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24-Epibrassinolide improves some physiological disorders in pistachio cultivars

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Abstract: The effect of 24-epibrassinolide foliar spray on some physiological disorders of pistachio cultivars was evaluated in two years. This factorial experiment was designed as a randomized complete block design with three replications. Factor A involved different pistachio cultivars and factor B involved the application time of hormone with four treatments including T1= bud swelling, T2= after full bloom, T3= T1 + T2, and T4= control. Different parameters were recorded. The application of hormone in both different stages caused the maximum fruit set percentage and minimum inflorescence bud and fruit abscission percentage in three sprayed cultivars. Despite of the highest percentage of inflorescence bud and fruit abscission in control treatment of cv. Kallehghoochi, the application of this hormone decreased these parameters in this cultivar in T3 treatment. Chlorophyll, protein, and proline contents of the leaves were increased in all brassinosteroide treatments, especially under two-stage application. Also, ion leakage and reduced sugar were decreased in treated leaves after using hormones. The effect of this hormone on antioxidant enzymes showed that all enzymes (CAT, SOD and APX) except POD were increased by the application of brassinosteroides. The loss of carbohydrate in one-year-old woods indicated the growth stimulating effect of this hormone. Thus, 24-epibrassinolide is a good suggestion to increase the quantity and quality of pistachio, especially in cv. Kallehghoochi.

1. Introduction

Pistachio (*Pistacia vera* L.) is an important horticultural crop that has high economic value. Unfavourable environmental conditions in recent years have resulted in the loss of fruit set and yield in commercial pistachio cultivars. Also, physiological disorders such as bud and fruit abscission, and blank and non-split nuts are the most important problems of this produce that have been deteriorated by the biotic and abiotic stresses in most pistachio-growing areas.

Plant antioxidant defence system consists of such enzymes as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and also, non-enzymatic components may include osmolytes like proline, glycine betaine, sorbital, and mannitol (Anjum *et al.*, 2010, 2012).

In these conditions, growth regulators are useful for the prevention of fruit abscission and increased yield. Nowadays, foliar spray of growth regulators in fruit orchards has become a usual practice, and their considerable effects are evident on fruit quantitative characteristics. Brassinosteroids (BRs) are steroid hormones that are widely distributed in the plant kingdom, with a regulatory function in normal plant growth and development (Mandava, 1988).

Exogenous application of BRs to roots of young tomato and radish plants induced the hypocotyls and petioles (Takatsuto *et al.*, 1983). Brassinosteroids are known as hormones with pleiotropic effects that influence different processes like cell elongation, senescence and xylem differentiation, growth, seed germination, rhizogenesis, flowering, stem elongation, pollen tube growth, leaf epinasty, ethylene biosynthesis, proton pump activation, gene expression, and photosynthesis (Clouse and Sasse, 1998; Dhaubhadel *et al*., 1999; Khripach *et al*., 2000; Steber and McCourt, 2001; Krishna, 2003; Yu *et al*., 2004; Vert *et al.*, 2006). Brassinosteroids also could induce resistance to various abiotic stresses (Rao *et al.*, 2002).

Commercial application of this hormone has been started since 1990 and 24-epiprassinolid and 28-homobrassinolid are more effective in garden conditions because of their stability (Khripach *et al.*, 2000). Effects of 28-homobrassinolide have been reported on yields of wheat, rice, groundnut, mustard potato, and cotton (Ramraj *et al.*, 1997). Foliar spray of brassinoides on watermelon seedling increased female flower, fruit set and yield considerably. In other experiments, effective role of brassinosteroid has been confirmed in vegetative and reproductive growth in strawberries and yellow passion fruit (Ramraj *et al.*, 1997; Gomes *et al.*, 2006). Also, the application of BRs could accelerate the ripening of tomato and grape fruits (Vardhini and Rao, 2002; Symons *et al*., 2006). Despite numerous studies on agronomic crops, there are few investigations into the effect of brassinosteroides on fruit trees.

Hosseinpur *et al.* (2014) used different concentrations (0.5, 0.75, 1 and 1.5 mg/l) of 24-epibrassinolid before flowering on Kallehghuchi pistachio and showed that 1.5 mg/l of epibrassinolid was the best treatment for increasing fruit set of this cultivar. Due to the increasing unfavourable environmental conditions during pistachio flowering, this hormone was tested before and after flowering for increasing fruit set. Numerous studied has showed that effect of this hormone depends on its application time. Thus, we compared different application times on three commercial cultivars to determine the best application time for brassinosteroides on pistachio trees. The objective of the study was to evaluate the effect of 24-epibrassinolid on quantitative and qualitative traits of pistachio fruit.

