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Effect of Spermine, Epibrassinolid and their interaction on inflorescence buds and fruits abscission of pistachio tree (*Pistacia vera* L.), "Ahmad–Aghai" cultivar

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Abstract. Several physiological problems such as abscission of inflorescence buds and fruit, incidence of blank, non–split and deformed nuts leads to diminish yield of pistachio trees. This research designed study the effects of epibrassinolid and spermine application on fruit disorders and abscission on *Pistacia vera* "Ahmad–Aghai" cultivar. Shoots, of pistachio trees sprayed with Epi–epibrassinolid and spermine, with three replicates. The hormonal treatments consisted of, 0.5 and 1 ppm Epi–Br and 100 and 200 ppm Spm and their combination. Some yield traits including inflorescence and fruit abscission were studied. Fruit abscission diminished significantly in all treatments in comparison with untreated hormonal trees. Epibrassinolid and spermine treatments decreased fruit blankness percentage and ethylene emission in compared with control. Simlutanouse application of epibrassinolid and spermine effectively raised split percentage in compared to each hormonal treatment alone. Chlorophyll content and carbohydrate content significantly increased by spermine 100 and 200 ppm, epibrassinolid 0.5 ppm and combination of spermine and epibrassinolid 0.5 in compared with the control. According to the results, it seems that spermine, epibrassinolid and combination of these hormones could significantly reduce fruit abscission and improve some of the physiological parameters in pistachio trees, probably through the antagonistic effects on ethylene production.

Keyword: Ethylene emission, Fruit and bud abscission, fruit disorder, pistachio yield.

Introduction

The genus *Pistacia* (Anacardiaceae) consists of eleven species. Among them, *Pistacia. vera* L. is economically important species due to its valuable nuts. Pistachio with about 200 tons production in a year is one of the most important products of Rafsanjan (Kerman, Iran). Pistachio trees display some physiological disorders including abscission of inflorescence buds, fruit abscission, blankness, non–splitting, early splitting and deformation of nuts [ACAR and ETI 2007]. Abscission defined as the separation of a plant part from the parent plant Abscission may occur during different flower and fruit developmental stages [ARTECA and ARTECA 2008].

Abscission of inflorescence buds during the heavy crop year and their maintenance during the light crop year result in the alternate cycle (on/off cycle) of pistachio trees [CRANE and NELSON 1971]. A portion of the flowers produce by pistachio trees are pollinated and set fruit but over time they exhibit significant fruit abscission. In many fruit trees, fruit abscission normally occurs during early fruit development.

This phenomenon is influenced by environmental factors, the species and the physiological state of the tree. Different speculations have been reported to explain the mechanism of abscission. It has been indicated that carbohydrate competition between developing fruits and inflorescence buds as well as nutrient deficiency are of the main causes of the phenomenon [SPANN *et al.*, 2008].

Thus, attention has been centered on possible involvement of plant growth substances in the abscission process.

Environmental conditions, competition for carbohydrates and plant growth substances are among the most important causes of activating abscission zone and fruit abscission in plants which might be adverted by different pollen parents, lack of pollination or fertilization and disturbances in embryogenesis [RODINO, *et al.,* 2014, BUTU, *et al.,* 2015, BUTNARIU, *et al.,* 2015a].

It has been found that exogenous application of plant growth regulators is a beneficial technique to improve the quality and the yield of fruit crops [PANAHI and KHEZRI 2011]. Polyamines (PAs such as *putrescine*, spermine, seprmidine); low molecular aliphatic polycations, are involved in many plant developmental processes.

The role of endogenous polyamines in fruit development and diminishment of fruit abscission and nut traits has been reported in plant species [KHEZRI *et al.,* 2010].

Exogenous polyamines application is a promising method in higher fruit set and fruit quality and lessen fruit abscission [MALIK and SINGH 2006].

Rousses et al found a precise relationship between endogenous content of polyamines and the inflorescence buds abscission in pistachio trees [ROUSSOS *et al.,* 2004]. It was reported that the bloom bud abscission is connected to PA content, In the lower PA concentration abscission and aging will be accelerates [PRITSA and VOYIATZIS 2005].

