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Effect of Gibberellic acid and progesterone on growth, vase life and volatile component of *Lathyrus odoratus* plant

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ABSTRACT

The objective of this study was to evaluate the response to exogenous application of progesterone (prog.) and/or Gibberellic acid (GA₃) with level of 10, 20 and 30 ppm and of 150,200 and 250 ppm, respectively grown in pot experiments. The obtained data showed promotive effect of progesterone in number of branches, dry weight/plant, number of flowers/plant and decreased the number of days in appearance of the first flower and oil %. Foliar application of GA₃ showed asignificant effect on plant height, lower concentration hasted flower induction, the higher concentration of GA₃ treatment caused the higher percentage of oil. Using (Ag-NPs) silver nitrate was more effective on shelf life when plants sprayed with GA₃ 250 ppm. The volatile oils of the most effective treatments from flowers were obtained by hydrodistillation and analyzed by GC and GC/MS. A total of twenty two volatile compounds were detected, the most abundant compounds were belonging to the class of terpenoids, (Z)- β -ocimene (E)- β -ocimene, geraniol and linalool. A significant increase in (Z)- β -ocimene, which were 27.52% in GA₃ at 200 ppm and 20.19% in progesterone at 20 ppm, compared to control treatment which represent 12.43%. An opposite trend was observed for geraniol which exhibited 9.42% in control treatment and a reduction in the treatments had occurred.

Keywords: Growth regulators, *Lathyrus odoratus*, Antioxidant, volatile

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INTRODUCTION

Lathyrus odoratus (Sweet pea) plant is well known as ornamental plants, belonging to family leguminosae (Fabace) which play an important role in the diet of millions of people. In recent year, attention has been focused upon the application of several plant growth regulators to improve quantitatively and qualitatively of growth and production of many plants. The beneficial effect of gibberellic acid (GA₃) on several plants has been reported by several investigators (Lincoln and Eduardo, 2012; AKash Kumar *et al* (2014) and Faisal and Azza (2015).

Progesterone is one of the steroidal hormone, the brassinosteroids hormone occur ubiquitously in the plant kingdom. The occurrence of brassinosteroids (BRs) has been demonstrated in almost every part of plants (Bajguz and Tretyn, 2003; Andrzej and Shamsul, 2009). Also, Hayat and Ahmed (2003) reported that (BRs) play a critical role in arrangement of developmental progress e.g. stem and root growth, floral initiation and the development of flower and fruits. The improving effect of (BRs) on plant growth were reported by Youssef and Talaat (1995) on *Lavandula officinalis*, Youssef (1998) on *Nigella Sativa*, Abd El-Wahed *et al.*(2000) on *Triticum aestivum*.

Silver nitrate nanoparticles (Ag-NPs) were used for treating the cut flowers. Several studies have been reported the effects of nanoparticles in enhancing the ability to absorb and utilize water (Lu *et al.*, 2002 and Abdelraouf *et al.*, 2013). The longevity of vase life resulting from the in preservative solution of (Ag-NPs) which make as inhibitor for C₂H₄ action near the base of flower cut stem (Kofranek and Paul, 1972 and Abdelraouf *et al* , 2013).

The fragrance of plant results from the production of volatile compounds, most volatile compounds are synthesized de novo in the tissues from which they are emitted (Dudareva and Pichersky, 2000). The major sites of volatile emission in rose flowers are the petals, specifically the adaxial and abaxial epidermis where volatiles are produced and emitted (Bergougnoux *et al.*, 2007). The sweet pea cultivars have been perceived by the public to have reduced levels of fragrance. Little information is currently available as to whether this is due to changes in the total amount of volatiles released by the flowers or to changes in the chemical constituents of the floral bouquet. Similarly, previous investigations on the vase life of sweet pea (Sexton *et al.*, 1995) have revealed that some postharvest treatments may significantly prolong the period over which the flowers continue to release detectable volatiles. Clearly in order to attain a better understanding of the fundamental mechanisms involved, detailed analyses of the constituent volatiles are required.

To the best of our knowledge, no literature is currently available on the effect of growth regulators on the growth, vase life and the volatile components of *Lathyrus odoratus*. Therefore, the objectives of this study are determination of the growth, vase life and volatile components of *Lathyrus odoratus*.

MATERIAL AND METHODS

The experiment was carried twice during the two successive seasons at the green house of National Research Centre, of Giza, Egypt.

Seeds were sown in earthenware pots of 30 cm² in diameter and 50 cm depths that were filled with 10 kg soil. Each pot was filled with media containing a mixture of clay plus sand as 2:1 by volume.

Seeds were sown in November and irrigated regularly with tap water, after 15 days from sowing thinning is done in each pot. During the period of experiment the moisture of the soil were kept at fixed percentage of available water.

