

Evaluation the Effects of Foliar Treatments of Polyamines and Some Organic Acids on Quantitative and Qualitative Traits in Some Pistachio Cultivars

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Abstract

Among the most important problems of pistachio are physiological disorders such as fruit abscission and production of blank nuts. Environmental stresses and inadequate nutrition during flowering time have increased these problems in recent years. In order to evaluate the effects of foliar treatments with polyamines and other compounds (amino acids and ascorbic acid) on quantitative and qualitative traits in pistachio cultivars, an investigation was conducted using a factorial experiment base on a completely randomized block design (CRBD) at an orchard condition in the Rafsanjan region. This experiment was performed to evaluate three factors, 1) type of treatment (spermine, spermidin, amino acids and ascorbic acid) at 6 concentrations, 2) cultivar ('Ohadi', 'Akbari' and 'Kaleghoochi') with three replications and 3) year (2013 and 2014). Different traits, such as yield per shoot, number of nuts per cluster, fruit abscission, blank nuts, split nuts, deformed nuts, length and diameter shoot were measured. The results indicated that all of the treatments increased yield and percentage of split, and decreased fruit abscission, blank and deformed nuts in comparison with the control. Spermine (1 mM) combined with ascorbic acid and amino acid, had the most significant effect, increasing yield 50 % and split nuts 18 % and also decreasing fruit abscission 20 %, and decreasing blank and deformed nuts 5 % in all cultivars, in comparison with controls. Ascorbic acid and amino acid treatment without polyamines had a lower effect in comparison with spermine treatment combined with these compounds. The combined use of these compounds, e.g., 1 mM spermine, ascorbic acid (0.5 g/L) and amino acids (3 g/L) suggests that they might act as a suitable compound fertilizer in order to reduce fruit abscission and increase the yield of pistachio crops without the impact of environmental pollution.

Keywords: Fruit abscission, Physiological disorders, Pistachio, Polyamines, Yield.

Introduction

Pistachio (*Pistachia vera* L.) is an important horticultural crop that has high economic value. Pistachio production in Iran has a long history and the Iranian people have valued the life-giving and intelligence-increasing properties of pistachio for a long time (Abrishami, 1995). 'Ohadi', 'Akbari' and 'Kaleghoochi' are some of the famous commercial pistachio cultivars and are compatible

with most pistachio-growing areas (Abrishami, 1995). From a horticultural point of view, pistachio trees have numerous physiological problems, for example, flower bud abscission, fruit abscission, blankness, non-splitting, deformities and early splitting of nuts (Ferguson *et al.*, 2005).

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Pistachio fruit abscission after the end of flowering, especially in the 'Kaleghoochi' cultivar is a physiological phenomenon whose main cause is probably lack of fertilization (Acar and Eti, 2007). Fruit abscission of pistachio cultivars even in high yielding (on) years approaches 80 percent. Pollen type, pollination and fertilization problems, unfavorable environmental conditions, impaired absorption of carbohydrates, minerals and growth regulators are reported as some reasons for this phenomenon (Crane and Iwakiri, 1985). On the other hand, reduction of water, increased salinity and unfavorable environmental conditions in most pistachio orchards, especially during flowering time has reduced fruit set. Free polyamines such as putrescine, spermine and spermidine are some low molecular weight polycationic compounds that have been shown to play an important role as growth regulators in different stages of growth and development of buds, flowers and fruits in citrus, grapes and plum trees (Eskandari, 1999). Polyamines have different roles in the plant such as control of the flowering, fruit set, growth and development of fruit, ovary evaluation, pollen germination and growth of pollen tube (Tiburcio, 1997). Kamiab *et al.* (2013) indicated that foliar application of polyamines on Badami zarand pistachio seedlings caused more growth under saline stress, in comparison with controls. The results of different studies has shown that foliar spraying of free polyamines decreases the severe abscission of fruits in grapes, mango and lychee (Aziz *et al.*, 2001). Numerous studies have shown that ascorbic acid and polyamines can reduce the bad effects of salinity in pistachio (Bastam *et al.*, 2013 and Kamiab *et al.*, 2013). Bastam *et al.* (2013) has stated that foliar application of ascorbic acid on leaves of 'momtaz' pistachio decreased the harmful effects of salinity and increased growth rates in seedling. On the other hand, in the early part of the growth season, when the temperature is low and the leaves have appeared, mineral absorption is low. At this time the plant starts to flower (increase in cell division and metabolic activities) and the materials stored from last year may not be enough to supply the energy needed for flowering and fertilization.

