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Effect of Pollination Time on Yield and Antioxidant Properties of Some Pistachio Cultivars

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Abstract
To obtain the best quality and quantity of pistachio fruits, knowing the most suitable time of pollination, is of paramount importance. This study was conducted to investigate the effect of pollination time, different stages of female flower development, including A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), and E (the stigma in the lowest and medial florets was pink) on quantitative and qualitative characteristics of fruits in three pistachio cultivars ('Ghermez Peste', 'Kal Khandan', and 'Kaleh Bozi'). Results showed that the highest yield in 'GhermezPeste' obtained when the manual pollination carried out at the stage that the cluster were green (early flowering period) while the highest yield In 'KalKhandan' and 'KalehBozi' were observed when the pollination carried out at the full bloom stage. The highest amount of total phenolics, total flavonoid, and antioxidant activity of fruits observed when pollination carried out at the stage that lower florets opened and clusters were green. The Lowest amount of total flavonoid and antioxidant capacity were obtained in the fruits that pollination carried out in the stage that the stigma in middle and lower florets were pink. As a first step towards identifying the best time for manual pollination of pistachio trees, our results showed manual pollination in the suitable blooming stage led to higher yield and quality of pistachio nuts.

Keywords: Antioxidant, Blooming, Flavonoid, Manual pollination, Nut, *Pistacia vera.*

Introduction

Pistachio (*Pistacia vera*L.) is one of the most important, widely grown commercial plant crops in Iran. Iran is one of the two major centers of diversity and the largest producer of pistachio in the world (Pazouki et al., 2010; Aliakbarkhani et al., 2017). Moreover, Iran has the largest cultivation area of pistachio in the world (FAO, 2016).

Pistachio is a valuable source of antioxidants including phenolic and flavonoid substances that are essential for human health (Bellomo and Fallico, 2007; Ghrab et al., 2012). Pistachio is a dioecious (Kardoush et al., 2009) and anemophilous tree, therefore the existence of wind at the time of pollination is critical (Talebi et al., 2016).

To get good nut production, sufficient male trees are required for pollination.

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Pistachio trees are dioecious and pollination is an important factor to obtain satisfactory yield. Unsuccessful pollination can be due to inadequate male to female ratio or non-overlapping periods of pollen and pistil reception (Karimi and Zeraatkar, 2016; Ozeker et al., 2006). In male trees, flower opening starts from the end of March while the female flower opening is from mid-April up to the end of April. Blooming time of female cultivars is coincident with the beginning of vegetative growth. Usually, bloom periods in male and female trees do not overlap sufficiently to obtain satisfactory pollination and fruit set. Evaluation of pistachio cultivars showed that flower opening in 'Ghermez Peste', 'Kal Khandan', and 'Kaleh Bozi' is later than the males. Therefore, these conditions reduce the fruit set and yield. On the other hand, flower opening in pistachio is gradual and all formed florets on the cluster do not open at the same time. Therefore, manual pollination and identifying the best time of manual pollination for enhancing yield and quality in pistachio is critical. To obtain an economical yield, knowing the most suitable time of pollination based on the blooming stage and pistil receptivity, is of paramount importance in deciduous fruit trees (Mohammadi et al., 2017). To the best of our knowledge, however, this is the first research that identifies the best time for manual pollination of pistachio trees in order to increase yield and quality of fruits. Therefore, the objectives of this study were to investigate the effect of pollination time on yield and antioxidant properties of some pistachio nuts and to identify the best pollination time for increasing yield and fruit quality of pistachio cvs 'Ghermez Peste', 'Kal Khandan', and 'Kaleh Bozi'.

