008 in earthen pots, 30 cm diameter (two plants/pot). Phosphorus and potassium fertilization were applied to soil before transplanting at the rate of 4g/pot calcium superphosphate (15.5 % P₂O₅) and 1g potassium sulphate (48 % K₂O), respectively. Nitrogen fertilization in form of ammonium nitrate (33.5 % N) was applied in two applications (1g/pot for each) with two weeks interval started 30 days after transplanting. Fertilization was repeated after the 1st cut. The experiment was set up in a completely randomized design with three replicates. Plants were foliarly sprayed with vitamin-B₂ (riboflavin) at 30, 60 and 90 mg/l, vitamin-C (ascorbic acid) at 50, 100 and 150 mg/l and active dry yeast at 2000, 4000 and 6000 mg/l. The composition of yeast solution was reported by Nagodawithana [15]. Spraying was done twice; the first one at 2 weeks before 1st cutting and the second was sprayed one week later. The same spraying technique was done before the second cut. The first cut was collected at November, 2007 and December, 2008 and the second one at June 2008 and 2009, for the two seasons respectively. The plant herbage was harvested by cutting above 10 cm over the soil surface and plant growth characters in terms of plant height (cm), number of branches/plant, fresh and dry weights of plant (g) were determined. Photosynthesis pigments including chlorophyll (a), (b) and carotenoids contents were determined in fresh leaves as mg/g fresh weight according to the procedure achieved by Moran [16].

Total soluble phenols were determined calorimetrically by using Folin Ciocaltea reagent according to Singleton *et al.* [17]. Total flavonoids were determined using the method reported by Chang *et al.* [18]. Antioxidant activity [DPPH radical scavenging activity] was determined according to the method

described by Brand *et al.* [19]. Essential oil percentage was determined using hydro-distillation method according to the Egyptian Pharmacopoeia [20]. The essential oil composition for the samples dehydrated over anhydrous sodium sulphate was determined using GLC and analysis with Agilent Technologies 6890 GC system model. The separation was carried out with 5 % phenyl methyl siloxane capillary, column J-413. The column temperature was programmed started from 60°C to 220°C at a rate of 4°C/min. The detector temperature was 30°C and flow rate of the carrier gas (N₂) was 30 ml/min., (H₂) was 30 ml/min and air at 300 ml/min. The identification of the constituents was determined by comparing their retention times (RT) with those of the available authentic oil samples injected in GLC under the same conditions.

Statistical Analysis: Data obtained were subjected to standard analysis of variance procedure. The values of L.S.D. were calculated at 5 % for significant F-test according to Snedecor and Cochran [21].

RESULTS AND DISCUSSION

Vegetative Growth: Data given in Table 1 clearly indicated that in cuttings I and II, foliar application of riboflavin (90 mg/l) dry yeast (4000 mg/l) and ascorbic acid (150 mg/l) caused the most promoting effect for increasing fresh and dry weights of geranium plants. The variation in dry matter accumulation at the two cuttings could be attributed to the seasonal changes in climatic conditions, especially air temperature prevailing during successive growth and developmental stages until the plant reached full blooming stage where the cutting of over ground part was done. The increases in vegetative growth as a result of vitamin and dry yeast treatments might essentially be due to ascorbic acid is currently considered to be a regulator on cell division and differentiation and involved in a wide range of important functions as antioxidant defense, photoprotection and regulation of photosynthesis and growth. Riboflavin (vitamin B₂) could serve as coenzyme in keto-glutamic acid which had its importance in the metabolism of carbohydrates [22]. Ascorbic acid plays an important role in the electron transport system [1]. Bolkhina *et al.* [3] stated that ascorbic acid is the most abundant antioxidant which protected plant cells. Application of ascorbic acid to wheat plants increased dry weight as compared with untreated plants [23, 24].

Table 1: Effect of dry yeast, ascorbic acid and riboflavin foliar spray on some growth characters of Geranium (average of two seasons)

Treatments (mg/l)	Plant height (cm)		Branches numbers		Fresh weights (g/plant)		Dry weight (g/plant)	
	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II
Control	48.3	44.6	11.6	7.3	167.1	164.6	48.1	36.0
Dry yeast (2000)	52.6	44.6	13.6	9.6	253.7	203.0	56.2	52.9
Dry yeast (4000)	55.6	47.6	14.6	11.0	279.0	247.6	65.0	54.0
Dry yeast (6000)	51.6	45.0	10.3	10.6	259.9	237.2	64.3	43.4
Ascorbic acid (50)	48.3	46.3	12.6	9.3	170.1	203.7	49.5	47.1
Ascorbic acid (100)	50.6	55.0	13.3	13.6	238.2	228.5	59.7	55.4
Ascorbic acid (150)	52.4	70.0	14.0	14.6	250.9	260.5	65.6	63.4
Riboflavin (30)	43.6	50.3	12.3	11.3	247.6	215.7	60.8	41.6
Riboflavin (60)	48.0	49.3	13.3	12.0	249.2	219.6	63.2	43.7
Riboflavin (90)	58.6	50.0	14.6	14.0	259.9	251.1	67.5	59.2
LSD 0.05	7.2	9.5	4.2	3.4	16.9	50.5	7.9	15.4

