



Foliar Application of Arginine Improves Vegetative and Reproductive Characteristics of Bearing Pistachio Trees

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ABSTRACT

Pistachio is one of the most important horticultural crops in Iran. There is a unique role for amino acids in the plant response to stressful conditions, and special physiological role for Arginine (Arg) compared to other amino acids. The effects of this compound were investigated on vegetative and reproductive characters of bearing pistachio trees of 'Ahmad-Aghaei'. The experiments were done as a factorial based on RCBD with four concentrations of Arg (0, 100, 200 and 300 μ M) and three spraying stages (post harvest, flower buds swollen and flowering). Based on the results, Arg improved the vegetative and reproductive characters. Arg increased length and diameter of shoot, leaf area, the number of floweringbuds, the number of nuts in cluster, fresh weight of cluster, nut splitting (%) and ounce, and decreased flower buds abscission percent and blankness. Arg also significantly increased photosynthetic pigments (chlorophyll a and b and total chlorophyll) in bearing pistachio trees. The best concentration of Arg was 200 μ M applied of the flowering stage, but the strongest effect on the number of nut in cluster, fresh weight of cluster and blankness were observed when spraying at the flower bud swollen stage.

Introduction

Pistachio (*Pistacia vera* L.), a member of Anacardiaceae family, is an important and exportable nut crop of Iran, and has a long history in Iran (Abrishami, 1995). Pistachio trees exhibit some physiological disorders such as abscission of inflorescence buds, fruit abscission and the production of blank, non-split, early-split and deformed nuts (Khezri *et al.*, 2010). Different hypotheses have been reported to explain the precise mechanism of these phenomena. Carbohydrate competition between

developing fruits and inflorescence buds (Nzima *et al.*, 1999; Spann *et al.*, 2008) as well as nutrient deficiency (Weinbaum *et al.*, 1994; Picchioni *et al.*, 1997) has been shown not to be the sole causes. In many fruit trees, natural fruit abscission occurs during early fruit development and is influenced by the species, the physiological state of the tree and environmental factors (Stephenson, 1981). Pistachio trees produce abundant flowers. Some of the flowers are pollinated and set fruit but there after exhibit significant fruit abscission.

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Although different pollen parents, lack of pollination or fertilization, disturbances in embryogenesis, adverse environmental conditions, competition for carbohydrates, plant growth substances and nutrients are among the most important causes of activating abscission zone(s) and fruit abscission (Stephenson, 1981). Few reports have interpreted this physiological problem in pistachio (Nzima *et al.*, 1997; Acar and Eti, 2007). Production of blank nuts in pistachio results from embryo abortion and under development of kernel to fill the shell (Crane *et al.*, 1971). Generally, the production of blank nuts has been attributed to improper pollination, lack of fertilization, untimely spring rainfall or hail during blooming stage as well as nutrition and drought stress during the kernel development (Crane and Iwakiri, 1981; Ferguson *et al.*, 2005).

L-Arginine (Arg) is one of the most functionally diverse amino acids in living cells. In addition to serving as a constituent of proteins, Arg is a precursor for the biosynthesis of polyamines, agmatine, and proline, as well as the cell-signaling molecules glutamate, amino butyric acid, and nitric oxide (Morris, 2002; Cederbaum *et al.*, 2004; Liu *et al.*, 2006). Arg has shown a lot of physiological roles than other amino acids (Kakkar *et al.*, 2000). Generally, Arg is a vital regulator for the developmental and physiological process in plants (Galston and Kaur-Sawhney, 1990). Polyamines and nitric oxide have variety of functions and play important roles in physiological, biological and developmental processes and responses to stresses in plants (Grun *et al.*, 2006). Polyamines are essential for cell division, growth, and differentiation (Alcázar *et al.*, 2006). Khezri *et al.* (2010) reported spraying of free polyamines on pistachio trees 'Kaleghochii' to decrease physiological problems (splitting, blanking and alternate bearing) and increase pistachio nut yield. The results of different studies have shown that foliar application of free polyamines decreases the severe abscission of fruits

in grapes, mango and lychee (Aziz *et al.*, 2001). Nassar *et al.* (2003) reported that the application of Arg (as a precursor for Putrescine) significantly promoted the growth of bean plants and increased the fresh and dry weights and lengths of roots and shoots, chlorophylls (a, b) and carotenoids compared with control plants. In another experiment, it was shown that amino acids can increase yield in 'Momtaz', and 'Fandoghi' cultivars, because the plants were able to use amino acids as nitrogen source (Bastam *et al.*, 2013).

The effect of Argon vegetative and reproductive characteristics of bearing pistachio trees were evaluated in this experiment. Therefore, the aim of the present study was to improve pistachio yields using Arg spraying.

Materials and Methods

Plant material and experiments

This study was conducted on mature 'Ahmad-Aghaei' pistachio trees in a commercial orchard located at pistachio research station in Rafsanjan, Iran during 2014-2016. The climate of the area is hot and dry with desert characteristics.

The study was carried out as factorial based on a randomized complete block design (RCBD) with three replications. Factor A, included four concentrations of Arg (0, 100, 200 and 300 μ M), and factor B, three application stages (post-harvest, flower buds swollen and flowering). There were 12 treatments with three replications in this experiment.

Vegetative characteristics

The length and diameter (at middle of shoot) of current season growth shoots were measured by ruler and calipers, respectively. Leaf area was measured by a Digital Leaf Area Meter (ADC, Hoddeston, UK). 10 leaves (at middle of shoot) were used for measuring leaf area in each replication.