Material and methods

Plant material and pollination process

Field pollination experiments were carried out in a commercial pistachio orchard during 2011-2012 in the Buin Zahra region (35°46′N, 50°4′E, elevation 1210 m) of Qazvin, Iran. The effect of pollination time was studied on 16-year-old pistachio trees cvs 'Ghermez Peste', 'Kal Khandan', and 'Kaleh Bozi'. All cultivars were grafted on the 'Badami' cultivar and spaced at 10 m × 10 m. Agricultural practices were performed according to standard practices in the area. Pollination was carried out on 45 uniform trees in 5 stages of female flower development as the follows: A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in lowest and medial florets was pink). Pollination was performed according to the Iisfendiyaroglu et al. (2001) method. Ten days before pollination, selected female flowers were covered with netting bags and the netting bags were covered by plastic bags. The pollination operations were carried out at 10 am. The inflorescences of the male tree at different stages were put on the cluster of female cultivars. After completion of the pollination period, plastic bags were removed.

Yield

Fruit weight was measured with a scale sensitive to 0.01 g. The dry weight of the kernel in each cluster considered as the yield.

Preparation of samples

The fruits were harvested after ripening, and the skin is separated from the kernel. Kernels and skins were oven-dried for 24 h at 70°C. The samples were powdered and kept at -20 °C until extracting time.

Total phenolics

Total phenolics of kernels and skins were analyzed spectrophotometrically using the modified Folin–Ciocalteu colorimetric method with minor modifications as described by Singleton et al. (1999). The amount of total phenolics were estimated by comparing the absorbance of each sample with a standard response curve

generated using gallic acid. Data were expressed as mg gallic acid equivalent per g of dry weight (mg GAE/g DW).

Determination of total flavonoids

The total flavonoids contents of kernels and skins were determined colorimetrically as described previously by Du et al. (2009) at 506 nm. The flavonoid content was quantified by a (+)-catechin standard curve and expressed as mean of mg of (+)-catechin equivalent (CE) per g of dry weight.

Antioxidant activity

The antioxidant activity of kernels and skins was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging method as described by Du et al. (2009) with minor modifications. Briefly, 50 µL of different pistachio extracts were added to 950 µL of a 6.25. 10^{-5} M solution of DPPH in methanol. A control sample containing the same volume of solvent in place of the extract was used to measure the maximum DPPH absorbance. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. The percentage of DPPH, which was scavenged (% DPPHsc), was calculated using:

$$\% \text{ DPPHsc} = [(A_{cont} - A_{samp}) / A_{cont}] \times 100$$

where A_{cont} is the absorbance of the control, and A_{samp} is the absorbance of the sample.

Statistical analysis

The experiment was conducted as a randomized complete block design in three replications.

Analysis of variance was performed by GLM procedures (SAS 9.1 for Windows). Significant differences were calculated according to Duncan's multiple range tests. The p value < 0.05 was considered statistically significant.

Results

Yield

Results showed that pollination time had a significant effect on yield. The highest yield in 'GhermezPeste' obtained when the manual pollination carried out at the stage that the lower and middle florets were open and clusters were green (B treatment) while the highest yield in 'KalKhandan' and 'Kaleh Bozi' were observed when the pollination carried out at the full bloom stage and the clusters were light green color (C treatment). The lowest yield in all cultivars was observed in the clusters that pollinated in the last stage of blooming (E treatment) (Fig 1).

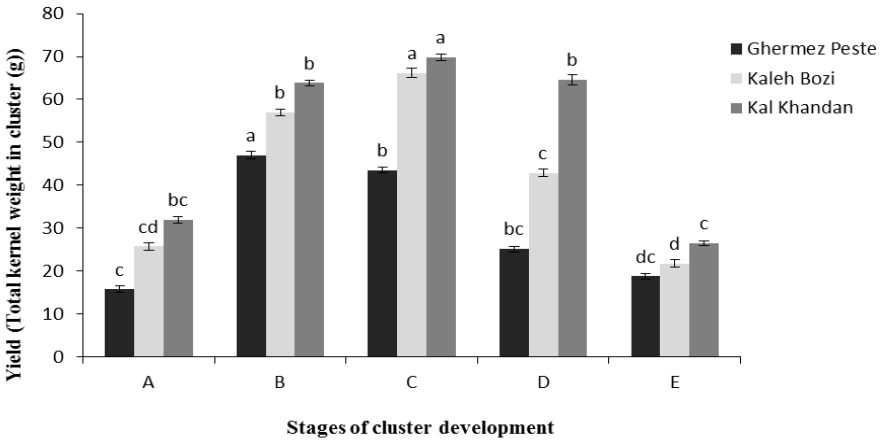

Fig. 1. Effect of manual pollination time on the yield of three pistachio cultivars ('GhermezPeste', 'KalKhandan' and 'Kaleh Bozi'). A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in the lowest and medial florets was pink). Means in the same column by the same letter (s) are not significantly different at p < 0.05 according to Duncan's multiple range test.

