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# Leaf temperature and peroxidase activity of bearing pistachio cultivars

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**ABSTRACT**

**Purpose:** Environmental stresses are a main disturbing factor influencing horticultural productivity around the world. It will affect all plants including resistant or non-resistant cultivars. So, it is important to find the better cultivars and to check the response to adverse environmental conditions. **Research method:** Thus, the present research was conducted to evaluate responses of different bearing status of pistachio cultivars including Kalleh-Ghochi (K), Ohadi (O) and Ahmad-Aghaii (A), grafted on Badami-Rize-Zarand as rootstock, for six consecutive years to soil salinity. **Findings:** ON-bearing trees of 'Ahmad-Aghaii' showed the highest yield, followed by 'Ohadi' and 'Kalleh-Ghochi'. In addition, the lowest leaf temperature was observed with this cultivar. Although the highest nitrogen, sodium, carbohydrate, peroxidase and leaf temperature was obtained in ON-bearing trees, however, the lowest potassium and total phenol content indicated in OFF-bearing status. It was found a negative correlation between leaf potassium content and ABI, between leaf peroxidase activity and ABI and between leaf temperature and ABI. On the other hand, leaf temperature increased as leaf sodium content increased. **Research limitations:** There was no limitation. **Originality/Value:** From data presented here, it is concluded that salinity and Na accumulation might be effective in changing the response of the pistachio cultivar under harsh environmental conditions which affects yield component and alternate bearing index.

## INTRODUCTION

*Pistacia vera* L. in Anacardiaceae (Ferguson & Kallsen 2016) is a dioecious plant (Crane & Iwakiri, 1985). The world production of pistachio is about 1 MT (FAOSTAT, 2020). Ashraf et al. (2008) stated that salinity accounted for 20 percent yield loss around the world, after high-temperature stress as a major environmental factor (40%). Currently, approximately 1,125 million hectares of land are salt-affected (Hossain, 2019). Generally, the pistachios are grown in areas with higher temperatures during growing season, where usually heat stress is dominant. In addition to heat and water scarcity, salinity stress should be added to cultivated areas. Plant metabolism activities are strongly affected by temperature fluctuations (Chaitanya et al., 2001; Levitt, 1980).

As literature shows, there are various recent reports regarding different aspects of pistachio trees from Iranian researchers, as Iran is one of the main producers of this valuable nut in the world (Azarmi-Atajan & Sayyari-Zohan, 2022; Pakdaman et al., 2023; Azarmi-Atajan et al., 2023; Miri et al., 2023; Eskandari Torbaghan, 2023). The alternate bearing disorder in fruit trees has been studied extensively (Barnett & Mielke 1981; Khezri et al., 2020; Goldschmidt & Sadka, 2021; Khayyat et al., 2018; El-Mardi et al., 2005). Genetic characteristics, environment, yield, and carbohydrate storage and mobilization are contributing factors to this habit. Khezri et al. (2020) evaluated pistachio alternate bearing in detail. Reports showed that ON-bearer pistachio leaves have more IAA and CKs compared with OFF-bearer trees (Okay et al., 2011). Moreover, ABA level was higher in most ON-bearing years compared with OFF-bearing status (Crane, 1986). Global warming is increasing and creating concerns about producing high-value products worldwide. The pistachio is categorized as a tolerant fruit tree to different harsh environments; however, we suppose that there are differences among pistachio cultivars. It is assumed that bearing intensity of pistachio cultivars might be influenced by stress conditions. However, there is no study regarding the correlation between leaf enzyme activities (peroxidase) and physiological performances in Iranian pistachios. In addition, there is no assessment regarding correlation between leaf temperature and alternate bearing index in pistachio cultivars. Thus, this study aimed to evaluate alternate bearing index (ABI) in pistachio cultivars including Kalleh-Ghochi, Ohadi and Ahmad-Aghaii grafted on Badami-Rize-Zarand and its correlation to leaf temperature, and to determine enzyme activity in parallel to leaf nutrient content during OFF and ON bearing statuses.