Relative water content (RWC)

After two months of treatment, ten leaves were randomly sampled from current season growth shoot of around each tree for measuring RWC. The RWC was calculated using the following equation (Wheatherley, 1950):

$$\text{RWC} = \frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}} \times 100$$

Where FW and DW represent the fresh and dry weight of leaf discs, respectively; and TW represent weight of leaf discs after soaked in distilled water for six hours in dark.

Chlorophyll (Chl) extraction

One gram of fresh leaves was grinded with 20 ml of 80% acetone and was centrifuged at 5000 rpm for five minutes and the supernatant was transferred to a 100 ml volumetric flask. After extraction, the supernatant was diluted to a final volume of 100 ml by 80% acetone. The absorbance was measured at 663, 645, 652 and 510 nm using a spectrophotometer (Cecil 3041). Chl a, b, total and carotenoid (Car) were calculated on an exponential basis, using the equations (Arnon, 1949).

$$\text{Chlorophyll a (mg/g FW)} = [12.7(\text{OD}_{663}) - 2.69(\text{OD}_{645})] \times [V/1000 \times W]$$

$$\text{Chlorophyll b (mg/g FW)} = [22.9 (\text{OD}_{645}) - 4.68(\text{OD}_{663})] \times [V/1000 \times W]$$

$$\text{Total chlorophyll (mg/g FW)} = [(\text{OD}_{652}) \times 1000] / [34.5 \times (V/1000 \times W)]$$

$$\text{Carotenoid (mg/g FW)} = [7.6 (\text{OD}_{480} - 1.49 \times \text{D}_{510})] \times V/1000 \times W$$

Where V and W represent the volume of used acetone and the weight of leaf samples, respectively, and OD represent absorbance.

Fruit characteristics

At the harvest stage, all the clusters were detached from each shoot. Then, fresh weights of fruits (with green hull before drying) were calculated on each marked branches. Nut splitting percentage and blank were determined by counting hulled nuts randomly collected from each tree (Zeng and Brown, 1998). Then, the dry weight of split nuts (without green hull after drying in sun for three days) was measured.

Flower bud formation and abscission

The total number of initiated flower buds and the number of abscised buds on the individual current season growth shoots were counted at rest period (January). The percentage of inflorescence bud abscission was calculated as: the number of abscised buds per total number of buds initiated on each shoot.

Statistical analysis

Data were analyzed using SAS 9.1 software. The significant differences among means were carried out by using Duncan test at $P < 0.05$. The diagrams were drawn with Excel software.

Results

The results of analysis of variance indicated that interaction of Arg concentration and stage of application on the total of flower buds, flower buds abscission, number of nut per fruit cluster, fresh fruit cluster weight, blankness, Chl b and carotenoid were significant at level of 0.01 P. Arg had no significant effect on the current season shoot length, nut splitting, RWC, Chl a and total Chl, whereas on current season shoot diameter, leaf area and nut ounce was significant at level of 0.05.

Vegetative and physiological characteristics of bearing pistachio trees**Effect of Arg on current season shoots length**

Different concentrations of Arg significantly increased current season shoot length comparing to the control. The highest increase of current season shoot

length was observed in concentration of 300 μM Arg (16.08 cm) that showed no significant difference with the concentration of 200 μM Arg (Fig. 1).

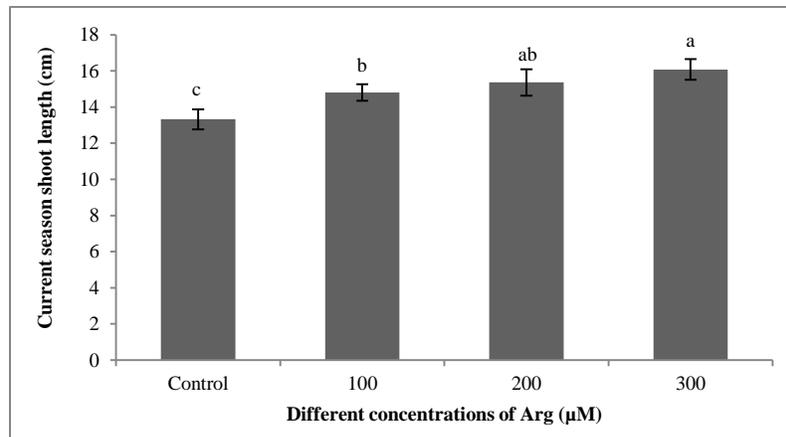


Fig. 1. The effect of different concentrations of Arg on current season shoot length in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effect of Arg on current season shoot diameter

Different concentrations of Arg significantly increased the current season shoot diameter at spraying of flowering and flower buds swollen stages comparing to the control. The highest increase of current season shoot diameter was recorded at concentrations of 200 and 300 μM of Arg (7.83 and 7.78 mm respectively) at spraying of flowering. Spraying higher concentrations of Arg (200 and 300 μM) at flower buds swollen stage

showed no significant differences with concentration of 100 μM . The best stage to spray Arg was the flowering stage. The next best effect was recorded at spraying of flower buds swollen. Spraying of Arg at the post-harvest stage (except 300 μM) showed no significant effect on increasing of current season shoot diameter (Fig. 2).

