International Journal of Horticultural Science and Technology
Vol. 6, No. 1; June 2019, pp 51-62
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2019.270762.265
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Effect of Exogenous Application of L-arginine and Sodium Nitroprusside on Fruit Abscission and Physiological Disorders of Pistachio (*Pistacia Vera* L.) Scions

Maryam Eslami[1], Fatemeh Nasibi[1], Khosrow Manouchehri Kalantari[1], Masoud Khezri[2] and Hakimeh Oloumi[3*]

1. Department of Biology, Faculty of Science, Shahid Bahonar University, Kerman, Iran

2. Department of Horticultural Sciences, Faculty of Agriculture, Shahid Bahonar University of Kerman

3. Department of Ecology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

(Received: 3 December 2018, Accepted: 8 March 2019)

Abstract
Pistachio yield are often negatively affected by some physiological problems such as abscission of inflorescence buds and fruits, deformed or blank nuts, and non-split shells. In the present study the effect of exogenous application of arginine (Arg) (a substrate for nitric oxide (NO) synthase) and sodium nitroproside (SNP), as a NO-donor was investigated on yield production, fruit and inflorescence buds abscission, and physiological parameters of *Pistacia vera*. The experiment was conducted in randomized complete block design with factorial structure in a commercial pistachio orchard. Factors were included two levels of Arg (0.5 and 1 mM), two levels of SNP (50 and 100 μM) and their combination applied at two distinct time; one week before full bloom (first stage) and five weeks after full bloom (second stage). Comparing to SNP-treated trees, Arg was more effective on growth and physiological parameters of shoots and on fruit and inflorescence buds abscission, and on fruit parameters such as split and non-split shells, blank nuts, number of nuts per ounce. Effects of these treatments were considerably depending on the time of application and the concentrations of applied compounds. It is proposed that the effects of Arg and SNP may be related to the NO signaling and polyamines production.

Keywords: Fruit abscission, Inflorescence buds abscission**,** *Pistacia vera*, Nut splitting.

Abbreviations: Arg, Arginine; **AVG,** Aminoethoxyvinylglycine; **NO,** Nitric oxide; **PAs,** Polyamines; **SNP,** Sodium nitroproside.

Introduction

Pistachio (*Pistasia vera* L.) is an economically important product that is cultivated in arid and semi-arid areas. Nowadays; those countries that are producing pistachio with better fruit characteristics are more successful. Therefore, there is a high demand for improving pistachio quality and production. Abscission of inflorescence buds and fruits, fruit blankness, non-splitting shells and early splitting and deformation of nuts are among the main concerns related to production of pistachio (Crane and Iwakiri 1981, 1985;

* Corresponding Author, Email: oloumi.ha@gmail.com

Fabbri et al. 1998; Nzima et al. 1997). It is proposed that competition between floral buds and developing nuts for carbohydrates and other resources are of main reasons for abscission in plants (Crane and Al-shalan 1977; Porlingis 1974; Takeda et al. 1980). Annual accumulation and mobilization of carbohydrates in tree fruits is greatly affected by availability of carbon reserves, supplied assimilate from photosynthesis and crop loading (Samach and Smith 2013; Wright 1999).

Abscission is a highly regulated process that involves structural, biochemical, and molecular changes, which ultimately resulting in the detachment of plant organs (Roberts et al. 2002; Lewis et al. 2006). In an agricultural context, abscission is a major limiting factor for crop productivity (Estornell et al. 2013). Fruit abscission negatively affects fruit production, harvest, and postharvest processes (Eo and Lee 2011). Natural fruit abscission in many fruit trees is influenced by the species, physiological state of the tree and the environmental factors (Stephenson, 1981). The importance of plant growth regulators on fruit abscission and yield production has been proved in many plant species. Fruit abscission is finely mediated by the signals originating from phytohormones, saccharides, polyamines (PAs), H_2O_2 and *etc* (Iglesias et al. 2006).

The application of benzyl adenines and urea was considered as a promising technique for controlling bud abscission in plants (Khezri et al., 2010). Arginine (Arg) is a precursor for biosynthesis of polyamines (PAs), agmatine and proline as well as cell signaling molecules such as glutamine and nitric oxide (NO) (Chen et al., 2004; Liu et al., 2006). The metabolites originated from Arg, especially NO, polyamines and proline are all multifunctional molecules in plant, mediating an array of physiological and biological processes, including responses to abiotic and biotic stresses, programmed cell death, stomatal closure, seed germination, and root development (Arasimowicz and Wieczorek 2007; Groppa and Benavides 2008). The effects of NO, PAs or proline have been reported on delaying of senescence and enhancing the longevity of some flowers and fruits (Kumar et al. 2010). It has been reported that polyamines and NO can decrease ethylene production through ethylene inhibition (Zhu and Zhou 2007). It was reported that application of spermine (SPM); a polyamine, decreased the abscission of inflorescence buds in "on" year pistachio trees. These finding supports the idea that free polyamines are able to trigger the abscission process of inflorescence buds in pistachio (Khezri et al., 2010).

Despite to the high importance of pistachio in terms of the economy and foreign exchange earnings, there is not enough information regarding hampering nut disorders in pistachio. In this research Arg (a substrate for nitric oxide (NO) synthase) and sodium nitroproside (SNP), as a NO-donor were exogenously applied on pistachio trees to identify their role on abscission, yield production and fruit quality. The related physiological traits were also investigated on the treated plants.

Materials and methods

The experiments were conducted on 14-year-old pistachio trees ('Ahmad-Aghaei' cultivars scions grafted on *Pistacia vera* Badami-Riz rootstocks) at the experimental orchard located in Sirjan county (36º39´N and 49º12´E, 1766 m above sea level) in southeast of Kerman province, Iran.

Seventy-two "on" trees were selected during 2016. Three uniform shoots from each tree were chosen during "on" status and labeled one week before foliar application. Each selected shoot included three clusters with no lateral shoots. Treatments were included Arg (0.5 and 1 mM) and SNP (50 and 100 μM) and their combination. The experiment consisted of nine treatments including: (1) control; (2) Arg (0.5 mM); (3) Arg (1mM); (4) SNP (50 μM); (5) SNP (100 μM); (6) Arg (0.5 mM) + SNP (50 μM); (7) Arg (0.5 mM) +

SNP (100 μM); (8) Arg (1mM) + SNP (50 μM); (9) Arg (1mM) + SNP (100 μM). These treatments were applied separately at two time-points: a week before full bloom and five weeks after full bloom. Full bloom was defined as the date when 80% of the flowers on each tree were open. Experiment was designed as a randomized complete block with three replications.

