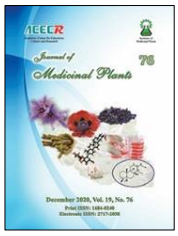
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**Research Article**

# Phytochemical and morpho-physiological variations in sea buckthorn (*Hippophae rhamnoides* L.) populations of Taleghan region in Iran

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ABSTRACT

**Background:** Sea buckthorn is a thorny shrub with nitrogen-fixing ability belonging to the Elaeagnaceae family. **Objective:** In this study, phytochemical and morpho-physiological traits in wild populations of sea buckthorn were investigated in two consecutive years in the Taleghan region of Iran. **Methods:** Some morpho-physiological traits in several parts of plants were measured. Also, some phytochemical analysis of fruit pulp (through spectrophotometric methods) and seed oil content was performed. **Results:** The results showed that the populations had significant differences ($P \leq 0.01$ or $P \leq 0.05$) in most of the main morpho-physiological traits and all phytochemical properties of leaves and fruits in growing seasons during two studied years. The highest and lowest amount of some more important traits of fruit were ranged from 16.02 to 48.55 mg/g, total phenol (Jostan-Bozaj), 0.71 to 1.65 mg/g carotenoid (Gelyard-Fashandak), 0.92 to 2.46 mg/g flavonoid (Dehdar-Shahrak), and 1.37 to 10.00 mg/g vitamin C (Gelyard-Shahrak). Factor analysis based on PCA revealed that the first three-component contributed about 70 and 76% of the total variation for phytochemical and morpho-physiological traits of populations, respectively. The first component (PC1) was contributed by some traits such as fruit glucose, total soluble solids (TSS), vitamin C, and leaf lycopene for phytochemical traits. **Conclusion:** The wide range of variation across the sea buckthorn populations in this region can be used for the selection of suitable genotypes for improvement and pharmaceutical exploitation of this plant in Iran.

## 1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L.: synonym of *Elaeagnus rhamnoides* (L.) A. Nelson) is a deciduous shrub native in temperate zone of Asia, Europe, and North America. It is a thorny nitrogen fixing shrub of Elaeagnaceae family with high nutraceutical, and therapeutical properties. The bioactive substances of this plant

*Abbreviations:* PCA, Principal Component Analysis; TSS, Total Soluble Solid

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are reputed to have considerable medicinal value and are frequently used for cure cough, skin wounds, cardiovascular diseases, improving blood circulation, and have antioxidant activity [1, 2]. Ascorbic acid (vitamin C) is the most important nutrient in raw juice of sea buckthorn fruits [3]. Sea buckthorn leaves and berries have been shown to have a high levels of phenolic compounds including flavonoids, flavones, phenolic acids, and tannins [1, 4, 5]. Its fruits contain carbohydrates (such as glucose and fructose) in the form of sugars [3]. Also, various carotenoids (such as lycopene and $\beta$-carotene) are the major substances exist in aerial part of Sea buckthorn especially in fruits pulp [6, 7]. The sea buckthorn grown widely in central and northern provinces of Iran and have used in folk medicine. Taleghan region in Alborz province is one of the main natural habitats of sea buckthorn in Iran.

Phytochemical and morpho-physiological traits are generally used as a tool for investigation of diversity and genetic relatedness. Growth and performance of plants are under influence of different factors such as genetic characteristics, regional climate and geographical location, etc. Furthermore, previous studies have demonstrated that medicinal plants produce various contents of secondary metabolites in different environments, resulting in differences in their medicinal qualities [8]. According to these facts, the phytochemical and nutritional composition of sea buckthorn flowers, leaves, and berries vary considerably because of genetic variation, parts analyzed, climate and growing conditions, variation between years, degree of ripening, storage conditions, time of harvesting and method of processing and analysis [7, 9, 6, 9, 10]. For example, large variability observed in *Hippophae salicifolia* D. Don populations, growing naturally in Garhwal Himalayas particularly in respect of number of the leaves, branch length, fruit length, 100 fruit weight, number of fruits per unit length of the fruiting branch, fruit yield, titratable acidity, total soluble solids and juice yield. Based on these morphological and phytochemical observations, it was evident that large diversity exists in naturally growing populations of *H. salicifolia* in the Indian Central Himalaya [11]. Some researchers studied the flavonoid content and composition in leaves and berries of sea buckthorn (*Hippophae* spp.) of different origins and clearly showed a difference between genotypes originating from China, Russia, Finland, and Canada [12]. In another study, the authors reported the genotypic effect on the chemical composition and antioxidant activity of sea buckthorn berry based on 10 wild genotypes from a single location in Turkey [13].

In order to adaptation to the environment, plant populations in different regions show genetic diversity which may have the influence on the phytochemical composition and biological activity of plants essential oil and extracts [14]. Nevertheless, no such studies were conducted to evaluate the variation pattern in natural wild populations of sea buckthorn in any region of Iran. The present study was carried to determine the variations in some phytochemical, physiological, and morphological traits of natural populations of sea buckthorn, growing in Taleghan region of Iran.