So if enough minerals are provided for pistachio flowers via foliar spray, they should promote fertilization (Swiet-like and Faust, 1984). Fruit number usually increases with an increased nitrogen supply if other factors are not limiting (Arias *et al.*, 2005). Inadequate nitrogen limits flowering and increases the trend toward alternate bearing, so an increased nitrogen supply increases the formation of fruit from flowers (Kelin and Weinbaum, 1984). The acceptance period of ovules for fertilization is short when the flowers are weak, and correction of flowering weakness via an increased nitrogen supply after harvesting and before the popcorn (balloon) stage, can increase the ovule life span (Zakinthions and Rouskas, 1995). In another experiment it has been shown that amino acids can increase yield in 'Momtaz' and 'Fandoghi' pistachio, because plants are able to use amino acids as a nitrogen source (Bastam *et al.*, 2013). Fruit abscission especially during the heavy crop year is one the most important of problems with pistachio trees (Acar and Eti, 2007). Nut blankness is another physiological pistachio problem. Blank nut production is seen not only in pistachio but it is also common in other nut trees. Blank nut formation is seen to different degrees among pistachio species. For domestic pistachio, blankness is a serious problem in bearing and production. The rate of blankness is dependent on cultivar and year (Alcázar *et al.*, 2006). Pollination and fertilization problems, unfavorable environmental conditions, nutritional stresses, salinity and drought can disturb the proper distribution of carbohydrates and growth regulators, and result in increased blank fruit production (Ferguson *et al.*, 2005). With regard to the non-splitting problem in pistachio, it is known that pollen type, high productivity, improper harvest time and water and nutritional stresses can play a role in development of this phenomenon (Polito and Pinney, 1999). Deformed fruit formation in pistachio is another problem and although some research has been done in this area, it seems that this phenomenon is related to productivity rate, mechanical damage and pest attack (Fabbri *et al.*, 1998). In connection with this issue, it has been confirmed recently that application of ascorbic acid

or polyamine alone on pistachio seedlings and amino acids on trees is very promising (Bastam *et al.*, 2013, Kamiab *et al.*, 2013 and Rahdari and Panahi, 2012). For this reason, we examined in the present study the effects of various concentrations of spermine and spermidine, in combination with amino acids and ascorbic acid, on the growth of different cultivars of pistachio.

The aim of this study was to consider the effect of polyamines, in combination with amino acids and ascorbic acid, on fruit set and yield in different cultivars of pistachio. These combinations of compounds have not been studied up to now. If they can decrease the harmful effects of environmental stress, especially at flowering time, fruit set and yield for this crop should increase.

Materials and Methods

This experiment was done in a commercial orchard in the Asadabad area of Rafsanjan in 2013 and 2014. The position of this area is 29.30° north and 56.05° east at a elevation of 1605 meters above sea level. Average temperature is 6 °C in the winter and 30 °C in the summer. The average annual precipitation is 145 mm. Irrigation in this orchard was carried out over a period of 40 days. The results of soil analysis of orchard are shown in Table 1.

Table 1. Chemical analysis of orchard soil

Texture soil	pH	Absorbable potassium (ppm)	Absorbable phosphorus (ppm)	Total nitrogen %N	Saturation (%)	Electrical conductivity (ds.m)	Depth (cm)
Sandy- clay	7.7	239	13.8	0.03	28	7	0-30

The trees in this experiment were 30 years old and grafted on Badami Riz rootstock. In this experiment, the effect of the polyamines, spermidine and spermine (Sigma-Aldrich Co.), and a mixture of 20 free amino acids [hydroxyproline (11.3%), aspartic acid (4.5%), threonine (3%), serine (3.9 %), proline (8.4 %), Glutamic acid (0.9 %), glycine (11.34 %), alanine (13.21 %), arginine (8.4 %), methionine (4.2 %), isoleucine (4.5 %), leucine (16.5 %), tyrosine (1.5 %), phenylalanine (5.1 %), lysine (5.1 %), histidine (3 %), valine (5.1 %), Cysteine (0.3 %), asparagine (0.3 %) and tryptophane (0.4 %) (Inagropars

Co.)) and ascorbic acid on commercial pistachio cultivars ('Ohadi', 'Akbari' and 'Kaleghoochi') were studied. The foliar spray of treatments was done with 6 concentrations of these compounds as shown in Table 2. Foliar application was done twice: one week before the end of flowering and two weeks after the end of flowering. Three factors concentration, cultivar and year were examined in this research. This experiment was performed as factorial base on a completely randomized block design with three replications in two consecutive years (2013 and 2014).