Inflorescence bud abscission and fruit abscission

The number of initiated inflorescence buds and the total number of abscised buds on the individual current-year shoots were counted six weeks after full bloom and also at the harvesting time. The percentage of inflorescence bud abscission was calculated using following formula:

$$\text{Inflorescence bud abscission\%} = \frac{\text{number of abscised buds}}{\text{total number of buds initiated on each shoot}}$$

The number of fruit set and the total number of abscised fruits on each cluster were counted two weeks after full bloom (first stage), five weeks after full bloom (second stage) and at the harvesting time, respectively. The percentage of fruit abscission was calculated based on the following equation:

$$\text{Fruit abscission\%} = \frac{\text{the number of abscised fruits}}{\text{the initial number of fruit set on each shoot}}$$

Fruit characteristics

At the harvesting time, all the clusters were detached from each shoot and hand sorted into blank, non-split and split nuts.

Fresh weight, yield per shoot and number of nuts per ounce

Total number of nuts per cluster was counted. Nuts with hulls were weighted for calculating the fresh weight in each shoot. Nuts without hulls were dried and weighted for calculating the number of nuts per ounce. The yield was calculated by weighting the total dried split and non-split nuts harvested from each shoot.

Growth parameters

The length, diameter and leaf area of current-year shoots were measured four months after the harvesting time (December, 2016).

Photosynthetic pigments

To determine the content of photosynthetic pigments, 0.1 g fresh leaves extracted in 80% acetone. After filtration, the absorption was spectrophotometrically read at wavelength of 646.8, 663.2 and 470 nm (Lichtenthaler, 1987). Chlorophyll *a*, chlorophyll *b* and carotenoids contents were determined using following formulas:

$$\text{Chla} = 12.25\ A_{663.2} - 2.79\ A_{646.8}$$
$$\text{Chlb} = 21.21\ A_{646.8} - 5.1\ A_{663.2}$$
$$\text{Carotenoids} = (1000A_{470} - 1.8\ \text{chla} - 85.02\ \text{chlb})/198$$

Total soluble sugar

Total soluble sugar was determined using anthrone reagent and glucose as standard (Roe 1955). 100 mg fresh tissue were pulverized in 2.5 ml of 80% ethanol and extracted for 60 min at 95ºC. The extract was filtered and kept for alcohol evaporation. The precipitate was dissolved in 2.5 ml distilled water. Test solution (1 ml) added into a 10-mL test tube and cooled on ice. Five mL ice-cold anthrone reagent was added and heated for 11 min at 100 ºC and cooled rapidly. The absorbance was recorded at 630 nm against water.

Total soluble proteins and enzyme extraction

Frozen shoot samples (0.5 g) were homogenized in 2.5 ml of 50 mM phosphate buffer (pH 7) containing 1 M EDTA, 1 mM PMSF, and 1% PVP. The homogenate solution was centrifuged at 20000g at 4°C for 20 min and the clear supernatant was used directly for the assay of enzyme activity and estimation of protein.

Protein content was determined according to the method of Bradford (1976), Bovine serum albumin used as standard. 5 ml Biuret reagent (coomassie

brilliant blue in ethanol 95% and ortho-phosphoric acid 85%) was added to 100 µl protein extract. The absorbance was read by spectrophotometer at 595 nm after 5 min (Bradford, 1976).

Determination of enzyme activities

Catalase (EC 1.11.1.6) activity was determined according to the method of Dhindsa et al. (1981). The reaction started after addition of 15 mM H_2O_2 to reaction mixture that was consisted of 50 mM potassium phosphate buffer (pH 7.0), and 100 ml of enzyme extract. CAT activity calculated based on absorbance reduction at 240 nm in a min and it was expressed as U/mg protein.

Ascorbate peroxidase (EC 1.11.1.11) activity was determined spectrophotometrically according to the method of Nakano and Asada (1981). The reaction solution contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2 and 150 µl enzyme extract. The oxidation of APX by H_2O_2 was followed by measuring the decrease in absorbance within 1min at 290 (extinction coefficient (ε) of 2.8 $mM^{-1}cm^{-1}$) (Nakano and Asada 1981).

The guaiacol peroxidase (EC1.11.1.7) activity was determined using guaiacol as a substrate and the method of Plewa et al. (1991). Reaction mixture consisted of 25 µl of enzyme extract, 2.77 ml of 50 mM phosphate buffer) pH 7.0), 0.1 ml of 1% H_2O_2 (V/V), and 0.1 ml of 4% guaiacol (V/V). The increase in absorbance at 470 nm was recorded for 3 min (Zhang et al. 2005). One unit of GPX activity was defined as the amount of enzyme that caused an increase in the absorbance of 0.01 min^{-1}.

Statistical analysis

The individual shoot was considered as the experimental unit. The analyses of variance were performed using the SAS software (SAS Institute Inc., Cary, NC, USA). Means were separated by Duncan's multiple range test at $P < 0.05$.

Results

Inflorescence bud and fruit abscission

Application of treatments one week before full bloom had no significant effect on abscission percentage of inflorescence buds compared with the control (Table 1). SNP (100 µM) and Arg 0.5 mM + SNP 100 µM significantly increased inflorescence buds abscission in the first stage. Arg (0.5 and 1 mM) effectively decreased the bud abscission five weeks after full bloom. Combination treatments of Arg 0.5 mM + SNP 100 µM; Arg 1 mM + SNP 50 µM and Arg 1 mM + SNP 100 µM remarkably decreased inflorescence buds abscission in the second stage.

Application of Arg (0.5 and 1 mM) one week before full bloom significantly decreased the percentage of fruit abscission in comparison with the other treatments (Table 1). The results showed that Arg (0.5 mM), SNP on concentrations of 50 100 µM decreased the fruit abscission in the second stage. Also, at this stage a significant decrease was observed in fruit abscission in Arg 0.5 mM + SNP 100 µM and Arg 1mM+ SNP 50 µM (Table 1).