Brassinosteroids (BRs, polyhydroxysteroids) play an essential role in plant development.

They elicit a broad range of physiological and morphological responses in plants, including stem elongation, leaf bending and epinasty, induction of ethylene biosynthesis and proton pump activation, nucleic acid and proteins synthesis, regulation of carbohydrate assimilation and allocation [KANG *et al.,* 2009].

BRs application can also accelerate the ripening of fruit plants and regulate carbohydrate content [HIWASA–TANASE *et al.,* 2014]. It has been reported that there is a crosstalk between brassinosteroids and ethylene responses affect yield production in plants [DESLAURIERS and LARSEN 2010].

This study was conducted to evaluate the effect of foliar application of epibrassinolid (epi–Br), spermine (SPM) and their combination on fruit and inflorescence bud abscission of pistachio trees Ahmad–Aghai cultivar as commercially important product of Iran.

Some physiological and morphological parameters were also studied in SPM and Br treated branches.

Material and methods

The experiments were conducted on 14–year–old 'Ahmad–Aghaei' pistachio trees grafted on *Pistacia vera* cv. Badami–Riz rootstock at the experimental field of orchard located in Rafsanjan, Kerman province, Iran.

The experiment was actually performed randomized complete block design with four replicates (blocks). Hormonal treatments including spermine (Spm; a group of polyamines) and 24–epi–brassinolid (*epi*–Br) were applied on 3 shots from each tree of the same age on all four replicate blocks.

Treatments were conducted in two stages, once a week before full bloom (Full bloom was defined as the date when 80 % of the flowers on each tree were open) in the mid–April and five weeks after full bloom in late May.

Hormonal treatments included: control (Spm 0, epi–Br 0), 100 ppm Spm, 200 ppm Spm, 0.5 ppm epi–Br,1 ppm epi–Br, 0.5 ppm epi–Br+ 100 ppm Spm, 0.5 ppm epi–Br+ 200 ppm SPM, 1 ppm epi–Br+ 100 ppm Spm, 1 ppm epi–Br+200 ppm Spm.

Some physiological and yield parameters such as fresh and dry fruit weight, fruit and inflorescence buds abscission, length and diameter of branches, leaves area and length, split and fruit blankness percentage, the amount of fruits ethylene, leaves carbohydrates content, total chlorophyll content, total protein content were assessed two months after the hormonal applications (September of the same year).

Fruit fresh weight and the yield:

Fruit fresh weight and yield (including split and non–split fruits in each treated branches) were measured at the time of harvest (September).

Hence, fresh weight of split and non–split fruits having fresh skin were calculated using digital scale.

After separation of the fruit skin, and complete drying of the fruit, the yield from each shoot was calculated using a digital scale.

Inflorescence bud abscission and fruit abscission: The number of initiated inflorescence buds and the total number of abscised buds on the individual current–year shoots were counted five weeks after full bloom and at harvesting time, respectively.

The percentage of inflorescence bud abscission was calculated using following equation:

$$\text{inflorescence buds abscission} = \left(\frac{\text{total number of abscised buds} \times 100}{\text{The number of initiated inflorescence buds}}\right) \times 100$$

The number of fruit set and the total number of abscised fruits on each cluster were counted six weeks after full bloom and at the harvest time, respectively. The percentage of fruit abscission was calculated using following equation:

$$\text{fruit abscission} = \left(\frac{\text{total number of abscised fruits} \times 100}{\text{The number of initiated fruits}}\right) \times 100$$

Fruit characteristics: All the clusters were detached from each shoot at the harvest time.

Blankness and split nuts were counted to measure fruit blankness% and split% on each branch.

Number of nuts in ounce: The number of nuts per ounce is a marketing index to show the size and weight of the pistachio nuts, the lower the number of nuts per ounce the larger the nut size.

It has been calculated using the following formula:

$$\text{number of nuts per ounces} = \frac{\text{split fruit number} \times 28.3}{\text{split dry weigth}}$$

Ethylene emission: Ethylene released from fruits detached at mid–May was measured using gas chromatography technique.