2. Materials and Methods

Plant material and experiments

This experiment was done in a commercial orchard (Lat. 29°30’ N., Long. 56°05’ E., Alt. 1605 m.) in the Asad Abad district, Rafsanjan, Iran in 2014 and 2015. The results of soil analysis of this orchard are shown in Table 1. Mineral deficiencies are compensated with the use of fertilizer during the growing season.

In 2014, twenty adjacent “on” trees were selected. On each “on” tree, five uniforms “on” shoots were chosen and labeled a week before full bloom. Each selected shoot included four clusters with no lateral shoots. Because of the alternate bearing habit of pistachio trees, “on” trees in 2014 were naturally “off” trees in 2015, so this experiment was repeated by applying the same treatments on the similar previous year’s trees, naturally going into the “off” year, but the treatments were applied on other one-year-old shoots to avoid any experimental effects of the previous year’s treatments. These selected shoots were also uniform in length and diameter similar to the previous year’s shoots, but each shoot included only one cluster. 24-epibrassinolide (1.5 mg/l, Sigma

Table 1 - Chemical analysis of some macro and micro elements of the soil of the pistachio orchard in Asad Abad village, Rafsanjan

Texture soil	pH	Saturation (%)	EC (ds/m)	Depth (cm)	K (ppm)	P (ppm)	N (%)
Sandy-clay	7.7	28	7	0-30	239	13.8	0.03

Company) was sprayed on crown of the trees.

The experiment was carried out on a factorial base with a randomized complete block design with three replications. Factor A involved different pistachio cultivars (Akbari, Kalehghoochi and Ohadi). Factor B involved the time of the application of 24-epibrassinolide in four treatments: T1= bud swelling stage, T2= two weeks after full bloom, T3= application at two stages (T1 and T2) and T4= control (spraying with water). There were 12 treatments with 3 replications in this experiment, and two trees in each experimental unit (totally 72 trees) were compared.

Inflorescence bud and fruit abscission and fruit set

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 (May) and at the harvest time (September), respectively. The percentage of inflorescence bud abscission was calculated by dividing the number of abscised buds by the total number of buds initiated on each shoot.

In order to detect the fruit set percentage and fruit abscission, four branches with almost equal buds in different geographical sides of each tree were selected and marked. These factors were measured by the following formula, respectively:

$$\text{Initial fruit set percentage} = \frac{\text{Numer of fruit set (30th day)}}{\text{Number of flower buds}} \times 100$$

$$\text{Harvest fruit set percentage} = \frac{\text{Numer of fruit set (120th day)}}{\text{Number of flower buds}} \times 100$$

Fruit characteristics

At the harvest time, all clusters were detached from each shoot and were hand-sorted into blank, non-split, and split nuts, fresh and dry weight of fruits were measured on each marked branches. Yield factor is reported in results as dry weight of nuts per each branch.

Biochemical characteristics

All biochemical parameters were measured about two weeks after foliar application of brassinosteroides and spectrophotometer was used for all biochemical measurement.

Assay of protein, APX and POD

Fresh leaf samples (1 g) from all treatments were ground in 5 ml Tris-HCl buffer (0.05 M). The homogenates were centrifuged at 10,000 *g* for 25 min at 4°C. The protein content in the supernatant was analyzed by the procedure of Bradford (1979). The supernatants were also used to analyze the activity of APX and POD enzymes. APX activity was determined by Nakano and Asada method (1981).

Assay of SOD, CAT and H_2O_2

Frozen leaf samples (1 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.8 for SOD and pH 7 for CAT). Then, these materials were centrifuged at 12,000 *g* for 20 min at 4°C. The supernatant was used to measure the activity of the enzymes.

SOD activity was measured according to the method described by Giannopolitis and Ries (1977) and CAT activity was determined by the procedure of Cakmak and Marschner (1992).

Reducing sugars and Proline analysis

Proline and reducing sugars content were extracted from the fresh leaf samples according to Bates *et al*. (1973) and Somogyi (1952), respectively.

Chlorophyll content of leaves

The Chlorophyll content was calculated using the method introduced by Meidner (1984) as:

$$\text{Total Chlorophyll (mg/ml}^{-1}\text{ g}^{-1}\text{): [(17.76} \times \text{OD646.6) + (7.37} \times \text{OD663.6)]} \times \text{V/1000W}$$

where, OD= the read absorbance , V= consumed acetone volume, W= fresh weight of sample (g).