Fruit characteristics

Exogenous application of Arg (0.5 and 1 mM) had significant effect on blank and split shells in the first stage and non-split shells in the both stages (Table 2). SNP (50 µM) significantly decreased the percentage of blank nuts five weeks after full bloom. 100 µM SNP decreased the non-split shells in both stages but had no significant effect on the percentage of blank and split nuts at different stages. The results showed that the combined treatments improved the blank, non-split and split nuts in different stages of application (Table 2).

Fresh weight, yield per shoot and number of nuts per ounce

Arg (0.5 mM) when applied one week before full bloom significantly increased the fresh weight and yield per shoot compared with the control and the other treatments (Fig 1-A,

B). The combined treatment of Arg 1 mM + SNP 50 µM increased the fresh weight in both stages and yield per shoot at the second stage (Fig 1-A, B).

Application of Arg (0.5 mM) five weeks after full bloom decreased the number of nuts per ounce. The effect of other treatments on number of nuts per ounce at different stages of application was not significant (Fig 1-C).

Scions and leaves growth parameters

The leaf area and shoot diameter did not show any considerable changes among treatments in the first stage of application (Table 3). Arg (1 mM), SNP (50 and 100 µM), Arg 0.5 mM + SNP 50 µM and Arg 1 mM + SNP 50 µM increased the leaf area when used five weeks after full bloom. Arg at both concentrations (0.5 and 1 mM) increased the length and diameter of current-year shoots five weeks after full bloom. Application of SNP (50 and 100 µM) significantly increased shoot length in first and second stages, respectively (Table 3).

The results showed that in the second stage application of Arg 1 mM + SNP 50 µM and Arg 1 mM + SNP 100 µM increased the scions length. Shoot diameter increased by application of Arg 0.5 mM + SNP 100 µM and Arg 1 mM + SNP 50 µM treatments (Table 3).

Table 1. Effect of exogenous application of Arginine (Arg) and sodium nitroprusside (SNP) on inflorescence bud and fruit abscission of 'Ahmad Aghaei' pistachio shoots.

Treatments	Inflorescence bud abscission (%)		Fruit abscission (%)	
	Stage 1*	Stage 2	Stage 1	Stage 2
Control	20.3±3.6 cd**	33.6±6.3 a	33.8±3.1 a	17.0±3.1 a
Arg 0.5 (mM)	8.4±5.5 d	13.8±1.9 de	19.1±6.4 b	2.2±0.6 b
Arg 1 (mM)	27.8±0.8 a-c	16.6±4.1 b-e	17.2±1.6 b	6.2±0.6 ab
SNP 50 (µM)	23.8±0.6 bc	23.8±9.6 a-d	40.8±2.1 a	3.7±2.1 b
SNP 100 (µM)	33.0±4.5 ab	26.4±4.9 a-c	36.0±15.1 a	3.5±1.0 b
Arg 0.5 + SNP 50	8.5±1.5 d	28.3±11.6 ab	36.8±5.4 a	13.1±2.3 ab
Arg 0.5 + SNP 100	36.7±1.2 a	15.0±5.9 c-e	33.6±1.5 a	1.9±0.4 b
Arg 1 + SNP 50	17.2±3.4 cd	7.2±1.9 e	36.2±3.9 a	3.8±2.9 b
Arg 1 + SNP 100	21.6± 0.8 bc	21.0±2.4 b-d	33.7±12.4 a	10.7±1.5 ab

* Stage 1 = one week before full bloom; Stage 2 = five weeks after full bloom.

** Different letters within a column indicate significant differences by Duncan's multiple range test at $P < 0.05$. Values are means±SE.

Table 2. Effect of exogenous application of Arginine (Arg) and sodium nitroprusside (SNP) on fruit characteristics of 'Ahmad Aghaei' pistachio shoots

Treatment	Blank nuts (%)		Non-split nuts (%)		Split nuts (%)	
	Stage 1*	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
Control	19.0±1.6 a**	19.8±8.1 a	25.2±4.2 b	26.1±4.7 a	49.1±11.7 c	57.1±1.3 c-e
Arg 0.5 (mM)	6.9±1.8 e	17.9±0.6 ab	10.7±0.7 d	14.0±7.9 bc	76.4±2.8 a	65.2±6.1 b-e
Arg 1 (mM)	11.6±0.9 c-e	12.2±1.8 c-e	10.0±0.6 d	14.3±4.8 bc	73.4± 4.8 a	69.2±2.3 b-d
SNP 50 (µM)	15.3±0.5 a-c	13.9±0.7 b-d	19.5±3.1 bc	28.9±1.5 a	63.3±3.4 a-c	49.6±5.3 de
SNP 100 (µM)	16.6±2.8 a-c	16.1±2.1 a-c	4.8±1.3 d	9.8±1.7 bc	65.4± 9.9 a-c	65.0±1.7 b-e
Arg 0.5 + SNP 50	12.3±0.3 b-e	10.6±1.9 c-e	8.3±0.8 d	25.2±2.2 a	74.3±4.1 a	45.3±13.5 e
Arg 0.5 + SNP 100	17.5±2.3 ab	7.4±0.6 e	12.3±4.6 cd	7.9±3.6 c	60.1±11.3 a-c	90.0±10.2 a
Arg 1 + SNP 50	9.6±0.9 de	10.3±6.6 de	34.7±2.1 a	9.4±0.2 bc	52.3±2.4 bc	72.0±5.4 bc
Arg 1 + SNP 100	12.8±1.3 b-d	7.4±0.1 e	9.1±1.2 d	16.3±2.5 b	71.4± 5.1 ab	79.5±4.2 b

* Stage 1 = one week before full bloom; Stage 2 = five weeks after full bloom.

** Different letters within a column indicate significant differences by Duncan's multiple range test at $P < 0.05$. Values are means±SE.