Total phenolics and flavonoids of fruits (Kernel and skin)

According to the results, the total amount of phenol in pistachio kernel differed between 3.045 and 7.962 mg g^{-1} DW (Fig. 2). Also the amount of total phenol in pistachio skin differed between 9.946 and 26.91 mg g^{-1} DW (Fig. 3). The skins of KalKhandan and GhermezPeste had highest and lowest amount of total phenolics. Results showed that total phenolics of fruits (kernel and skin) were affected significantly by pollination time (Fig. 2, 3). The highest amount of total phenolics, (kernel and skin) in all pistachio cultivars belonged to the clusters that pollinated at the stage that lower florets opened and clusters were green (A treatment). The lowest amount of total phenolics of fruits belonged to the clusters that pollinated at the stage that the stigma color in lowest florets was light pink (E treatment) (Fig. 2, 3).

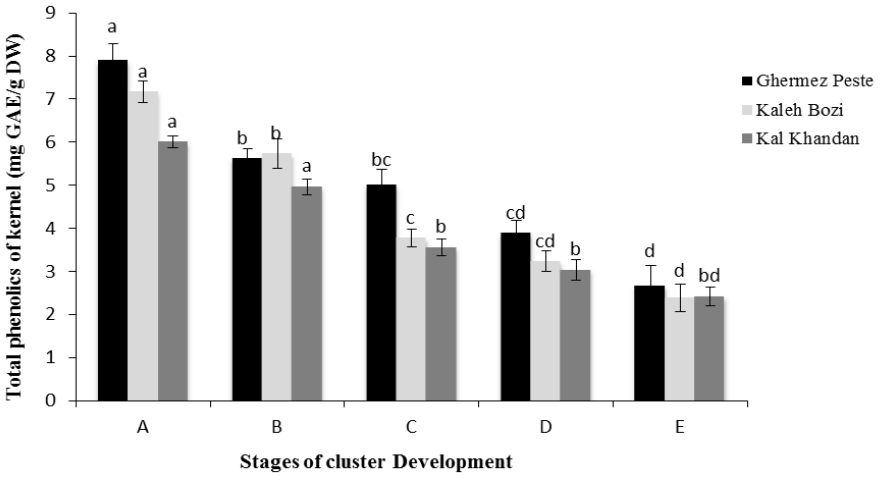

Fig. 2. Effect of manual pollination time on total phenolic contents of the kernel in three pistachio cultivars ('GhermezPeste', 'KalKhandan' and 'Kaleh Bozi'). A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in the lowest and medial florets was pink). Means in the same column by the same letter (s) are not significantly different at p < 0.05 according to Duncan's multiple range test.

