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Determination of Cold Hardiness of Pistachio (*Pistacia vera* L.) Cultivars Flower Buds during Rest Season

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A R T I C L E I N F O

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A B S T R A C T

Frost injury is a very widespread phenomenon occurring in both deciduous and ever-green trees such as pistachio. The cold hardiness levels of four cultivars of pistachio (*Pistacia vera* L.), 'Kalle-Ghuchi', 'Owhadi', 'Ahmad-Aghaei' and 'Akbari' were determined. Samples were collected from November 2007 to March 2008 and from November 2008 to March 2009, with three week intervals during dormant season. Then, single- bud cutting sections were placed in incubator and subjected to temperature, +5 (control), -10, -15, -20, -25 and -30°C, held for 24h at each temperature. The critical temperature for survival among the 4 cultivars was -15°C and more than 70% of the buds of all cultivars were killed once they were subjected to -30°C. All cultivars achieved a maximum cold hardiness in December and January. These four cultivars were classified as hardy ('Akbari'), semi-hardy ('Owhadi' and 'Ahmad-Aghaei') and sensitive ('Kalle-Ghuchi'). The tested pistachio cultivars exhibited higher cold hardiness levels in 'OFF' trees, rather than 'ON' trees.

Introduction

Pistachio tree (*Pistacia vera* L.) originates from Central Asia. It is an important commercial nut tree in Iran and has adapted to the native climate (Shamshiri and Hasani, 2015; Sharifkhah *et al*., 2010). Iran ranks No.1 in pistachio production and harvest area in the world while few research activities concerning the morphology and physiology of the pistachio trees have been carried out in this country. Therefore, the knowledge of the hardiness responses of pistachio trees is essential for economic production. Freeze injury to pistachio trees has severely decreased yield in many commercial orchards during the recent years in Iran. However, susceptibility to freeze damage appears to vary considerably between cultivars and should therefore, be amenable to breeding efforts (Ameglio *et al*., 2004, Arora *et al*., 1992, Ashworth, 1990, Aslamarz *et al*., 2010, Fuller and Wisniewski 1998, Lindsrom and Dirr 1991, Thomashow 1999).

Low winter temperatures are a major limiting factor in fruit production in the world, a major challenge is that adaptation to climate change is not a one-size fits all phenomenon; adaptation strategies and farmer responses will vary across regions (Ameglio 1990, Ashworth 1996, Aslamarz *et al*., 2009, Flinn and Ashworth 1998). Temperature is a primary factor affecting the rate of plant development and economic losses can occur due to very low temperatures during dormancy or due to untimely freezing temperatures before acclimation in the fall or after de-acclimation in the spring, the flower bud remains dormant for several months in pistachio (Bordelon *et al*., 1997, Morin *et al*., 2007). Due to the importance of cold hardiness in fruit production, this characteristic is often a selection criterion in cultivation fruit trees (Beck *et al*., 2007, Ishikawa and Arata 1997). Low temperatures decrease biosynthetic activity of plants, inhibit the normal function of physiological processes and may

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cause permanent injuries, finally leading to death (Ashworth and Wisniewski 1991, Aslamarz and Vahdati, 2010, Badaruddin and Meyer 2001, Canny 1997, Griffith and Antikienen 1996, Rahemi *et al*., 2016).

This study determined the level of cold hardiness of the flower buds of some pistachio cultivars in fall and winter (rest season) using a laboratory freezing test.

Materials and Methods

The experiment was conducted with four commercial pistachio cultivars: 'Kalle-Ghuchi' (early-bloom),'Owhadi'(middle-bloom), 'Ahmad-Aghaei' (middle bloom) and 'Akbari' (late-bloom) a Pistachio Research Station, Iran, during 2018-2019. This station is located in the northeast of Kerman, Iran. The trees were 30 years old at the beginning of the study and all trees received similar cultural practices such as irrigation and fertilization. The hardiness of pistachio cultivars was assesed by subjecting excised 1-year old twigs to a controlled freezing stress, the cuttings were evaluated from November 2017 to March 2018 and from November 2018 to March 2019, with three weeks intervals during dormant season. The single bud cuttings were placed in plastic bags and were kept humid. Then, single bud cutting sections were kept in ethylene-glycol (E-G) bath (Neslab, Model LT-50DD) at 1^{o}C for 24h. Samples were kept in incubator and subjected to sequential freezing temperature, +5 (control), -10, -15, -20, -25 and -30^{o}C. Sample temperatures were monitored using copper-constantant thermocouples inserted into the foil pouch and cuttings cooled at the rate of 5-7^{o}C/h. The bud cuttings held for 24h at each temperature before being removed for experiment. Individual buds were sectioned through the tip with razorblade and examined under a binocular microscope, checking for necrosis and brown and evaluated percentage of dead buds. The buds which appeared bright and green were considered alive and that appearing brown discoloration and straw and light brown was considered dead (Rekika 2004, Stergio and Howell 1977).