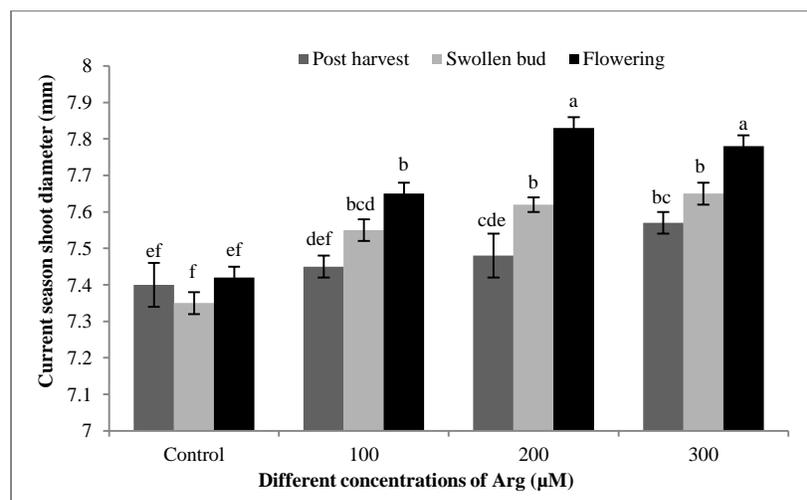


Fig. 2. The interaction effect of different concentrations of Arg and spraying stages on current season shoot diameter in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effect of Arg on leaf area

Different concentrations of Arg significantly increased leaf area by spraying at flowering and flower buds swollen stages comparing to the control. The highest increase of leaf area was recorded at a concentration 300 μM (96.77 cm^2). Spraying at flower stage showed no significant differences between

concentrations. Spraying of Arg at buds swollen stage was in the next rank. The best stage for spraying Arg was flowering stage. Spraying of Arg at post-harvest showed the lowest effect on an increase of leaf area (Fig. 3).

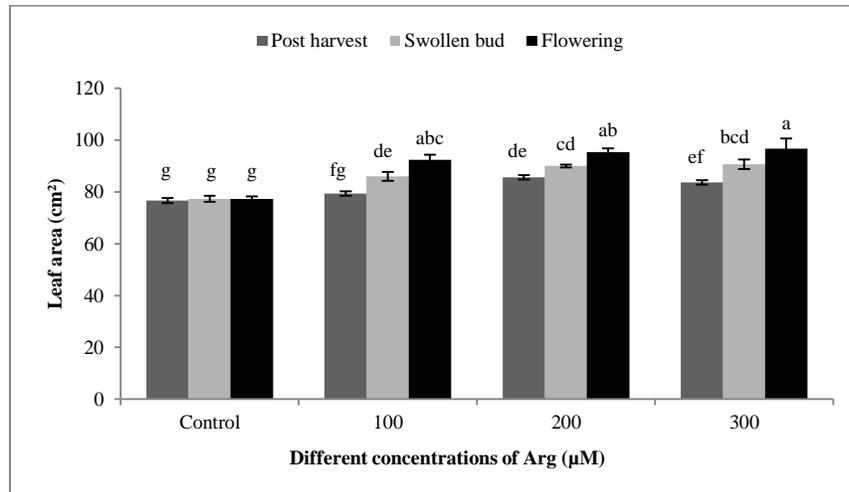


Fig. 3. Interaction between concentrations of Arg and application stages on leaf area in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effect of Arg on RWC

Different concentrations of Arg significantly increased RWC of leaves comparing to the control. The highest increase of RWC was recorded in concentration

of 300 μM Arg (61.68%) that showed no significant difference with 200 μM (Fig. 4).

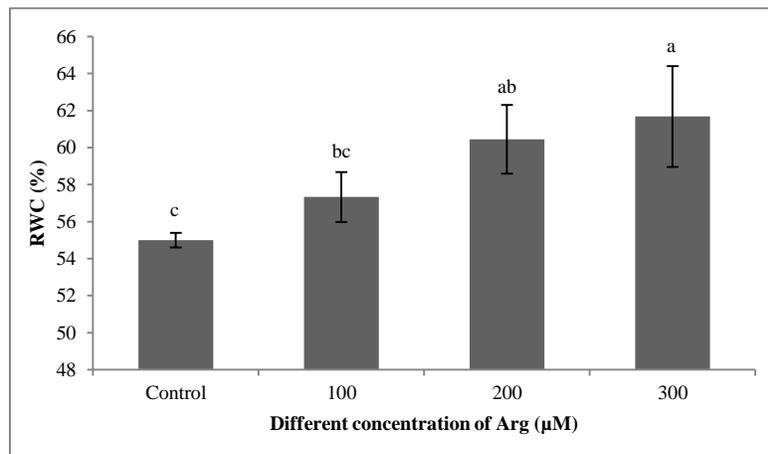


Fig. 4. The effect of concentrations of Arg on RWC in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effects of Arg on Chl a

Different concentrations of Arg significantly increased Chl a when spraying of flowering and flower buds swollen stages compared to the control. The highest increase of Chl a was observed in concentration of 300 μ M (0.92 mg/g FW) by spraying at flowering stage that show edno significant difference with 200 μ M

at this stage. There were no significant differences among concentrations of Arg by spraying at flower buds swollen stage. Spraying of Arg at post-harvest stage showed no significant effect on increasing Chl a (Fig. 5).

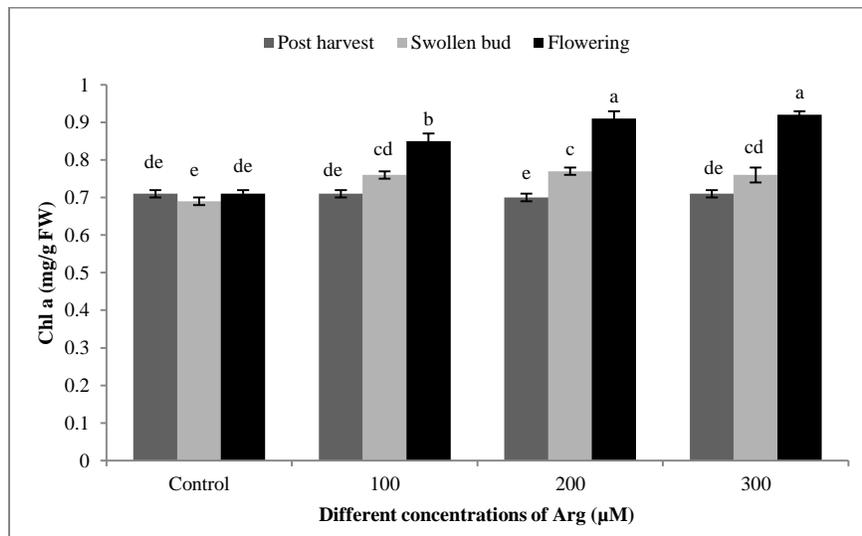


Fig. 5. Interaction of Arg concentrations and application stages on Chl a in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRTat P<0.05.