After 24 days from sowing two of hormones were used progesterone at conc. of 10, 20 and 30 ppm and GA₃ at conc. of 150,200 and 250 ppm the hormones sprayed twice during the plant life cycle, one after 24 days from planting and the other two weeks later. The experiment consists of 7 treatments replicated 5 times for each one and arranged in complete randomized design.

At flowering stage 120 days from sowing, samples were taken from each treatment and the following data were recorded.

plant height (cm), number of leaves, number of branches, stem diameter (cm), fresh weight of plant (g), fresh weight of leaves (g), dry weight of leaves (g), root length (cm), number of flowers, number of days from planting to flower(days), Vase life (days), oil percentage and oil chemical constituents.

Silver nitrate solution prepared and distilled water was used to make the dilutions. The same deionized water was used as control. The concentrations 0, 10, 20 and 30 ppm silver nitrate nanoparticles (Ag-NPs) were used for treating the cut flowers. The bottom of cut flowers immersed throughout the trail arranged at room temperature. The flowers were observed daily till the senescence of the petals flowers.

Extraction of volatile oils

The flower parts of *Lathyrus odoratus* in selected treatments were subjected to hydro distillation using a Clevenger-type apparatus for 3 h. After decanting and drying the oil over sodium sulfate anhydrous. The oils were stored in sealed vial in a refrigerator (6°C) before being analyzed by GC and GC/MS.

Determination of total Phenol Content

The total phenol concentration of the samples was determined by folin reagent as mentioned by (Lin and Tang, 2007). Briefly, 100 µL of extract in methanol (1.0 mg/mL) were mixed with 1.0 mL of distilled water and 0.5 mL of folin-ciocalcu's reagent (1:10 v/v). After mixing, 1.5 mL of 2% aqueous sodium bicarbonate were added, and the mixture was allowed to stand for 30 min with intermittent shaking. The absorbance was measured at 765 nm using a spectrophotometer. Total phenolic concentration is expressed as gallic acid equivalent (GAE) in mg per gram of extract. All assays were carried out in triplicate.

Radical scavenging of 1,1'-Diphenyl-2-Picrylhydrazyl (DPPH)

Sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 100, 150 and 300 µg/mL in methanol. Samples were added to 3 mL of methanolic DPPH (0.1 mM), prepared daily. The mixture was shaken and left to stand at room temperature in the dark. After 30 min, absorbance was measured at 517 nm against a blank containing all reagents except the test samples (Harborne and Williams, 2000). Assays were carried out in triplicate. The percentage of inhibition of DPPH (%) was calculated using the following equation:

$$I\% = (A_0 - A/A_0) \times 100$$

A_0 is the absorbance of the blank solution and A is the absorbance of the methanolic extract.

β-Carotene/Linoleic Acid assay

The β-carotene solution was prepared by dissolving 2 mg β-carotene in 10 mL chloroform; 1 mL of this β-carotene-chloroform solution was mixed with 20 mg linoleic acid and 0.2 g Tween 40. Subsequently, the chloroform was removed by a rotary evaporator at 45 °C. Distilled water (50 mL) was slowly added with vigorous agitation to form an emulsion. Emulsion aliquots (5 mL) were transferred with 0.2 mL of the extracts different concentrations (100-300 µg/mL, sample stock 1.0 mg/mL). Control samples were prepared with 0.2 mL methanol devoid of extract (Shahidi *et al.*, 2001). As soon as the emulsion was added to each tube, absorbance was read at 470 nm against blank (zero time). Tubes were placed in a water bath at 50 °C, and oxidation was monitored by absorbance at 15 min intervals until the color of β-carotene in the control sample had disappeared (105 min). The analyses were performed in triplicate.

Antioxidant activity (AA) was calculated as percent inhibition relative to the control:

$$\%AA = [1 - (A_i - A_t)/(A'_i - A'_t)] \times 100$$

A_i = absorbance of sample at zero time, A_t = absorbance of sample after incubation (105 min) at 50 °C, A'_i = absorbance of control at zero time, and A'_t = absorbance of control after incubation (105 min) at 50 °C. The IC_{50} , the concentration giving 50% inhibition of DPPH or β-carotene, were read off a graph of I% or AA% (percentage inhibition) versus extract concentration.

Chromatographic analysis

Gas Chromatography

GC analysis was performed on a Hewlett-Packard gas chromatograph (GC-5890 II; Hewlett-Packard GmbH, Bad Homburg, Germany) equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). Helium was used as a carrier gas (0.6 mL/min) and the capillary used was DB-5 (50m×0.2mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 2.5°C/min rate and kept constant at 220°C for 5 min.

Gas Chromatography/Mass Spectrometry

The chemical composition of the essential oils for both samples was analyzed using GC/MS technique. The mass spectrometer employed for GC/MS analysis was an HP 5973 mass selective detector in the electron impact (EI) ionization mode (70 eV); Hewlett-Packard 5890 gas chromatograph; capillary column HP-5 MS (60 m x 0.25 mm; film thickness: 0.25 µm capillary column, coated with phenyl methyl siloxane). The initial temperature of the column was 60°C and then was heated to 280°C with a 3°C/min rate. Injector and transfer line temperatures were set at 220°C and 280°C, respectively. Helium was used as the carrier gas at a flow rate of 0.6 mL/min. Sample size: 1µL; split 1:20; mass range: 40–400 amu, 3.99 scans/sec.