Table 2. Concentration of treatments which have used in this experiment

Treatments (Concentrations)
1- polyamine (0 mM) + Ascorbic acid (0 g/L)+Amino acids (0 g/L)
2-polyamine (0 mM) + Ascorbic acid (0.5 g/L)+Amino acids (3 g/L)
3- polyamine : Spermine (0.5 mM) + Ascorbic acid (0.5 g/L)+Amino acids (3 g/L)
4-polyamine : Spermine (1 mM) + Ascorbic acid (0.5 g/L)+Amino acids (3 g/L)
5-polyamine : Spermidine (0.5 mM) + Ascorbic acid (0.5 g/L)+Amino acids (3 g/L)
6-polyamine : Spermidine (1 mM) + Ascorbic acid (0.5 g/L)+Amino acids (3 g/L)

Four yielding branches on different geographic sides of each yielding tree were selected and labeled early in the flower-opening period. These branches were one year old, almost equal in length, lacked side branches and in four bunch. The number of fruits on each branch was counted two weeks after flowering ended. At the time of harvesting, namely late September, the total numbers of fruits were counted again and the percentage of fruit abscissions was calculated. At the time of harvesting, all the fruits of each branch were harvested and the yield was calculated by weighting the total dried split nuts separated from each shoot. Also the percentages of blank, non-splitting, splitting and deformed fruits were determined. The lengths and diameters of current year branches were measured by about six months after harvest in late winter (February). Data analysis was done using statistical software SAS (version 9.2) and Duncan's multiple range tests was used to compare means.

Results

The results of analysis of variance (Table 3) indicates that interaction effect of concentrations and cultivars in

different years (A*B*C) on yield and deformed nuts, concentration and cultivars (A*B) on number of nuts per cluster, split and deformed nuts and also cultivars and year (B*C) on split nuts have been significant in levels of 0.01 and 0.05. On the other hand, each of the factors A or B or C (alone) had significant effect on fruit abscission and Blank nuts in 0.01 and 0.05 levels. All of the treatments hadn't significant effect on length and diameter of Shoot except effect of year factor on shoot length.

Effect of interaction exogenous application of treatments (polyamines, ascorbic acid and amino acid) and cultivars on yield per shoot (g) in two continuous years

Evaluation of the effect of different treatments of three cultivars of pistachio shows that ascorbic acid and amino acid treatment significantly increased yields of all cultivars, in comparison with the control, but when they were applied with polyamines the increase in yield was considerably more. Both of concentrations of spermine (0.5 mM and 1 mM) were effective in increasing pistachio yields in each of the two consecutive years. However the higher concentration (1 mM) of spermine was significantly better in the Ohadi cultivar (Table 4).

Table 3. Analysis of variance (ANOVA) of the effect of foliar treatment of polyamines with other compounds on quantitative and qualitative traits in 'Ohadi' 'Akbari' and 'Kaleghoochi' pistachio in two years.

Source of variation	df	Mean square							
		Yield per shoot (g)	No. of nuts per cluster	Fruit abscission (%)	Blank nuts (%)	split nuts (%)	Deformed nuts (%)	Shoot length (cm)	Shoot diameter (mm)
Replication	2	3.7	73.2	2.7	2.4	24.6	0.8	0.8	70.4
Factor A (cultivar)	5	8623.6**	103.6**	613.6**	17.8**	220.8**	17.1**	6.6**	111.7 ^{ns}
Factor B (concentration)	2	7151.1**	828.4**	1114.5**	41.3**	1224.5**	110.1**	0.6 ^{ns}	74.6 ^{ns}
A*B	10	218.1**	30.1**	28.7 ^{ns}	0.7 ^{ns}	68.8**	5.7**	0.5 ^{ns}	66.4 ^{ns}
Factor C (year)	1	624.1**	176.4**	260.1**	77.2**	457.8**	329.4**	2.6*	13.2 ^{ns}
A*C	5	200.6**	6.4 ^{ns}	39.2 ^{ns}	0.4 ^{ns}	14.2 ^{ns}	2.6 ^{ns}	0.05 ^{ns}	69.5 ^{ns}
B*C	2	476.4**	14.4 ^{ns}	6.4 ^{ns}	0.4 ^{ns}	297.6**	2.3 ^{ns}	0.06 ^{ns}	69.2 ^{ns}
A*B*C	10	205**	6.4 ^{ns}	13 ^{ns}	0.4 ^{ns}	14.5 ^{ns}	3.5 ^{ns}	0.07 ^{ns}	69.7 ^{ns}
Error	56	12.6	10.8	22.2	1.4	12.5	2.2	0.5	69.8
CV%	4		12.2	7.6	11.9	6.1	29.6	10.4	8.5

*, ** represents effects significant at probability levels of 0.05 and 0.01 respectively; ns means non-significant (P<0.05).