Fruits were removed from branches and placed in glass vials. Vials were then immediately sealed with caps incubated for 24 h.

A 1mL sample of air was extracted through the insulin syringe and injected into a gas chromatograph (Agilent, 115–34H, USA) fitted with a flame ionization detector and a glass column packed with (30–100 mesh) activated alumina (180 cm × 0.34 cm o.d.). The GC was operated at injector, detector and oven temperatures of 90, 200 and 250 ºC, respectively.

Nitrogen was used as the mobile phase. Pure ethylene (99.9 %) was used as a standard.

Leaf area and shoot growth: Leaf area (including the beginning to the end of the measured leaf area) from the fully expanded leaves were calculated using millimeter paper.

The length and diameter of current–year shoots were measure at the harvest time.

Determination of soluble sugar content: 0.1 g Frozen leaves were grinded and extracted with 2.5 mL of 80 % (v/v) ethanol at 90 °C for 60 min, followed by centrifugation at 10 000×g at 4 °C for 10 min. Total soluble sugar content was determined using Anthrone reagent and glucose as standard. Results expressed as mg soluble sugar g^{-1}DW.

Total Soluble Proteins: Protein content of leaves was determined according to BRADFORD method by using Bovine serum albumin as standard. Results expressed as mg g^{-1}fw [BRADFORD, 1976].

Total chlorophyll content: To measure the amount of total chlorophyll, the pigment extract was measured against a blank of 80% (V/V) acetone at wavelengths of 646.8 and 663.2 nm for chlorophyll assays.

Statistical Analysis: GLM procedure of SAS statistical software was used for data analysis. Group data means were separated by Duncan's multiple range test ($P < 0.05$).

Results and discussion

Application of spermine and epi–brassinolid and their combination improved yield traits such as fruit abscission and fresh weight, nut blankness and splitting on pistachio, were studied.

Fruit characteristics; Inflorescence bud and fruit abscission:

The results from Duncan's multiple range of inflorescence bud abscission and fruit abscission are given in table 1.

Based on table 2, minimum buds abscission was observed at 100 ppm Spm, while Epi–Br 0.5 ppm+ Spm 100 ppm treatment minimized fruit abscission among the other groups (P<0.05).

Table 1.

Effects of exogenous application of spermine, epibrassinolid and their combination on inflorescence bud abscission and fruit abscission in *Pistacia vera* L., Ahmad–Aghai cultivar

Treatment	Inflorescence bud abscission (%)	Fruit abscission (%)
control	55.7±8.2 a	27.3±1.3 a
Epi 0.5 ppm	37.5±4.7 ab	12.2±1.0 cd
Epi 1 ppm	39.1±3.9 b	21.6±0.7 ab
Spm 100 ppm	30.5±2.4 b	11.5±0.5 cd
Spm200 ppm	43.8±6.1 ab	22.2±5.1 ab
Epi 0.5 ppm+ Spm 100 ppm	32.9±2.1 b	10.3±1.7 d
Epi 0.5 ppm+ Spm 200 ppm	31.0±2.4 b	11.2±1.1 cd
Epi 1 ppm+ Spm 100 ppm	43.5±5.7 ab	18.4±2.9 bc
Epi 1 ppm+ Spm 200 ppm	41.0±7.4 ab	20.6±2.8 ab

Different letters within a column indicate significant differences by Duncan's multiple range test (P < 0.05); no letters within a column indicate no significant differences. It was proposed that endogenous polyamines may regulate the process of inflorescence bud abscission in plants.

Spm as predominant endogenous polyamine in buds and shoots of pistachio exhibits a negative correlation with the inflorescence bud abscission.

Table 2.