Compounds identification

Identification of the components was based on the comparison of their spectral data and retention indices with Wiley Registry of Mass Spectral Data 8th edition, NIST Mass Spectral Library and those described by Adams (2007). The percentage compositions of the oils are based on peak areas obtained without FID factor correction.

Data were subjected to analyses of variance according to Snedecre and Cochran (1990) and the means were compared using L.S.D. at 5 % probability.

RESULTS

Effect of GA₃ and progesterone on vegetative growth

Data presented in Table (1) showed significant decrease in plant height, no of leaves, stem diameter, root length in progesterone sprayed plants accompanied by significant increase in number of branches in response to 10, 20, 30 ppm progesterone. The highest increment reached 160% compared that of the control plants. In GA sprayed plants, 150 and 200 ppm increased only plant height by about 18.97% and 15.32% respectively over the control plants. However the higher concentration of GA showed lower performance than the lower concentration.

Table (1) also show that 20 ppm prog. Increased fresh weight/plant, fresh weight of leaves and dry matter gain by leaves. On the other hand, the GA₃ treatments showed decrease in this respect compared with the control plants.

Table (1) Effect of GA₃ and progesterone on vegetative growth of *Lathyrus odoratus* plant (Mean of two successive seasons)

Characteristics	Plant height (cm)	No. of leaves	No. of branches	Stem diameter (mm)	Root length (cm)	Fresh weight of leaves (g)	Dry weight of leaves (g)	Fresh weight of plant/(g)
Treatment								
Control	1.37	33-33	5.00	2.33	6.33	9.49	5.31	23.40
prog.10ppm	1.18	26.66	7.33	1.73	5.60	6.52	4.39	14.62
Prog.20ppm	1.17	31.33	13.00	2.33	5.00	21.80	6.09	31.70
Prog.30ppm	0.78	17.66	5.33	1.40	8.66	7.23	4.40	14.63
GA 150 ppm	1.63	25.00	2-33	1.20	3.00	4.60	3.93	9.52
GA 200 ppm	1.58	23.33	3-50	1.03	2.36	3.95	3.23	7.59
GA 250 ppm	1.25	19.66	2.33	1.30	3.83	5.94	3.81	7.42
L.S.D.	0.06	2.82	1.52	0.50	1.04	2.62	0.87	4.13

Prog.: progesterone GA₃: gibberellic acid

Effect of GA₃ and/or progesterone on flowering characteristics

The results reported in Table (2) also showed that 20 ppm concentration of prog. Induced the higher number of flowers compared with that of the control and other treatments. The lowest number of GA flowers was attained for 250 ppm sprayed plants.

Table (2) Effect of GA₃ and progesterone on flowering and oil % of *Lathyrus odoratus* plant (Mean of two successive seasons)

Characteristics	No. of Flowers/plant	No. of days from planting to appearance of the first flower	Oil %
Treatment			
Control	15.00	98.66	0.06
prog.10ppm	15.00	100.00	0.12
Prog.20ppm	17.37	96.66	0.35
Prog.30ppm	7.33	97.66	0.16
GA 150 ppm	9.00	95.00	0.46
GA 200 ppm	6.00	101.33	1.97
GA 250 ppm	4.33	103.33	1.27
L.S.D. at 0.05	1.06	1.23	0.06

Data in Table (2) also reported that, for plants sprayed with 20 ppm prog., in general significant earliness of flowering, whereas the lower concentration showed a delaying effect reached 1.35% less than the control plants.

Data in Table (2) indicated that 150 ppm GA₃ sprayed plants hastened flowers induction but GA 200 and 250 ppm sprayed plants induced retardation of flowers which reached 2.70% and 4.73% above the control plants.

Oil percentage

The observed results of oil% of *Lathyrus odoratus* plant in response to GA₃ and/or prog. Treatments appeared to be depended mainly on the conc. used. In general, oil % found to be positive and showed significant increase in all treatments compared with the control plants.

Prog. Treatments induced marked change in oil % where 20 ppm sprayed plants induced significant increments reached 583% over the control. The pronounced increments of oil % in GA₃ treatments 200 and 250 ppm sprayed plants were reached 3183.33% and 2016.66% respectively over the control plants.

Effect of silver nitrate nanoparticles (Ag-NPs) on vase life of cut flowers

Generally, we can indicated from data presented in Fig (1) that, all preventative solution of (Ag-NPs) treatments (0, 10, 20 and 30 ppm) recorded increases in shelf life as compared with control. The highest values were obtained from treated plants by 250 ppm GA + 30 ppm (Ag-NPs) solution. It recorded 41.34 % pass the control. Followed by (10 and/or 30 ppm prog. +20 ppm (Ag-NPs) solution. This recorded 83.25 % over the control.