Ion leakage of leaves

The relative permeability of cell membranes was calculated using a slight modification of the method introduced by Zhang *et al.* (2006) as:

$$\text{Relative permeability (\%)} = \frac{EC_1\text{-}EC_0}{EC_2\text{-}EC_0} \times 100$$

Total nonstructural carbohydrate of 1-year old wood

One-year-old stems were immediately placed on ice and were taken to the laboratory. In the laboratory, the stem tissue were dried at 60°C, weighted, ground to pass a 40-mesh screen and analyzed for total nonstructural carbohydrate (TCN). The concentration of starch was measured by the method of Hedge and Hofreiter (1962). Dissolved sugar was calculated using the phenol and sulphuric acid method introduced by Hellebust and Craige (1978). The absorbencies were measured at 485 and 630 nm for dissolved sugar and starch, respectively. The concentration of total sugar and starch were summed to give an estimate of total nonstructural carbohydrate.

Statistical analysis

Finally, the collected data were analysed using SAS software package, the means were compared by Duncan test at 5% level, and the diagrams were drawn in MS-Excel software package.

3. Results

Interaction of brassinosteroides and cultivars for inflorescence bud, fruit abscission, and fruit set in pistachio

The result indicated that the two-stage application of this hormone decreased bud abscission by about 20% in Kalleghuchi cultivar but by about 10% in other cultivars (Akbari and Ohadi) (Table 2). In the "on" year, the highest percentage of fruit abscission was observed in control in Kalleghuchi cultivar and the application of this hormone especially in two stages decreased fruit abscission by about 30% while fruit abscission was decreased by 20% and 18% in Akbari and Ohadi cultivars, respectively (Table 2) and also the foliar spray of this hormone in two stages increased the fruit set significantly so that it was doubled as compared to control.

Interaction of brassinosteroides application time and cultivars for blank and split nuts in Pistachio

The highest blank nut and the lowest split nut were observed in control in 'Kallehghoochi' in two experimental years and in the "on" year, they were about 6% and 54%, respectively. The lowest blank nut and the highest split nut were observed in Kallehghoochi and Ohadi cultivars and brassinosteroides spray at two stages in the "on" year at about 4% and 80%, respectively. The percentage of blank nut was decreased in all cultivars in the "off" year by about 50% when the hormone was applied at two stages and by 25% when it was applied at one stage. In the "off" year, the percentage of split nut was significantly increased only in 'Kallehghoochi' at two stages of brassinosteroides application (Table 3).

Table 2 - Interaction of stage of brassinosteroides application and cultivar for fruit and bud abscission and fruit set percentage of pistachio in two years

	'Ohadi'				'Akbari'				'Kallehghoochi'			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
During 2014 "on year"												
Fruit set (%)	9.5 d	13 c	14 c	16 bc	11 d	14.5 c	18 b	21 a	7.1 f	8.5 e	9.2 d	13.5 c
Friut abscission (%)	58 b	50 c	47 c	40 d	62 ab	54 bc	55 b	42 C	68 a	47 c	43 c	36 d
Bud abscission (%)	68 c	63 d	60 d	58 d	88 a	83 b	80 b	78 b	90 a	78 b	72 c	68 c
During 2015 "off year"												
Fruit set (%)	11 d	12.5 c	13 c	14 c	15 b	16 b	17 b	19 a	9 d	9.5 d	10 d	12 c
Fruit abscission (%)	44 c	42.5 cd	40 d	38 d	50 b	46 c	47 bc	42 cd	54 a	50 b	48 bc	45 c
Bud abscission (%)	50 a	45 ab	43 ab	40 b	30 c	28 c	28 c	22 d	25 cd	24 cd	22 d	22 d

C= Control, T1= Bud swelling phase, T2= Two weeks after full bloom, T3= T1 and T2.
Different letters within a row indicate significant differences by Duncan's multiple range tests at P<0.05.