Table 4. The effect of interaction of treatments (polyamine, ascorbic acid and amino acid) and cultivar on yield per shoot (g) in two continuous years.

Cultivars																	
Kalleghoochi						Ohadi						Akbari					
Concentration																	
Ascorbic Acid (0.5)+ Amino Acid (3) (g/L)						Ascorbic Acid (0.5)+ Amino Acid (3) (g/L)						Ascorbic Acid (0.5)+ Amino Acid (3) (g/L)					
polyamine						polyamine						polyamine					
Control	0	Spr0	spr1	spd0.5	spd1	Control	0	spr 0.5	spr1	spd0.5	spd1	Control	0	spr0.5	spr1	spd0.5	spd1
	mM	.5 mM	mM	mM	mM		mM	mM	mM	mM	mM		mM	mM	mM	mM	mM
Yield per shoot (g), (2013 and 2014)																	
70	90	115	120	83	92.5	47.5	77	70	97.5	59.5	71.5	75.5	95	120	125	84	94
fg	bcd	a	a	de	bcd	h	ef	fg	b	g	f	ef	bc	a	a	cde	bcd

Spd (spermidine), Spr (spermine). Different letters within a column indicate significant differences by Duncan's multiple range test at P<0.01.

Effect of exogenous application of polyamines, Ascorbic acid and Amino acid on yield (weight) per shoot in different pistachio cultivars

All of the treatments improved yields in comparison with controls. Higher concentrations (1 mM) of each polyamine, especially spermine in combination with ascorbic acid and amino acids were more effective

in increase the yields. The average yield in controls was 60 g but increased to 120 g (maximum) for spermine treatment and 80 g for ascorbic acid and amino acid treatment without any polyamine (Fig. 1).

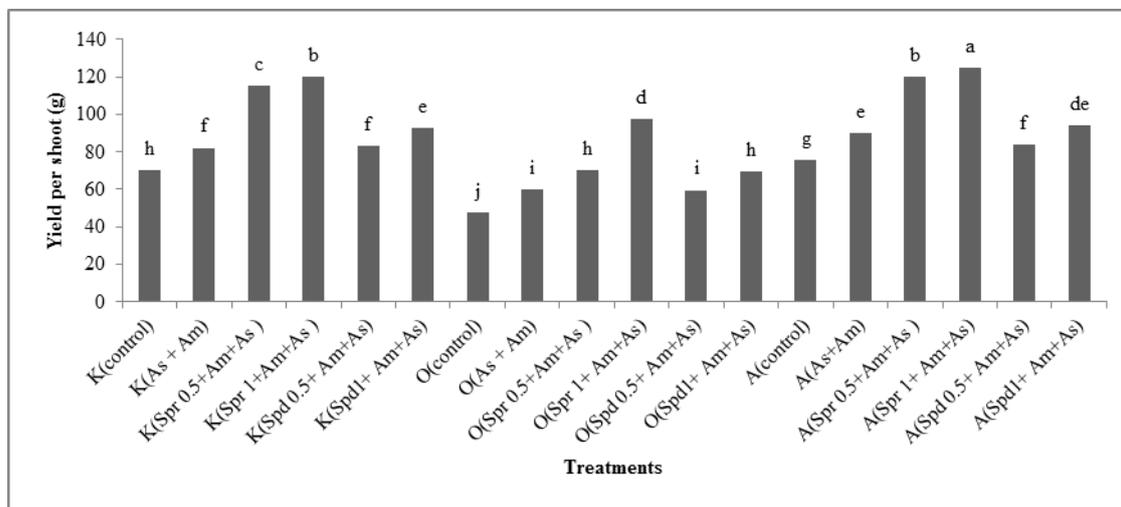


Fig. 1. The effect of interaction of different treatments (polyamine (mM), Amino acid (05 g. l) and Ascorbic acid (3 g. l)) and cultivar on Yield per shoot (g). K (Kalleghuchi), O (Ohadi), A (Akbari), As (Ascorbic acid), Am (Amino acid), Spd (spermidine), Spr (spermine)). Different letters within a column indicate significant differences by Duncan’s multiple range test at P<0.01.

Effect of exogenous application of polyamines, Ascorbic acid and Amino acid on Number of nuts per cluster in different pistachio cultivars

Treatment with spermine at both concentrations (0.5 mM and 1 mM) was more effective with spermidine. The higher concentration (1 mM) of spermine in combination with ascorbic acid and amino acids, especially in Akbari and Ohadi cultivars, gave the best yields. Ascorbic acid and amino acids alone gave the next best results. The average number of nuts per cluster in

controls was 23 but rose to 38 for spermine in combination with ascorbic acid and amino acids and 29 in ascorbic acid and amino acid treatment without any polyamine (Fig 2). It should be noted that the lower concentration (0.5 mM) of spermidine was not significantly better than the control (Fig. 2).