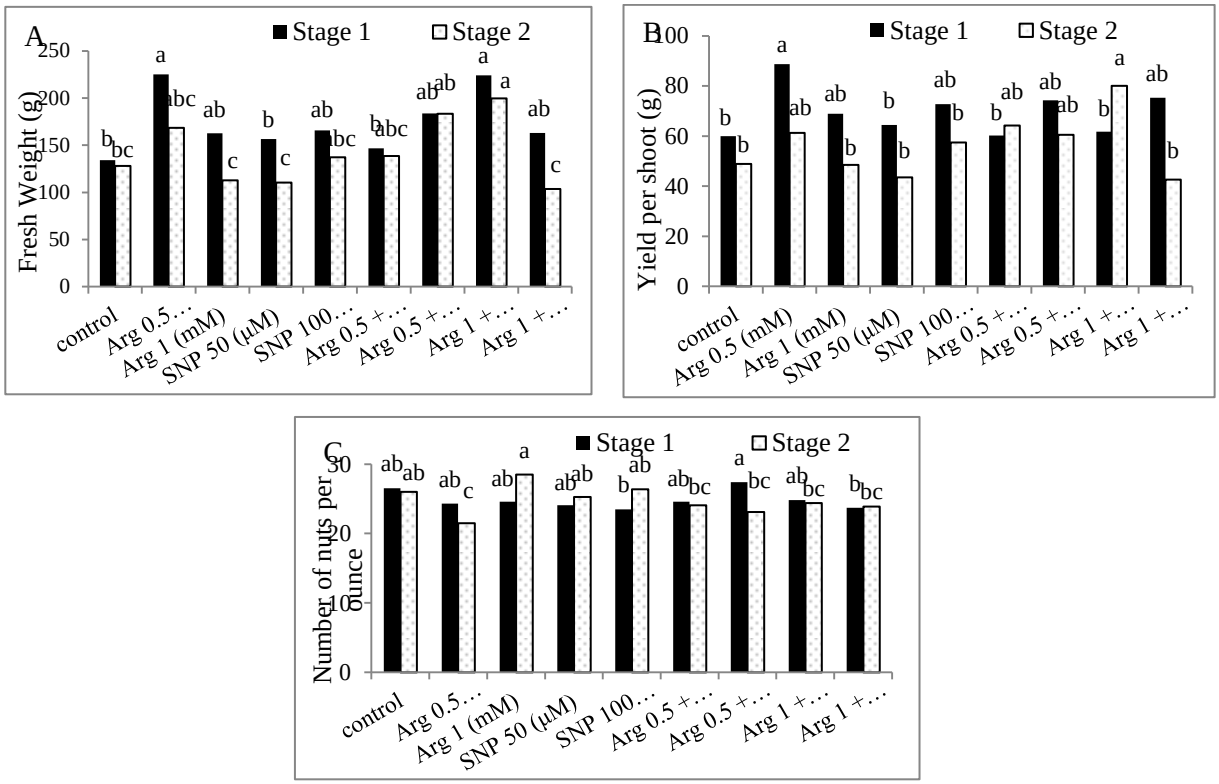

Fig. 1. Effect of exogenous application of Arginine (Arg) and sodium nitroprusside (SNP) on fresh weight (A), yield per shoot (B) and number of nuts per ounce (C) of 'Ahmad-Aghaei' pistachio shoots. Stage 1 = one week before full bloom; Stage 2 = five weeks after full bloom. Different letters within columns indicate significant differences by Duncan's multiple range test at P < 0.05. The vertical bars are the mean values ± SE. (Ounce = 28.30 g)

Table 3. Effect of exogenous application of Arginine (Arg) and sodium nitroprusside (SNP) on growth parameters of current-year 'Ahmad Aghaei' pistachio shoots.

Treatment	Leaf area (cm2)		Shoot length (cm)		Shoot diameter (mm)	
	Stage 1*	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
Control	86.7±1.8 a**	76.7±2.8 c	17.2±2.7 c	13.5±4.2 bc	6.0±0.4 a-c	5.7±0.1 d
Arg 0.5 (mM)	90.9±6.0 a	81.8±0.3 bc	23.5±2.0 a-c	23.7±1.2 a	6.0±0.2 a-c	7.1±0.1 a
Arg 1 (mM)	97.9±3.1 a	98.7±3.6 a	24.4±0.6 ab	20.3±2.0 a	6.4±0.1 a	6.5±0.1 b
SNP 50 (µM)	95.0±2.6 a	95.6±0.9 ab	27.0±1.1 a	17.1±1.2 a-c	5.5±0.2 c	6.2±0.3 b-d
SNP 100 (µM)	97.1±4.7 a	94.0±3.1 ab	17.9±0.6 bc	20.4±2.4 a	5.5±0.1 c	5.8±0.2 cd
Arg 0.5 + SNP 50	86.5±4.4 a	93.2±8.9 ab	17.0±1.7 c	11.5±0.8 c	6.2±0.1 a	6.1±0.1 b-d
Arg 0.5 + SNP 100	95.0±5.4 a	88.2±0.3 a-c	24.3±2.5 ab	18.1±2.0 ab	5.8±0.1 bc	6.3±0.2 bc
Arg 1 + SNP 50	95.9±4.9 a	93.9±2.3 ab	23.1±0.3 a-c	23.3±2.5 a	6.5±0.3 a	6.4±0.1 b
Arg 1 + SNP 100	89.7±1.1 a	80.2±9.9 bc	18.1±1.6 bc	23.7±5.0 a	6.1±0.1 ab	6.2±0.2 b-d

* Stage 1 = one week before full bloom; Stage 2 = five weeks after full bloom.

** Different letters within a column indicate significant differences by Duncan's multiple range test at P < 0.05. Values are means±SE.

Photosynthetic pigments

Arg (0.5 mM) increased the content of chlorophyll *a* and *b* in both stages of applications. Application of Arg (1 mM) and SNP (50 µM) five weeks after full bloom significantly increased the chlorophyll *a* and *b* (Table 4). Arg 0.5 mM + SNP 100 µM increased both chlorophyll *a* and *b* in five weeks after bloom (Table 4).

Among all treatments, application of Arg 0.5 mM one week after full bloom significantly increased the carotenoids content in comparison with the control and the other treatments (Table 4).

Table 4. Effects of exogenous application of Arginine (Arg) and sodium nitroprusside (SNP) on photosynthetic Pigments of 'Ahmad Aghaei' pistachio shoots.