DISCUSSION

Our results showed increase in *Lathyrus odoratus*, plant height, stem diameters, fresh and dry weight of leaves in response to 150 and 200 ppm GA₃. In previous studied Kaur *et al* (1998) reported that addition of exogenous GA cause an increase in seedling growth by enhancing the availability of endogenous gibberellic acid. However in this respect gibberellins are most often associated with the promotion of stem growth and the application of gibberellins to intact plants can induce large increases in plant height, this may be attributed to the stimulation effect of the lower concentration 150 and 200 ppm on the cell division and /or cell expansion and cell extensibility. These results hold true with the finding of AKash Kumar *et al* (2014).

The observed decrease in leaves number, stem diameter, fresh and dry weight of GA₃ sprayed plants in agreement with Lincoln and Eduardo (2012), who reported that GA₃ treatments increased plant height and is accompanied by a decrease in stem thickness, leaves number and fresh, dry weight of leaves.

Progesterone treatments has promotive effect on number of branches and 20 ppm progesterone sprayed plants induced the highest plant fresh weight and fresh and dry weight of leaves. These results hold true with the finding of Ebtihal (2008) on *Triticum aestim* and El- Sherbeny *et al.* (2008) on *Calondula officinalis*, Hagagy *et al.* (1999) on papaga seeds and El-Shamy (2002) on bougainvilla and Sami (2010) on gerbera plants.

In this respect Mandava and Thonson (1983) and Hamada (1986) recorded that BRs may be regarded as a new group of plant hormone with regulatory function in cell elongation and cell division and may also have a role in the control of RNA synthesis. Sami (2010) also reported that 10 ppm progesterone led to increase in endogenous hormone cytokinine, IBA and higher concentrations 20 and 30 ppm showed apromotive effect on both GA₃ and IAA.

Data of our Experiments also showed that GA₃ treatments decreased flowers number these results are harmony with the finding Faisal and Azza (2015) on *Euphorbia milii*.

The promotive effect of 10 ppm progesterone treatments on number of flowers of *Ladyrus odyratus* plants and decrease the number of days in appearance of the first flowers were in agreement with Anna Janeezka *et al* (2003) who mentioned that progesterone stimulated flowering induction in vitro in *Arabidopsis thaliana*. The same results was recorded by El-Sherbeny *et al.* (2009).

Thus, it is evident that progesterone is a steroidal sex hormone. These results hold true with finding of Sami (2010) on gerbera.

Our results also showed that, GA₃ treatments gave a pronounced increase in oil % compared with control and other treatments. These results are in harmony with El-Sahhar *et al.* (1984) and Bedour, *et al* (1994) on basil plants.

Data of our vase life experiment showed that, the beneficial effect of Ag-NPs vase life solution on longevity of cut flowers the same results reported by Halevy and Mayak (1980) and they added also, Ag-NPs has widely been assumed to be the result of powerful biocidal activity of the ion.

The active moiety of presentitive solution of (Ag-NPs) in improving longevity is clearly that the Ag⁺ ion is a potent inhibitor of action of C₂H₄ (David and Micheal,. 1983). Ag No₃ moves very poorly in cut stem and Ag⁺ ion had highly affinity for anionic groups in xylem wall. Therefore, the longevity of vase life resulting from the in

presentitive solution of (Ag-NPs) which make as inhibitor for C₂H₄ action near the base of flower cut stem. The obtained results in agreement with Koframk and Paul, (1972) and Abdlelraouf *et al* (2013).