Table 2: Effect of dry yeast, ascorbic acid and riboflavin foliar spray on chlorophyll and carotenoids content of Geranium (average of two seasons)

Treatments (mg/l)	Chl. a (mg/g)		Chl. b (mg/g)		Total Chl. (mg/g)		Carotenoids (mg/g)	
	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II
Control	0.97	0.88	0.68	0.56	1.63	1.44	0.29	0.30
Dry yeast (2000)	1.08	1.02	0.79	0.80	1.88	1.82	0.58	0.36
Dry yeast (4000)	1.48	1.12	0.92	0.96	2.40	2.08	0.41	0.25
Dry yeast (6000)	1.10	1.13	0.79	0.78	1.90	1.91	0.31	0.40
Ascorbic acid (50)	0.81	0.80	0.55	0.56	1.36	1.37	0.26	0.42
Ascorbic acid (100)	1.05	1.18	0.60	0.75	1.66	1.93	0.31	0.29
Ascorbic acid (150)	1.70	1.11	1.09	0.89	2.80	2.01	0.58	0.38
Riboflavin (30)	1.21	1.13	0.86	0.82	2.08	1.95	0.40	0.34
Riboflavin (60)	1.47	1.20	0.92	1.00	2.40	2.21	0.45	0.35
Riboflavin (90)	1.57	1.44	0.98	1.03	2.55	2.47	0.41	0.38
LSD 0.05	0.29	0.24	0.18	0.28	0.47	0.45	0.09	0.11

Photosynthetic Pigments: Table 2 showed that the promoting effect of the applied vitamins and dry yeast on chlorophyll (a), (b) and total chlorophyll can be arranged in the following descending order; ascorbic acid (150 mg/l), riboflavin (90 mg/l) and dry yeast (4000 mg/l). This was true at cutting (I). However, at cutting (II) there were significant effects between the different concentrations of all applied vitamins on chlorophyll (a). The increases in chlorophyll (b) were more pronounced as compared with their controls at both cuttings, especially that of ascorbic acid (150 mg/l) at cutting I and those of riboflavin (90 mg/l). The increase in chlorophyll (b) could be considered as an advantage to increase the out put of photosynthetic materials and thus increased the dry matter of geranium (Table 1). Carotenoids content (Table 2) was significantly increased at (2000 mg/l) of dry yeast, ascorbic acid (150 mg/l) and riboflavin at (60 mg/l) at cutting (I). The increases in carotenoids content were in advantage for protecting chlorophylls from oxidation.

In support, Foyer and Harbinson [25] reported that carotenoids could indirectly protect photosynthetic apparatus from photoinhibitory damage by singlet oxygen, which produced by the excited triplet state of chlorophyll. The present results of ascorbic acid effects on vegetative growth and photosynthetic pigments of geranium plants contributed understanding the role of ascorbic acid in plant growth. Ascorbate was reported by Smimoff [26] and Blokhina *et al.* [3], to be involved in wide range of important physiological functions as antioxidant defense, photoprotection of regulation of photosynthesis and growth regulation.

Essential Oil Percent and Yield: Table 3 showed that oil percent and yield were significantly increased at treatments of dry yeast (4000 mg/l), ascorbic acid (150 mg/l) at both cutting. Moreover, riboflavin (90 mg/l) treatment showed the highest oil percent and yield at both cuttings. For other essential oil containing plants,

Table 3: Effect of dry yeast, ascorbic acid and riboflavin foliar spray on oil percent and oil yield (ml/fresh wt./plant) of Geranium (average of two seasons)

Treatments (mg/l)	Oil %		Oil yield (ml/fresh wt./plant)	
	Cutting I	Cutting II	Cutting I	Cutting II
Control	0.20	0.19	0.31	0.31
Dry yeast (2000)	0.21	0.21	0.54	0.42
Dry yeast (4000)	0.25	0.24	0.63	0.59
Dry yeast (6000)	0.23	0.21	0.59	0.48
Ascorbic acid (50)	0.20	0.19	0.34	0.39
Ascorbic acid (100)	0.21	0.20	0.50	0.46
Ascorbic acid (150)	0.24	0.22	0.57	0.58
Riboflavin (30)	0.21	0.20	0.52	0.42
Riboflavin (60)	0.24	0.23	0.60	0.48
Riboflavin (90)	0.29	0.27	0.75	0.66
LSD 0.05	0.01	0.01	0.05	0.12

Table 4: Effect of dry yeast, ascorbic acid and riboflavin foliar spray on major constituents of essential oil from Geranium (average of two seasons)

Components	Control		Dry yeast (4000 mg/l)		Ascorbic acid (150 mg/l)		Riboflavin (90 mg/l)	
	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II
α -Pinene	0.75	0.63	-	0.61	1.04	0.52	0.73	0.69
β -pinene	4.43	3.51	5.74	5.22	3.25	4.51	5.36	6.02
1,8-cineol	1.52	1.21	1.56	1.23	0.31	0.62	1.62	1.23
Linalool	6.86	7.15	9.82	9.31	8.75	8.32	10.09	8.95
Citronellol	25.13	26.55	35.81	33.52	28.52	30.21	31.45	33.51
Geraniol	5.72	5.11	6.93	6.32	6.30	6.51	5.60	6.53
Carvon	3.43	3.10	3.93	3.86	2.87	3.21	3.86	3.69

