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Influence of ecological variation across *Pistacia altantica* on fruit oil content

Bayzid Yousefi

Kurdistan Agriculture and Natural Resources Research Center
PO Box 714, Sanandaj, Kurdistan, Islamic Republic of Iran

ABSTRACT

In the western and central parts of Iran, there are more than eight million female trees of *Pistacia atlantica* Desf. with approximately 20,000 tons of fruit oil production ability. In order to evaluate the influence of select ecological characteristics, fruit samples of seven *P. atlantica* habitats in Kurdistan (Iran) were collected in late October over two years (2013-14). Fruit oil was extracted using soxhlet extraction. The results showed a relatively small variation in oil content in fruit across plants of *P. atlantica*. The fruit oil content ranged from 25.4% to 28.4%, with a mean of 27.3%. Most habitats belonged to temperate and semi-arid regions, with higher longitude and altitude but less latitude and slope, higher soil electrical conductivity, total neutralizing value, organic carbon and clay, medium yearly rainfall, evaporation and sunny hours and also trees with medium ages such as Borban and Kashtar producing higher fruit oil yield than those that belonged to cool and semi-humid regions. Cluster analysis classified habitats into four distinct groups at 50% similarity; four habitats belonging to forest areas were located in the first, Borban and Kashtar in the second and third and Abdolmomen, with the coldest climate and the oldest trees, was in the fourth group. The results of principal component analysis (PCA) revealed that habitats with low fruit oil showed extreme values of PC_1 and PC_2 but ones with high fruit oil were located mainly in the central zones of the bi plot, which suggest that habitats with medium ecological conditions produce higher fruit oil.

Key words: environmental conditions, fruit quality, habitat, pistachio tree

INTRODUCTION

The genus *Pistacia* includes many species widely distributed in the Mediterranean and Middle Eastern areas and belongs to the Anacardiaceae family (Ben Douissa et al. 2005). Large populations of *Pistacia* grow wild in different parts of Iran. There are three species of the *pistacia* genus in Iran: *Pistacia avera, P. atlantica* and *P. khinjuk* (Karimi et al. 2009). The *atlantica* species has been postulated to have three subspecies: *mutica* in the middle and south of the Zagros mountains, *kurdica* mainly in Kurdistan (Zoh.) Rech. and *cabulica* in the northeast of the country (Khatamsaz 1988). *Pistacia atlantica* Desf., which is called "Baneh" in Iran, is a multipurpose tree, a drought-tolerant species that is adapted to semi-arid and arid areas (Rowshan et al. 2013). Because of its economic properties, *P. atlantica* is one of the most economically and ecologically important wild species in many rural areas. It plays a considerable role in the rural society's extension, especially in the west of Iran (Razavi 2006, Pourreza et al. 2008). *P. atlantica* is long-lived and dioecious, with male and female trees producing different types of flowers. Both types are small and greenish and

Corresponding author.
Tel.: +98 9188717049;
e-mail: bayzidyousefi@gmail.com, bayzidyousefi@yahoo.com (B. Yousefi).