## 2. Material and Methods

### *2.1. Plant material and collection*

A total of 10 sea buckthorn populations were evaluated from different natural habitat in Taleghan region in mid-October 2014 and 2015. Voucher specimens have been deposited in the Medicinal Plants Institute Herbarium (MPIH), ACECR, Karaj, Iran. Taleghan is located about 140 km northwest of Tehran in mountainous



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altitudes of Central Alborz province in Iran. Its climate is the cold mountainous type and geographical origins of the 10 sea buckthorn populations and their GPS coordinates are shown in table 1. The areas range between longitudes 36° 08′ E and 36° 14′ E, latitudes 50° 42′ N and 51° 03′ N, and altitudes 1830 and 2339 m. In this study for assessing morphological and phytochemical traits, we evaluated mean results of leaf and fruit samples from five randomly individual shrubs in each population with triple replications. Some measurements were done in natural habitat and laboratory with fresh fruits and leaves, some of them with frozen fruit (such as vitamin C and TSS) and other traits were measured in air dried fruits and leaves. Collected leaf samples were dried at room temperature (27 °C) for one week and fruits samples were also dried in an air-dryer apparatus for 72 hours at 40 °C, and all of them were grounded prior to chemical analyses in an electric mill. All kinds of solvents and acids used in the present study included gallic acid standard, quercetin standard, Folin-Ciocalteu reagent, polyvinyl-polypirrolidone (PVP) and methanol were purchased analytical grade from Merck, Germany.

**Table 1.** Geographical origins of *H. rhamnoides* populations

| Population No. | Herbarium No. | Region originated | Latitude (N) | Longitude (E) | Altitude (m) |
|---|---|---|---|---|---|
| 1 | MPIH-4515 | Bozaj | 36° 12' 42.02" N | 50° 48' 35.09" E | 2298 |
| 2 | MPIH-4511 | Parachan | 36° 14' 45.65" N | 50° 56' 49.31" E | 2339 |
| 3 | MPIH-4509 | Jostan | 36° 10' 55.74" N | 50° 54' 21.00" E | 1973 |
| 4 | MPIH-4517 | Khodkavand | 36° 08' 35.77" N | 50° 49' 59.79" E | 2231 |
| 5 | MPIH-4510 | Dehdar | 36° 11' 22.23" N | 51° 03' 06.04" E | 2328 |
| 6 | MPIH-4512 | Shahrak | 36° 10' 32.92" N | 50° 46' 47.87" E | 1830 |
| 7 | MPIH-4513 | Fashandak | 36° 09' 07.55" N | 50° 42' 35.42" E | 1972 |
| 8 | MPIH-4516 | Gelyard | 36° 09' 11.25" N | 50° 50' 47.43" E | 2178 |
| 9 | MPIH-4514 | Gooran | 36° 10' 31.81" N | 50° 50' 52.51" E | 1893 |
| 10 | MPIH-4508 | Nesa | 36° 10' 57.80" N | 50° 52' 52.38" E | 1933 |

*2.2. Morpho-physiological traits*

The morpho-physiological measurements included the plant height (cm), canopy width (cm), trunk diameter (cm), leaves, fruits and seeds length and width (mm), fruits peduncle length (mm), petiole length (mm), 1000 seed weight (g), 100 fresh fruit weight (g) and fresh fruit moisture (%). These morpho-physiological traits were measured by using laboratory equipment such as digital caliper and precise electronic balance.

*2.3. Total phenolic content*

The amount of total phenolics in methanol extracts of dry fruits and leaves was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). The concentration of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml of gallic acid were prepared in methanol. Thus, the calibration curve of gallic acid was drawn. The concentration of 0.1 and 1 mg/ml of plant extract was also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10 fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760 nm (UV-2601 double beam UV/VIS spectrophotometer) spectrometrically [15].



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*2.4. Tannin content*

Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVP), which binds tannins as described by Makkar et al. [16]. Briefly, 1 ml of extract dissolved in methanol (1 mg/ml), in which the total phenolics were determined, was mixed with 100 mg PVP, vortexed, kept for 15 min at 4 °C and then centrifuged for 10 min at 3000 rpm. In the clear supernatant, the non-tannin phenolics were determined the same way as the total phenolics. Tannin content was calculated as a difference between total and non-tannin phenolic content.

*2.5. Total flavonoid content*

The total flavonoid content of each fruit and leaf extract was estimated by the method described by Zhishen et al. [17]. Based on this method, each prepared sample (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.30 ml of a $NaNO_2$ solution (10 %). After 5 min, 0.30 ml $AlCl_3$ solution (10 %) was added fallowed by 2.0 ml of NaOH solution (1 %) to the mixture. Immediately, the mixture was thoroughly mixed and absorbance was then determined at 510 nm versus the blank. The standard curve of quercetin was prepared (1-12 mg/ml) and the results were expressed as quercetin equivalents (mg quercetin.$g^{-1}$ dried sample).