Effects of exogenous application of spermine, epibrassinolid and their combination on fruit fresh weight and yield in *Pistacia vera* L., Ahmad–Aghai cultivar

Treatment	fresh weight (gr)	Yield (gr)
control	95.8±3.0 c	38.3±1.2 c
Epi–Br 0.5 ppm	127.7±27.3 ab	51.7±1.9 ab
Epi–Br 1 ppm	113.3±21.3 bc	40.8±7.6 bc
Spm 100 ppm	128.2±18.4 ab	57.4±9.3 ab
Spm200 ppm	126.2±12.2 ab	50.2±4.9 ab
Epi–Br 0.5 ppm+Spm 100 ppm	126.1±15.4 ab	50.6±5.1 ab
Epi–Br 0.5 ppm+Spm 200 ppm	128.9±12.7 ab	56.3±7.0 ab
Epi–Br 1 ppm+ Spm 100 ppm	114.6±9.6 bc	45.4±3.8 bc
Epi–Br 1 ppm+ Spm 200 ppm	113.6±11.1 bc	42.2±1.7 bc

The higher Spm concentration of the shoot may cause the lower inflorescence buds abscission [ROUSSOS *et al.*, 2004]. The decrease in the percentage of fruit abscission by application of Spm is ascribed to the inhibitory role of free polyamines in fruit abscission.

Exogenous Spm was shown to be effective in controlling fruit abscission in mango [MALIK and SINGH 2006, BUTNARIU, 2012, PETRACHE, *et al.*, 2014, BUTU, *et al.*, 2014b].

These results support the findings from the current study, which fruit abcission decreased by Spm application. The main reasons for fruit abscission in pistachio are not clearly known. However, environmental influences and competition for resources such as photo–assimilates and plant hormones are likely causal factors of fruit abscission.

Ethylene, is the most dominant hormonal factor promoting senescence and exogenous application of ethephon (an ethylene producer) enhanced bud abscission on non–fruiting branches [ROUSSOS *et al.*, 2004].

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Figure 1 shows fruit blankness and fruit splitting under hormonl treatments.

Nut blankness % decreased in all SPM and Epi–Br treatments in compared with control. 100 ppm spermine was the most effective treatment for diminishment of fruit blankness (Figure 1 A). Both Epi–Br and Spm treatments significantly increased fruit splitting of pistachio. The lowest splitting rate was observed at control nuts (P<0.05). Combined treatment of Spm and Epi–Br on all concentrations effectively increased fruit splitting in pistachio trees (Figure 1 B).

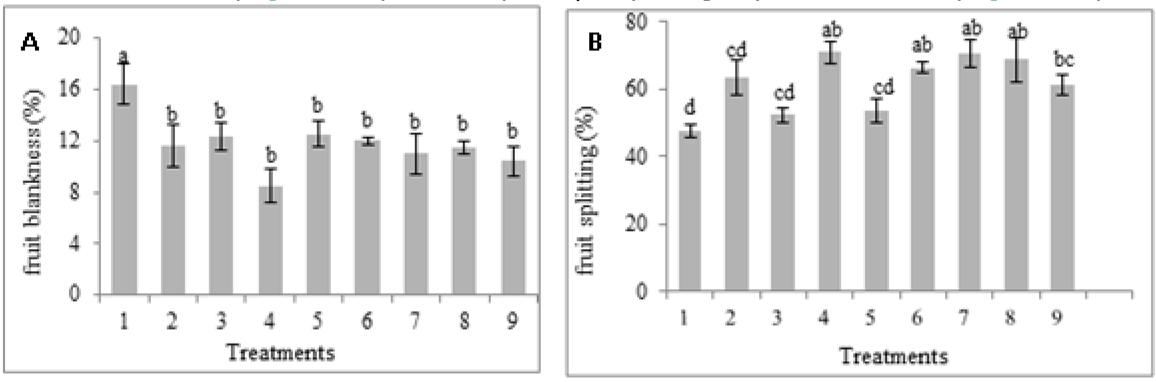

Figure 1. Effects of exogenous application of spermine (Spm) and epibrassinolid (Epi–Br) and their combination on fruit blankness (A) and fruit splitting (B) in *Pistacia vera* L., Ahmad– Aghai cultivar. Stages of application are as follows. Treatments consist of 1–control (0 Spm, 0 Epi–Br), 2–Epi–Br 0.5 ppm, 3–Epi–Br 1 ppm, 4–Spm 100 pmm, 5– Spm 200 ppm, 6– Epi–Br 0.5 ppm+ Spm 100 pmm, 7–Epi–Br 0.5 ppm+ Spm 200 pmm, 8–Epi–Br 1 ppm+Spm 100 pmm and 9–Epi–Br 1 ppm+Spm 200 pmm. Different letters within a column indicate significant differences by Duncan's multiple range test (P < 0.05); no letters within a column indicate no significant differences. The vertical bar is SE.