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are located in simple or compound clusters and fall away quickly. Monoecious and hermaphrodite trees have been seen but are unusual (Isfendiyaroglu and Ozekera 2009). The trees grow up to 7 m (23 ft.) in height, with branches spreading and growing erect to form a dense crown. The oblong, fleshy, oily and aromatic fruit borne by the female tree are 6 to 8 mm, long and pink in color, ripening blue. In Iran and Kurdistan province, late October to November is the ripening time of *P. atlantica* fruit. There are many uses for this plant. The resin and fruit oil were historically used for a variety of medicinal purposes. It is the source of traditional medicinal agent "gum" mastic, an oleoresin exudate from the stem of this plant (Dogan et al. 2003). Investigations have shown some pharmacological effects such as reducing blood pressure (Villar et al. 1987), upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer, anti-inflammatory and antimicrobial action (Farhoosh et al. 2009, Ghalem and Mohamed 2010, Tohidi et al. 2011). *P. atlantica* is often used as a rootstock for pistachio (*P. vera*) (Ibrahim et al. 1984, Ruelas Garcia 1990). The fruit of *P. atlantica* is an important source of food, although the fruit are smaller and not as commercially valuable as those produced in orchards (Zangeneh 2003). Farhoosh et al. (2011) noted that the fruit oil of *P. atlantica*, which is called Bene Hull Oil (BHO), has been recently introduced to the world as a highly stable compound with anti-oxidative properties. The components of BHO include 6.5% unsaponifiable matter (common in vegetable oil), carotenes, tocopherols and alcohols. Tocopherols and tocotrienols have antioxidant activity and act similarly to vitamin E, which is beneficial for human health (Mezni et al. 2014). Tanideh et al. (2014) showed that a high dose of *P. atlantica* fruit oil, administered orally and rectally, can improve colitis physiologically and pathologically in a rat model and may be efficient for ulcerative colitis. The studies of Arefi et al. (2003 and 2006) have shown that *P. atlantica* fruits contain valuable oils, including 19 to 39%, especially unsaturated oils, and is suitable for human consumption. Benhassaini et al. (2007) stated that the fruit oil content of *P. atlantica* is more than 39%, with valuable fatty acids such as oleic (54.2%), linoleic (28.8%) and palmitic (12.2%) acids. Oleic acid was the major fatty acid (more than 40%), followed by palmitic (22.3-28.6%) and linoleic (13.6-20.5%) acids (Mezni et al. 2014). The fruit oil of *P. atlantica* has been reported by Hossein-Khan and Farhang (1996) to be about 30%. In the food industry, there is a tendency to search for new sources of oil that may have nutritional value. *P. atlantica* fruit oil has potential uses as a source for edible oil, as well as for the pharmaceutical and oleo chemical industries (Soad et al. 2014).

In Iran, the use of *P. atlantica* is usually focused on its resin or gum and the oily and aromatic fruits have not been used considerably. Large populations of *P. atlantica* grow wild in different parts of Iran; for example, there are more than 400,000 over 10-year-old female trees only in Kurdistan province (Yousefi 2002), thus, the project of *P. atlantica* fruit oil extraction can provide a new medicinal and edible oil source. Considering this, the present research was performed to compare *P. atlantica* fruit oil production in various habitats to evaluate the influence of select important ecological characteristics on the oil contents of fruits.

MATERIAL AND METHODS

The research was conducted in seven *P. atlantica* habitats in Kurdistan province in the west of Iran (Fig. 1) including: Marivan, Sarv-Abad, Kamyaran, Sanandaj, Saghez, Baneh1 (Allout) and Baneh2 (Khori-Abad) during two years (2013-14). After recording climate factors such as temperature, rainfall, freezing days etc., some chemical characteristics of soil were recorded by analysis of the soil samples (at 0-60 cm depth) in every habitat by the soil science laboratory of the Agricultural and Natural Resources Research Center of Kurdistan province. For this, the soil's level of acidity (pH) was measured using a pH meter, soil organic carbon (OC) using the Walkley-

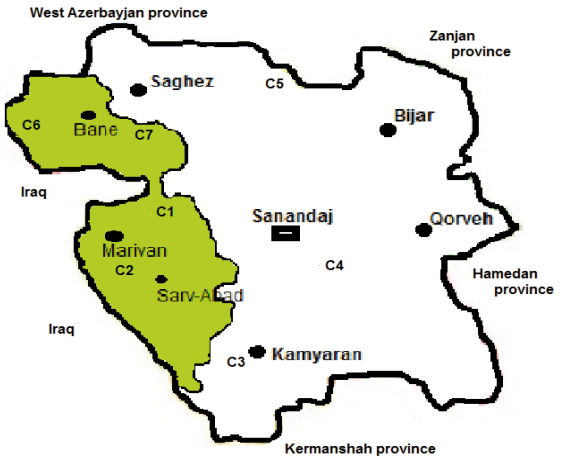

Figure 1. The *P. atlantica* habitats (C1-C7) on the map of Kurdistan province (green color denotes forest areas)