Statistical analysis

The experimental design was a factorial randomized complete-block with three replications. Analysis of variance using SPSS 8.0 windows was conducted for each year during the test. The data were statistically analyzed and the means were compared by Dancans's Multiple Rang Test (DMRT). Differences between means at 5% ($p<0.05$) level were considered as significant.

Results

The present study indicated that significant differences in the survival of flower buds were observed among cultivars within each sampling data ($p<0.05$) (Tables 1, 2). The critical temperature for survival among the four cultivars was -15^{o}C, while more than 70% of the buds of all cultivars were killed once being subjected to -30^{o}C (Table 3, 4). The critical temperature for survival among the four cultivars was -15^{o}C, and more than 70% of the buds of all cultivars were killed once while being subjected to -30^{o}C (Fig. 1). These four cultivars were classified as hardy ('Akbari'), semi-hardy ('Owhadi', 'Ahmad-Aghaei') and sensitive ('Kalle-Ghuchi') (Fig. 2). Hardiness of the buds increased during the sampling period, from November to January and then that decreased from February to March (Fig. 3). During all sampling period, almost 60 to 90% of the buds of all cultivars were killed at -30^{o}C (Fig.4). The most severe damage was in November and March sampling in all cultivars (Figs. 5 and 6).

Table 1. Variance analysis of freezing temperature, month and cultivar effects on percent dead flower buds of pistachio cultivars during dormant season (2008-2009, 'OFF' Trees).

S.O.V	df	percent dead flower buds	SS	MS	F	$F_{0.05}$	$F_{0.01}$
Block	2	0.12**	0.12**	0.06	2.696629 ns	3.02	4.69
freezing temperature	5	0.71**	0.71**	0.142	6.382022**	2.25	3.07
month	4	22.11**	22.11**	5.5275	248.427**	2.4	3.38
cultivar	3	11.02**	11.02**	3.673333	165.0936**	2.63	3.84
freezing temperature× month	20	3.02**	3.02**	0.151	6.786517**	1.6	1.94
freezing temperature × cultivar	15	10.11**	10.11**	0.674	30.29213**	1.7	2.1
month× cultivar	12	2.41**	2.41**	0.200833	9.026217**	1.75	2.23
freezing temperature× month× cultivar	60	0.09**	0.09**	0.0015	0.067416 ns	1.3	1.51
Error	240	5.34	5.34	0.02225			
cv%	-	20.68	20.68				

ns, * and **: non-significant, significant at 5% and 1%, respectively

Table 2. Variance analysis of freezing temperature, month and cultivar effects on percent dead flower buds of pistachio cultivars during dormant season (2007-2008, 'ON' Trees).

S.O.V	df	percent dead flower buds	SS	MS	F	$F_{0.05}$	$F_{0.01}$
Block	2	0.28**	0.28**	0.14	2.364545 ns	3.02	4.69
freezing temperature	5	1.34**	1.34**	0.268	4.526415**	2.25	3.07
month	4	0.98**	0.98**	0.245	4.137954**	2.4	3.38
cultivar	3	12.54**	12.54**	4.18	70.59857**	2.63	3.84
freezing temperature× month	20	3.11**	3.11**	0.1555	2.626334**	1.6	1.94
freezing temperature × cultivar	15	0.10**	0.10**	0.006667	0.112597 ns	1.7	2.1
month× cultivar	12	1.14**	1.14**	0.095	0.287123 ns	1.3	2.23
freezing temperature× month× cultivar	60	1.02**	1.02**	0.017	0.287123 ns	1.3	1.51
Error	240	14.21	14.21	0.059208			
cv%	-	31.05	31.05				

ns, * and **: non-significant, significant at 5% and 1%, respectively

Table 3. Interaction effects of freezing temperature, month and cultivar on percent dead flower buds of pistachio cultivars during dormant season (2007-2008, 'ON' Trees).