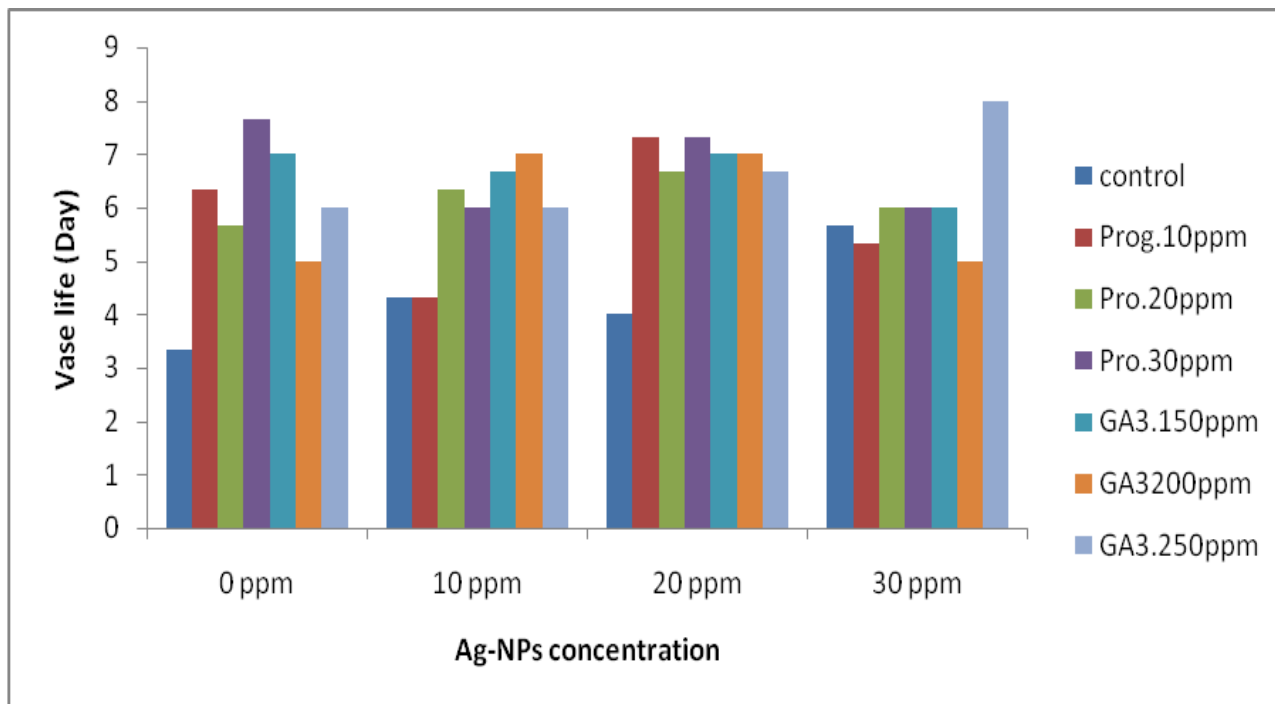


Fig. (1) Effect of AgNO₃ (Ag-NPs) as a persentitive solution on vase life cut flowers of *Lathyrus odoratus*

The total phenolic content and antioxidant activity

The total phenolic content mg GAE/g of essential oil extracted from *L. odoratus* ranged between 81.2 and 112.8 mg/g in treatments of progesterone at 10 ppm and gibbrellic acid at 300 ppm, respectively (Table 3). The highest phenolic content was obtained from gibbrellic acid treatment at 300 ppm applications, which was approximately 10 folder higher than that of control treatment.

Table (3) Effect of progesterone and gibbrellic acid (GA₃) concentrations (ppm) of essential oil yield, total phenol content and antioxidant activity

Treatment	Control	Progesterone			GA ₃		
		10	20	30	150	200	300
T.PH (mg/g)	11.8±0.13	81.2±0.78	95.7±0.82	98.4±0.18	88.6±0.52	103.7±0.46	112.8±0.92
IC ₅₀ DPPH			180.81			34.23	
IC ₅₀ β-Carotene		473.67				52.79	

Phenolic compounds exhibited their beneficial health effects mainly through their antioxidant activity. These compounds are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (Heim *et al.*, 2002). Therefore, determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts (Canadanovic-Brunet *et al.*, 2005). The obtained results (Table 3) revealed that an increase in phenolic content with increase the growth regulator under investigation. However, a remarkable increase in gibbrellic acid treatment had occurred compared to progesterone (Table 3). Our results in good agreement with Giannakoula *et al.* (2012) who found that gibberellic acid, indole-3-acetic acid and kinetin significantly increased specific phenolic compounds (gallic acid and rutin).

Evaluation the antioxidant activity is complex, and no standard antioxidant assay has yet been agreed (Frankel and Meyer 2000). Therefore, in the present study the selected treatments antioxidant activity were evaluated using DPPH and β-carotene assays. The DPPH method is a preferred method because it is fast, easy

and reliable and does not require a special reaction and device. DPPH is a stable, synthetic radical that does not disintegrate in water, methanol, or ethanol. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components.

Highest DPPH radical scavenging activity, i.e. lowest IC₅₀ value, was observed in gibberlic acid treatment with 34.23 ug/mL compared to progesterone which exhibited 180.81ug/mL (Table 3). Our results in good agreement with Eraslan et al. (2007) who reported that exogenous application of salicylic acid, enhanced growth, physiological process and antioxidant activity of carrot plants grown under salinity stress. Table (3) showed a significant positive correlation between phenolic content and antioxidant activity in *Lathyrus odoratus* essential oil and these results in agreement with previous studies (Giovaneli and Buratti 2009; Koca and Karadeniz 2009).