Treatment	Chlorophyll *a* (mg/gFW)		Chlorophyll *b* (mg/gFW)		Carotenoids (mg/gFW)	
	Stage 1*	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
Control	1.18±0.14 b**	0.66±0.02 d	0.48±0.05 bc	0.31±0.01 d	0.33±0.01 b	0.39±0.06 a
Arg 0.5 (mM)	1.60±0.23 a	1.41±0.13 b	0.74±0.15 a	0.58±0.01 ab	0.58±0.12 a	0.44±0.01 a
Arg 1 (mM)	1.47±0.01 ab	1.42±0.20 b	0.60±0.10 a-c	0.50±0.07 bc	0.38±0.03 b	0.34±0.02 a
SNP 50 (µM)	1.12±0.21 b	1.25±0.20 bc	0.53±0.08 bc	0.52±0.03 bc	0.37±0.01 b	0.38±0.01 a
SNP 100 (µM)	1.05±0.16 b	1.02±0.26 b-d	0.41±0.07 c	0.41±0.09 b-d	0.35±0.03 b	0.33±0.05 a
Arg 0.5 + SNP 50	1.43±0.10 ab	1.03±0.18 b-d	0.63±0.04 ab	0.43±0.07 b-d	0.38±0.06 b	0.37±0.04 a
Arg 0.5 + SNP 100	1.15±0.10 ab	1.82±0.08 a	0.47±0.02 bc	0.73±0.06 a	0.38±0.01 b	0.38±0.06 a
Arg 1 + SNP 50	1.06±0.16 b	0.98±0.18 cd	0.45±0.05 bc	0.53±0.02 bc	0.35±0.01 b	0.40±0.01 a
Arg 1 + SNP 100	1.30±0.26 ab	0.64±0.12 d	0.60±0.03 a-c	0.37±0.07 cd	0.43±0.03 b	0.33±0.01 a

* Stage 1 = one week before full bloom; Stage 2 = five weeks after full bloom.

** Different letters within a column indicate significant differences by Duncan's multiple range test at $P < 0.05$. Values are means±SE.

Total soluble sugar

Combination of Arg 1mM + SNP 50 µM when applied one week before full bloom, significantly increased the total soluble sugar (Fig 2-A). Other treatments did not showed any significant changes on total soluble content of leaves compared to the control.

Total soluble protein

Exogenous application of Arg (0.5 mM) had significant effect on total soluble protein in the second stage of application. Arg (1 mM) increased the protein content in both stages (Fig 2-B). Application of SNP (50 µM) and Arg 0.5 mM + SNP 100 µM one week before full bloom, and Arg 1mM + SNP 50 µM five weeks after full bloom significantly increased the total soluble protein (Fig 2-B).

CAT, APX and GPX enzymes activities

Application of Arg 1 mM + SNP 100 µM significantly increased the CAT activity in comparison with the control and the other treatments in the first stage. Exogenous application of Arg (0.5 mM) and SNP (50 µM) increased the activity of CAT in the second stage (Fig 3-A).

The results showed that exogenous application of either Arg, SNP nor their combination had no significant effect on APX enzyme activities (Fig 3-B). However application of SNP (100 µM), Arg 0.5 mM + SNP 50 µM, Arg 0.5 mM + SNP 100 µM and Arg 1 mM + SNP 50 µM treatments significantly increased the GPX activity in the first stage. The results showed that Arg 0.5 mM + SNP 50 µM and Arg 1mM + SNP 100 µM significantly increased but SNP (50 µM) and Arg 1 mM + SNP 50 µM significantly decreased the GPX activity in the second stage (Fig 3-C).

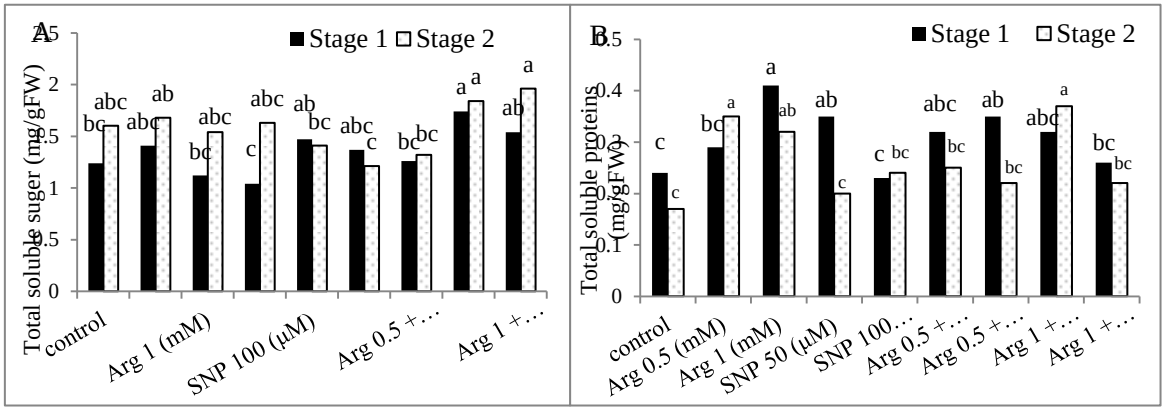

Fig. 2. Effects of exogenous application of Arginine and sodium nitroprusside (SNP) on total soluble sugar (A) and total soluble protein (B) of 'Ahmad-Aghaei' pistachio shoots. Stage 1= one week before full bloom; Stage 2= five weeks after full bloom. Different letters within columns indicate significant differences by Duncan's multiple range test at $P < 0.05$. The vertical bars are the mean values ± SE.