Cultivar	Temperature (°C) / Month	-30	-25	-20	-15	-10	+5
Kalle-Ghuchi	November	90.2ab*	55.23j-m	27q-u	5xz	0z	0z
	December	86.33abc	45.67no	18uvw	0.69z	0z	0z
	January	81.25bcd	45.35no	13.6vwx	0z	0z	0z
	February	92.6a	55j-m	24.3q-u	2.66z	0z	0z
	March	94a	68.6e-h	39op	18uvw	1.3z	0z
Owhadi	November	85.62abc	32pq	12.56v-y	0.61z	0z	0z
	December	69efgh	22r-v	7.43xyz	0z	0z	0z
	January	69.21e-h	21tuv	6.69xyz	0z	0z	0z
	February	82bcd	52k-n	31p-s	5xyz	0z	0z
	March	92a	55j-m	30p-s	8xyz	0z	0z
Ahmad-Aghaei	November	80.71cd	27q-u	13y-v	0.33z	0z	0z
	December	65.67f-i	21.24s-v	5.33xyz	0.30z	0z	0z
	January	73.65def	21.7r-v	3.23yz	1z	0z	0z
	February	77.65cde	51mn	7.55xyz	3.62yz	0z	0z
	March	85.31abc	53.8j-n	25.67q-u	7.59xyz	0z	0z
Akbari	November	60.67h-k	36.3p	10wxyz	0z	0z	0z
	December	58.29i-l	30p-t	3yz	0z	0z	0z
	January	58ijkl	20uv	4xyz	0.39z	0z	0z
	February	62.27g-j	31.39pqr	11w-z	4xyz	0z	0z
	March	71efg	46mno	20uv	5xyz	0z	0z

*Means separation in each column by Dancans's Multiple Rang Test (DMRT) at 5% level.

Table 4. Interaction effects of freezing temperature, month and cultivar on percent dead flower buds of pistachio cultivars during dormant season (2008-2009, 'OFF' Trees).

Cultivar	Temperature (°C) / Month	-30	-25	-20	-15	-10	+5
Kalle-Ghuchi	November	81.3abc*	50.4klm	20q-t	2.6yz	0z	0z
	December	80.32abc	38.7no	11.7s-y	0.4z	0z	0z
	January	79bc	43.21mn	10t-z	0z	0z	0z
	February	89a	52j-m	20.2q-t	3.4yz	0z	0z
	March	87ab	57h-k	26.4pqr	8.3u-z	0z	0z
Owhadi	November	82abc	29pq	7.6w-z	0.36z	0z	0z
	December	66e-h	20q-t	5.35xyz	0z	0z	0z
	January	64e-i	18q-u	7w-z	0z	0z	0z
	February	77bcd	48klm	27.61pqr	2.3yz	0z	0z
	March	86ab	51klm	26.29pqr	8v-z	0z	0z
Ahmad -Aghaei	November	73.34cde	21.7p-s	3.7yz	0.32z	0z	0z
	December	61.62f-j	18.3q-v	10.71t-z	0z	0z	0z
	January	68.7def	18.6q-v	4xyz	0z	0z	0z
	February	72.71cde	45.7lmn	12s-y	2yz	0z	0z
	March	79c	48klm	17.7r-w	6xyz	0z	0z
Akbari	November	55i-l	31.25op	7.62w-z	0z	0z	0z
	December	54i-l	29pq	6xyz	0z	0z	0z
	January	52j-m	25.7pqr	6.64w-z	0z	0z	0z
	February	58.27g-k	27pqr	14.5s-x	1.5yz	0z	0z
	March	68efg	43mn	21q-t	5xyz	0z	0z

*Means separation in each column by Dancans's Multiple Rang Test (DMRT) at 5% level.