Effect of GA₃ and progesterone on volatile oil composition

The volatile oils extracted using hydrodistillation from flower parts of *Lathyrus odoratus* were subjected to GC and GC-MS analysis and the obtained results are summarized in Table (4). Identification of the components of the oils was performed using the retention indices on DB-5 and by comparison of its mass spectrum with data in the literature (Swigar and Silverstein, 1981 and Mac-Lafferty, 1993) or those of our own database. A total of twenty two volatile compounds were detected, the most abundant compounds were consistently found to be (Z)-ocimene (E)-ocimene, geraniol and linalool. A significant increase in (Z)-ocimene which were 27.52% in GA₃ and 20.19% in progesterone treatment compared to control one which represent only 12.43%. An opposite trend was observed for geraniol which exhibited 9.42% in control treatment and a reduction in the treatments had occurred. (Z) and (E)-ocimene belong to monoterpenes hydrocarbons were the most abundant volatile compounds with values 12.43% and 15.37%, respectively in control treatment.

Table (4) Volatile composition of the oils of *Lathyrus odoratus* treated with GA₃ and progesterone hormone

Volatile compound	KI ^a	Control	GA ₃ 200 ppm	Progesterone 20 ppm	Method of identification ^c
Methyl butanoate	728	3.73 ^b	0.80	0.72	MS,KI,St
⌊-Thujene	925	1.71	7.76	0.31	MS,KI
⌊-Pinene	937	6.40	0.47	2.72	MS,KI,St
Methyl hexanoate	975	1.48	2.65	2.11	MS,KI
Sabinene	972	0.71	4.46	4.77	MS,KI
⌊⌊ Pinene	980	0.79	0.06	0.62	MS,KI,St
⌊-Myrcene	994	0.38	0.16	0.93	MS,KI
(Z)-⌊-ocimene	1064	12.43	27.52	20.19	MS,KI
(E)-⌊-ocimene	1086	15.37	16.59	19.83	MS,KI,St
Benzaldehyde	1092	1.61	0.09	1.78	MS,KI,St
Linalool	1104	9.00	10.02	11.92	MS,KI,St
Methyl benzoate	1217	2.64	0.02	0.52	MS,KI
Citronellal	1249	2.53	2.04	2.53	MS,KI,St
Decanal	1298	0.43	0.15	1.85	MS,KI
⌊-Citronellol	1325	0.47	0.41	3.89	MS,KI
Nerol	1334	8.10	9.61	10.43	MS,KI
Geraniol	1360	9.42	4.21	4.59	MS,KI
(Z)-citral	1374	1.40	0.05	0.53	MS,KI,St
(E)-citral	1419	0.55	1.21	0.82	MS,KI
Neryl acetate	1438	6.98	1.91	1.64	MS,KI
Geranyl acetate	1452	7.65	3.82	4.42	MS,KI
Caryophyllene	1508	0.49	1.50	1.38	MS,KI

^a: KI: = "Kovat Index" determined in reference to a homologous series of *n*-alkanes on DB-5 column

^b: The percentages of each component are reported as raw percentages based on total percentages without standardization,

^c; Compounds identified by GC-MS(MS) and/or by comparison of MS, St: standard and KI of standard compound run under similar conditions

(E)-ocimene has previously been reported as the major constituent of floral scents from other leguminous flowers including faba beans (*Vicia faba*) (Sutton *et al.*, 1992). On the other hand, the ⌊-Myrcene was detected with trace amount in all treatments as following: in the control treatment with a concentration

0.38% while a reduction in GA₃ with a concentration 0.16% compared to increase in progesterone treatment with a concentration 0.93% (Table 4).

The obtained results in contrast to Bartk *et al.* (2003) who found that the monoterpenes (C₁₀ compounds, such as myrcene) was detected as the main fragrance components of *Lathyrus vernus* (L.), these differences may be due to the method of extraction.

The monoterpene alcohols such as linalool, nerol and geraniol accounted on average for 26.52% of the total volatiles detected in the investigated samples. This alcohol has been detected in the floral scents of a wide range of plant species (Kndsen *et al.*, 1993) including alfalfa (Loper, 1972) as well as in the head space of faba bean foliage (Blight *et al.*, 1984). The first two-monoterpene alcohols showed significant increase after the treatments, in contrast to geraniol, which reduced from 9.42% in control treatment, compared to 4.21% and 4.59% in GA₃ and progesterone treatments, respectively. This results in good manner with Sudria *et al.* (1999) who mentioned that quantitative changes in the major monoterpene components (1,8-cineole, fenchol, borneol and camphor) and sesquiterpene content of plantlet oil of *Lavandula dentata*, were also observed in response to the effect of varying growth regulator concentration in the culture medium. Generally, our treatments promoted the increase in volatile compounds except esters; e.g.: neryl acetate which represent 6.98% in control treatment compared to 1.91% and 1.64% in GA₃ and progesterone, respectively (Table 4). Our results in good agreement with Jiang *et al.* (2011) who found that jasmonic acid promoted the emission of floral volatiles of from the flowers of chinese wisteria (*Wisteria sinensis*) and japanese wisteria (*W. floribunda*). In addition, a significant reduction had occurred in methyl butanoate and α -Pinene which exhibited 3.73% and 6.40%, respectively in control while they were 0.80%, 0.47% in GA₃ and 0.72%, 2.72% in progesterone, respectively (Table 4). In cut sweet pea flowers, endogenous and exogenous ethylene accelerated senescence and loss of fragrance (Sexton *et al.*, 2005).