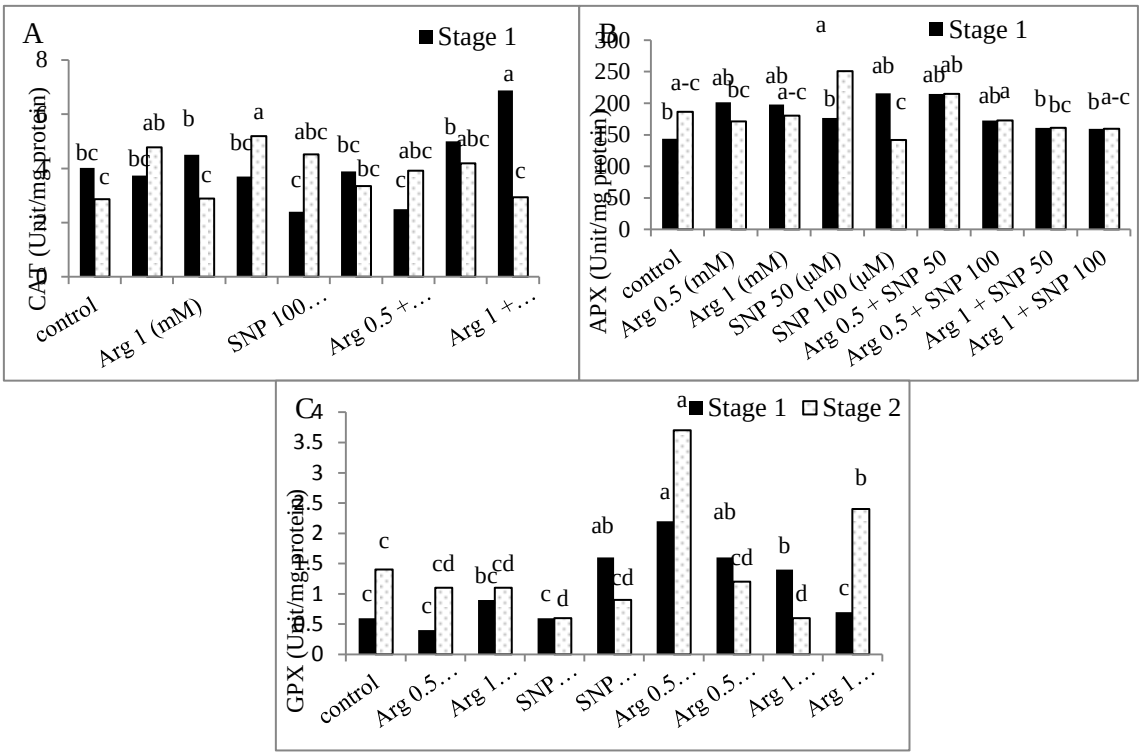

Fig. 3. Effects of exogenous application of Arginine (Arg) and sodium nitroprusside (SNP) on CAT (A), APX (B) and GPX (C) enzymes activities of 'Ahmad-Aghaei' pistachio shoots. Stage 1= one week before full bloom; Stage 2= five weeks after full bloom. Different letters within columns indicate significant differences by Duncan's multiple range test at $P < 0.05$. The vertical bars are the mean values is ± SE.

Discussion

Our results indicated that exogenous application of Arg and SNP decreased the percentage of fruit and inflorescence bud abscissions in pistachio scions. Both Arg and SNP treatments improved the blank nuts, non-split and split shells in different stages of application. These effects were considerably related to the stage that treatments were applied on the scions. Kernel development and flower buds abscission have been associated with competition for resources among fruitlets and between reproductive and vegetative organs (Byersand Wolf 1991; Stephenson 1983). It has been reported that there is a correlation between the kernel development and splitting (Ferguson et al. 2005). Urea as a nitrogen supply compound has been found to delay the senescence stages of buds and increased the entrance of photosynthetic compounds, hormones and other metabolites to the inflorescence buds, which are very important for preventing bud abscission in pistachio (Talaie et al. 2006). Since Arg serves as important nitrogen reserve, it can be considered for the reasons for decrease in abscission percentages in our study. It seems that the improvement of fruit characteristic in SNP or Arg treated tree may be related to the NO signaling. The effects of SNP supposed to be related to the direct NO emission from this substance, while the effect of Arg may be related to the NO synthesis from this amino acid. It has been reported that NO can decrease ethylene production and delay senescence in fruits such as strawberry through inhibition of ethylene synthesis (Zhu and Zhou 2007). Ethylene is one of the plant hormones that regulates abscission, accelerates senescence and increases by environmental tensions (Estornell et al. 2013). It has been shown that NO exposure effectively prevents the

autocatalytic ethylene biosynthesis in climacteric peach fruit, through the binding of NO to 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), forming a binary ACO–NO complex, which is then chelated by ACC to produce a stable ACC–ACO–NO complex (Parra-Lobato and Gomez-Jimenez 2011).

Application of Arg (0.5 mM) and Arg 1 mM + SNP 50 μM combination significantly affected the fresh weight and yield production per shoot in comparison with the control. In previous studies, it has been reported that application of Arg significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophyll a and b and carotenoids in bean (Nassar et al. 2003), wheat (El-Bassiouny et al. 2008) and rice (Lin and Kao1995). Exogenous application of Arginine and SNP was very effective on growth parameters of current-year 'Ahmad Aghaei' pistachio shoots. Spann et al. (2009) reported that individual short-shoots produced significantly less yield and fewer fruit clusters per shoot compared with long-shoots. It has been reported that Arg and SNP pre-treatment significantly increased the length of plants under non-stress and salinity conditions (Nejadalimoradi et al. 2014). It has been reported that inflorescence buds abscission in pistachio shoots are affected due to decreasing the leaf area. Leaves are the main source of hormones, metabolites and carbohydrate synthesis and these products are necessary for preventing bud drop (Esmaeilpour and Khezri 2006). Crane et al. (1973) reported that any factors that decrease the leaf area would increase the percentage of abscised buds (Crane et al. 1973).

The abscission of young fruits and flowers depends on assimilate supply to the developing organs, or both competition and dominance of source-sink strength (Marcelis et al. 2004). Measurement of photosynthetic pigments showed that Arg and combination of Arg with SNP increased the amounts of chlorophyll content. These results are in agreement with those obtained by Nejadalimoradi et al. (2014) who observed that SNP and Arg increased the chlorophyll content in leaves of control and stressed Sunflower plants (Nejadalimoradi et al., 2014). It has been reported that NO can scavenge the ROS and thus decreased the oxidative damage in photosynthetic apparatus and increased the chlorophyll content (Lei et al. 2007). The promoting effect of Arg on chlorophyll concentration may be explained by its potential to retard the deterioration of chlorophyll and/or increase chlorophyll biosynthesis by stabilizing the thylakoid membrane (El-Bassiouny et al. 2008).