## MATERIALS AND METHODS

The experiment was conducted in a commercial plantation in Bajestan suburban (34.5221° N, 58.1722° E), Razavi Khorasan, Iran, on 20 years old pistachio trees grafted on Badami-Rize-Zarand as rootstock in 2015 (OFF), 2016 (ON), 2017 (OFF), 2018 (ON), 2019 (OFF) and 2020 (ON) growing seasons. The study was done on three cultivars (V), including Kalleh-Ghochi, Ohadi and Ahmad-Aghaii, as uniform trees (190 ± 20 cm height), planted at a spacing of 6m between rows and 3m on rows. The selected trees were treated according to conventional farm management, for example, pruning, thinning, irrigation (ECw=6.01 $dsm^{-1}$ as moderate salinity; pH=8.01), fertilization, and manuring. The soil type (Table 1) was deep, loamy and plants were fertilized using composted manure (2Kg per tree), urea (0.3Kg per tree), ammonium phosphate (0.2Kg per tree) and potassium sulphate (0.3Kg per tree) at essential amount regarding soil analysis results.

ABI evaluated based on Hoblyn et al. (1936) and Wood (1989) using the following formula (1):

$$I = (1/n-1)\,(a2-a1/a2+a1) + (a3-a2/\,a3+a2) + \cdots\,(an-1-n/an-1+) \qquad (1)$$

Where a = yield in corresponding years and n = number of years. If I = 0, there is no alternate bearing; if I = 1.0, there is total alternate bearing.

The total leaf-soluble carbohydrates were determined on the fully expanded leaves in July, August and September, according to Irigoyen et al. (1992) and glucose (0–100 mg $l^{-1}$, from MERCK) was used as a standard. The absorption at 625 nm was determined by a spectrophotometer (SHIMADZU AA-670, Japan). The equation used for standard curve preparation was y = 424.65 x – 13.176 ($R^2$ = 0.92). Total phenols were evaluated on the fully expanded leaves in July, August and September, based on Folin–Ciocalteu method (Singleton & Rossi, 1965), and the absorbance was measured at 725 nm using a spectrophotometer. Leaf temperature was measured in July, August and September, by a compact infrared thermometer with a laser pointer (Extech Instruments, Model 42500, Mini IR Thermometer, USA), on the newest fully expanded leaves. To measure the surface temperature of the leaves, five leaves were selected in each tree and the compact was placed 5cm from the surface and shot the gun to flash. Values represent the results for an average of 10 typical days each month with a clear sky from 12:00 to 2:00 pm. Enzyme activities were evaluated in July, August and September, in which all experiments were performed at 4°C. The leaf blades (10 g) were homogenized based on the method by Chaitanya et al. (2002). Peroxidase (POD, EC 1.11.1.7) activities were determined by the Putter (1974) using guaiacol at 436 nm. To reach this, 0.1 ml of the enzyme extract was added to the reaction mixture containing 0.05 ml guaiacol solution and 0.03 ml hydrogen peroxide solution in 3 ml phosphate buffer solution (pH 7.0). Then, the solution was mixed and the absorbance read at 436 nm using a spectrophotometer. Time was then noted for the absorbance to increase by 0.1. The enzyme activity was calculated using the extinction coefficient of guaiacol dehydrogenation product under the conditions specified. Chemical analysis (Chapman & Pratt, 1982) was carried out with oven-dried samples of leaves and inflorescence tissues in late July, which were ground separately and ashed at 550°C for 90 min in a porcelain crucible. The ash was re-suspended in hot 2 M HCl, filtered, made up to 50 ml with distilled water, and then used for $K^+$ and $Na^+$ analysis with a flame photometer (CORNING 405, Cambridge, UK). Nitrogen content was evaluated with Kjeldahl method (Zeraatgar et al., 2019). Fruit was harvested at the maturity stage in October, and the yield was assessed after hulling and drying, using a digital balance with an accuracy of 0.001g under consecutive bearing (B) statuses (OFF vs. ON).

**Experimental design and data analysis**

The experiment was conducted as factorial based on a complete randomized block design, with different cultivars (Kalleh-Ghochi, Ohadi and Ahmad-Aghaii) and bearing status (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON) as main and alternative factors, respectively. Three replications with six trees in each were used (18 trees for each cultivar). Statistical analysis of data was performed using ANOVA to determine statistically different values at significance levels of 0.05 and 0.01 based on LSD. All statistical analyses were performed using SAS version 9.2. The figures developed in Excel software.