Black method (1934), electrical conductivity of the soil extract (EC) using an EC meter (CDM 210), lime material or soil total neutralizing value (TNV) using titration by hydrochloric acid (HCl) and soil texture was plotted across a triangle that measues the proportions of sand, silt, and clay after separation by sedimentation (pipet method). Five uniform female trees with fruit production ability were selected in each habitat (as replications) and so five 200 g whole fruit samples were harvested in late October. Also, the height and diameter at 1.3m height and crown diameter of trees in habitats were measured separately in every habitat and year. After drying the whole fruit samples (in an oven at 105ºC for 12 hours), fruit oil was extracted using a soxhlet extractor for 5 h using petroleum ether as a solvent. The solvent was evaporated under reduced pressure, using a rotary evaporator at 50°C. The oil content was determined as the difference in the weight of the dried fruit sample before and after the extraction (AOCS 1989). Oil was weighed and stored at −20°C. All of the analyses were conducted in triplicate.

Statistical analyses

Analysis of variance for *P. atlantica* fruit oil contents performed using two years' average data as the nested analysis method and means were compared using Duncan's multiple range test. Cluster analysis (using an agglomerative hierarchical method with standardized variables by subtracting the means and dividing by the standard deviation and dendrogram with the average linkage method and squared Euclidean distance) and principal component analysis (PCA) (using a correlation matrix and score plot for the first two components) were performed for the grouping and characterizing of *P. atlantica* habitats and the correlation coefficients between fruit oil content with recorded characteristics were also estimated.

RESULTS AND DISCUSSION

As shown in Table 1, the studied *P. atlantica* habitats were located at 1290-1750 m altitude, with 14-55% slope, 1.9-7.9ºC average minimum temperature (T_{min}), 14.1-23.4ºC average maximum temperature (T_{max}), 10.3-16.7ºC average optimum temperature (T_{opt}), 44-58% humidity, 442.1-788.4 and 1241-1371 mm annual rainfall and evaporation, 80-114 freezing days, 2725-2881 annual sunny hours, 7.1-7.4 soil pH, 0.32-1.05 mmhos cm^{-1} soil electrical conductivity (EC), 5.1-29% soil total neutralizing value (TNV), 0.8-7.1% soil organic carbon (OC), 20.2-43.2% soil clay and trees with 5.21-12.33 m height, 9.9-45.5 cm diameter at 1.33 m height and 4.79-10.15 m crown diameter. In addition, *P. atlantica* was developed in both forest areas associated with major species of Zagros such as *Quercus* spp*., Amygdalus* spp*., Pyrus* spp., etc. and in non-forest areas as a pure colony. According to the results, the ranges of ecological characteristics of *P. atlantica* habitats were wider than those of many other tree species, which suggests a high range tolerance of this plant against a range of ecological characteristic changes and an important cause for remaining across the hard dry climate in Iran during the last decades. In accordance to this, Rowshan et al. (2013) stated that *P. atlantica* is a drought-tolerant species that has adapted to semi-arid and arid areas.