REFERENCES

- [1] Abd El Wahed M. S. A., Farahat M. M. and El Habba E. (2000). Response of wheat (*Triticum aestivum*) to seed rates and stigmaterol. *J. Agric. Sci. Mansoura Univ.* 25(12):7649-7658.
- [2] Abdelraouf R.E., Metwally S.A and Mehana H.M. (2013). Reuse of Treated Wastewater for Cultivation of Roses for Decoration Only and Not for Food Industries. Proceedings of BGL, International Symposium on Botanical Gardens and Landscape 59-71, August 5-8, Golden Tulip Sovereign Hotel, Bangkok, Thailand.
- [3] Adams R.P. (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, Edn4, Allured Publishing, Carol Stream, Illinois, USA.
- [4] Akash K., Tarunk B., Neha S. and Dr.E.P.Lal. (2014). Effect of gibberellic acid on growth, quality and yield of tomato (*Lycopersicon esculentum* Mill.). *IOSR. Journal of Agriculture and Veterinary Science (IOSR-JAVS)*.
- [5] Andrzej B. and Shamsul H. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiology and Biochemistry*, 47, 1-8.
- [6] Anna J., Wladyshow F., Jolanta B. K., Izabela M. and Zbigniew J. (2003). The influence of animal sex hormones on the induction of flowering in *Arabidopsis thaliana* comparison with the effect of 24 epibrassinolide. *Plant Cell, Tissue and Organ Culture*, 72:147-151.
- [7] Bajguz A. and Tretyn A. (2003). The chemical characteristic and distribution of brassinosteroids in plants, *Phytochemistry*, 62, 1027-1046.
- [8] Bartk P., Petr, B., Lubomr, C., Lenka O. and Zdenek S. (2003). SPME – A valuable tool for investigation of flower scent. *J. Sep. Sci.* 26: 715-721
- [9] Bedour H. Abou-Leila, Aly M.S. and Nagwan F. Abdel-Hady (1994). Effect of foliar Application of GA and Zn on *Ocimum basilicum* L. Grown in different soil type. *Egypt. J. Physiol. Sci* 18 (2): 265-3380.
- [10] Bergougnoux V., Caissard J.C., Jullien F., Magnard J.L., Scalliet G., Cock J.M., Huguency P. and Baudino S. (2007). Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds. *Planta* 226, 853-866.
- [11] Blight M. M., Pickett J. A., Smith M. C. and Wadhams L. J. (1984). An aggregation pheromone of *Sitona lineatus*. *Naturwissenschaften* 71: 480-487
- [12] Canadanovic-Brunet J.M., Djilas S.M. and Cetkovic G.S. (2005). Free-radical scavenging activity of wormwood (*Artemisia absinthium*) extracts. *J Sci Food Agric* 85: 265-272.