## RESULTS

Soil analysis showed high salinity (Table 1) and SAR value (29.65) for studied site. Soil status was not suitable for the mentioned cultivars. Leaf temperature showed significant changes with bearer and non-bearer trees, and ON-bearings showed the highest level of this variable. 'Ahmad-Aghaii' showed the lowest leaf temperature compared with other cultivars; however, the highest level of this variable was obtained with 'Ohadi' and 'Kalleh-Ghochi', during ON status (Table 2). Data showed a linear correlation between ABI and leaf temperature and it was clear that any increase in leaf temperature increases this index ($R^2$=0.61). Peroxidase activity (PXR) was evaluated for four consecutive years and data indicated higher values in ON-bearing trees. Moreover, 'Ahmad-Aghaii' showed the highest levels of activity compared with other cultivars. Regarding to sampling time, September showed the highest amounts of activity (data not shown). Interactive evaluation of Bearing× Cultivar (B×V) indicated the highest PXR in 'Ahmad-Aghaii' during ON-bearing status, compared with others (Table 2). A negative correlation was observed between ABI and PXR rate ($R^2$=-0.78). The highest total phenols were observed with OFF-bearing status, however, Ahmad-Aghaii cultivar showed the highest level of this variable in both bearing status (Table 2). Sampling times indicated that September with higher phenol contents compared with other times (July and August) (data not shown). Moreover, an exponential correlation was observed between ABI and total phenols ($R^2$=0.90), in which increasing leaf phenol contents raises the ABI.

**Table 1.** Chemical and physical characteristics of experimental soil.

| ECe (dS $m^{-1}$) | pH | Total N(%) | P | $K^+$ | $Zn^{2+}$ | $Cu^{2+}$ |
|---|---|---|---|---|---|---|
| | | | (mg $kg^{-1}$) | | | |
| 6.05±0.2 | 8.49±0.10 | 0.36±0.03 | 5.37±0.3 | 153.04±0.21 | 1.91±0.50 | 1.63±0.41 |
| $Mn^{2+}$ | $Fe^{2+}$ | $Na^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $Cl^-$ | $HCO_3^-$ |
| (mg $kg^{-1}$) | | (meq $L^{-1}$) | | | | |
| 2.14±0.57 | 2.58±0.37 | 97.85±0.59 | 12.67±0.32 | 9.15±0.34 | 3.04±0.61 | 0.63±0.33 |

Data presented as mean± SD. The soil texture was sandy-loam. P (phosphorus), K (potassium), Zn (zinc), Cu (copper), Mn (manganese), Fe (Iron), Na (sodium), Mg (magnesium), Ca (calcium), Cl (chlorine) and $HCO_3^-$ (bicarbonate).

**Table 2.** Interactive effects of bearing status and cultivar on total phenols and total carbohydrates within pistachio leaves tissue during consecutive years (2017: OFF; 2018: ON; 2019: OFF; 2020: ON).

| Bearing | Cultivar | Total phenols | Total carbohydrate | PXR | Leaf Temp. |
|---|---|---|---|---|---|
| | | mg $g^{-1}$ D.W. | mg $g^{-1}$ D.W. | (Units $mg^{-1}$ chl $min^{-1}$) | (°C) |
| | Ohadi | 11.37 | 163.86 | 214.90 | 41.67 |
| OFF | Ahmad-Aghaii | 23.38 | 189.83 | 478.16 | 39.46 |
| | Kalleh-Ghochi | 16.32 | 81.86 | 308.72 | 42.86 |
| | Ohadi | 9.70 | 208.83 | 294.77 | 47.30 |
| ON | Ahmad-Aghaii | 11.78 | 211.49 | 572.42 | 41.91 |
| | Kalleh-Ghochi | 9.70 | 148.89 | 396.47 | 46.83 |
| | Ohadi | 14.55 | 161.67 | 218.24 | 39.72 |
| OFF | Ahmad-Aghaii | 25.81 | 169.68 | 490.57 | 41.02 |
| | Kalleh-Ghochi | 21.03 | 91.41 | 331.69 | 41.14 |
| | Ohadi | 11.51 | 219.93 | 306.22 | 51.56 |
| ON | Ahmad-Aghaii | 16.51 | 218.82 | 552.69 | 43.40 |
| | Kalleh-Ghochi | 16.71 | 148.08 | 394.40 | 45.13 |
| LSD | | 1.541 | 7.258 | 9.538 | 1.816 |
| Bearing status (B) | | <.001 | <.001 | <.001 | <.001 |
| Cultivar (C) | | <.001 | <.001 | <.001 | <.001 |
| B × C | | <.001 | <.001 | <.001 | <.001 |

ON: Bearing trees; OFF: Non-bearing trees. Total phenols assessed based on Galic acid; PXR: Peroxidase. Three replications each containing six trees were evaluated.