Effect of Arg on Chl b

Foliar application of Arg at flowering and flower buds swollen stages significantly increased Chl b comparing to the control. The highest increase of Chl b was observed by foliar application of 200 μ M Arg (0.6 mg/g FW) when spraying during the flowering stage.

There were significant differences among spraying stages for any concentration of Arg. The best stage for Arg application was flowering stage and after that, flower buds swollen stage (Fig. 6).

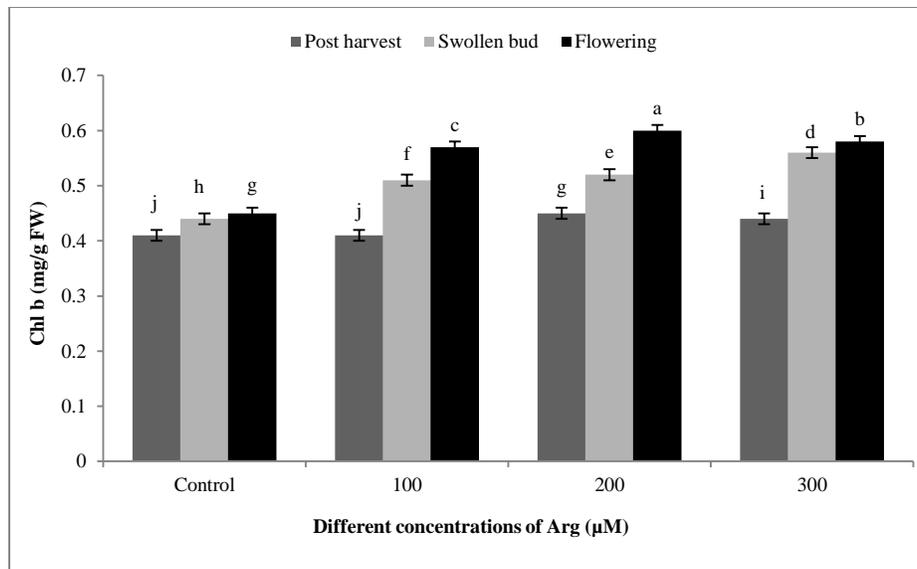


Fig. 6. Interaction of Arg concentrations and application stages on Chl b in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effect of Arg on total chlorophyll

Different concentrations of Arg significantly increased total Chl by spraying at flowering and flower buds swollen stages comparing to the control. The highest increase of total Chl was observed in concentration of 300 µM (1.51 mg/g FW) when spraying during the flowering stage, which showed no significant difference with 200 µM concentration at this

stage. There were no significant differences among different concentrations of Arg spraying at flower buds swollen stage. Spraying of Arg at post-harvest stage showed no significant effect to increase total Chl. The best stage to spray Arg was flowering stage and after that, flower buds swollen stage (Fig. 7).

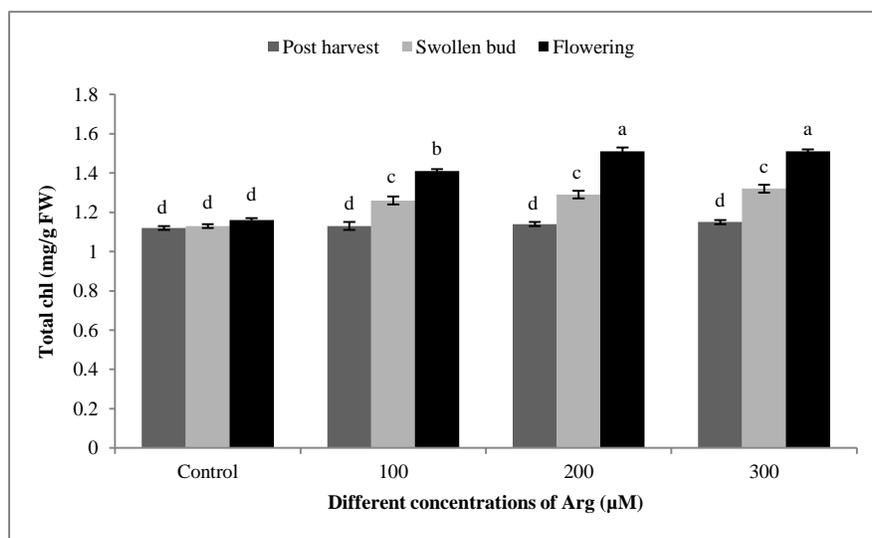


Fig. 7. Interaction of Arg concentrations and application stages on total Chl in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effect Arg on Carotenoid content of leaves

Different concentrations of Arg significantly increased carotenoid at spraying of flowering and flower buds swollen stages in comparison with the control. The highest increase of carotenoid was recorded in concentration 300 μM (0.68 mg/g FW) when spraying

during the flowering stage. It showed no significant differences with spraying of that at flower buds swollen stage and 200 μM at spraying of flowering stage. Spraying of Arg at post-harvest stage showed no significant effect on the increase of carotenoids (Fig. 8).