The analysis of variance revealed significant differences ($p \leq 0.05$) in the fruit oil content among locations (L) (Tab. 2). Because of the varying ecological conditions of the studied locations (seven areas involving almost all parts of the Kurdistan province), and also *P. atlantica* is a deciduous tree and highly heterozygous observed differences among locations were expected. The existence of significant differences among *P. atlantica* populations for fruit oil content was also reported by Hossein-Khan and Farhang (1996) and Arefi et al. (2003 and 2006). This variation can be due to both environmental and genetic factors and provide a suitable base for the selection of a superior population from the view point of fruit oil content. The mean of whole fruit oil was 27.3% with a range of 23.2-31.4% (Tab. 3). The results correspond to those reported by Arefi et al. 2003 and 2006 (19 to 39%) and Hossein-Khan and Farhang 1996 (about 30%) but are less than the results of Benhassaini et al. 2007 (39%). The studied *P. atlantica* populations belonged to *P. atlantica* subsp. *Kurdica,* whose fruits are wider than *P. atlantica* subsp. *mutica*, thus the differences in fruit related traits, e.g. fruit dimensions and oil content are naturaly less and more, respectively. The fruit oil content data (with $n = 35$, $SD = 1.89$, $\bar{Y} = 27.25$, Anderson-Darling test (AD) = 0.55 and $p = 0.10$) follow a normal distribution. Among the studied locations, *P. atlantica* trees from the habitat of Borban produced the highest fruit oil, with 28.4%, while trees from Allout produced the lowest fruit oil, with 25.4%. *P. atlantica* habitats were divided in two high and low yield groups for the entire fruit oil content. The habitats of Borban, Khori-Abad, Kashtar and Gelah with 28.4, 28.4, 28.1 and 28.0%, respectively, produced the highest whole fruit oil content, while

Table 1. Select ecological characteristics of the research locations

P. atlantica habitat			Geographic factors					
Area	Sub area	Code	Position in Kurdistan province	Longitude (East)	Latitude (North)	Altitude (meters)	Habitat slope (%)	Slope direction
Marivan	Gelah	C1	West	46º 24´	35º 36´	1750	45	Southeast
Sarv-Abad	Dezli	C2	West	46º 09´	35º 23´	1290	55	Southwest
Kamyaran	Kashtar	C3	South	46º 40´	35º 01´	1430	14	Eastern
Sanandaj	Borban	C4	Center	47º 02´	35º 08´	1575	25	Northern
Saghez	Abdolmomen	C5	Northern East	46º 43´	36º 17´	1480	30	Southern
Baneh1	Allout	C6	Northern West	45º 33´	36º 01´	1310	17	Northwest
Baneh2	Khori-Abad	C7	Northern west	46º 01´	35º 53´	1630	44	Southern

P. atlantica habitat			Climatic factors								
Area	Sub area	Code	Average temperature (º C)			Relative Humidity (%)	Annual rainfall (mm)	Number of freezing days	Annual evaporation (mm)	Total sunny hours	Climate**
			T_{Min}	T_{Max}	T_{Opt}						
Marivan	Gelah	C1	6.9	21.6	13.7	58	788.4	85	1352	2725	C, SH
Sarv-Abad	Dezli	C2	7.9	23.4	16.7	52	519.5	80	1371	2881	T, SA
Kamyaran	Kashtar	C3	5.1	21.3	15.2	45	442.1	105	1348	2859	T, SA
Sanandaj	Borban	C4	4.7	21.9	14.8	47	474.3	107	1347	2866	T, SA
Saghez	Abdolmomen	C5	1.9	14.1	10.3	44	497.4	114	1241	2823	C, SA
Baneh1	Allout	C6	7.2	18.5	14.9	51	633.3	82	1334	2795	CT, SH
Baneh2	Khori-Abad	C7	6.2	17.5	13.8	48	502.8	90	1340	2811	C, SA

P. atlantica habitat			Soil factors					
Area	Sub area	Code	pH	EC * (dS m^{-1})	TNV* (%)	Organic Carbon (%)	Clay (%)	Soil Texture
Marivan	Gelah	C1	7.3	0.32	7.7	1.00	29.4	Clay Loam
Sarv-Abad	Dezli	C2	7.2	0.44	5.1	1.34	27.4	Clay Loam
Kamyaran	Kashtar	C3	7.1	1.05	18.7	7.11	22.3	Loam
Sanandaj	Borban	C4	7.4	0.36	29.0	0.80	31.4	Clay Loam
Saghez	Abdolmomen	C5	7.4	0.54	5.7	1.80	20.2	Sand Clay Loam
Baneh1	Allout	C6	7.3	0.37	7.3	1.39	31.6	Clay Loam
Baneh2	Khori-Abad	C7	7.2	0.38	6.3	1.84	43.2	Clay