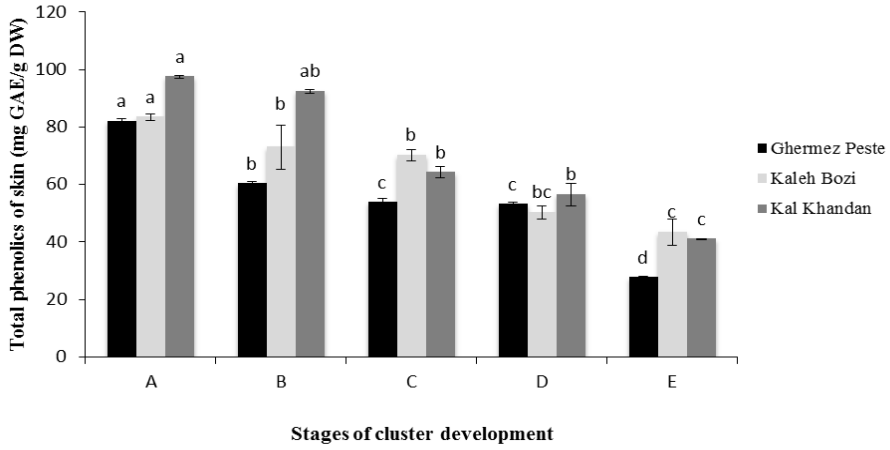

Fig. 3. Effect of manual pollination time on total phenolic contents of skin in three pistachio cultivars ('GhermezPeste', 'KalKhandan' and 'Kaleh Bozi'). A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in the lowest and medial florets was pink). Means in the same column by the same letter (s) are not significantly different at p < 0.05 according to Duncan's multiple range test.

The amount of total flavonoid in pistachio kernel differed between 17.06 and 26.317 mg CAT g^{-1} DW and for total flavonoid ranged between 94.117 and 117.115 (Fig. 4, 5). GhermezPeste cultivar had the highest amount of total flavonoid and Kal Khandan cultivar had the lowest amount of it. The highest amounts of total flavonoid were obtained in the fruits that pollination processes carried at the stage that lower florets opened the clusters were green. The lowest amount of total flavonoid belonged to the clusters that pollination carried in the stage that the stigma color in lowest florets was light pink.

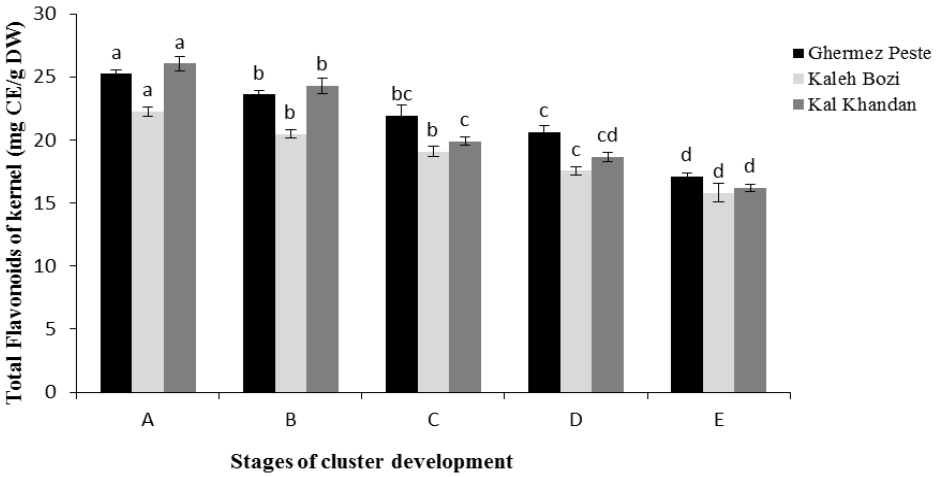

Fig. 4. Effect of manual pollination time on total flavonoid contents of kernel in three pistachio cultivars ('GhermezPeste', 'KalKhandan' and 'Kaleh Bozi'). A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in the lowest and medial florets was pink). Means in the same column by the same letter (s) are not significantly different at p < 0.05 according to Duncan's multiple range test.