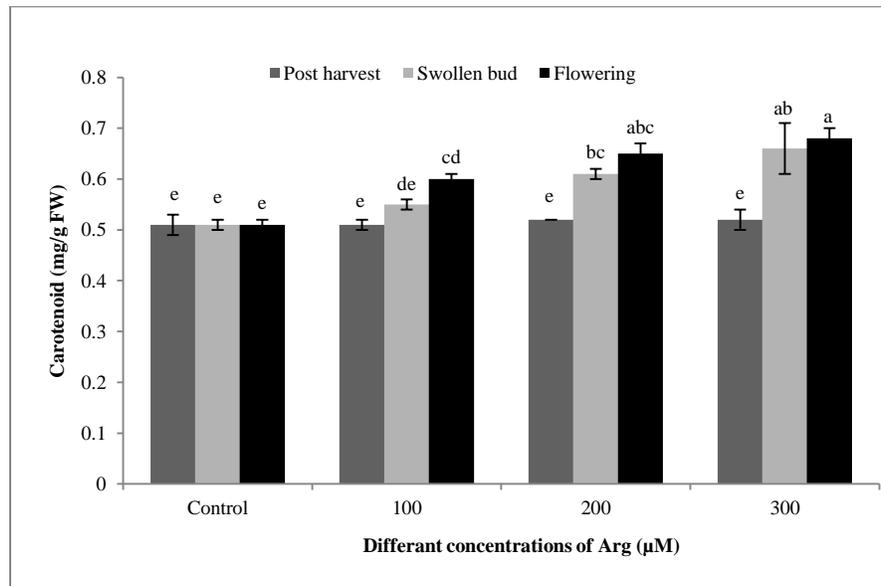


Fig. 8. Interaction of Arg concentrations and application stages on carotenoid content of leaves in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Reproductive characteristics of bearing pistachio trees

Effect of Arg on the number of nut per fruit cluster

Different concentrations of Arg significantly increased the number of nut per fruit cluster at spraying of flower buds swollen and flowering stages comparing to the control. The highest increase in the number of nut per fruit cluster was recorded at concentrations of 300 and 200 μM of Arg (18.2 and 18.1 respectively) when spraying during the flower buds swollen stage. Spraying

at flower buds swollen and flowering stages showed a significant difference with each other. Spraying of Arg at post-harvest stage showed no significant effect to increase the number of nut per fruit cluster. The best stage to spray Arg was flower buds swollen stage and after that, flowering stage (Fig. 9).

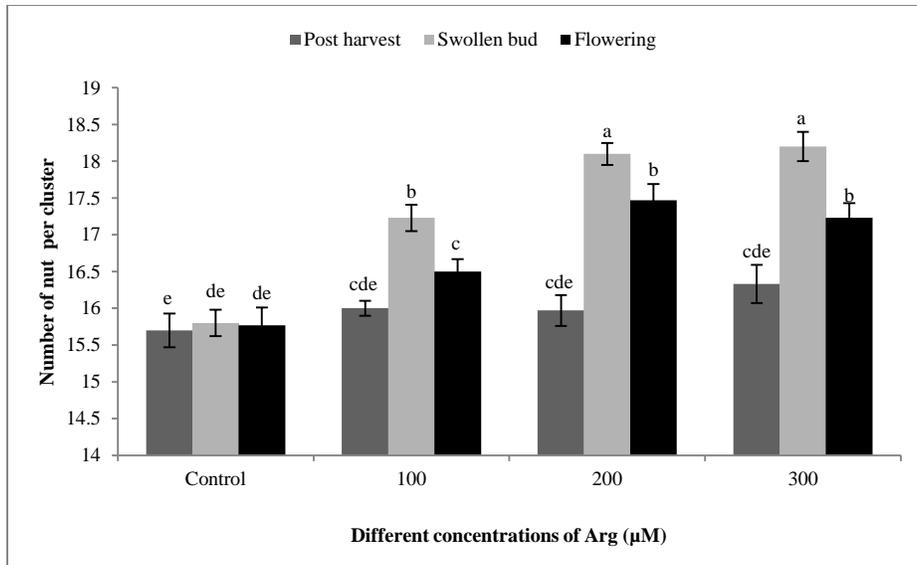


Fig. 9. Interaction of Arg concentrations and application stages on the number of nut per fruit cluster in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at P<0.05.

Effect of Arg on fruit cluster fresh weight

Different concentrations of Arg significantly increased fruit cluster fresh weight at spraying of flower buds swollen and flowering stages comparing to the control. The highest increase of fruit cluster fresh weight was recorded in concentrations of 200 and 300 µM Arg at spraying on flower buds swollen and

flowering stages. Spraying at flower buds swollen and flowering stages showed no significant difference with each other. Spraying of Arg at post-harvest stage showed no significant effect in increasing the fruit cluster fresh weight (Fig. 10).

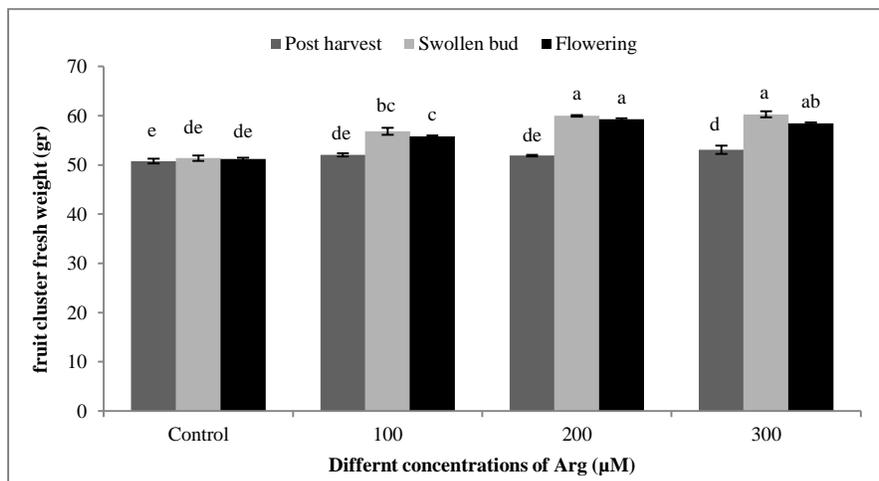


Fig. 10. Interaction of Arg concentrations and application stages on fruit cluster fresh weight in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at P<0.05.