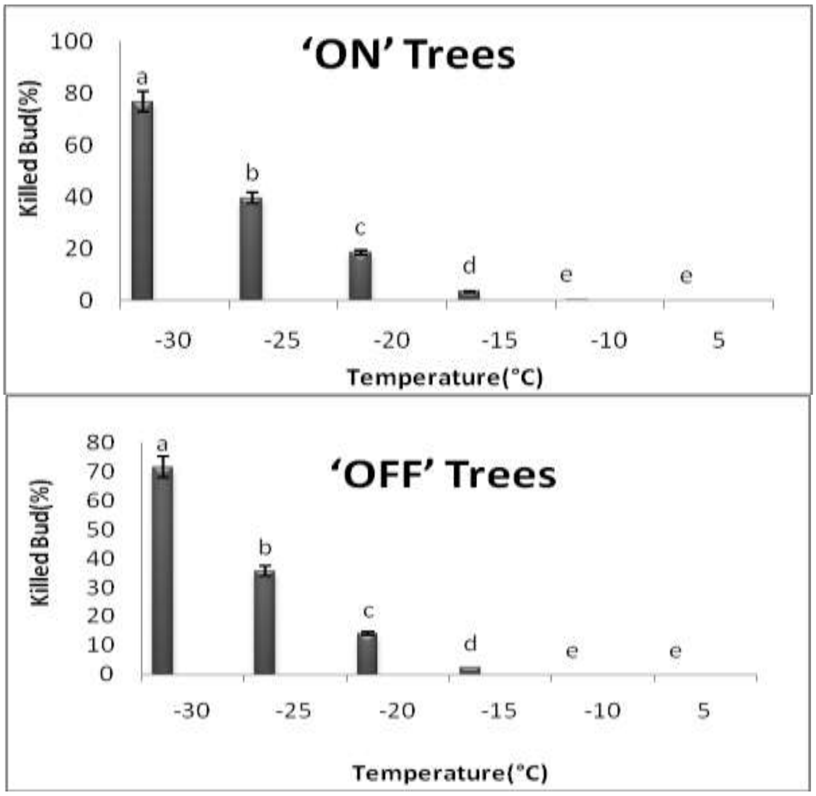

Fig. 1. Effects of subzero temperature on percent dead flower buds of pistachio cultivars during dormant season.

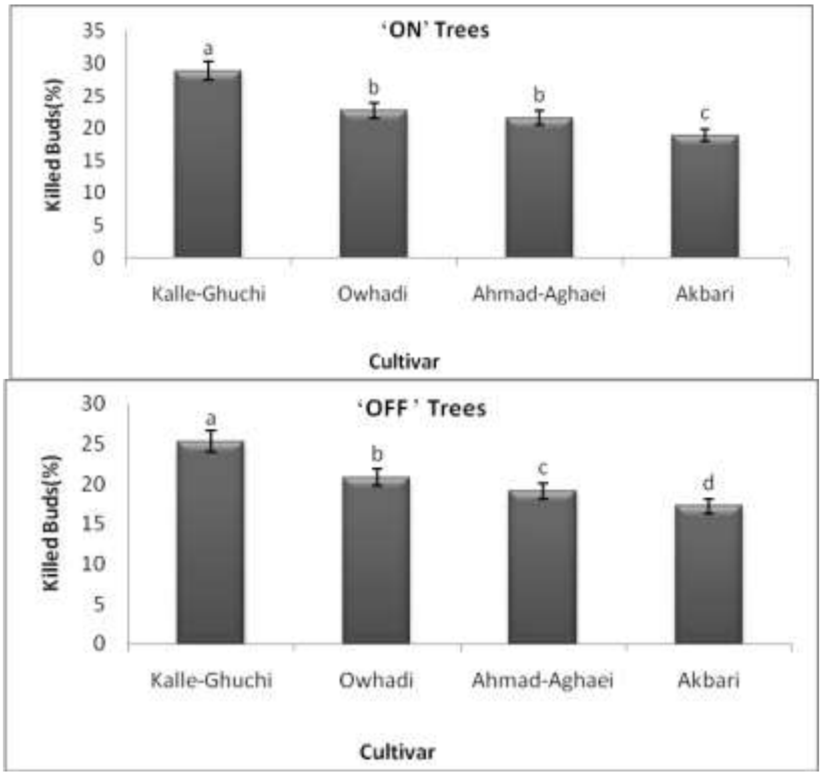

Fig. 2. Effects of subzero temperature on percent dead flower buds of pistachio cultivars during dormant season.