Table 3 - Interaction of stage of brassinostroides application and cultivar for blank, split, fresh and dry weight of nut, and yield per shoot of pistachio in two years

	'Ohadi'				'Akbari'				'Kallehghoochi'			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
During 2014 "on" year												
Blank nut (%)	4.5 bc	4.2 c	4 c	3.2 d	5.5 ab	5 b	4.5 bc	4 c	6 a	5 b	4.5 bc	4 c
Fresh weight nut (g)	2.1 e	2.1 e	2.4 d	2.9 c	2.9 c	3 c	3.2 b	3.8 a	2.9 c	3 c	3.2 b	3.9 a
Dry weight nut (g)	0.67 d	0.7 d	0.8 cd	0.95 c	0.9 c	1 bc	1.1 b	1.2 ab	0.9 c	1 bc	1.1 b	1.3 a
Split nut (%)	50 d	70 b	65 bc	75 ab	65 bc	70 b	75 ab	79 a	54 d	63 c	70 b	80 a
Yield per shoot (g)	45 e	75 c	80 c	100 b	60 d	90 b	95 c	120 a	50 ed	65 d	75 c	90 bc
Yield per tree (kg)	2 f	2.5 ef	3 e	3.5 de	4 d	5 c	6.1 b	7 a	3 e	3 e	4.1 d	5.2 c
During 2015 "off" year												
Blank nut (%)	10 b	8.5 c	8 c	5.5 d	12 a	8.5 c	9 bc	6d	11 ab	7 cd	6.5 d	6 d
Fresh weight nut (g)	2.8 d	3 cd	3 cd	3.8 a	3.2 c	3.7 ab	3.6 b	3.9 a	3.3 c	3.5 b	3.5 b	3.9 a
Dry weight nut (g)	0.9 e	1 d	1 d	1.2 b	1.1 c	1.2 b	1.2b	1.3 a	1.1 c	1.2 b	1.2 b	1.3 a
Split nut (%)	75 bc	76 bc	78 b	80 b	8 ab	87 a	87 a	90 a	73 c	80 b	80 b	80 b
Yield per shoot (g)	14 e	20 b	18 c	22 a	15 ed	20 b	20 b	22 a	16 d	20 b	18 c	22.1 a
Yield per tree (kg)	1 d	1.1d	1.5 c	1.8 b	1.5 c	1.8 b	2.1 b	2.8 a	1.3 c	1.3 c	1.7 bc	2 b

C= Control, T1= Bud swelling phase, T2= Two weeks after full bloom, T3= T1 and T2.
Different letters within a row indicate significant differences by Duncan's multiple range tests at P<0.05.

Interaction of brassinosteroides application time and cultivars for fresh and dry weight of pistachio nut

The highest fresh weight and dry weight of fruit were observed in 'Kallehghoochi' and in brassinosteroides spray at two stages, which were about 3.9 and 1.3 g, respectively. The lowest fresh and dry weights of fruit in the "on" year were observed in control in 'Ohadi' with no hormone application, which were about 2.1 and 0.67 g, respectively (Table 3).

Interaction of brassinosteroides application time and cultivars for yield per shoot and tree in pistachio

Table 3 showed that brassinosteroides significantly increased the yield per shoot and tree, especially when they were applied at two stages in the "on" year. The interaction between cultivar and the stage of brassinosteroides spray for the yield per shoot showed that the highest yield of branch and tree were observed in 'Akbari' and in brassinosteroides spray at two stages, which were about 120 g and 7 kg, respectively. The lowest yield of branch and tree were observed in control of Ohadi cultivar and they were about 45 g and 2 kg, respectively in the "on" year (Table 3).

Interaction of brassinosteroides application time and cultivars on proline, reduced sugar, and protein of pistachio leaves

Table 4 reveals that the highest amount of reduced sugar and the lowest amount of protein and proline was observed in control in 'Kallehghuchi' in both experimental years. The application of brassinosteroides (at two stages) increased protein and proline contents of the leaves in all pistachio cultivars significantly, especially in the "on" year, and also the lowest amount of reduced sugar was observed in all cultivars in both "on" and "off" years when the hormone was applied at two stages (Table 4).

Interaction of the time of brassinosteroides application and cultivars for chlorophyll and ion linkage of leaves

According to Table 4, the chlorophyll content was significantly increased in all brassinosteroides treatments as compared to control. Maximum chlorophyll content was observed in two-stage application of this hormone in all cultivars. Maximum ion leakage was related to the control in all cultivars and all brassinosteroides treatments showed significantly lower ion leakages. Minimum ion leakage was observed in two-stage application of this hormone in all cultivars (Table 4).

Interaction of the time of brassinosteroides application and cultivars for antioxidant enzyme activities

Figures 1 and 2 shows that activity of all antioxidant enzymes was significantly higher in the "on" year than in the "off" year. The application of brassinosteroides suppressed the activity of all enzymes (CAT, SOD and APX) except POD. The lowest activity of all enzymes was observed in control of Kallehghuchi cultivar in two trial years so that they were remarkably boosted with application of this hormone as compared to other cultivars. Maximum enzyme activity was observed in two-stage application of this hormone in all cultivars.