Effect of Arg on nut splitting (%)

Different concentrations of Arg significantly increased the nut splitting percent comparing to the control. The highest increase of nut splitting (%) was

recorded in concentration of 300 μ M Arg (74.6%), which showed no significant difference with concentration of 200 μ M (72.6%) (Fig. 11).

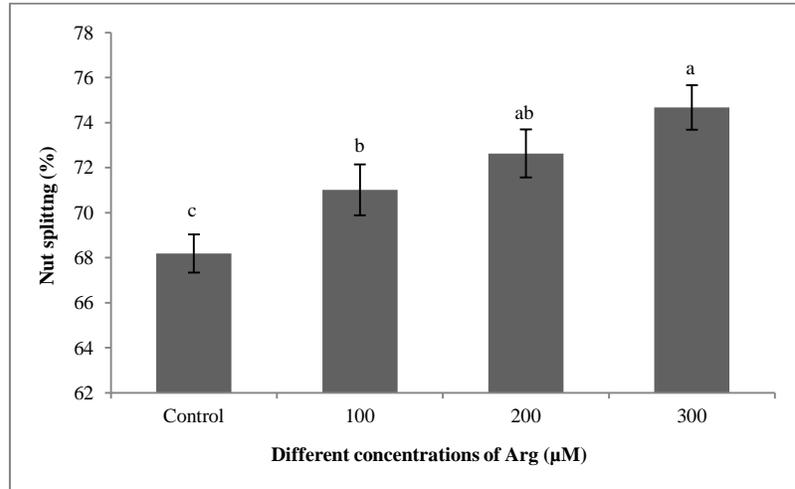


Fig. 11. The effect of different concentrations of Arg on nut splitting (%) in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effect of Arg on blankness (%)

Application of 200 and 300 μ M of Arg significantly decreased blankness (%) when spraying at the flower buds swollen stage spraying by 200 and 300 μ M at post harvest stage also showed significant effective on

decreasing the blankness (%). Spraying of Arg at flowering stage showed even on increase the blankness (%) (Fig. 12).

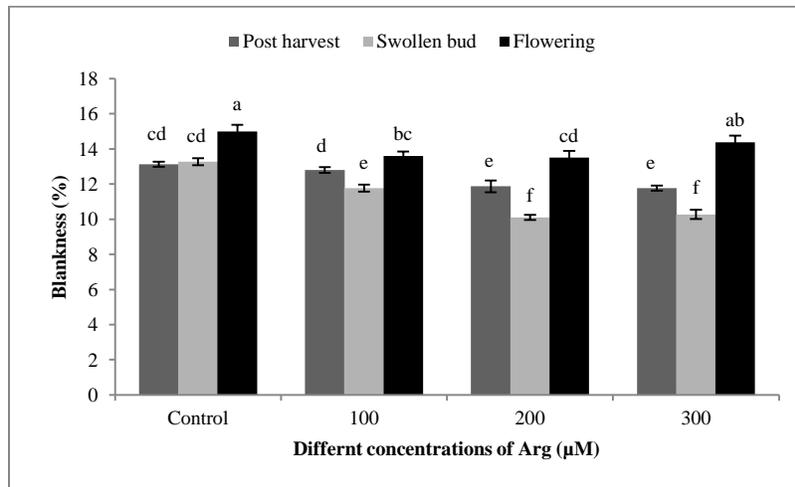


Fig. 12. Interaction concentrations of Arg and application stage on blankness (%) in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at $P < 0.05$.

Effect of Arg on nuts ounce

Different concentrations of Arg significantly increased the nut ounce when spraying at flower buds swollen and flowering stages in comparison with the control. The highest increase of nuts ounce was recorded in concentration of 200 μ M when spraying at flower buds swollen and flowering stages, 25.77 and

25.7, respectively, which showed no significant differences with 300 μ M. spraying at flower buds swollen and flowering stages showed no significant difference with each other. Spraying of Arg at post-harvest stage showed no significant effect on increasing the pistachio ounce (Fig. 13).

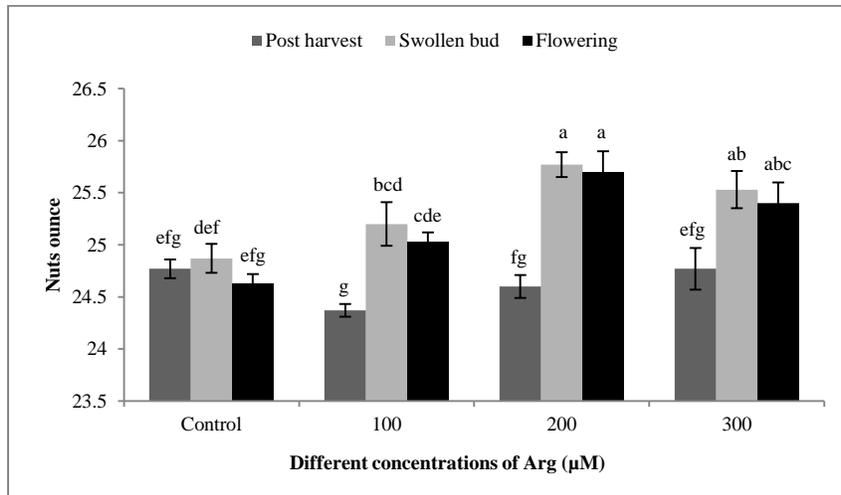


Fig. 13. Interaction of Arg concentrations and application stage on nuts ounce in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at P<0.05.