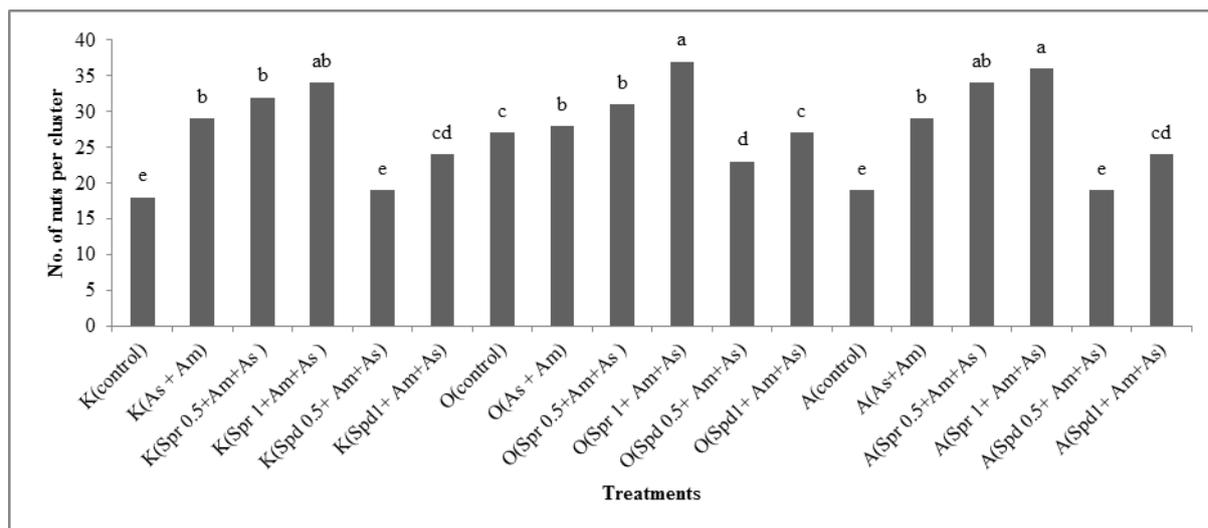


Fig. 2. The effect of interaction of different treatments (polyamine (mM), amino acid (0.5 g. l) and ascorbic acid (3 g.l) and cultivar on no. of nuts in cluster. K (Kalleghuchi), O (Ohadi), A (Akbari), As (Ascorbic acid), Am (Amino acid), Spd (spermidine), Spr (spermine). Different letters within a column indicate significant differences by Duncan’s multiple range test at P<0.01.

Effect of exogenous application of polyamines, ascorbic acid and amino acid on the percentage of split nuts in different pistachio cultivars

The highest increase in split nuts was seen for 1 mM spermine plus ascorbic acid and amino acids, followed by, 0.5 mM spermine plus ascorbic acid and amino acids and then ascorbic acid and amino acids alone for all of the cultivars. The average of split nut percentage rose from 52 % in the control to around 65 % for the

higher spermine plus ascorbic acid and amino acids treatment and about 62 % in ascorbic acid and amino acids alone. But there were no significant differences between the spermidine-treated branches and the controls (Fig. 3).