Total carbohydrate (TC) showed higher levels in leaves of ON-bearing trees, and the highest value was observed with ‘Ahmad-Aghaii’. Higher TC is evidence of higher nut demand for this variable when the seed is grown. September sampling showed the highest level of this variable compared with others (July and August) (data not shown). Interaction between B×V showed the highest total carbohydrate in ON-bearer ‘Ahmad-Aghaii’, although there was no significant difference with ‘Ohadi’ (Table 2).

All studied cultivars showed alternate bearing and a remarkable yield fluctuation was observed. OFF-bearing ‘Kalleh-Ghochi’ showed a lower yield in the first two years (2015 and 2017), compared with others. On the other hand, ON-bearer ‘Ohadi’ and ‘Ahmad-Aghaii’ showed higher yield components (years 2016, 2018, and 2020; Fig. 1). Alternate bearing index (ABI) was evaluated (Fig. 2) and ‘Kalleh-Ghochi’ was indicated as a high alternate bearer, compared with others, followed by ‘Ohadi’ and ‘Ahmad-Aghaii’.

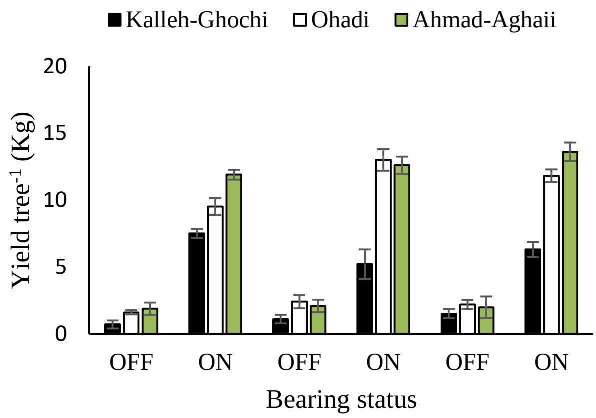


**Fig. 1.** Yield of different pistachios in ON and OFF bearing statuses from 2015 to 2020. Data represents means± SD. ON: Bearing trees; OFF: Non-bearing trees. Three replications each containing six trees were evaluated. (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON).

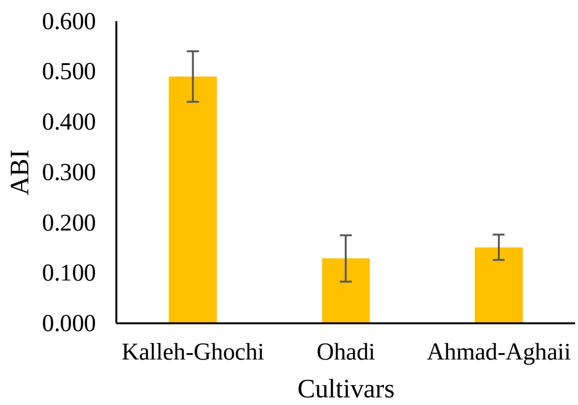


**Fig. 2.** Alternate bearing index **(**ABI) in different pistachio cultivars. Data represents means± SD. Three replications each containing six trees were evaluated. (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON)

Different leaf nitrogen contents were obtained based on bearing status (OFF vs. ON), and approximately higher levels of this variable were observed in ON bearer trees (data not shown). There was a significant difference in this variable when interactions were evaluated. In the second year of study (ON bearing status), all cultivars showed higher nitrogen content within leaf tissues (Table 3).

Regarding B×V, and in general, the highest potassium (K) content was observed within OFF-bearer leaf tissues followed by ON-bearers. Moreover, data showed that 'Kalleh-Ghochi' accumulated higher K levels within leaves compared with other cultivars (Table 3).

Evaluation of correlation showed a negative correlation between ABI and K contents within leaves ($R^2$=-0.60, data not shown).