Interaction of the time of brassinosteroides application and cultivars on annual changes in total non-structural carbohydrate (TCN) concentration of one-year-old wood of pistachio

Figures 3 and 4 reveal that carbohydrate storage

Table 4 - Interaction of stage of brassinostroides application and cultivar for some physiological traits of pistachio in two years

	'Ohadi'				'Akbari'				'Kallehghuchi'			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
During 2014 "on" year												
Proline (µm/g FW)	20 c	22.6 c	25.7 bc	35 ab	20 c	23 c	28 b	38 a	18 c	20 c	30 b	40 a
Reducing sugars (mg/g FW)	30 b	25 bc	24 bc	18 c	35 b	30 b	26 bc	20 c	40 a	30 b	25 bc	12 d
Protein (mg/g FW)	18 bc	19.1 b	20 ab	21 a	19 b	19 b	19 b	21 a	15 d	16.8 c	19 b	2 ab
Ion Leakage (%)	38 b	34 c	35 bc	31 cd	34 c	30 d	30 d	29 d	43 a	40 ab	39 b	34 c
Chlorophyll (mg/ml FW)	21 c	25 b	28 ab	29.9 a	19.5 c	26 b	27 ab	30.3 a	16.3 d	26.5 b	27.3 ab	30.2 a
During 2015 "off" year												
Proline (µm/g FW)	15 c	16 c	16.2 c	20 b	14.5 c	14.5 c	15c	19 b	14.2 c	17.5 bc	20.5 b	25.8 a
Reducing sugars (mg/g FW)	7.7 ab	7.56 b	7.5 b	6.9 c	6.5 d	6.5 d	6 e	6 e	7. 9 a	7.75 ab	7.75 ab	7.42 b
Protein (mg/g FW)	17.1 bc	17.1 bc	17.5 b	17.7 b	19 ab	19 ab	19.1 ab	20.1 a	16 c	16.5 c	18.3 b	20 a
Ion Leakage (%)	40 b	37 c	37 c	32 d	36 c	33 d	31 d	29 e	45 a	43 ab	41 b	38 bc
Chlorophyll (mg/ml FW)	25 c	28 bc	32 ab	34 a	22 d	29 b	30 b	33 a	20 d	29 b	30 b	33 a

C= Control, T1= Bud swelling phase, T2= Two weeks after full bloom, T3= T1 and T2.
Different letters within a row indicate significant differences by Duncan's multiple range tests at $P<0.05$.

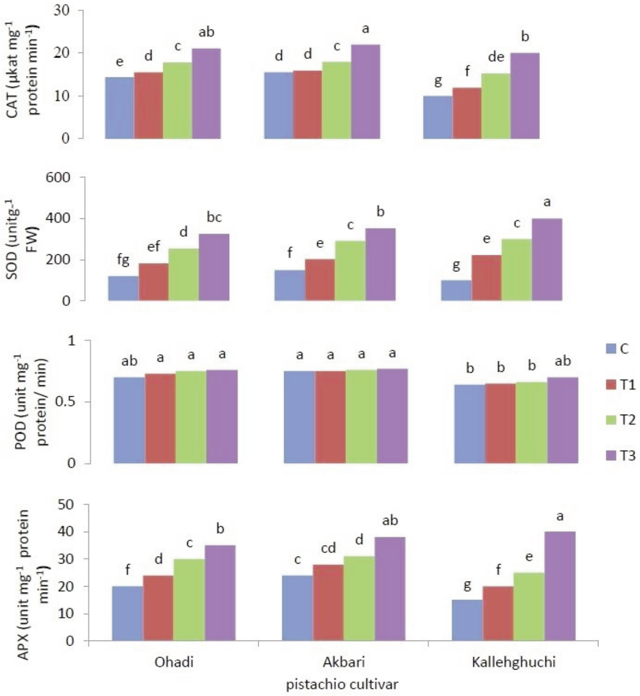

Fig. 1 - Interaction of stage of brassinostroides application and cultivar for the activities of some antioxidant enzyme of pistachio in the "on" year. C= Control, T1= Bud swelling phase, T2= two weeks after full bloom, T3= T1 and T2. Different letters within a row indicate significant differences by Duncan's multiple range tests at P<0.05.