P. atlantica habitat			Vegetative characteristics				
Area	Sub area	Code	Landscape	Associated forest tree	Tree height (m)	Tree diameter in 1.3 m height (cm)	Tree crown diameter (m)
Marivan	Gelah	C1	Forest	*Querqus* spp. *Pyrus, Amygdalus, Acer*, etc.	12.33	35.3	9.95
Sarv-Abad	Dezli	C2	Forest	*Querqus*spp,.*Amygdalus, Acer*,*Pyrus,* etc.	7.62	12.3	7.78
Kamyaran	Kashtar	C3	*P. atlantica* colony	-	6.38	35.9	5.02
Sanandaj	Borban	C4	*P. atlantica* colony	-	9.85	36.8	7.74
Saghez	Abdolmomen	C5	*P. atlantica* colony	-	11.10	45.4	10.15
Baneh1	Allout	C6	Forest	*Querqus* spp. *Pyrus, Amygdalus, Acer*, etc.	9.13	37.4	8.54
Baneh2	Khori-Abad	C7	Forest	*Querqus* spp. *Amygdalus, Pyrus, Acer*, etc.	5.21	9.9	4.79

*EC: Electrical Conductivity (dS/m or deci Siemens per meter), TNV: Total Neutralizing Value (Lime material) (Percentage)
**T: Temperate: C: Cool, W: Warm, A: Arid, SA: Semi-arid, H: Humid. Yearly mean temperatures in warm, temperate and cool climates are 15-25°C, 10-15°C and 0-5°C, respectively. Yearly mean rainfalls in semi-humid, semi-arid and arid climates are 600-1400, 300-600 and 100-300 mm, respectively

the habitats of Allout, Abdolmomen and Dezli with 25.4, 26.0 and 26.4%, respectively, produced the lowest content among the studied locations (Tab. 3). There was wide ecological variation both between and within the two groups of high and low fruit oil yield locations. *P. atlantica* habitats

Table 2. Analysis of fruit oil content variance over seven locations

Sources of variation (SV)	Degree of freedom (DF)	Sum of squares (SS)	Mean squares (MS)
Replication (R)	4	3.50	0.87 ns
Locations (L)	6	47.28	7.88 *
Error (E)	24	71.38	2.97

* and ns denote significant at 5% and nonsignificant, respectively

with higher fruit oil yield were mainly located in a higher longitude and altitude but with less latitude and slope and moderate temperature, both in a pure *P. atlantica* colony and associated with forest species (e.g. *Querqus* spp. *Pyrus* spp*., Amygdalus* spp*., Acer* spp., etc.), with medium yearly rainfall, evaporation and sunny hours but with more freezing days than that recorded for lower fruit oil yield trees. Yousefi (2002) stated that *P. atlantica* trees in forest areas such as the Marivan and Baneh populations in Kurdistan province produced larger leaflets, leaves and fruits than those in non-forest areas. It seems that the smallness and narrowing of leaves, leaflets and fruits in cold and dry areas is a tolerance mechanism to drought stress. The soil of high fruit oil yield habitats showed a higher salt concentration in the soil solution expressed as EC values, total neutralized value (% TNV), organic carbon content and clay and also medium vegetative traits, while trees of low fruit oil yield were larger and older. In accordance to this, longitude, altitude, maximum temperature, total neutralizing value and optimum temperature were significantly and positively correlated with fruit oil yield while latitude and tree crown diameter were significantly and negatively correlated with fruit oil yield (Tab. 4).