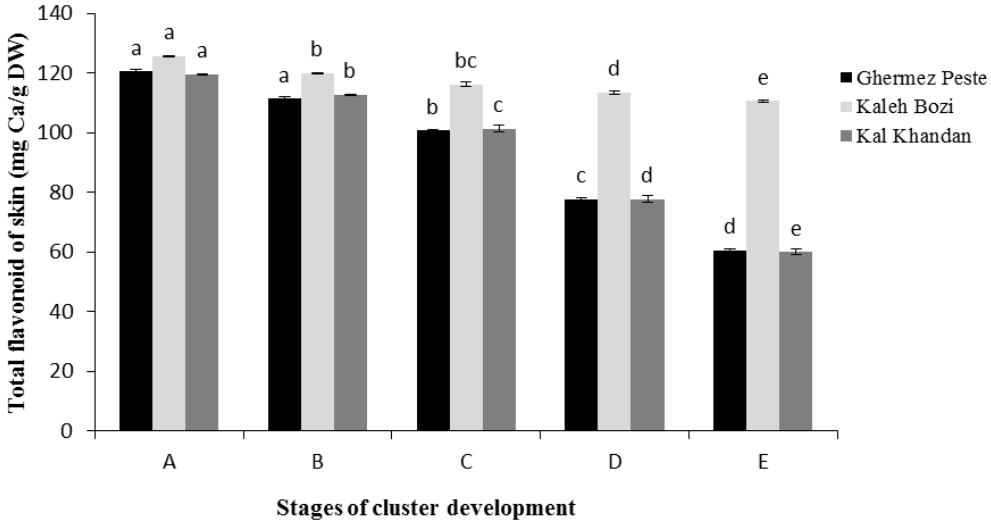

Fig. 5. Effect of manual pollination time on total flavonoid contents of skin in three pistachio cultivars ('GhermezPeste', 'KalKhandan' and 'Kaleh Bozi'). A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in the lowest and medial florets was pink). Means in the same column by the same letter (s) are not significantly different at p < 0.05 according to Duncan's multiple range test.

Antioxidant activity of fruits

The antioxidant activity in pistachio kernel differed between 65.618 and 74.149 and in pistachio skin between 71.20 and 75.35 (Fig 6, 7). The earlier pollinated fruits had more antioxidant activity compared to later pollinated fruits. The highest and lowest antioxidant activity of fruits (kernel and skin) in all pistachio cultivars belonged to A (the clusters which pollinated at the stage that lower florets opened and clusters were green) and E (clusters which pollinated at the stage that the stigma color in lowest florets was light pink) treatments, respectively (Fig. 6, 7).

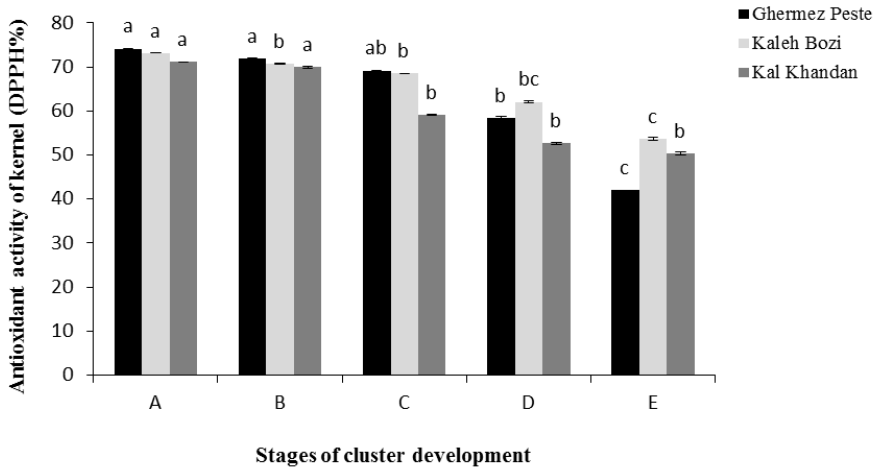

Fig. 6. Effect of manual pollination time on antioxidant activty of kernel in three pistachio cultivars ('GhermezPeste', 'KalKhandan' and 'Kaleh Bozi'). A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in the lowest and medial florets was pink). Means in the same column by the same letter (s) are not significantly different at p < 0.05 according to Duncan's multiple range test.