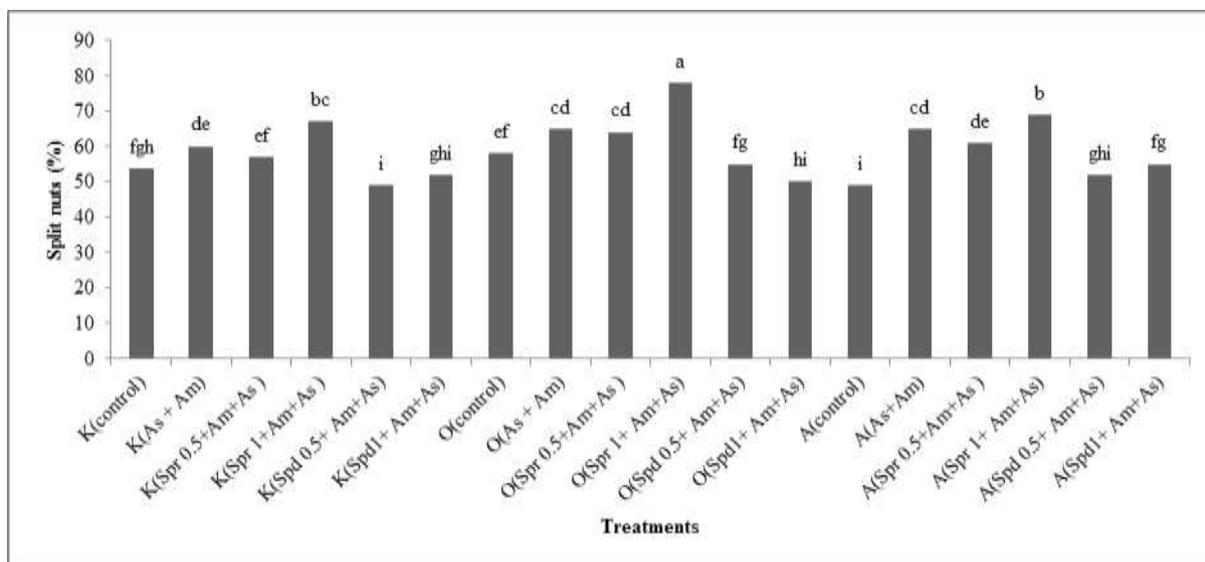


Fig. 3. The effect of interaction of different treatments (polyamine (mM), amino acid (0.5 g. l) and ascorbic acid (3 g. l) and cultivar on split nut percentage. K (Kalleghuchi), O (Ohadi), A (Akbari), As (Ascorbic acid), Am (Amino acid), Spd (spermidine), Spr (spermine). Different letters within a column indicate significant differences by Duncan’s multiple range test at P<0.01.

Effect of exogenous application of polyamines, Ascorbic acid and Amino acid on the percentage of deformed nuts in different pistachio cultivars

All of the treatments decreased the percentage of deformed nuts significantly in comparison with controls. The best results were obtained with 1 mM spermine plus

ascorbic and amino acids, followed by 1 mM spermidine plus ascorbic and amino acids. The average percentage of deformed nuts decreased from about 9 % for

the controls to an average of about 4 % for the other treatments (Fig 4).

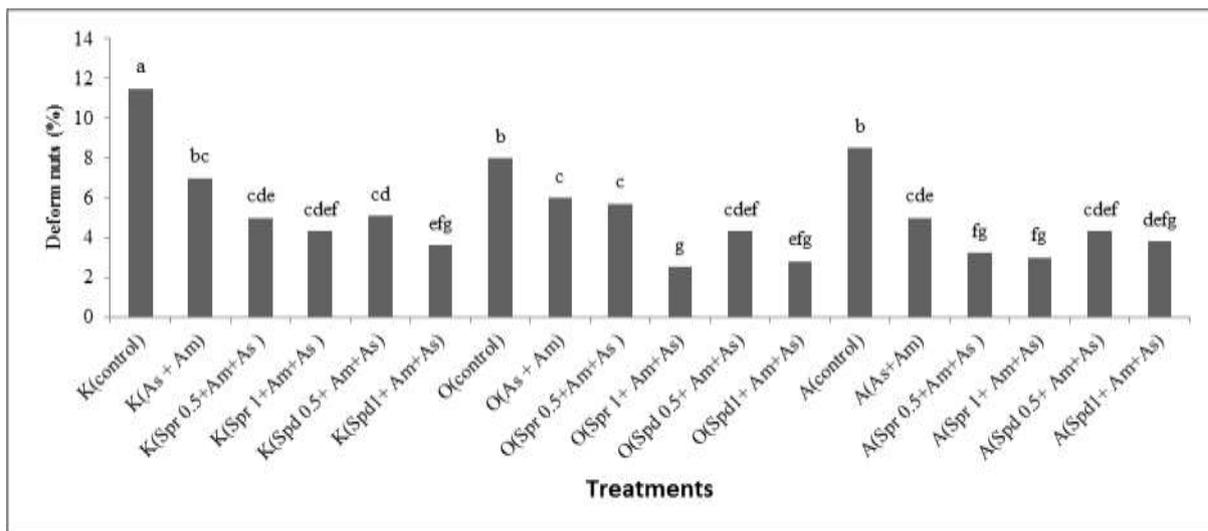


Fig. 4. The effect of interaction of different treatments (polyamine (mM), Amino acid (3 g. l) and Ascorbic acid (0.5 g. l) and cultivar on deformed nuts percentage. K (Kalleghuchi), O (Ohadi), A (Akbari), As (Ascorbic acid), Am (Amino acid), Spd (spermidine), Spr (spermine). Different letters within a column indicate significant differences by Duncan's multiple range test at P<0.01.