Seven *P. atlantica* habitats were grouped based on their ecological characteristics by using cluster analysis and the results is summarized are a dendrogram (Fig. 2). The sum of squares within clusters, average and maximum distances from the centroid were 126, 4.2 and 5.4, respectively. Based on the cluster analysis results, two habitats of Allout and Dezli showed the highest similarity among locations, with about 65%. Finally, the habitats were classified into four distinct groups at 50% similarity. The habitats of Allout and Khori-Abad, Dezli and Gelah were located in the first group, Borban in the second, Kashtar in the third and Abdolmomen, with the highest distance from the other habitats, in the fourth group. All of the habitats in the first group were located in the forest areas of Baneh and Marivan in the west of Kurdistan and Iran on the border of Iraq with warmer temperatures, higher humidity and rainfall but fewer sunny hours and medium fruit oil content. The habitats of Borban

Table 3. *P. atlantica* fruit oil percentages and descriptive statistics in seven habitats

Area	Sub area	Code	Max	Min	Range	Mean	CV (%)
Marivan	Gelah	C1	29.1	25.9	3.2	28.0 a	5.1
Sarv-Abad	Dezli	C2	27.2	25.8	1.4	26.4 ab	2.4
Kamyaran	Kashtar	C3	30.2	25.7	4.5	28.1 a	6.6
Sanandaj	Borban	C4	31.4	26.8	4.6	28.4 a	6.7
Saghez	Abdolmomen	C5	28.4	24.4	4.0	26.0 ab	7.3
Baneh1	Allout	C6	27.4	23.2	4.2	25.4 b	7. 9
Baneh2	Khori-Abad	C7	30. 2	26.8	3.4	28.4 a	4.5
Total	–		31.4	23.2	3.6	27.3	5.8

Means with different letters show significant differences at 5% probability

Table 4. Correlation between fruit oil percentage and other characteristics

	Longitude	Latitude	Altitude	Slope	TMin	TMax	TOpt	Humidity	Rainfall	Freezing days	Evaporation	sunny hours	pH	EC	TNV	OC	Clay	Tree H	Tree 1.3D	Tree CD
Fruit oil	0.61**	-0.74**	0.72**	0.09 ns	-0.01 ns	0.57 **	0.46 *	-0.01 ns	-0.13 ns	0.22 ns	0.41 ns	-0.01 ns	-0.24 ns	0.14 ns	0.57 **	0.23 ns	0.36 ns	-0.23 ns	-0.22 ns	- 0.51 *

**,* and ns denote significant at1%, 5% and nonsignificant, respectively

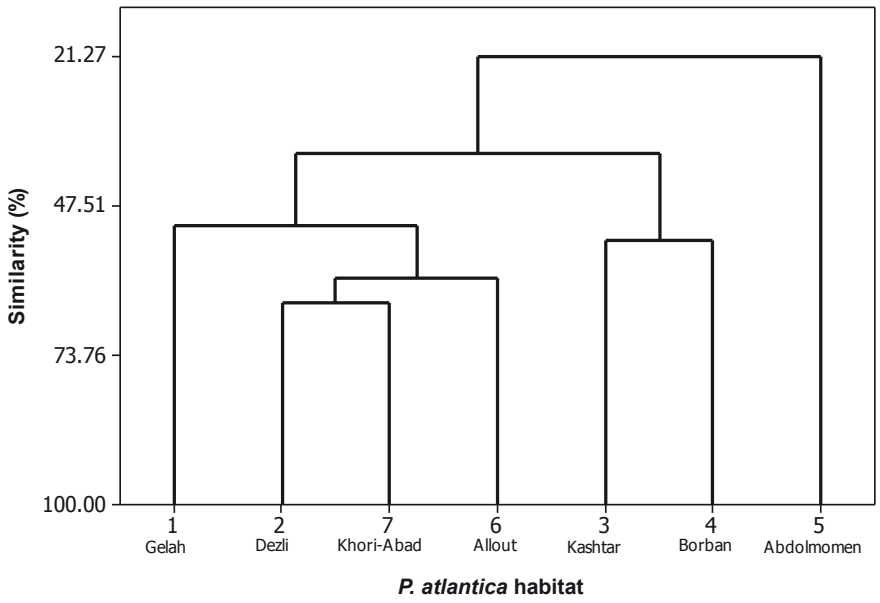

Figure 2. Dendrogram of cluster analysis based on the ecological characteristics of *P. atlantica* habitats