Table 5: Effect of dry yeast, ascorbic acid and riboflavin foliar spray on biochemical constituents of Geranium (average of two seasons)

Treatments (mg/l)	Total phenols (mg/g D.W.)		Total flavonoids (mg/g)		Antioxidant activity	
	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II
Control	0.73	0.63	19.51	14.63	79.64	80.50
Dry yeast (2000)	1.19	0.68	16.71	15.93	83.10	84.03
Dry yeast (4000)	1.24	1.13	24.29	19.77	84.23	85.38
Dry yeast (6000)	1.09	0.65	21.07	10.63	82.33	83.02
Ascorbic acid (50)	1.34	0.58	23.61	18.21	81.49	82.36
Ascorbic acid (100)	1.39	0.82	26.58	19.04	82.23	83.59
Ascorbic acid (150)	1.48	1.22	33.74	25.12	83.24	84.41
Riboflavin (30)	1.06	0.69	23.61	16.86	81.24	82.20
Riboflavin (60)	1.33	0.75	27.40	23.09	80.94	83.95
Riboflavin (90)	1.34	0.97	29.12	34.15	82.23	84.49
LSD 0.05	0.07	0.01	0.38	0.09	0.30	0.06

foliar application of ascorbic acid caused pronounced increases in the percent and yield of essential oil of lemongrass [27] and Rosemary [28]. In this concern, Eid *et al.* [6] reported that ascorbic acid at (150 mg/l) pronouncedly increased the major components of the essential oil in the flowers of *Jasminum grandiflorum* L. However, no report could be traced in the literature for the effect of ascorbic acid, riboflavin and dry yeast on essential oil of Geranium, especially *Pelargonium graveolens* L.

Major Essential Oil Constituents: Data in Table 4 represented the major constituents of essential oil at the most effective promoting treatments of vitamins and dry yeast on the percent and yield. Gas liquid chromatography revealed that citronellol was

quantitatively the major constituent of oil in all treatments at both cuttings, other detected fractions of the oil were α -pinene, β -pinene, 1, 8-cineol, linalool, geraniol and carvon at all treatments. Dry yeast (4000 mg/l) was the most effective treatment for increasing citronellol followed by riboflavin (90 mg/l) then ascorbic acid (150 mg/l). Other constituents of the oil were slightly increased at the abovementioned treatments, especially at dry yeast (4000 mg/l).

Total Phenols Content: Significant pronounced increases in total phenols content were obtained at the highest levels applied in the following descending order: ascorbic acid (150 mg/l), riboflavin (90 mg/l) and dry yeast (4000 mg/l) as shown in Table 5. In accordance, other plants treated with ascorbic acid contained higher

content of total phenols as gladiolus [5] and chamomile [29]. Recently, Wu *et al.* [30] concluded that *Geranium sibiricum* represented a valuable natural antioxidant source after determination of polyphenolic compounds of the extract of this plant.

Total Flavonoids Contents: Data presented in Table 5 indicated that ascorbic acid (150 mg/l) was the most effective vitamins for significant and pronounced increase in total flavonoids content followed by that of riboflavin (90 mg/l) then that of dry yeast (4000 mg/l) at cutting (I). However, at cutting II, riboflavin (90 mg/l) treatment showed the highest total flavonoids. The importance of the flavonoids was known to possess significant antimicrobial activities and was utilized as natural plant protectants [31, 32]. It could be suggested that geranium flavonoids content may be an alternative to conventional fungicides in the control of storage grains (as wheat) and protein-rich seeds (as beans) against some fungi especially in developing countries.

Antioxidant Activity: Data in Table 5 showed that the antioxidant activity (as DPPH-radical scavenging capacity) of Geranium pronouncedly increased only at 4000 mg/l of dry yeast and at 150 mg/l ascorbic acid, this was true at both cuttings. In addition, treatment 90 mg/l riboflavin showed significant increase in antioxidant activity, especially at cutting II. Noctor and Foyer [33] reviewed the action of ascorbate and glutathione for keeping active oxygen under control. The increase in scavenging activity can be considered an advantage of ascorbic acid treatment. This could be attributed to the increases in total phenols and total flavonoids. In this concern, Neagu *et al.* [13] reported that concentrated *Geranium robertianum* extracts had a high antioxidant capacity (92 % of DPPH inhibition).

In conclusion, ascorbic acid (150 mg/l), riboflavin (90 mg/l) and dry yeast (4000 mg/l) pronouncedly increased vegetative growth, photosynthetic pigments, essential oil percent and yield as well as total phenols and total flavonoids. The antioxidant activity of *Pelargonium graveolens* L. proved to be increased under the effect of dry yeast (4000 mg/l) and at 150 mg/l ascorbic acid at both cuttings.

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