Effect of exogenous application of polyamines, ascorbic acid and amino acid on percentage of Fruit abscission

These experiments showed that treatment with 1mM spermine or 1mM spermidine, plus ascorbic and amino acids, reduced the percentage of fruit abscission from about 72 % in the control to around 52 % . A smaller

effect was seen when lower concentrations (0.5 mM) of spermine and spermidine (plus ascorbic and amino acids) were used (Fig 5).

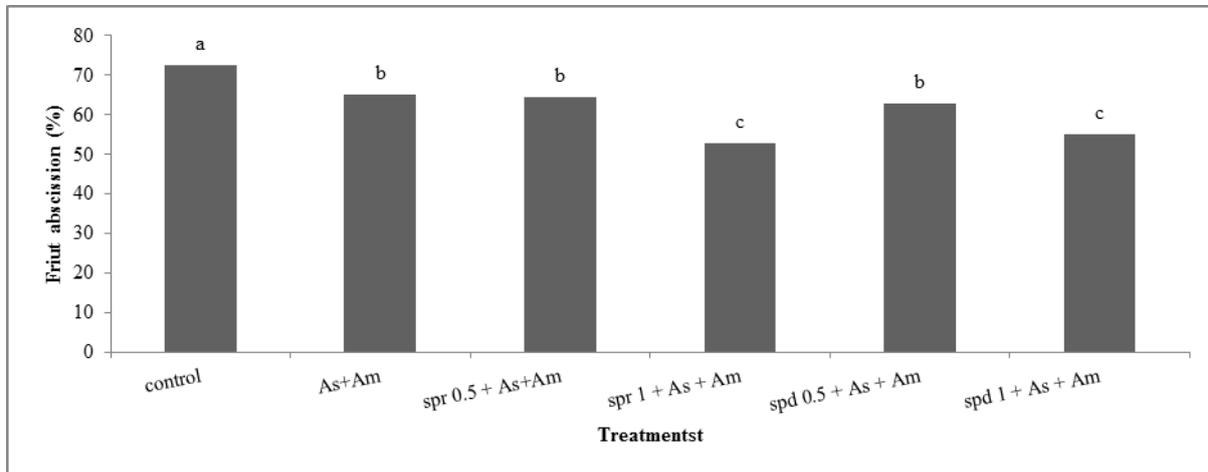


Fig. 5. Effect of different concentrations treatments (polyamine (mM), Amino acid and Ascorbic acid (g.l) on percentage of fruit abscission. Spd (spermidine), Spr (spermine). Different letters within a column indicate significant differences by Duncan's multiple range test at P<0.01.

Effect of exogenous application of polyamines on the percentage of blank nuts

All of the treatments decreased the percentage of blank nuts, but treatment with 1mM spermine plus ascorbic and amino acids was the most effective, followed by ascorbic and amino acids alone. The average

percentage of blank nuts decreased from 12 % in controls has reached to an average of 8 % for the 1 mM spermine plus ascorbic and amino acids treatments and 9 % for the other treatments (Fig 6).

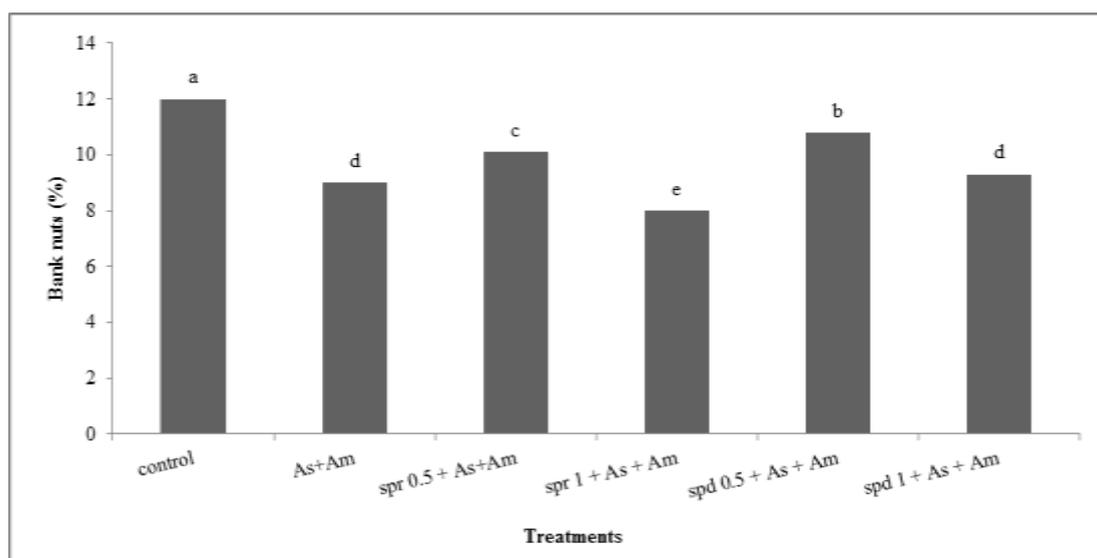


Fig. 6. Effect of different concentrations treatments (polyamine (mM), Amino acid and Ascorbic acid (g. l)) on percentage of blank nuts. Spd (spermidine), Spr (spermine). Different letters within a column indicate significant differences by Duncan's multiple range test at $P < 0.01$.