Duncan's analysis of yield and fruit fresh weight (Table 2) point that both Spm and Epi–Br significantly increased the branch yield and fruit fresh weight in compared with the control trees (P<0.05).

Inflorescence bud abscission and fruit abscission showed a remarkable decrease in trees treated by Spm, Epi–Br and Spm+Epi–Br treatment.

Different letters within a column indicate significant differences by Duncan's multiple range test (P < 0.05); no letters within a column indicate no significant differences.

Degeneration of the ovary segments especially funicle have been proposed as the major causes of blanking in pistachio. It was found that there is a correlation between the kernel development and splitting [FERGUSON *et al.*, 2005, BUTU, *et al.*, 2014c, CAUNII, *et al.*, 2015, BUTNARIU, *et al.*, 2016]. Thus, lower percentage of blank and non–split nuts by Spm treatment observed in this study, might be correlated to the role of Spm in improving the growth and development of reproductive organs and preventing ovary degeneration as it was reported by Galston and collab. The polyamines led to reduce blankness percentage probably by maintaining different part of the embryo sac [GALSTON *et al.*, 1997]. It has been reported that brassinosteroids depending on concentration, developmental stage of the plant and the environmental condition may cause increasing fruit weight, length and its size, and improvement of the yield [YUAN *et al.*, 2010].

Fruit ethylene emission: Based on results in figure 2, Spm and Epi–Br treatments significantly supressed ethylene emission from pistachio fruits in comparison with control (P<0.05).

The correlation between ethylene production and fruit abscission in plants was reported before [WAGSTAFF *et al.*, 2005]. Rahemi and Ramezanian reported that the application of ethephon increased the fruit abscission of pistachio [RAHEMI and RAMEZANIAN, 2007]. It seems that polyamines by an antagonistic effect with ethylene possibly through competing for their common precursor; SAM, reduce the abscission of inflorescence buds, flowers and fruits [ALCáZAR *et al.*, 2006].

It has been shown that BR–ET signaling pathways interact antagonistically which is in consistent with the negative BR–ET relationship observed on growth responses [DESLAURIERS and LARSEN 2010]. Our study indicated that ethylene emission from fruits decreased either by Spm or Epi–Br, so it seems that treatments exhibiting antagonistic relationship with ethylene may causes reduced ethylene production and nut/fruit abscission and amplified yield production. However, this effect was more significant in the combined hormonal treatment.

The results of this study confirmed an antagonistic relationship between epibrassinolid exogenous treatment and ethylene effects.

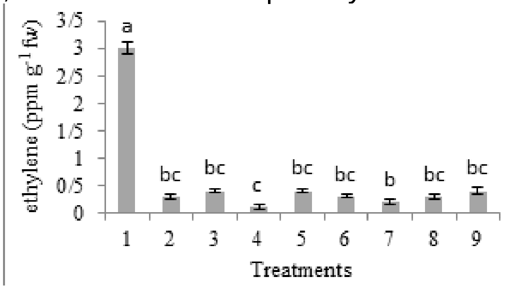

Figure 2. Effects of exogenous application of spermine and epibrassinolid on ethylene emission of fruits in pistachio treated branches (*Pistacia vera* L.), Ahmad–Aghai cultivar in. Treatments consist of 1– control (0 Spm, 0 Epi–Br), 2–Epi–Br 0.5 ppm, 3– Epi–Br 1 ppm, 4– Spm 100 pmm, 5–Spm 200 ppm, 6– Epi–Br 0.5 ppm+Spm 100 pmm, 7–Epi–Br 0.5 ppm+ Spm 200 pmm, 8– Epi–Br 1 ppm+Spm 100 pmm and 9–Epi–Br 1 ppm+Spm 200 pmm. Different letters within a column indicate significant differences by Duncan's multiple range test (P < 0.05); no letters within a column indicate no significant differences. The vertical bar is SE. The vertical bar is SE.