Results of this research also showed that our treatments had no significant effects on soluble sugar content. Saccharide shortage promotes fruit abscission in concomitant with ascending content of ABA and 1-aminocyclopropane-1-carboxylic acid (ACC) (Iglesias et al. 2006). The importance of sucrose has been confirmed by Baninasab and Rahemi, (2006), who also found a negative correlation between carbohydrate content and bud abscission. However, Crane et al. (1976) and Crane and Al-Shalan, (1977) reported that there is no relationship between carbohydrates and bud abscission. In accordance, our results did not show any remarkable relationship between carbohydrates level and abscission in pistachio trees.

In this study, Arg (1 mM) caused a considerable increase in total soluble protein. These results are in agreement with those obtained by Mostafa et al. (2010) who observed that, use of either Arg or putrescine induced significant increases in protein percentage of wheat grains. It has been also reported that Arg pretreatment reduced the oxidation of protein in tomato plant. Therefore, the increase of protein content in such treatments may be related to the protective effect of Arg (Nasibi et al. 2013).

Inhibition of ROS production and/or

scavenging suppresses continuous H_2O_2 production, prevents expression of the cellulase gene, and consequently inhibits abscission. Conversely, application of H_2O_2 enhances cellulase gene expression and abscission, indicating that production of H_2O_2 is important to induce abscission (Sakamoto et al. 2008). Antioxidant enzymes such as catalase (CAT) and peroxidases (POX) are involved in the scavenging of ROS (Shanker et al., 2004). It has been reported that Arg and SNP pretreatment increased the activity of CAT and APX in root and leaves of Sunflower plants under salinity (Nejadalimoradi et al., 2014). It has been also reported that Arg increased the postharvest longevity of tuberose flower which was accompanied with the elevation of antioxidant enzymes activity. The effects of Arg and SNP on the reduction of abscission observed in the current study could be attributed to the activity of SOD antioxidant enzymes that decompose the H_2O_2 and inhibit cellulose expression, however the future studies need to confirm this hypothesis.

Conclusion

Exogenous application of Arg and SNP in pistachio shoots used in this study alleviates the physiological disorders and improved the yield of pistachio trees. However, the positive effects of treatments were considerably depending on the time and the applied concentrations. In addition, Arg showed greater positive impact on fruit and physiological parameters than SNP. The positive effects of Arg on alleviation of fruit abscission might be contributed to the production of other compounds such as polyamines which synthesized from Arg. However, the mode of action of these compounds in relation to abscission, physiological disorders and yield is not well understood so far and more researches are imperative to elucidate the relationships.

References

1. Arasimowicz M, Wieczorek J. 2007. Nitric oxide as a bioactive signaling molecule in plant stress responses. Plant Science 172, 876-887.

2. Baninasab B, Rahemi M. 2006. Possible role of non-structural carbohydrates in alternate bearing of pistachio. European Journal of Horticultural Science 71, 277-282.

3. Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochemistry 72, 248-254.

4. Byers R.E, Wolf T.K. 1991. The influence of low light on apple fruit abscission. Journal of Horticultural Sciences 66, 7-17.

5. Canton F.R, Suarez M.F, Canovas F.M. 2005. Molecular aspects of nitrogen mobilization and recycling in trees. Photosynthesis Research 83, 265-278.

6. Chen H, McCarig B, Melotto M, Yang S, He S, Howe G.A. 2004. Regulation of plant arginase by wounding, Jasmonate and the phytotoxin coronatine. The Journal of Biological Chemistry 279, 45998-46007.

7. Crane J.C, Al-shalan I.M. 1977. Carbohydrate and nitrogen level in pistachio branches as related to shoot extension and yield. Journal of the American Society for Horticultural Science 102, 396-399.

8. Crane J.C, Al-shalan I, Carlson R.M. 1973. Abscission of inflorescence buds as affected by leaf area and number of nuts. Journal of the American Society for Horticultural Science 98, 591-592.

9. Crane J.C, Catlin P.B, Al-Shalan I. 1976. Carbohydrate levels in the pistachio as related to alternate bearing. Journal of the American Society for Horticultural Science 101, 371-374.

10. Crane J.C, Iwakiri B.T. 1981. Morphology and reproduction of pistachio. Review Horticulture 3, 376-393.

11. Crane J.C, Iwakiri B.T. 1985. Vegetative and reproductive dominance in pistachio. Journal of Horticultural Sciences 20, 1092-1093.

12. Crane J.C, Nelson M.M. 1971. The unusual mechanism of alternate bearing in the pistachio. Journal of Horticultural Sciences 6, 489-490.

13. Dhindsa R.S, Dhindsa P, Thorpe A.T. 1981. Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decrease levels of superoxide dismutase and catalase. Journal of Experimental Botany 32, 93-101.

14. El-Bassiouny H.M.S, Mostafa H.A, El-Khawas S.A, Hassanein R.A, Khalil S.I, Abd El-Monem A. 2008. Physiological responses of wheat plant to foliar treatments with arginine or putrescine. Australian Journal of Basic and Applied Sciences 2, 1390-1403.

15. Eo J, Lee B.Y. 2011. Anatomical and Histological Changes in the Fruit Abscission Zone of Water Dropwort (*Oenanthe stolonifera* DC.). Horticulture, Environment, and Biotechnology 52(3), 315-320.

16. Esmaeilpour A, Khezri M. 2006. Abscission of Inflorescence Buds as Affected by Genetic Characteristics in Some Iranian Commercial Pistachio Cultivars. International Journal of Agriculture and Biology 8(3), 360-362.

17. Estornell L.H, Agusti J, Merelo P, Talón M, Tadeo F.R. 2013. Elucidating mechanisms underlying organ abscission. Review. Plant Science 199-200, 48-60.

18. Fabbri A, Ferguson L, Polito V.S. 1998. Crop load related deformity of developing *Pistacia vera* cv `Kerman' nuts. Scientia Horticulture 77, 219-234.

19. Ferguson L, Beede R.H, Freeman M.W, Haviland D.R, Holtz B.A, Kallsen C.E. 2005. Pistachio Production Manual (4th ed.). Fruit and Nut Research and Information Center, University of California, Davis, California.