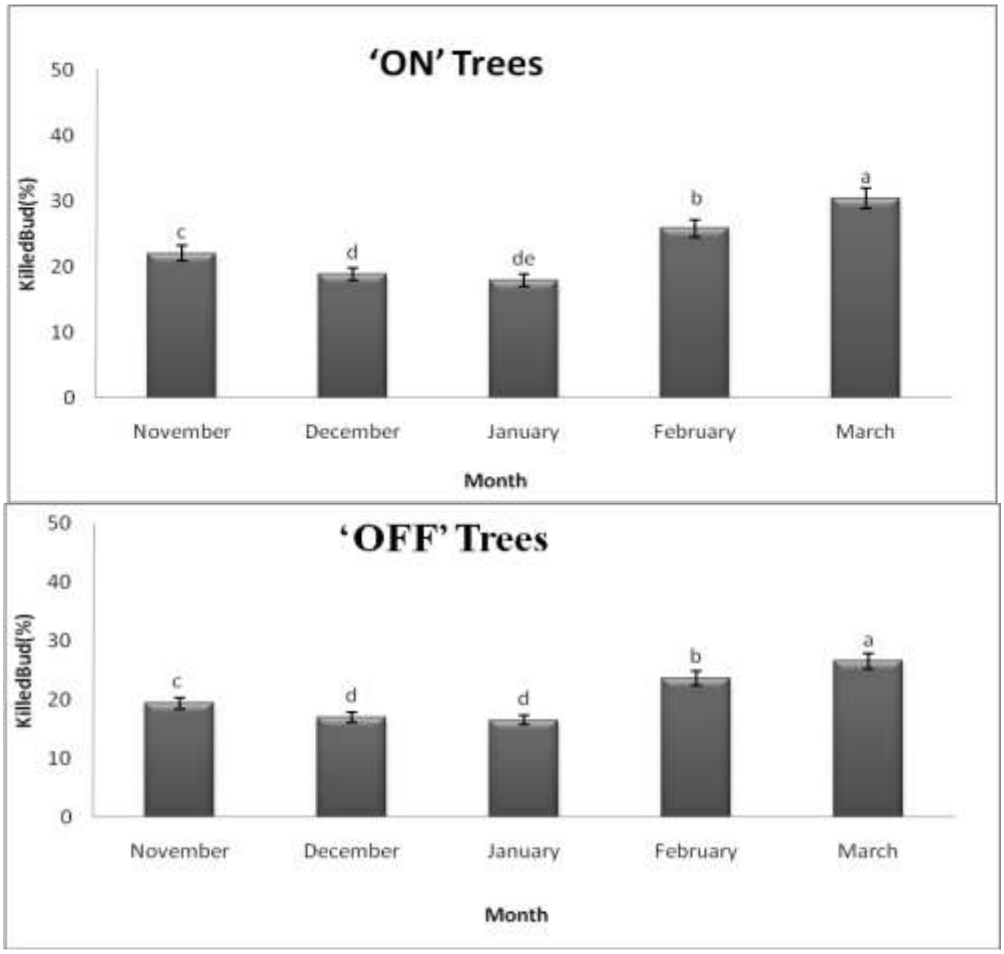

Fig. 3. Effects of subzero temperature on percent dead flower buds of pistachio cultivars during dormant season.

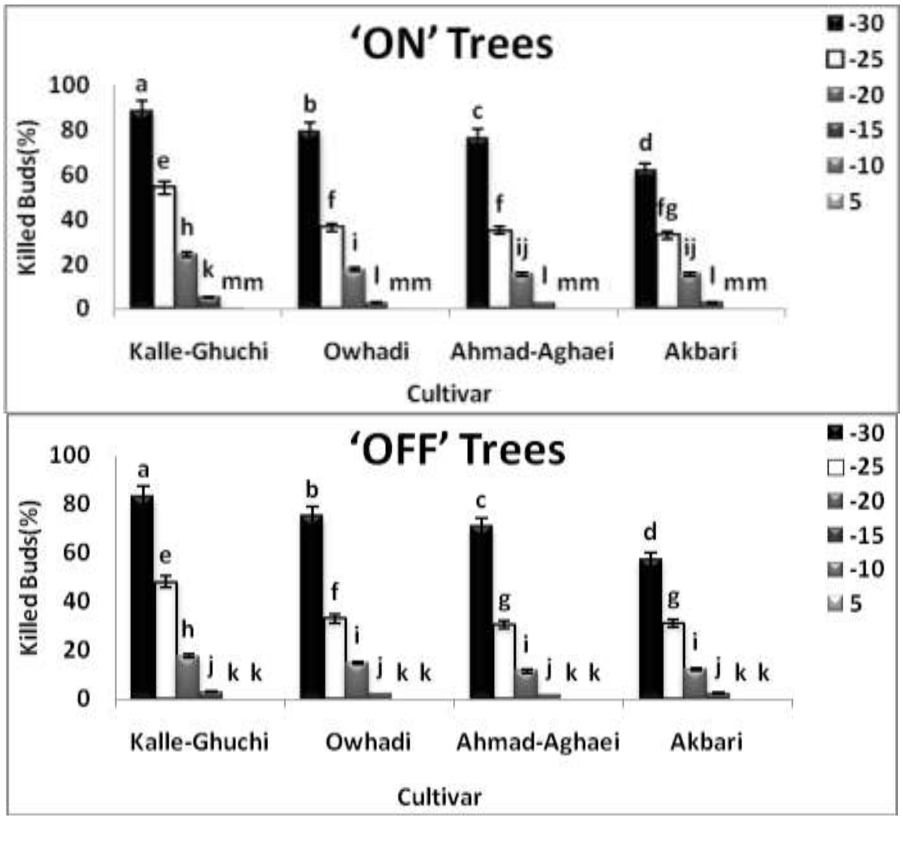

Fig. 4. Interaction effects of subzero temperature and cultivar on percent dead flower buds of pistachio cultivars during dormant season.