Discussion

Application of spermine increased the yield of pistachio crop in both years possibly due to the role of polyamines in improving the growth and development of reproductive flowers and fruits (Galston *et al.*, 1997). However, polyamines are also known as polycationic nitrogenous and anti-senescence compounds (Aziz *et al.*, 2001). It is very likely that these bio-regulators 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. Bastam *et al.*, 2013 indicated that application of a foliar spray of ascorbic acid resulted in an increase in salinity stress tolerance in pistachio seedlings. Other research by Molamhamadi (2014) showed that foliar application of 550 mg/L of ascorbic acid at flowering time increased pistachio fruit set. Ascorbic acid is one of the most important and abundant water-soluble anti-oxidants in plants and is responsible for inducing tolerance to a number of biotic and abiotic stresses (Bastam *et al.*, 2013). Studies carried out on organic compounds containing amino acids have reported the successful absorption of these materials by leaves. For instance, foliar application of 5% urea to apple trees increased yields by 62% in comparison with the use of soil nitrogen (Molaei *et al.*, 2013). Zakinthinos and Rouskas (1995) were successful in increasing

the pistachio weight and rate of shell dehiscence by 85-95 percent through two foliar applications of a sugar mixture containing 3% sucrose, 2% glucose, 1% rhamnose and 1% inositol. 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 and Rahdari and Panahi, 2012). In the present research, we applied the results of previous work on foliar application of amino acids and ascorbic acid and selected the most effective concentrations from those studies for inclusion in our studies with polyamines. Thus we observed that fruit abscission decreased considerably when we used these three compounds together on pistachio trees at flowering time. The decrease in the percentage of fruit abscission by application of spermine is ascribed to the inhibitory role of free polyamines in fruit abscission (Arias *et al.*, 2005 and, De Dios *et al.*, 2006). Exogenous application of spermine has shown to be effective in controlling fruit abscission in mango (Malik and Singh, 2006), which we also found in this study. 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). It has been suggested that polyamines improve fertiliza-

tion, and subsequent embryo and fruit development (Galston, *et al.*, 1997), and application of nitrogen has been reported to increase fruit set in fruit trees and to increase the longevity of the ovule in the ovary (Marjani, 2000). In this research the percentage of blank and non-split nuts decreased with application of polyamines. It has been reported that degeneration of the ovary segments, especially of the funicle, is the major cause of blanking in pistachio (Shuraki and Sedgley, 1996) and it has been found that there is a correlation between kernel development and splitting (Ferguson *et al.*, 2005). Thus, decreasing the percentage of blank and non-split nuts by application of spermine might be attributed to the role of spermine in improving the growth and development of reproductive organs and preventing ovary degeneration (Galston *et al.*, 1997). On the other hand, the applications of amino acids increase the production of carbohydrate and assimilation into the plants and, as a result, the growth of kernel increases (Crane and Iwakiri, 1985). Our results showed that both of the polyamines especially spermine decreased the percentage of deformed nuts. If deformation of nuts in pistachio is the result of disease, insect or other physical damages (Niven *et al.*, 1994), it seems that polyamines could possibly increase the resistance of the pistachio hull or shell to these attacks or potentially improve wound healing, as reported in other plants (Williams, 1965). If the fruit cropping load is the main cause of deformation (Fabbri *et al.*, 1998), a balanced growth of hull, shell and kernel may result from the application of polyamines. The application of ascorbic acid as an antioxidant and anti-stress (biotic and abiotic) compound can increase quantity and quality of fruits in pistachio trees (Molamohamadi *et al.*, 2014).

Conclusions

Two exogenous applications, before and after flowering, of spermine, in combination with ascorbic acid and amino acids, resulted in improved quantity and quality of pistachio. Because of reduction of the harmful effects of environmental stress and supplementary nitrogen. Comparison of the effects of polyamines plus

ascorbic and amino acids vs. ascorbic and amino acids alone has shown that the combination with spermine was more effective in increasing the quantity and quality of the pistachio crop. Therefore, 1 mM spermine in combination with amino acids (3 g/L) and ascorbic acid 0.5 g/L) can be used to decrease physiological disorders in this crop.

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