Growth parameters: Figure 3 reveal the effect of Spm and Epi–Br application some growth parameters of pistachio trees. 0.5 ppm Epi–Br, 100 ppm Spm and combination treatment of 0.5 ppm Epi–Br+200 ppm SPM) had a significant (P<0.05) effect on shoot diameter compared to control (Figure 3–A). 100 ppm Spm and its combination with Epi–Br 0.5 had a significant effect (P<0.05) on shoot length compared to control trees (Figure 3–B).

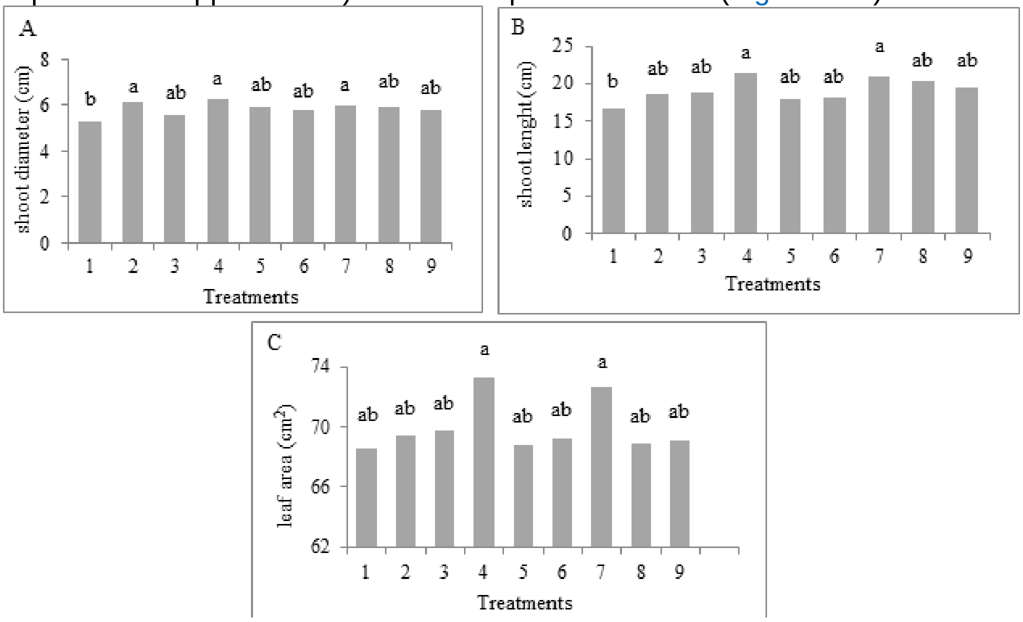

Figure 3. Effects of exogenous application of spermine (Spm) and epibrassinolid (Epi–Br) and their combination on shoot diameter (A), shoot length (B), and leaf area (C) in *Pistacia vera* L., Ahmad–Aghai cultivar. Treatments consist of 1–control (0 Spm, 0 Epi–Br), 2–Epi–Br 0.5 ppm, 3–Epi–Br 1 ppm, 4–Spm 100 pmm, 5–Spm 200 ppm, 6–Epi–Br 0.5 ppm+Spm 100 pmm, 7–Epi–Br 0.5 ppm+Spm 200 pmm, 8–Epi–Br 1 ppm+ Spm 100 pmm and 9–Epi–Br 1 ppm+Spm 200 pmm.

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As it is shown in figure 3–C, none of the treatments had significant effect on leaf area of pistachio trees.

Different letters within a column indicate significant differences by Duncan's multiple range test ($P < 0.05$); no letters within a column indicate no significant differences. The vertical bar is SE. Growth of cell and tissue depends on the synthesis of proteins and nucleic acids. It was reported that polyamines are involved in the process of growth. Growth often increases by higher cell division [HANZAWA *et al.*, 2000, BUTNARIU, 2014, BARBAT, *et al.*, 2013, BUTU, *et al.*, 2014a, BUTNARIU, *et al.*, 2015b].

It has been suggested that polyamines improve fertilization, embryo and fruit development [GALSTON *et al.*, 1997].