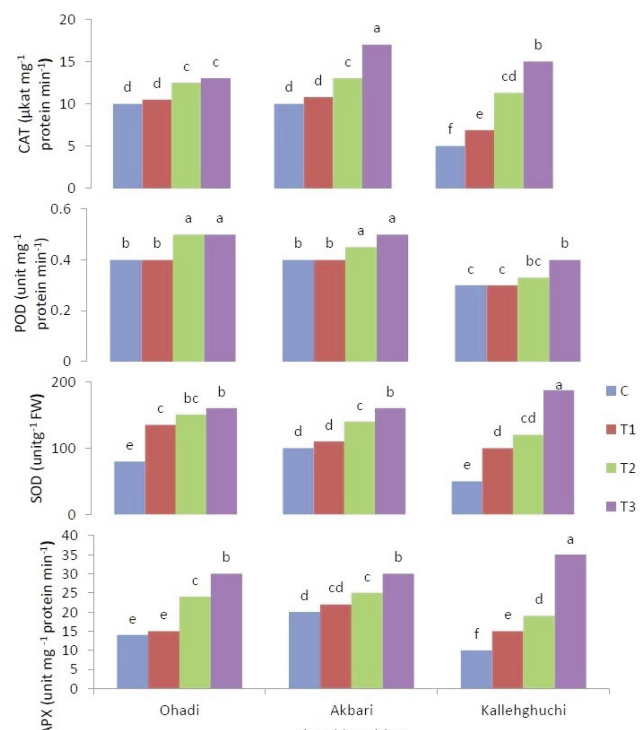

Fig. 2 - Interaction of stage of brassinostroides application and cultivar on activities of some antioxidant enzyme of pistachio in the "off" year. C= Control, T1= Bud swelling phase, T2= two weeks after full bloom, T3= T1 and T2. Different letters within a row indicate significant differences by Duncan's multiple range tests at P<0.05.

in shoots of "on" and "off" trees decreased following the spring growth flash. Figure 3 shows that, in "on" trees, the lowest amount of carbohydrate was observed in Jun and Aug in all cultivars. After harvest (Sep), amount of carbohydrate increased again. Figure 4 shows that, in "off" trees, stored carbohy-

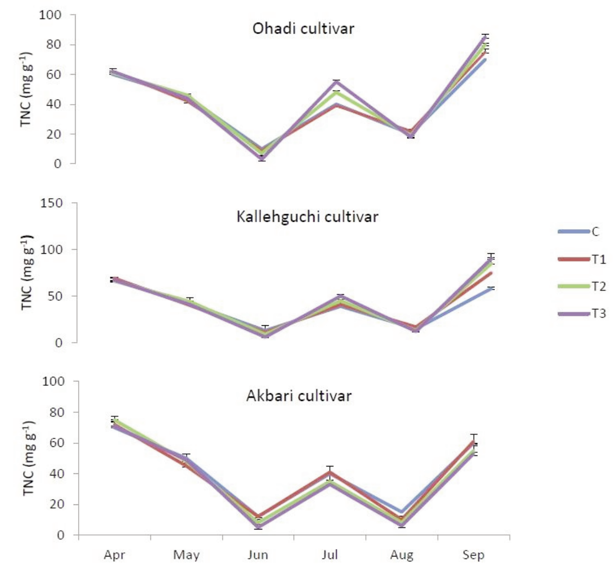

Fig. 3 - Interaction of stage of brassinostroides application and cultivar on annual changes in total non-structural carbohydrate (TCN) concentration of one-year-old wood of pistachio in the "on" year. C= Control, T1= Bud swelling phase, T2= two weeks after full bloom, T3= T1 and T2.

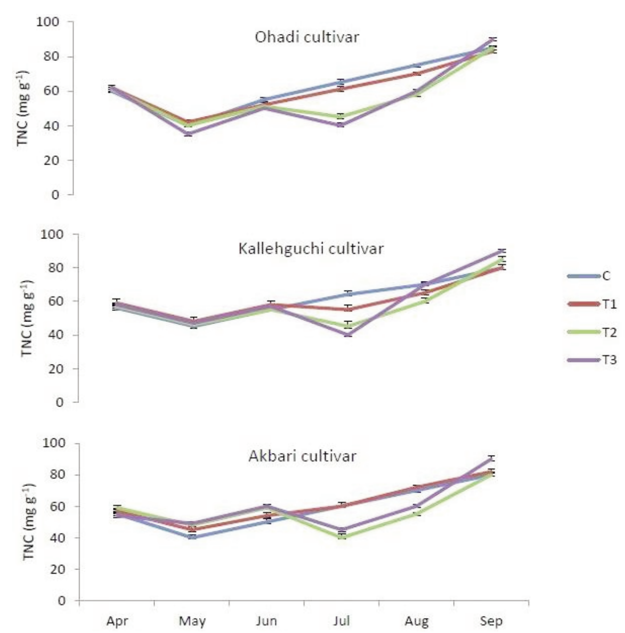

Fig. 4 - Interaction of stage of brassinostroides application and cultivar on annual changes in total non-structural carbohydrate (TCN) concentration of one-year-old wood of pistachio in the "off" year. C= Control, T1= Bud swelling phase, T2= two weeks after full bloom, T3= T1 and T2.

drate of control and T1 treatments increased and remained high after initial growth flash but, in T3 and T4 treatments, it decreased in July and then started to increase again.