**Table 3.** Interactive effects of bearing status and cultivar on nutrient concentration within pistachio leaves tissue during consecutive years (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON).

| Bearing | Cultivar | Nitrogen | Potassium | Sodium | K/N | K/Na |
|---|---|---|---|---|---|---|
| | | mg $g^{-1}$ D.W. | | | | |
| OFF | Ohadi | 2.260 | 8.940 | 66.360 | 3.975 | 0.1355 |
| | Ahmad-Aghaii | 2.120 | 10.700 | 16.780 | 5.035 | 0.6686 |
| | Kalleh-Ghochi | 2.280 | 9.088 | 40.000 | 4.015 | 0.2289 |
| ON | Ohadi | 3.040 | 5.460 | 84.340 | 1.811 | 0.0664 |
| | Ahmad-Aghaii | 3.220 | 6.740 | 20.280 | 2.087 | 0.3334 |
| | Kalleh-Ghochi | 3.300 | 5.920 | 50.100 | 1.796 | 0.1186 |
| OFF | Ohadi | 2.240 | 8.270 | 62.800 | 3.694 | 0.1319 |
| | Ahmad-Aghaii | 2.200 | 10.840 | 17.400 | 4.937 | 0.6258 |
| | Kalleh-Ghochi | 2.360 | 8.730 | 37.240 | 3.772 | 0.2344 |
| ON | Ohadi | 2.320 | 4.546 | 87.040 | 1.963 | 0.0527 |
| | Ahmad-Aghaii | 2.320 | 6.700 | 22.300 | 2.889 | 0.3022 |
| | Kalleh-Ghochi | 2.240 | 5.760 | 54.020 | 2.577 | 0.1067 |
| OFF | Ohadi | 2.320 | 8.782 | 69.540 | 3.791 | 0.1265 |
| | Ahmad-Aghaii | 2.300 | 10.000 | 21.160 | 4.361 | 0.4799 |
| | Kalleh-Ghochi | 2.280 | 8.470 | 46.080 | 3.727 | 0.1844 |
| ON | Ohadi | 2.340 | 4.766 | 74.220 | 2.049 | 0.0646 |
| | Ahmad-Aghaii | 2.200 | 7.600 | 18.260 | 3.465 | 0.4279 |
| | Kalleh-Ghochi | 2.360 | 5.850 | 46.040 | 2.503 | 0.1284 |
| LSD | | 0.2211 | 0.7352 | 6.0720 | 0.4138 | 0.0702 |
| Bearing status (B) | | <.001 | <.001 | <.001 | <.001 | <.001 |
| Cultivar (C) | | 0.235 | <.001 | <.001 | <.001 | <.001 |
| B × C | | 0.038 | 0.013 | <.001 | 0.003 | <.001 |

ON: Bearing trees; OFF: Non-bearing trees. Three replications each containing six trees were evaluated.

Regarding to sodium (Na) content, the simple effects of bearing, cultivar and interaction of B×V were significantly different. Results showed the highest Na levels in ON-bearing trees, specifically with ‘Ohadi’ and ‘Kalleh-Ghochi’ (Table 3). ‘Ahmad-Aghaii’ showed the lowest sodium accumulation within leaves. There was a positive correlation between ABI and Na accumulation within leaves ($R^2$=0.84, data not shown). Moreover, a positive correlation was observed between sodium accumulation in the leaf and leaf temperature ($R^2$=0.92, data not shown).

The potassium to nitrogen (K/ N ratio) was the highest in OFF-bearing status, compared with ON status, which might be resulted from lower N content in bearing trees. Moreover, ‘Ahmad-Aghaii’ showed a high level for this ratio compared with others (Table 3). There was an exponential correlation between ABI and K/N ratio ($R^2$=0.54).

The K to Na ration showed the highest values for the OFF-bearer Ahmad-Aghaii cultivar, compared with others. ON-bearer trees showed a lower value of this ratio (Table 3). The evaluation indicated a negative correlation between ABI and K/Na ratio ($R^2$=-0.64).