Exogenous application of free polyamines resulted in decreasing physiological disorders and increasing yield of 'Kaleh–Ghoochi' pistachio shoots [KHEZRI *et al.*, 2010]. Previous studies also suggested that BRs act synergistically with auxin to promote cell elongation [HALLIDAY 2004]. Brassinolide application on bean *(Phaseolus vulgaris* L. var. Pinto) and mung bean *(Phaseolus aureus* Roxb.) plants increased the RNA and DNA polymerase activities and the synthesis of RNA, DNA, and protein.

These observations suggest the involvement of brassinosteroids in transcription and replication during tissue growth [BAJGUZ and HAYAT 2009].

Our results showed that some of treatments are able to increase of growth parameters of pistachio shoot under hormonal treatment.

Biochemical parameters: Soluble sugar content of pistachio leaves significantly increased ($P<0.05$) by 100 and 200 ppm Spm, and 0.5 ppm Epi–Br +Epi–Br 0.5 ppm in comparison with control (Figure 4).

Protein content was also increased at 100 ppm spermine treatment, Epi–Br 0.5 ppm+ Spm 100 ppm and Epi–Br 0.5 ppm+ Spm 200 ppm ($P<0.05$) in leaves (Figure 5).

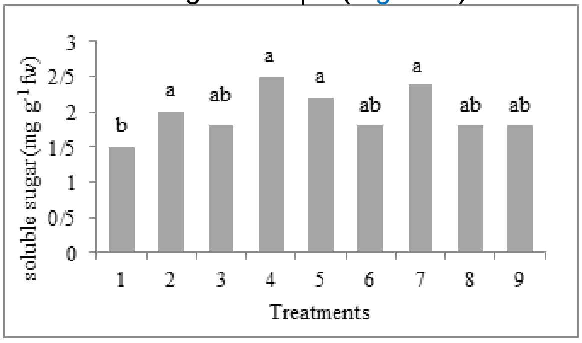

Figure 4. Eeffects of exogenous application of spermine (Spm) and epibrassinolid (Epi–Br) on soluble sugar content of leaf in *Pistacia vera* L., Ahmad– Aghai cultivar. Treatments consist of 1–control (0 Spm, 0 Epi–Br), 2–Epi–Br 0.5 ppm, 3–Epi–Br 1 ppm, 4–Spm 100 pmm, 5–Spm 200 ppm, 6–Epi–Br 0.5 ppm+Spm 100 pmm, 7–Epi–Br 0.5 ppm+Spm 200 pmm, 8–Epi–Br 1 ppm+Spm 100 pmm and 9–Epi–Br 1 ppm+ Spm 200 pmm.

Different letters within a column indicate significant differences by Duncan's multiple range test ($P < 0.05$); no letters within a column indicate no significant differences. The vertical bar is SE. The vertical bar is SE.

Figure 6 summarized the effect of hormonal application on chlorophyll content of pistachio leaves.

Epi–Br 0.5 ppm, Spm 100 and 200 ppm and the combination of Epi–Br 0.5 and Spm 200 caused higher total chlorophyll content in leaves compared to control trees (Figure 6).

The highest chlorophyll content was observed on Spm 100 ppm treatment.

It has been shown that the carbon shortage drop increases abscisic acid (ABA) and ethylene, both are involved in the induction of the abscission of different organs [GóMEZ–CADENAS *et al.*, 2000].

Carbohydrate content and soluble sugars are highly correlated to fruit abscission. BRs lead to a specific enhancement of leaf carbohydrate content [GOETZ *et al.*, 2000, CERNEA, *et al.*, 2015, SAMFIRA, *et al.*, 2015, BUTNARIU and GIUCHICI, 2011].