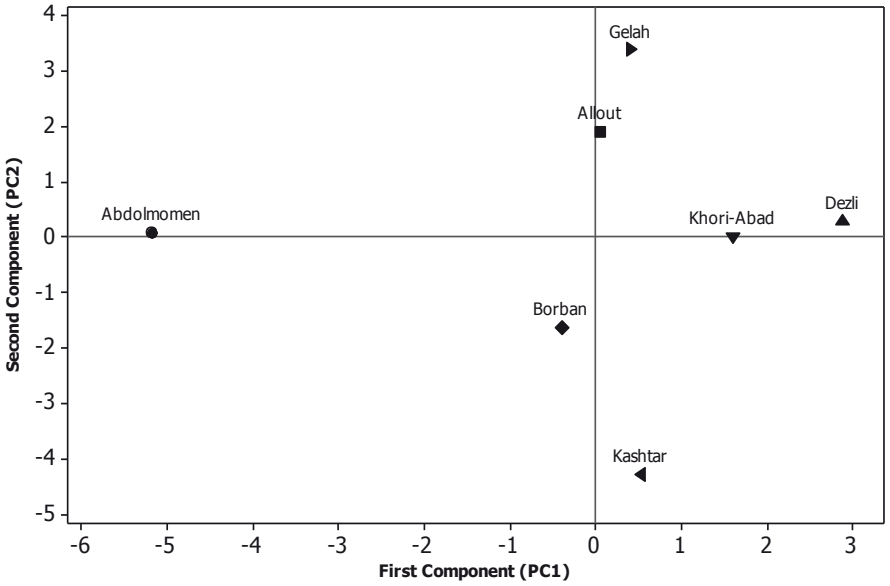

Figure 3. Score plot for the first two components of principal component analysis (PCA)

and Kashtar in groups two and three were located in non-forest areas in the center of Kurdistan with a higher longitude, altitude, maximum and optimum temperature, TNV and produced the highest fruit oil content, with more than 28%. The habitat of Abdolmomen (group four) showed less than 22% similarity with the other habitats, and was located in the northern non-forest areas of Kurdistan with the coldest climate, the least soil TNV and clay with the oldest *P. atlantica* trees and low fruit oil yield.

The result of principal component analysis (PCA) revealed that the five first PC with 6.54, 6.02, 3.29, 2.27 and 1.77 eigen values, respectively, covered 95% of total variance. The score plot for the first two components (Fig. 3) is in accordance to the dendrogarm of cluster analysis. All of the *P. atlantica* habitats of the first group had positive PC_1 and PC_2. The habitats with low fruit oil content such as Allout, Abdolmomen and Dezli showed extreme values (positive or negative) of PC_1 and PC_2, but the habitats with high fruit oil content such as Borban, Khori-Abad and Ksshtar, located mainly in central zones of the bi plot, suggest that the *P. atlantica* habitats with medium ecological

conditions (medium temperatures, rainfall, slope, freezing days, sunny hours, tree height, diameter and age, etc.) produce higher fruit oil.

CONCLUSIONS

1. A relatively small variation in oil content in fruits across plants of *P. atlantica* was found. The fruit oil content ranged from 25.4% to 28.4%.
2. Most of the *P. atlantica* habitats in the western and central part of Iran belonging to temperate and semi-arid regions, with higher longitude and altitude but less latitude and slope, higher soil electrical conductivity, total neutralizing value, organic carbon and clay, medium yearly rainfall, evaporation and sunny hours, and also trees with medium ages such as Borban and Kashtar produced higher fruit oil yields than those that belonged to cool and semi-humid regions.
3. Cluster analysis classified habitats into four distinct groups at 50% similarity. Four habitats belonging to forest areas were located in the first, Borban and Kashtar in the second and third and Abdolmomen, with the coldest climate and the oldest trees, was in the fourth group.
4. The results of principal component analysis (PCA) revealed that habitats with lower fruit oil showed extreme values of PC1 and PC2 but ones with higher fruit oil were located mainly in the central zones of the bi plot, which suggest that habitats with medium ecological conditions produce higher fruit oil.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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