Effect of Argon total flower buds number per shoot

Different concentrations of Arg significantly increased the total number of flower buds per shoot at spraying of flowering and flower buds swollen stages in comparison with the control. The highest increase total of flower buds number was recorded at concentration of 300 μ M (5.92) when spraying at flowering stage which showed no significant difference with concentration of

200 μ M at this stage. There were significant differences among spraying stages in different concentrations of Arg. The best stage to spray Arg for number of flower buds was the flowering stage. Spraying of Arg at post-harvest stage showed no significant effect in increasing the total number of flower buds (Fig. 14).

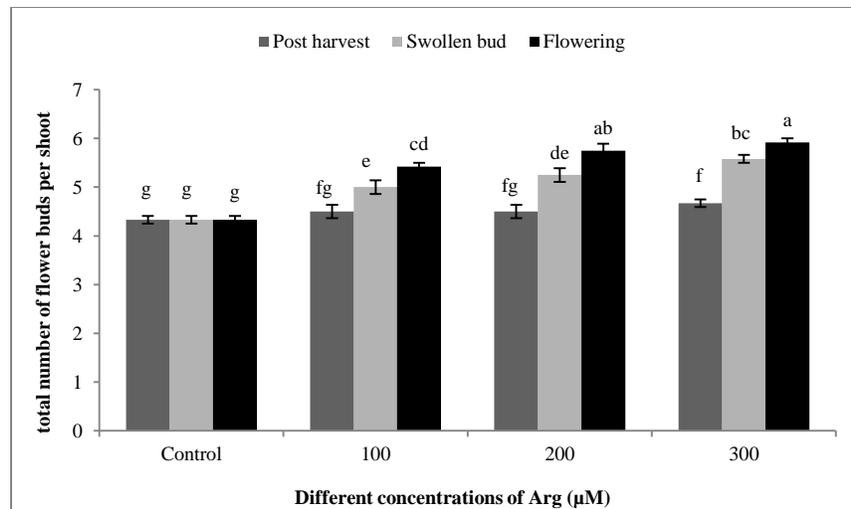


Fig. 14. The interaction of different Arg concentrations of and spraying stages on total number of flower buds per shoot in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at P<0.05.

Effect of Argon flower buds abscission percent

Different concentrations of Arg significantly decreased flower buds abscission percent by spraying at flowering and flower buds swollen stages in comparison with the control. The highest decrease of flower bud abscission percent was recorded in concentrations of 300 and 200 μM (45.07 and 44.4%, respectively) when spraying at flowering stage, which did not show a

significant difference with concentration of 300 μM spraying at flower buds swollen stage. Spraying of Arg at post-harvest stage showed no significant effect in decreasing the flower buds abscission percentage. The best stage to spray Arg for this character was at flowering stage and the flower buds swollen stage (Fig. 15).

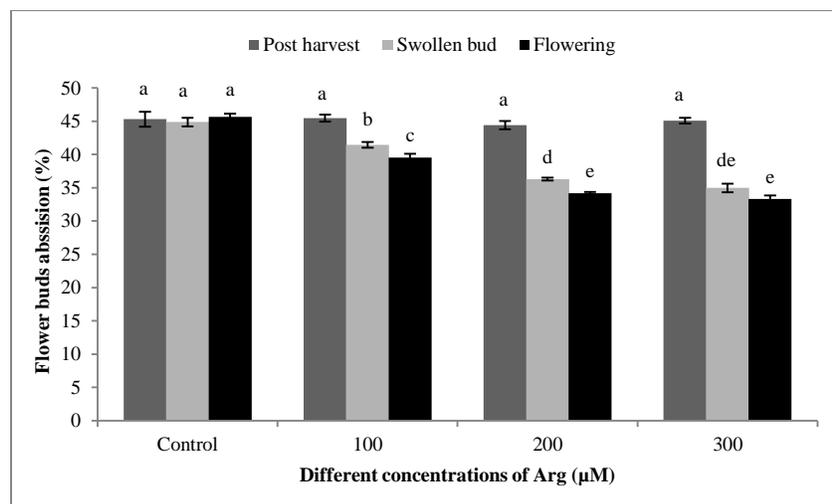


Fig. 15. The interaction of different Arg concentrations and spraying stages on flower buds abscission percent in bearing pistachio trees. Different letters on the columns indicate significant differences by DMRT at P<0.05.

Discussion

In this study, foliar application of Arg increased the length and diameter of shoot and leaf area. These results were confirmed by results of other studies. Khezrii *et al.* (2010) reported spermine (0.1 and 1mM) applied two weeks after full bloom significantly increased the leaf area of pistachio. Arg improved vegetative characters in pistachio seedlings under *in vitro* conditions and increased root and stem length (Barand *et al.*, 2012). Kamiab *et al.* (2013) indicated that foliar application of polyamines on “Badami Zarand” pistachio seedlings caused more growth under saline stress comparing to the control. The application of Arg increased growth, fresh and dry weight and endogenous growth regulators in bean plants (Nassar *et al.*, 2003) and wheat (El-Bassiouny *et al.*, 2008). Seedlings growth of bean was improved by application of Arg, which was associated with increased content of the polyamines, spermine and spermidin (Zeid *et al.*, 2009). Exogenous application of polyamines had significant effects on the length and diameter of current-year shoots of pistachio across the years and stages of application (Khezrii *et al.*, 2010). In this study, improvement of vegetative characters was related to the role of Arg as precursor of polyamines.