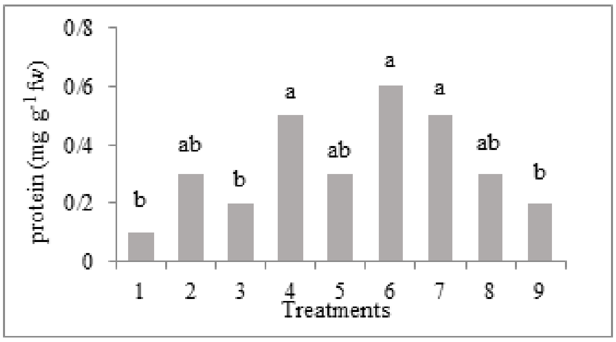

Figure 5. Effects of exogenous application of spermine (Spm) and epibrassinolid (Epi–Br) on protein content of leaf in *Pistacia vera* L., Ahmad– Aghai cultivar. Treatments consist of 1– control (0 Spm, 0 Epi–Br), 2–Epi–Br 0.5 ppm, 3– Epi–Br 1 ppm, 4–Spm 100 pmm, 5–Spm 200 ppm, 6–Epi–Br 0.5 ppm+Spm 100 pmm, 7– Epi–Br 0.5 ppm+Spm 200 pmm, 8–Epi–Br 1 ppm+ Spm 100 pmm and 9–Epi–Br 1 ppm+ Spm 200 pmm.

In this study, epibrassinolid treatments significantly increased the amount of carbohydrates in leaves while it reduced fruit and bud abscission.

So it is probable that Br treatments reduce abscission by increasing the amount of carbohydrates content.

It has been reported that Epi–Br may cause higher carbon fixation efficiency through higher chlorophyll content in soybean [ZHANG *et al.,* 2008]. Spm application also caused higher sugar content in leaves of treated branches.

It has been reported that exogenous polyamines increased soluble sugar content in both leaves and inflorescences of grapevine.

It was proposed that polyamines regulate fruitlet abscission in grapevine by modulating the levels of sugars and amino acids [AZIZ, 2003, BUTNARIU and CORADINI, 2012, PENTEA, *et al.,* 2015, BUTNARIU, *et al.,* 2012].

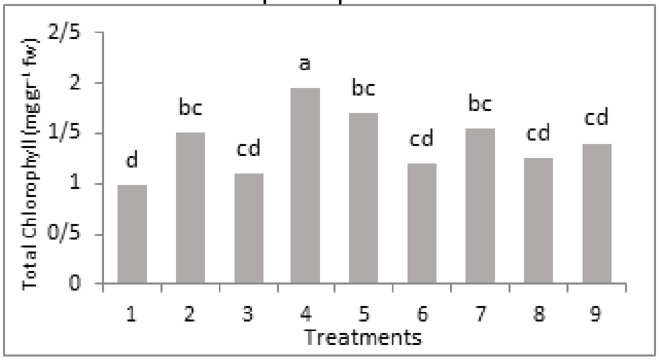

Figure 6. Effects of exogenous application of spermine (Spm) and epibrassinolid (Epi–Br) on chlorophyll content of leaf in *Pistacia vera* L., Ahmad– Aghai cultivar. Treatments consist of 1– control (0 Spm, 0 Epi–Br), 2–Epi–Br 0.5 ppm, 3–Epi–Br 1 ppm, 4–Spm 100 pmm, 5–Spm 200 ppm, 6–Epi–Br 0.5 ppm+Spm 100 pmm, 7–Epi–Br 0.5 ppm+Spm 200 pmm, 8–Epi–Br 1 ppm+ Spm 100 pmm and 9–Epi–Br 1 ppm+Spm 200 pmm.

Different letters within a column indicate significant differences by Duncan's multiple range test ($P < 0.05$); no letters within a column indicate no significant differences.

The vertical bar is SE. The vertical bar is SE.

Conclusions

According to the result, it seems that exogenous application of spermine, epibrassinolid are good candidate to improve fruit and yield commercially important traits of *Pistacia vera* "Ahmad–Aghai". Simultaneous application of Spm and Epi–Br has also the positive significant effects on abscission suppress and improvement of fruit traits possibly through the antagonistic effects on ethylene production.

Hormonal treatment caused reinforced chlorophyll; protein and carbohydrate content in pistachio trees thereby diminish physiological disorders during abscission process of pistachio plants. In this research the 100 ppm spermine showed the greatest impact on physiological parameters and yield trait of "Ahmad–Aghai" *Pistacia vera*.

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