## DISCUSSION

Soil analysis cleared harsh environmental conditions for the studied site, and also for mentioned cultivars, although, all of them were grafted on ‘Badami-Rize-Zarand’ as a resistant pistachio rootstock to drought and salinity (Adish et al., 2010; Rahneshan et al., 2018). There was a difference between leaf temperature of bearer and non-bearer statuses, with the highest values in ON-bearings trees. Comparing cultivars, ‘Ahmad-Aghaii’ showed the lowest value, and the highest level obtained with ‘Ohadi’ and ‘Kalleh-Ghochi’. Higher leaf temperature influences photosynthetic apparatus and chlorophyll content, normal cellular homeostasis, electron transport, function of PSII because of enzyme degradation and inhibit Rubisco activase (Rca), carbohydrate assimilation and growth and development, glycolate pathway and caused $H_2O_2$ production, malondi-aldehyde (MDA) production due to lipid peroxidation, photo-inhibition, protein denaturation, enzymes and nucleic acid denaturation and accumulation of compatible solutes (Sade et al., 2011; Hasanuzzaman et al., 2013; Song et al., 2014; Bi et al., 2016; Moore et al., 2021). The oxidative stress further leads to cellular injury, including membrane protein breakage (Sade et al., 2011). An increase in leaf temperature will result in increases in VPD in natural environment, which affects photosynthetic induction by itself (Kaiser et al., 2017). It is suggested that ‘Ahmad-Aghaii’ exhibits different mechanisms to face high temperatures, and maintain PSII activity under harsh environments. ABI showed a positive linear correlation with leaf temperature ($R^2$=0.61), which may be because of running a lower rate of assimilation processes. Higher PXR activity was observed in ON-bearing trees and ‘Ahmad-Aghaii’ showed the highest levels of activity compared with two others. A negative correlation observed between ABI and PXR ($R^2$=-0.78), and higher PXR activity improved leaf photosynthetic assimilation which increases carbohydrate resources. We suggest that lower leaf temperatures might be resulted from higher PXR activity that was disagreement with results by Chaitanya et al. (2002), and Gulen and Eris (2004). More specifically, there is a correlation between PRX and catalase (CAT) enzyme activity and the appearance of physiological injuries caused by thermal stress, and its activity was enhanced by high-temperature stress (Kumar et al., 2012; Chalanika et al., 2017). In a study under heat stress, Gulen and Eris (2004) found an increase in PXR and decreased total protein content of strawberry plants. Peroxidase triggers the conversion of $H_2O_2$ to water and oxygen as an enzymatic defense of plant cells (Gaspar et al., 1982). Moreover, many other performances are related to PXR, including removing $H_2O_2$ and other toxic reductants, lignin production in cell walls, auxin catabolism, defensive responses to wounding, defense against pathogen or insect attack, and some respiratory processes (Gaspar et al., 1982). CAT and PXR are chloroplast or cytosolic enzymes, which scavenge $H_2O_2$ generated primarily through SOD action. Hydrogen peroxide stimulates several different genes related to abiotic and biotic stress tolerances (Prasad et al., 1994). Under high temperatures, the reduction of $H_2O_2$ by ascorbate- glutathoine cycle is useful for dissipating energy and aids in adjusting ATP: NADPH ratios at times. Totally, Liu and Huang (2000) stated that any decrease in antioxidant enzyme activities under heat stress could contribute to damage of cell membranes.

OFF-bearer trees showed the highest total phenols, however, Ahmad-Aghaii cultivar showed the highest level of this variable in both bearing statuses. Certain PXR utilize the phenolic compounds and $H_2O_2$ to start the biosynthesis of several secondary metabolites that are essential for the plant growth, development and differentiation (Gaspar et al., 1991). Khayyat et al. (2018) stated that total phenols increase in vegetative organs of ON-bearing seedless barberry. It is suggested that bearer trees supply fruits with different substances to maintain sufficient yield, thus, lower phenols are not far away. Sampling times indicated the September with the higher phenol contents compared with other times (July and August) (data not shown) that was disagreement with Khayyat et al. (2018) on seedless barberry. An exponential correlation observed between ABI and total phenols ($R^2$=0.90).

Total carbohydrate (TC) showed higher levels in leaves of ON-bearing trees that was in agreement with Khayyat et al. (2018) but disagreed with Vemmos (2010), and the highest value observed with 'Ahmad-Aghaii'. Higher TC is evidence of higher nut demand for this variable when the seed is grown. At high temperatures, photosynthetic decline because of increased photorespiration and mitochondrial respiration, inactivation of Rubisco, decreased activity of photosystem II (PSII), and damage to the thylakoid membrane, which results in a reduction in ATP synthesis and increased thylakoid membrane permeability (Hozain et al., 2010). September sampling showed the highest level of this variable compared with others (July and August) (data not shown) that was disagreement with Khayyat et al. (2018). Research indicated that high temperatures also cause a rapid consumption of carbohydrates for maintenance (Teskey et al., 2015). The mentioned reasons might be the leading causes of lower TC with other cultivars in our experiment. It is thought that respiration might be the main cause of decrease in the TC reservoirs within leaves. Moreover, lower PXR levels in mentioned cultivars might be the cause of higher TC consumption under harsh environments.