- [13] David W.F. and Michael S.R. (1983). Factors affecting the vase life of fronds of maidenhair Fren. *Scientia Horticulturae*. 21:181-188.
- [14] Dudareva N. and Pichersky E. (2000). Biochemical and molecular genetic aspects of floral scents. *Plant Physiol*. 122, 627–633.
- [15] Ebtihal M. AE. (2008). Physiological effects of some phytohormones on growth, productivity and yield of wheat plant cultivated in new reclaimed soil. Ph.D. Thesis. Collage of Women for Arts, Science and Education. Ain Shams Univ.
- [16] El Sahhar K.F., Fouad M.K., Fahmi R. and Reiad F. (1984). Effect of gibberellic acid (GA_3) on some botanical and chemical characteristics of basil (*Ocimum basilicum* L.) *Annals Agric.Sci.Fac.Agric.Ain Shams Univ*.
- [17] El Shamy H.A. (2002). In vitro culture studies on bougainvillea plant. Ph.D. Thesis Faculty of Agriculture, Zagazig University.
- [18] El Sherbeny, M.R; Mohamed, S.A and Aboubaker, A.A. (2009). Effect of β -sitosterol and gibberellic acid on leaf angle, growth, flowering and biochemical constituents of marigold *Calendula officinalis* L. *Medicinal and Aromatic Plant Science and Biotechnology*, 3(1):21-27.
- [19] Eraslan F., Inal A, Gunes A. and Alpaslan M. (2007). Impact of exogenous salicylic acid on growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Sci. Hort*. 113: 120-128.
- [20] Faisal S. and Azza M. Abdel.Moniem (2015). Effect of some factors on growth and development of *Euphorbia nilli* var, *Longifolia*. *Middle East Journal of Agriculture Research*. ISSN 2077-4605.
- [21] Frankel E. N. and Meyer, A. S. (2000). The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric*. 80: 1925-1941.
- [22] Giovanelli G. and Buratti S. (2009). Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. *Food Chem*. 112: 903-908.
- [23] Hagagy N. A. , Zaied N.S. and Khafagy S.A. (1999). Preliminary studies on the effect of steroid sex hormones on papaya plants. *Zagazig J. Agri. Res*. Vol. 26, No. (3A):725-741
- [24] Halevy A.H. and Mayak S. (1980). Senescence and postharvest physiology of cut flowers. Part 2. In: J. Janick (Editor), *Horticulture Reviews 2*. AVI Publishing, Westport, CT. pp: 59-143.
- [25] Hamada K. (1986). Brassinolide some effects for crop cultivations. *Conf. Proc. Int. Seminar Plant Growth Regul*. Tokyo, Japan, Oct.15.113-114.
- [26] Harborne J.B. and Williams C.A. (2000). Advances in flavonoid research since 1992. *Phytochem*. 55: 481–504.
- [27] Hayat S. and Ahmad A. (2003). *Brassinosteroids: Bioactivity and Crop Productivity*, Kluwer Academic Publishers, Dordrecht.
- [28] Jiang Y. , Xinlu, C. , Hong, L. , Fei W. and Feng C. (2011). Floral Scent in *Wisteria*: Chemical Composition, Emission Pattern, and Regulation. *J. AMER. SOC. HORT. SCI*. 136(5):307–314
- [29] Kaur S. , Gupta A.K. and kaur N. (1998). Gibberellin A_3 reverses the effect of soft stress in chick pea (*Cicer arietinum* L.) seedlings by enhancing amylase activity and mobilization of starch in cotyledons. *Plant Growth Regul*. 26, 85-90.
- [30] Koca I. and Karadeniz B. (2009). Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey. *Sci. Hort*. 121: 447-450.
- [31] Kofranek A.M. and Paul J.L. (1972). Silver-impregnated stems aid carnation flower longevity. *Florists' Review*. 151(3913):24-25.
- [32] Lin J.Y. and Tang C.Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem*. 101, 140–147.
- [33] Lincoln T. and Eduardo, Z. (2012). *Plant Physiology (Fifth Edition)*. Sinauer Associates Inc., Publishers Sunderland, Massachusetts. U.S.A.
- [34] Loper, G.M. (1972). *Medicago sativa* and *Citrus depressa* flower volatiles. *Phytochem*. 11:1865-1869
- [35] Lu, C.M., Zhang C.Y., Wen J.Q., Wu G.R. and Tao M.X. (2002). Research of the effect of nanometer materials on germination and growth enhancement of glycine max and its mechanism. *Soybean Science*. 21:168-172.
- [36] Mac-Lafferty F.W. (1993). *Registry of Mass Spectral Data*. John Wiley & Sons., New York, NY.
- [37] Mandava, N.B. and Thomson, M.J. (1983). Chemistry and functions of brassinolide. In *Proceedings of the Isopentenoid Symposium*, ed. W.D. Nes, G.Fuller, L.S.Tsai pp.28:401-431. New York: Dekker.

- [38] Sami, A.M. (2010). Physiological and anatomical studies on the effect of gamma and laser irradiation and some bioregulators treatments on the growth, flowering and keeping quality of gerbera. Ph.D. Thesis. Fac. of Agric., Zagazig Univ.
- [39] Sexton R., Stopford A.P., Moodie W.T. and Porter A.E.A. (2005). Aroma production from cut sweet pea flowers (*Lathyrus odoratus*): the role of ethylene. *Physiol. Plant.* 124, 381–389.
- [40] Shahidi F., Chavan U.D., Naczek M. and Amarowicz, R. (2001). Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). *J. Agric. Food Chem.* 49, 926–933.
- [41] Snedecor G.W. and Cochran W.G. (1990). *Statistical Methods* 8th Ed Iowa State Univ., Iowa, U.S.A.
- [42] Sudria C., Pinol M.T., Palazon J., Cusido R.M., Vila R., Morales C., Bonfill M. and Canigueral S. (1999). Influence of plant growth regulators on the growth and essential oil content of cultured *Lavandula dentata* plantlets. *Plant Cell, Tissue and Organ Culture* 58: 177–184.
- [43] Sutton C.J., Keegans, S.J., Kirk W.D.J. and Morgan E.D. (1992). Floral volatiles of *Vicia faba*. *Phytochem.* 31:3427–3428.
- [44] Swigar, A.A. and Silverstein, R.M. (1981). *Monoterpenes*. Milwaukee, WI, USA: Aldrich Chemical Company.
- [45] Youssef A. A. (1998). Influence of brassinosteroid and zinc on growth, yield and seed composition of *Nigella sativa* L. *J. Agric. Sci. Mansoura Univ.*, 23(10):4551-4558.
- [46] Youssef A. A. and Talaat I. M. (1998). Physiological effect of brassinosteroid and kinetin on the growth and chemical constituents of *Lavandula officinalis* L. *Plants. Annals Agric. Ain Shams Univ., Cairo*, 43 (1):261-272.