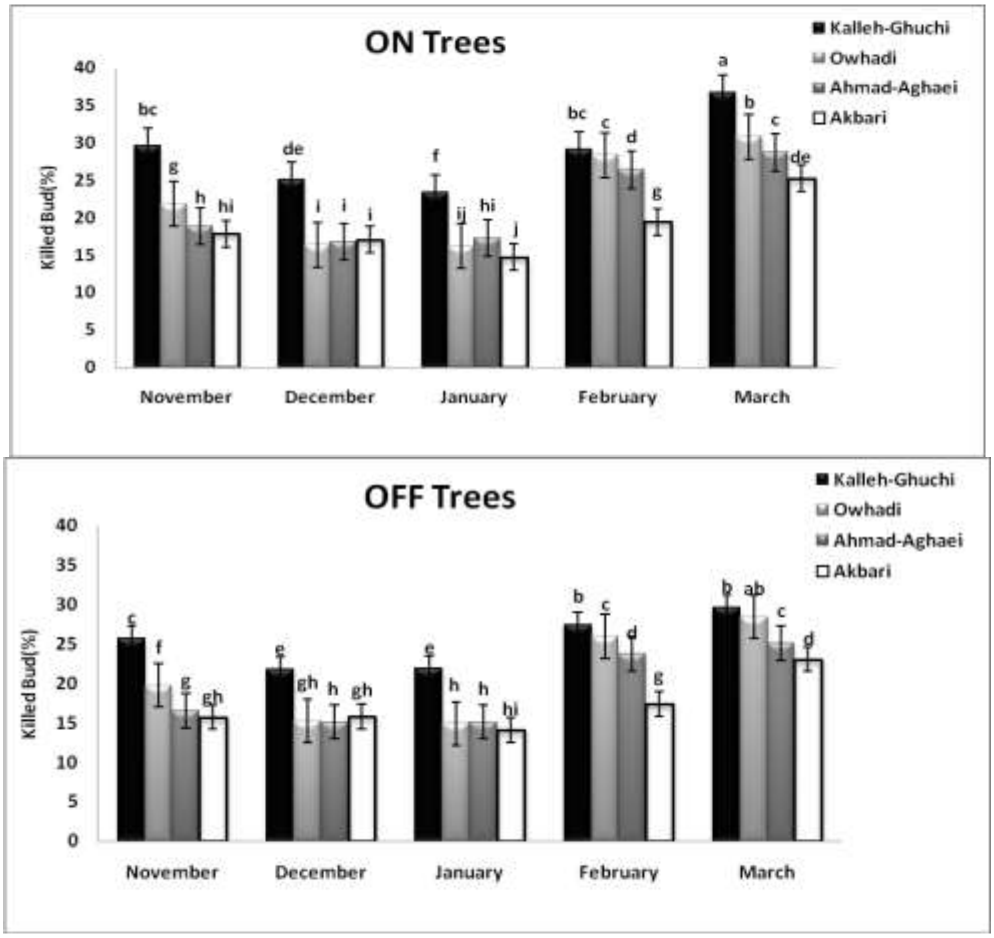

Fig. 5. Interaction effects of month and cultivar on percent dead flower buds of pistachio cultivars during dormant season.