4. Discussion and Conclusions

The effect of 24-epibrassinolid application on yield and quantitative traits of pistachio in three cultivars was considerable in this experiment. Maximum yield was observed in Akbari cultivar and brassinosteroides spray at two stages so that it was doubled as compared to the control. Also, the application of this hormone at two stages increased fruit set significantly as compared to control. This finding confirms those reported by Heidari *et al.* (2015) who showed that the application of epi-brassinolid increased fruit set in Alberta peach. Brassinosteroides regulate fruit number and are also able to induce cell division and elongation (Rao *et al.*, 2002). Gomes *et al.* (2006) reported that the use of Brassinosteroides in yellow passion after flowering has led to an increase in fruit number and yield. Foliar application of brassinosteroids resulted in an increase in the number of flowers in strawberry (Leubner-Metzger, 2001), and also in grape fruits, the foliar application of brassinosteroids in autumn increased the number of flowers (Pipattanawong *et al.*, 1996). These results show that the increased yield in this experiment could be due to the increase in pistachio flowers induced by this hormone.

Another problem in pistachio is fruit abscission whose causes are not clearly known. However, environmental influences and competition for resources such as photo assimilates and plant hormones are the likely causal factors of fruit abscission (Thompson, 1996). fruit abscission in this experiment fell by half in Kallehghuchi cultivar at two-stage hormone application. Foliar spray of epibrassinolid on grape (0.01 mg/l) during flowering reduced fruit abscission (Pozo *et al.*, 1994). Brassinosteroides can increase carbohydrate content because of the increase in photosynthesis capacity and translate assimilate from source to sink (Goetz *et al.*, 2000).

Evaluation of the carbohydrate storage in one-year-old wood of pistachio in spring and summer season in this research showed that the amount of carbohydrate decreased in spring (April and May).

This reduction of stored carbohydrate could be related to spring growth flash. In “on” year, there were other two steps of reduction of carbohydrate, the first and second steps were due to summer growth flash and period of kernel fill, respectively. This finding confirms the results of Timothy *et al*. (2008). But it should be noted that the application of this hormone, especially in T3 treatment, decreased significantly the amount of stored carbohydrates in first step of reduction as compared to control. This seems to be due to stimulation of growth after application of this hormone. Stored carbohydrates, in one-year-old wood, after harvest of pistachio were significantly higher in this treatment. In “off” trees, stored carbohydrate of control and T1 treatments increased and remained high after initial growth flash but stored carbohydrate in T3 and T4 treatments decreased in July and then started to increase again. It seems that decreased carbohydrate in July could be caused by the stimulation of growth by the use of hormone. Thus, other reason for higher yield in this experiment is the enhanced growth after application of this hormone caused by the increased number of leaves and shoot and, consequently, more assimilation synthesis for kernel growth and flower bud development.

Chlorophyll content of leaves was increased in the treated plants. Thus, the increased chlorophyll could be the reason for the increase in assimilates in pistachio, resulting in the increased fruit set, fresh and dry weight of nuts and yield. This is consistent with the results of Hassan Zadeh (2013) who showed that the application of epi-brassinolid increased chlorophyll in cantelop.

Unfavourable environmental stresses such as drought and high temperature conditions during flowering and after it decrease fruit set and, on the other hand, increase fruit abscission in most pistachio gardens in Rafsanjan. Plant stress tolerance requires the activation of complex metabolic activities including anti-oxidative pathways, especially ROS-scavenging systems within the cells that in turn can contribute to continued plant growth under stress conditions (El-Mashad and Mohamed, 2012). One of the roles of Brassinosteroids is the increased resistance of plants against various abiotic stresses as confirmed by the results of this experiment. So, APX, SOD and CAT enzymes were increased with the application of this hormone especially its two-stage application. It should also be noted that in ‘Kallehghuchi’ as the most sensitive cultivar to physiological disorder, the amount of antioxidant enzymes were lower than other cultivars and that it was increased with the application of brassinosteroides hormone significantly. There are numerous reports in this regard, some of which are reviewed here. Brassinosteroid-treated tomato and rice plants grew better than control plants under low-temperature conditions (Kamuro and Takatsuto, 1991). In rice, 24-epibrassinolide increased the resistance against chilling stress (1-5°C) and the tolerance was associated with the increased ATP, proline levels and SOD activity, thus indicating brassinosteroid involvement in membrane stability and osmo regulation (Wang and Zang, 1993). Brassinosteroids increased tolerance to high temperature in wheat leaves (Kulaeva *et al.*, 1991).