Arg increased the number of flowering buds, the number of nut in cluster, fresh weight of cluster, nut splitting percent and ounce, and decreased flower bud abscission percent and blankness. Roussos *et al.* (2004) reported that endogenous polyamines have a regulatory role in the process of inflorescence bud abscission, which is in accordance with this study. Moreover, the exogenous application of free polyamines was shown to be a promising technique in increasing fruit set, fruit quality and reduction of fruit abscission in litchi (Stern and Gazit, 2000) and mango (Malik and Singh, 2006). Application of free polyamines decreased the inflorescence bud abscission (Baninasab and Rahemi, 2008). Environmental influences and competition for resources such as photo assimilates and plant hormones

are likely causal factors of fruit abscission (Thompson, 1996). Exogenous application of spermine has shown to be effective in controlling fruit abscission in mango (Malik and Singh, 2006). Lovatt and Ferguson (1994) reported the amount of cytokines decreased when there was the highest flower bud abscission. Kamiab *et al.* (2013) reported that foliar treatments by polyamines (spermine and spermidin), amino acids and ascorbic acid on pistachio increased yield and percentage of nut splitting, and decreased fruit abscission, blanks and deformed nuts comparing to the control. Research by Molamhamadi (2014) showed that foliar application by 550 mg/L ascorbic acid at flowering stage increased the pistachio fruit set. Weinbaum *et al.* (1994) stated that cytokinin decreased the flower bud abscission percentage. These researchers reported these compounds increased buds vigor to absorb photosynthetic products, which is also shown in the studies by Lovatt and Ferguson *et al.* (1994) and Alizadeh and Rahemi (2003).

Since initial fruit abscission of pistachio is also assumed to be related to improper fertilization, the dominance of fruits in a cluster or abnormalities of reproductive organs (Crane and Iwakiri, 1985), Arg can improve subsequent embryo and fruit development. It has been reported that degeneration of the ovary segments, especially funicle degeneration, is the major cause of blankness in pistachio (Shuraki and Sedgley, 1996), and it was found that there is a correlation between the kernel development and splitting (Ferguson *et al.*, 2005). Thus, a decrease in the percentage of blankness by application of Arg might be attributed to the role of Arg in improving the growth and development of reproductive organs and preventing ovary degeneration. Galston *et al.* (1997) mentioned that spermine improved the development of reproductive organs. Khezrii *et al.* (2010) reported application of spermine increased the yield of pistachio crop possibly due to the role of polyamines in improving the growth

and development of reproductive flowers and fruits (Galston *et al.*, 1997). It is very likely that these compounds could be suited to serve either as nitrogenous sources or as signal molecules regulating the abscission process as well as fruit growth and development, consequently improving regenerative characters of pistachio crop.

The application of Arg increased the leaf area and this might lead to more photo assimilate synthesis and thus, greater carbohydrate availability to decrease the abscission of in florescence buds and to enhance the number of nut in cluster, fresh weight of cluster, nut splitting and the ounce. It has been suggested that polyamines improve fertilization, and subsequent embryo and fruit development (Galston, *et al.*, 1997), and the application of nitrogen has been reported to increase the fruit set in fruit trees and to increase the longevity of the ovule in the ovary (Marjani,2000). Therefore, Arg as precursor of polyamines can play this role. The highest increase of the number of flowering buds and decreased flower bud abscission percentage by Arg were observed in spraying at flowering stage. Thus, it was suggested that Arg to be used in this stage to improve studied characters. The using of Arg at post-harvest stage had no effect on the measured characters.

In this study, Arg significantly increased photosynthetic pigments (Chl a and b, total Chl and carotenoids) in bearing pistachio trees. Arg increased all photosynthesis pigments almost equally. The best concentration of Arg was 200 μ M. Hare *et al.* (1997) stated that cytokinins as plant growth regulators enhanced chlorophylls in plants. The application of Arg increased chlorophylls (a, b) and carotenoids in bean plants (Nassar *et al.*, 2003) and wheat (El-Bassiouny *et al.*, 2008). In this research, the effect of Arg was greatly dependent on both the stage of application and the concentrations. Since Arg decreased the percentage of blanked nuts and flower bud abscission, it seems that

Arg dominantly played a key role in the growth and development of pistachio nuts.

The results indicated the possible direct role of Arg in decreasing the physiological disorders and increasing the yield in pistachio. Also, it might be assumed that Arg effect on these physiological processes is indirectly via altering the concentrations of other plant growth substances like auxin, abscisic acid, ethylene as well as carbohydrates and nutrients. On the other hand, amino acids can also be an important source of available nitrogen for plants, and it has been reported that exogenous application of amino acids are effective in increasing yields in pistachio (Niven *et al.*, 1994; Rahdari and Panahi, 2012). It is very likely that these compounds could be suited to serve either as nitrogen sources or as signal molecules regulating both the abscission process and fruit growth and development, consequently affecting the yield of pistachio crop.

Conclusions

Spraying Arg improved the vegetative and reproductive characteristics of bearing pistachio trees. The foliar application of 200 μ M Arg improved the studied characters by spraying at flowering stage; but the highest efficiency of Arg on the number of nut in cluster, fresh weight of cluster and blank nut were observed by spraying at flower buds swollen stage. Spraying of Arg at post-harvest showed no effect on the vegetative and generative characteristics of bearing pistachio trees.

Acknowledgements

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