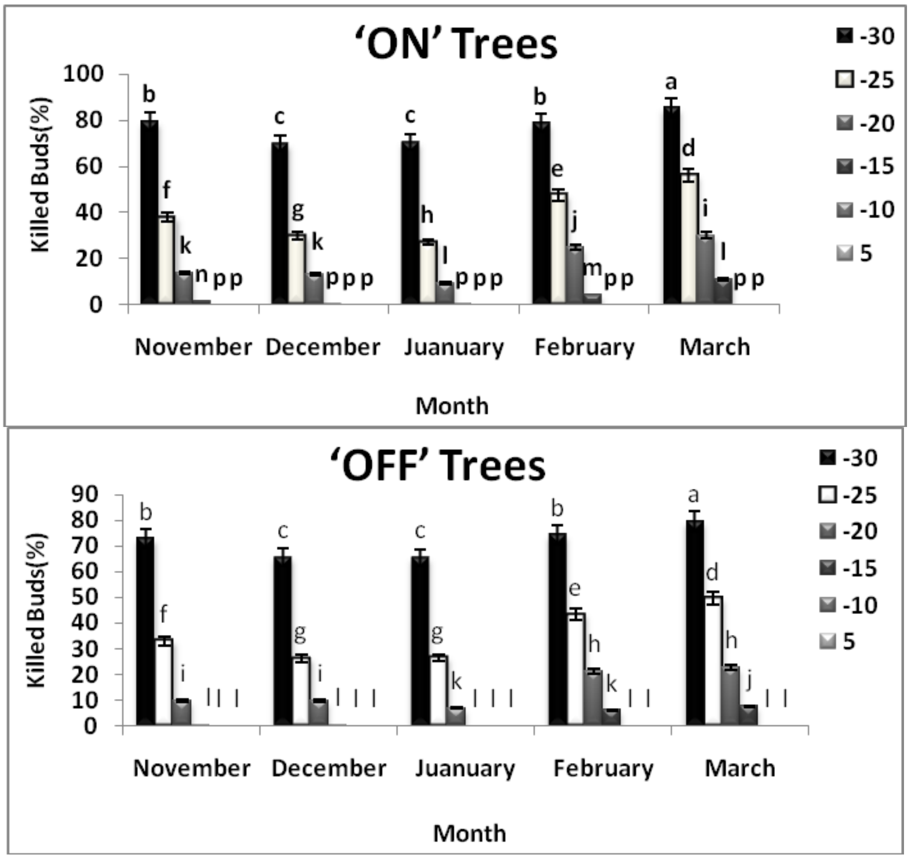

Fig. 6. Interaction effects of month and subzero temperature on percent dead flower buds of pistachio cultivars during dormant season.

Discussion

The present study indicated that significant differences in the survival of flower buds were observed among cultivars within each sampling data and temperate woody perennials survive to low temperatures in winter entering a dormant stage. Rate of plant growth and development is dependent upon the temperature surrounding the plant and each species has a specific temperature range represented by a minimum, maximum, and optimum, the various cultivars of fruit trees show cold hardiness with different degree and this difference depends on genetical and environmental factors and cultural practices (Lindstrom and Dirr 1991; Rekika *et al*., 2004; Miraboolbaghi *et al*., 2010; Ghasemi Soluklui *et al.*, 2014).

Responses to temperature differ among crop species throughout their life cycle and are primarily the phenological responses, for example the stages of plant development. For each species, i.e., pistachio a defined range of maximum and minimum temperatures form the boundaries of observable growth. The tested pistachio cultivars exhibited higher cold hardiness levels in 'OFF' trees, from 'ON' trees. Pistachio trees have been shown alternative bearing (Crane and Nelson 1971, Ferguson *et al*., 1995, Monselise and Goldschmidt 1982, Weinbaum *et al*. 1994). Both qualitative and quantitative changes in hydrocarbons, proteins, minerals and others compounds contents have been involved during acclimation and de-acclimation in various woody perennials (Aslani Aslamarz *et al.*, 2011, Badaruddin and Meyer 2001. Foyer *et al*., 1994, Guy 1990, Morin *et al*., 2007), always total storage in fruit trees are higher in 'OFF' trees from 'ON' trees (Crane and Nelson 1971, Ferguson *et al*., 1995, Monselise and Goldschmidt 1982, Weinbaum *et al*. 1994). Therefore, soluble carbohydrates, proteins, minerals and others compounds were closely related to environmental stress adaptation (Dehghanipoodeh *et al.*, 2018; Norozi *et al.*, 2019) especially cold hardiness (Morin *et al*., 2007, Sivacia 2006). Whereas, the storages and nutrient sources of tree increase, leading to increased cold hardiness. Since 'ON' pistachio trees had lower total storage than 'OFF' pistachio trees, it an increase has been indicated in frost damage. This result is consistent with many other studies (Badaruddin and Meyer 2001, Crane and Nelson 1971, Ferguson *et al*., 1995; Morin *et al*., 2007, Sivacia 2006).

The results of the present study and others researchers indicate that maximum cold hardiness is attained following periods of cold weather, late autumn, early and mid winter, (December and January) while cold hardiness is lost with warm temperatures (late winter, February and March), (Figs. 3, 5 and 6) (Ameglio *et al*., 2004, Ashworth 1990, Ashworth 1996, Bite and Drudze 2000, Lichev and Papachazis 2006, Lind 2002, Wolf and Warren 2000). The fruit trees lose hardiness more rapidly once the chilling requirement is complete. The maximum hardiness can be attained after the chilling requirement has been completed, late winter, (February and March). Our findings in Figs. 3, 5, and 6 were in agreement with the previous reports (Arora *et al*., 1992, Ashworth and Wisniewski 1991, Pearce 2001, Rajashekar *et al*., 1982).