All studied cultivars showed alternate bearing and a remarkable fluctuation of yield was observed. The yield components of trees might be resulted from genetic background and environmental stresses influence on tree performance (Goldschmidt & Sadka, 2021). In our study, similarity on soil conditions and rootstock evidence that genetic background might be a main cause of any differences among cultivars. It is believed that plants whose fruit ripens early in the growing season or plants having a longer PRFP (post-ripening foliation period) and vegetative growth will accumulate a larger pool of assimilates and thus show lower ABI compared with those that ripen late in the season (Khayyat et al., 2018).

Higher levels of leaf nitrogen observed in ON bearer trees (data not shown) that was in disagreement with Elmardi et al. (2005) and in agreement with Brown et al. (1995) findings. Brown et al. (1995) found a huge depletion of nitrogen accumulation in OFF bearer pistachios, after ON status.

The highest potassium (K) content observed in OFF-bearing trees followed by ON status, that was disagreement with Brown et al. (1995). Elmardi et al. (2005) stated that more potassium and sodium observed within ON-tree leaves compared with OFF status. We suppose that higher K accumulation in OFF bearing leaves might be the prerequisite for its translocation and accumulation in perennial plant part for next season (ON status). Evaluation of correlation showed an exponential correlation between ABI and K contents within leaves ($R^2$=0.60). Regarding to potassium content, it is assumed that leaf potassium sufficiency led to lower leaf temperature, may be because of higher RWC, specifically under hot environments, which could be a suitable characteristic for screening heat-tolerant cultivars.

Results showed the highest Na levels in ON-bearing trees that were in agreement with Elmardi et al. (2005). There was a significant difference among cultivars and 'Ahmad-Aghaii' showed the lowest sodium accumulation within leaves. There was a negative correlation between ABI and Na accumulation within leaves ($R^2$=-0.84, data not shown). Moreover, a positive correlation observed between sodium accumulation in leaf and leaf temperature ($R^2$=0.92, data not shown). Increased leaf temperature might be related to lower RWC or lower content of osmolites within photosynthetic tissues. Thus, lower entrance of $Na^+$ could be applied as a good character for breeding or selection.

The potassium to nitrogen (K/ N ratio) was the highest in OFF-bearing status, compared with ON status, which might be resulted from lower N content in bearing trees. There was an exponential correlation between ABI and K/N ratio ($R^2$=0.54). The K to Na ration showed the highest values of OFF-bearing status of Ahmad-Aghaii cultivar, compared with others. Evaluation indicated an exponential correlation between ABI and K/Na ratio ($R^2$=0.64). Potassium ions are highly involved in the activation of several enzymes essential for cellular functions (Tester and Davenport 2003). When plants are exposed to sodium chloride (NaCl) induced salinity, the influx of $Na^+$ and $Cl^-$ impairs the transport of other ions, such as $K^+$ and $Ca^{2+}$ (Binzel et al., 1988). In such conditions, protein synthesis and enzyme activation processes are directly affected by the ability of plants to select $K^+$ at the expense of $Na^+$ ions (Kamiab et al., 2012). We supposed that bearer trees are highly demanding for potassium accumulation in fruits that decrease the K/ Na ratio within leaves.

## CONCLUSION

Data showed the ON-bearing 'Ahmad-Aghaii' with the highest yield, followed by 'Ohadi' and 'Kalleh-Ghochi'. The lowest leaf temperature observed with this cultivar. ON trees showed the highest nitrogen, sodium, carbohydrate, peroxidase and leaf temperature, however, the lowest potassium and total phenol content observed with OFF-bearers. ABI showed an exponential correlation with potassium and peroxidase activity. It is concluded that Na accumulation within leaves influence the growth response of pistachio trees under harsh environmental conditions, might be related to enzyme activity and leaf temperatures, and lead to change in yield and alternate bearing index.

### Competing interests and declarations

All authors declare that they have no competing financial interests and non-financial interests related to this work and its publication. We emphasis that we did not receive any grants from any organization.

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