20. Groppa M.D, Benavides M.P. 2008. Polyamines and abiotic stress, recent advances. Amino Acids 34, 35-45.

21. Iglesias D.J, Tadeo F.R, Primo-Millo E, Talon M. 2006. Carbohydrate and ethylene levels related to fruitlet drop through AZ A in citrus. Trees 20, 348-355.

22. Khezri M, Talaiea A, Javanshah A, Hadavi F. 2010. Effect of exogenous application of free polyamines on physiological disorders and yield of 'Kaleh-Ghoochi' pistachio shoots (*Pistacia vera* L.). Scientia Horticulturae. 125, 270-276.

23. Kumar N, Pal M, Singh A, Sairam R, Srivastava G.C. 2010. Exogenous proline alleviates oxidative stress and increase vase life in rose (*Rosa hybrida* L. 'Grand Gala'). Scientia Horticulture 127, 79-85.

24. Lei Y, Yin C, Ren J, Li C. 2007. Effect of osmotic stress and sodium nitroprusside pretreatment on proline metabolism of wheat seedlings. Journal of Plant Physiology 516, 386-390.

25. Lewis MW, Leslie ME, Liljegren SJ. 2006. Plant separation, 50 ways to leave your mother. Current Opinion in Plant Biology 9, 59-65.

26. Li J, Yuan R .2008. NAA and ethylene regulate expression of genes related to ethylene biosynthesis, perception, and cell wall degradation during fruit abscission and ripening in 'Delicious' apples. Journal of Plant Growth Regulation 27, 283–295.

27. Lichtenthaler H.K .1987. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. Methods in enzymology 148, 350-382.

28. Lin C.C, Kao C.H .1995. Levels of endogenous polyamines and NaCl inhibited growth of rice seedlings. Plant Growth Regulation 17, 15-20.

29. Liu J.H, Nada K, Honda C, Kitashiba H, Wen X.P. 2006. Polyamine biosynthesis of apple callus under salt stress. Importance of the arginine decarboxylase pathway in stress responses. Journal of Experimental Botany 57, 2589-2599.

30. Marcelis L.F.M, Heuvelink E, Baan Hofman-Eijer L.R , Den Bakker J, Xue L.B. 2004. Flower and fruit abortion in sweet pepper in relation to source and sink strength. Journal of Experimental Botany 55, 2261-2268.

31. Mostafa H.A.M, Hassanein R.A, Khalil S.I, El-Khawas S.A, El- Bassionny H.M.S, Abd El-Monem A.A. 2010. Effect of arginine or putrescine on growth, yield and yield components of late sowing wheat. Journal of Applied Science and Research 6(2), 177-183.

32. Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach choloroplast. Plant Cell Physiology 22, 867-880.

33. Nasibi F, Barand A, Kalantari K M Rezanejad F. 2013. The effect of arginine pretreatment on germination, growth and Physiological parameters in the increase of low temperature tolerance in *Pistaci avera* L. in vitro culture. International Journal of Agriculture and Crop Sciences 5, 1918-1925.

34. Nassar A.H, El–Tarabily K.A, Sivasithamparam K. 2003. Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine producing isolate of *Streptomyces griseoluteus*. Plant Growth Regulation 40(2), 97-106.

35. Nejadalimoradi H, Nasibi F, Kalantari K.M, Zanganeh R. 2014. Effect of Seed Priming with L-arginine and sodium nitroprusside on some physiological parameters and antioxidant enzymes of sunflower plants exposed to salt stress. Agricultural Communications 2(1), 23-30.

36. Nzima M, Martin G, Nishijima C. 1997. Leaf development, dry matter accumulation, and

distribution within branches of alternate bear 'Kerman' pistachio trees. Journal of the American Society for Horticultural Science 122, 31–37.

37. Parra-Lobato M.C, Gomez-Jimenez M.C. 2011. Polyamine-induced modulation of genes involved in ethylene biosynthesis and signaling pathways and nitric oxide production during olive mature fruit abscission. Journal of Experimental Botany 62, 4447-4465.
38. Plewa M.J, Smith S.R, Wanger E.D. 1991. Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. Mutation Researches 247, 57-64.
39. Porlingis I.C. 1974. Flower bud abscission in pistachio as related to fruit development and other factors. Journal of the American Society for Horticultural Science 99, 121-125.
40. Roberts J.A, Elliott K.A, Gonzalez-Carranza Z.H. 2002. Abscission, dehiscence, and other cell separation processes. Annual Review of Plant Biology 53, 131-158.
41. Roe JH. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. Journal of Biological Chemistry 212, 335-343.
42. Sakamoto M, Munemura I, Tomita R, Kobayashi K. 2008. Reactive oxygen species in leaf abscission signaling. Plant Signaling and Behaviors 3, 1014-1015.
43. Samach A, Smith H.M. 2013. Constraints to obtaining consistent annual yields in perennials. II, Environment and fruit load affect induction of flowering. Plant Science 207, 168-176.
44. Shanker A.K, Djanaguiraman M, Sudhagar R, Chandrashekar C.N, Pathmanabhan G. 2004. Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO 4) roots. Plant Science 166, 1035-1043.
45. Spann T.M, Beede R.H, Dejong T.M. 2009. Contributions of short- and long-shoots to yield of 'Kerman' pistachio (*Pistacia vera* L.). Scientia Horticulturae 121, 495-500.
46. Stephenson A.G. 1983. Cost of over initiating fruit. Am mid nat 112, 379-86.
47. Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Syst. 12, 253-279.
48. Takeda F, Ryugo K, Crane J.C. 1980. Translocation and distribution of c14-photosynthates in bearing and non bearing pistachio branches. Journal of the American Society for Horticultural Science 105, 642-4.
49. Talaie A, Seyedi M, Panahi B, Khezri M. 2006. Effects of Shoot Girdling and Urea Combined with 6 –BenzylAdenine on Abscission of Inflorescence Buds in "Ohadi" Pistachio Cultivar (*Pistacia vera* L.). International Journal of Agriculture and Biology 08(4), 474-476.
50. Wright G.C. 1999. The carbohydrate economy of horticultural crops: introduction to the colloquium. Scientia Horticulturae 34, 1014-1015.
51. Zhu S, Zhou J. 2007. Effect of nitric oxide on ethylene production in strawberry fruit during storage. Food Chemistry 100(4), 1517-1522.