Results of this experiment showed that the reduction of ion leakage in leaves confirms that this hormone impacts membrane permeability, resulting in resistance to environmental stress. Thus, the increased fruit set and decreased fruit abscission in treated trees could be due to the increased resistance to a biotic stress because of hormone application. On the other hand, the application of epi-prassinolid increased proline content and decreased the reduced sugar in pistachio leaves. It seems 24-epi brassinolid could alleviate the adverse effects of abiotic stress on pistachio cultivars, especially on Kallehghuchi arguably by increasing the activities of ant oxidative enzymes and the contents of proline. The increase in proline and anti-oxidant enzymes are the natural mechanisms to induce tolerance to abiotic stress in plants. In a similar study, Rady (2011) reported that the spray of 5µM 24-epi brassinosteroides to NaCl-exposed *Phaseolus vulgaris* improved the elevation of the activities of anti-oxidative enzymes and proline content. Aghdam *et al.* (2012) reported that the treatments with 0.3 and 0.6 µM BRs to tomato fruits stored at 1°C for 21 days enhanced proline. Foliar application of brassinosteroides was reported to improve Cd-tolerance in *Brassica juncea* through the increase in activity of antioxidative enzymes (such as CAT, POD, SOD) and the content of osmolyte such as proline (Hayat *et al.,* 2007). On the other hand, lower amount of reduced sugar in brassinosteroides treatments could reflect the alleviation of the adverse effects of abiotic stress after the application of this hormone.

Dry and fresh weight of pistachio nuts was increased by about 30% with the application of 24-epibrassinolid at two stages. The increase of nut weight may be another reason for the increased yield in this experiment. Brassinosteroides have important role in such phenomenon as cell division and elongation, photosynthesis rate, transfer material, regulation of enzyme activity and hormone balance, each of which can increase nut weight and yield in pistachio crop. Increased DNA and RNA polymerase and increased synthesis of protein by the use of Brassinosteroides can induce growth (Kalinich *et al.*, 1985). Vardhini and Rao (1998) showed the relationship between increased growth and DNA, RNA and protein synthesis in peanut. Yu (1999) found significant increases in the initial activity of Rubisco and in the sucrose, soluble sugars, and starch contents followed by substantial increases in sucrose phosphate synthase, sucrose synthase and acid invertase activities after BRs treatment. Impaired carbohydrate metabolism and reduced biomass were found in a brassinosteroid-deficient *Arabidopsis* mutant (Schluter *et al.*, 2002).

Increasing of blank nut especially in Kallehghoochi and Akbari cultivars is one of the most important disorders in pistachio trees. It has been reported that the degeneration of the ovary segments, especially funicle degeneration, is the major cause of blanking in pistachio (Shuraki and Sedgley, 1996). Brassinosteroids are considered as hormones with pleiotropic effects as they influence various developmental processes like growth, flowering, pollen tube growth, tissue differentiation, proton pump activation, gene expression and photosynthesis (Khripach *et al.*, 1998, 2000). Therefore, brassinosteroide can improve growth and development of reproductive organs and prevent ovary degeneration and, on the other hand, embryo abortion could be decreased by the application of this hormone because of the inducing protein, carbohydrate synthesis and, consequently, the blank nuts can be decreased.

In this research the percentage of non-split nuts was decreased with the application of epibrassinolid. A correlation has been found between kernel development and splitting (Ferguson *et al.*, 2005). Thus, the decrease in the percentage of non-split nuts by the application of this hormone might be attributed to its role in improving the growth and development of pistachio trees and, on the other hand, the application of epibrassinolid increases the production of carbohydrate and assimilation into the plants and, as a result, the growth of kernel increases (Crane and Iwakiri, 1985).

In conclusion, exogenous application of 24-epibrassinolide indicated the possible direct role of this hormone in alleviating the physiological disorders and increasing the quality and yield in pistachio. Thus, 24-epibrassinolide is a good suggestion for increasing quantity and quality of pistachio, especially in Kallehghoochi cultivar that has the most disorder physiological problems.

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