Hardy fruit trees characteristically undergo a series of changes in the autumn which enable them to withstand freezing stress. In nature, the first stage of acclimation appears to be induced by short days (Ameglio *et al*. 2006, Ashworth 1996, Welling *et al*. 1997). The second stage of acclimation in nature is apparently induced by low temperatures, in fact, frost often appears to be the triggering stimulus (Heino and Palva 2003, Lind 2002, Xiong *et al*. 2002b). There appears to be a third stage of acclimation in hardy woody species being induced by low temperatures. Prolonged exposure to temperatures in this rang can ultimately cause hardened twigs to attain a state of hardiness which may not commonly be attained in nature. This kind of hardiness is quickly lost (Ameglio *et al*. 2006, Ashworth and Wisniewski 1991, Rekika *et al*., 2004).

Hardiness and dormancy tended to be synonymous, as fruit tree become dormant, hardiness increase. It was recognized that all parts of the tree do not become hardy in unison (Ashworth 1996). In November, the order of severity of freeze damage between tissues may be quite different from that in January, and the observations of the present study confirm this report (Tables 3, 4 and Figs. 3,5, and 6) (Ashworth 1996, Thomashow 1999). During the rest period, the tree is unable to grow and consequently remains hardy, after the chilling requirement has been satisfied, warm weather promotes growth and with the resumption of growth comes a loss of hardiness. The hardiness period occurs with lowering temperatures and shorting days. It could be prolonged by warm weather or hastened by cold. Such lack of hardiness was attributed to mild winter conditions which satisfy the chilling requirement by mid winter and allow the plants to grow during February and early March. This period increased frost damage and the results of the present study were in agreement with the pervious findings (Ameglio *et al*., 2006, Ashworth 1996, Ashworth and Wisniewski 1991, Rekika *et al*., 2004, Thomashow 1999).

In the dormant buds of many fruit trees species, water appeared to be withdrawn from the developing floral organs and migrate to the growing ice crystals within the scales and axis. In subzero temperature, freezing induced cellular dehydration is the widest spread cause damage. However, damage in organs and species may occur for other reasons. For example, the cells which supercool will die if their capacity for supercooling is exceeded (Aslamarz *et al*., 2010, Lindstrom and Dirr 1991, Morin *et al*., 2007, Rajashekar *et al*., 1982). The water potential of ice is lower than that of liquid water consequently, extracellular ice crystals grow by drawing water from cells until the water potential of ice and cell are equal, thus dehydrating the cell contents. The water potential of ice falls as temperature falls and thus cellular dehydration becomes progressively greater as temperature falls down to a limit set by vitrification (Hara *et al*., 2001, Heino and Palva 2003, Palta 1990). In some species, cell walls partially resist the collapse in cellular volume, creating a divergence from equilibrium and reducing the extent of dehydration. However, substantial cellular dehydration still occurs. The ice crystals spread and then lead to death cell, tissue, and organ and finally the whole plant (Ashworth 1990, Canny 1997, Foyer *et al*. 1994, Griffith and Antikienen 1996, Hara *et al*., 2001, Ishikawa *et al*., 1997, Pearce 2001).

Conclusions

Frost is the temperature that causes freezing. Hardening is a physiological change of a plant with subzero temperature. The factors evaluated in studies indicated that the cold hardiness of pistachio cultivars could be modified significantly by changing the cultivar, sampling month, and subzero temperatures. These 4 cultivars were classified as hardy (‘Akbari’), semi-hardy (‘Owhadi’, ‘Ahmad-Aghaei’) and sensitive (‘Kalle-Ghuchi’). All cultivars showed sensitivity to temperature -30^{o}C. All of the cultivars showed highest hardiness in November and January. The hardening period occurs with lowering temperatures and short days. Cultural practices have a considerable effect, prolonging growth tending to retard hardening. The period of deepest dormancy and greatest hardiness depends upon the rest phenomenon. However, the response of pistachio trees to sub-zero temperature is both varied and complex. Plants have evolved various strategies to cope with low temperatures and, in the case of deciduous fruit trees such as pistachio tree, different tissues within the same plant respond differently. The mechanisms by which plant cells respond to a freezing stress are influenced by the unique physiological and anatomical features of the tissue.

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