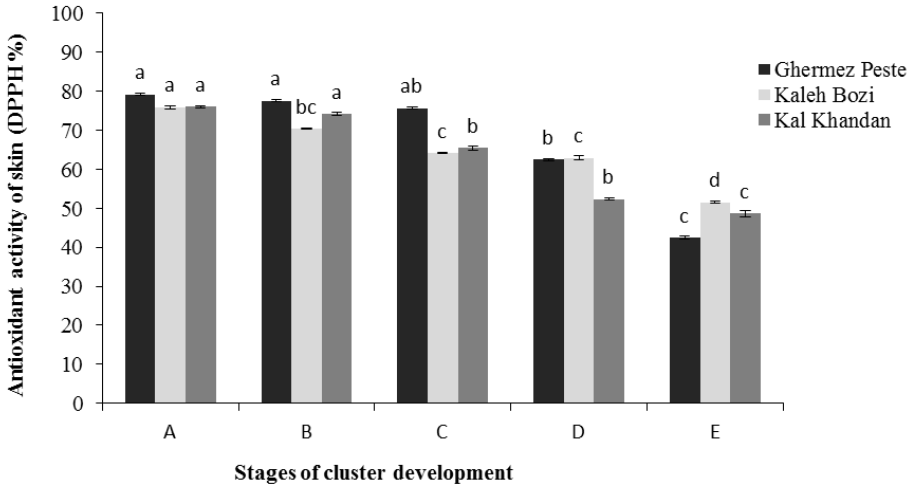

Fig. 7. Effect of manual pollination time on antioxidant activty of skin in three pistachio cultivars ('GhermezPeste', 'KalKhandan' and 'Kaleh Bozi'). A (lower florets opened and clusters were green), B (lower and medial florets opened and clusters were green), C (all florets opened and clusters were light green), D (the stigma in the lowest florets was light pink), E (the stigma in the lowest and medial florets was pink). Means in the same column by the same letter (s) are not significantly different at p < 0.05 according to Duncan's multiple range test.

Discussion

Delaying of manual pollination (after fullbloom) significantly decreases fruit yield. Therefore, the identifying suitable time of hand pollination in pistachio is important and it is necessary to determine the best time of hand pollination for each specific cultivar. Our results are in accordance with the results found by Mohammadi et al. (2017) and Iqbal et al. (2014). They found that pollination at suitable times increases yield and fruit set in date palm. There are no reports about the effect of pollination time on the yield of pistachio fruits.

Phenolics substances are important components of fruit quality because of their sensory properties (Pourghayoumi et al., 2016). Furthermore, these substances inhibit lipid auto-oxidation by acting as radical scavengers and consequently are important antioxidants that protect the body from cancers, diabetes, cardiovascular problems, degenerative diseases, and infections (Navarro et al., 2006; Pourghayoumi et al., 2016). According to the findings of Bellomo and Fallico (2007), with increasing fruit maturity grade the accumulation of anthocyanin and phenolic substances in pistachio kernel increases. Also, florets that pollinated at primary stage could absorb more nutrients and carbohydrate and subsequently produce more phenolic substances. Therefore, pollination in the primary stage of blooming is an effective factor for increasing antioxidant substances in pistachio fruits. Although, a specific pollen source by transferring carbohydrates to the endosperm and embryo tissues could affect phenolic substances of fruit (Pourghayoumi et al., 2012; Barzamini and Fotouhi Ghazvini, 2017). There are no reports about the effect of pollination time on phenolic substances of pistachio fruits.

Many factors such as climate, soil and altitude, genetic diversity, methodology, extraction solvents, and measuring status are effective on the amount of secondary metabolits including antioxidant qualities (Mirzaei et al., 2011; Murugan and Parimelazhagan, 2014). The clusters that pollinated at the primary stage of blooming had the highest contents of antioxidant substance while the clusters that pollinated at the final stage of blooming had the lowest amount of it. In pistachio, flower opening is a gradual process. In fact, the lower florets of inflorescences firstly open, based on our findings pollination at this stage could increase antioxidant substances in pistachio fruits. There are no reports about the effect of pollination time on antioxidant substances of pistachio fruits.

The current investigation clearly revealed that a significant improvement in pistachio quantity and quality could be achieved through the scheduling the hand pollination time. In fact, for obtaining fruits with high quality and quantity hand pollination at the primary and final stage of blooming in the evaluated cultivar is essential.

The findings suggest that for improving fruit quality (fruits with high antioxidant substances), the cultivars should be manually pollinated at the stage that lower florets opened and clusters were green. Moreover, for increasing yield and obtaining economical yield the manual pollination at full bloom stage should also be considered (C treatment). Pollination after full bloom stages when stigma color was light pink significantly decreased yield. Therefore, delaying manual pollination reduces yield and leads to economic losses.

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Conflict of interest: The authors declare that there is no conflict of interests.

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