*2.6. Total carotenoids, lycopene and β-carotene content*

Total carotenoids extraction and determination were conducted as described by Lee [18]. The method used a mixture of hexane: ethanol: acetone (2: 1: 1 by vol.) containing 0.05 % butylated hydroxytoluene (BHT). For total carotenoid quantification, the absorbance of the hexane extract was read at 450 nm using a UV-2601 double beam UV/VIS spectrophotometer. *β*-Carotene and lycopene were determined according to the method of Nagata and Yamashita [19]. The dried methanol extract (100 mg) was vigorously shaken with 10 ml of acetone-hexane mixture [4:6] for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of *β*-carotene and lycopene were calculated according to the following equations:

Lycopene (mg/100 ml) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$

*β*-Carotene (mg/100 ml) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$.

*2.7. HPLC quantification of vitamin C*

1 g of sample (frozen fresh fruit) was homogenized repeatedly with 0.01 mol/L metaphosphoric acid (6 × 20 ml) and centrifuged at 2200 × g for 5 min (Cooling Centrifuge C-30, REMI). The supernatant was filtered through a 0.45 µm filter and analyzed immediately. Juice samples were also treated with metaphosphoric acid, centrifuged and filtered. A 20 µL sample was injected into a Knauer Wellchrom HPLC (Germany) equipped with a K-1001 pump Phenomenex C-18 ODS-2 column (5 µm, 250 mm × 4.60 mm; Luna) and a K-2501 UV detector set at 246 nm, and 3.7 mmol/L phosphate buffer (pH 4) at a flow rate of 1 mL/min was used as mobile phase. Authentic standards of ascorbic acid (0.5 - 5 µg/ml) were used for optimizing the HPLC conditions [20].

*2.8. Total soluble solid* (*TSS*)

Its contents in the fruits of each population were expressed by the Brix of fresh juice. The measurement was taken by placing a drop of filtered juice on the prism of a digital refractometer (KRUSS Co. Germany, HR Series).



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*2.9. Fruits and leaves sugar (according to glucose)*

Total sugar determination was done with phenol-sulphuric method [21]. The total sugar concentration is determined by spectrophotometry at 490 nm wavelength. The sensitivity of this method range from 10 to 100 µg of total sugar and the quantification is made from a calibration curve using glucose as standard and calculation are performed by the equation of the linear regression obtained from the calibration curve.

*2.10. Seed oil extraction*

10 grams of the dried seeds were milled and placed in an extraction thimble and extracted with organic solvent, n-hexane, using a 250 ml capacity soxhlet apparatus for 8 h (60 °C) in 3 replications [22]. The solvent was then separated by rotary-evaporated under reduced pressure at 35 °C.

*2.11. Data analysis*

Analysis of variance was performed for all traits by SPSS statistics (ver. 22) software. ANOVA analysis and mean comparison of the morpho-physiological and phytochemical traits were done by using Duncan multiple range tests at $P \leq 0.05$ significant level. In order to determine the most variable characters among the populations, factor analysis based on the principal component analysis (PCA) was performed. Hierarchical cluster analysis of studied populations was based on the Euclidean distances of traits using wards method.

## 3. Results

According to obtained results, some of the studied traits such as fruit length, peduncle length, leaf length and width, petiole length, fruit moisture, leaf and fruit phenol and tannin, fruit glucose, carotenoid, lycopene, *β*-carotene, flavonoid, TSS and vitamin C, and seed oil changed significantly ($P \leq 0.01$ or $P \leq 0.05$) in the experimental years (Table 2). Year of the experiment had no significant effects on other existing traits. Also, variance analyses showed that the various populations had significant differences in respect of the more studied morpho-physiological and phytochemical traits ($P \leq 0.01$ or $P \leq 0.05$) in two studied years and mean of them (Table 2). Of course, some traits (plant height, fruit width and petiole length in 2014, 2015, and the mean of these years, seed width in 2014 and 2015, and the fruit peduncle length and leaf length in 2015) were not significantly affected by population treatment. The wide spectrums of variations were observed for different studied characters. The samples were phenotypically diverse, which trunk diameter, petiole length, fruit tannin, leaf carotenoid, leaf *β*-carotene, and fruit vitamin C were characteristics with the highest variation.

*3.1. Morpho-physiological traits*

In this study, the average height of trees was 393 and 409 cm in 2014 and 2015, respectively. The minimum and maximum canopy width in mean results of two years were observed in Bozaj (221 cm) and Jostan (447 cm) populations, respectively. The trunk diameter varied between 6.64 cm (Khodkavand) and 15.27 cm (Gelyard) in the mean of two studied years. The highest and lowest amounts of fruit length were in Fashandak in 2015 (4.97 mm) and Gooran in 2014 (8.47 mm) respectively. Also, the average fruit width among populations in the mean of two years was 4.45 mm. The seed length varied between 4.15 mm (Nesa) and 6.22 mm (Gooran) in the mean result of two years. Also, the average seed width was 3.21 mm in the mean of two years. Fruit peduncle length had a higher average in the 2015 year (4.43 mm) in comparing with the 2014 year (3.93 mm). Mean comparison results showed, the average leaf length in the second year (65.8 mm)



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was higher than the first year (61.8 mm). Maximum leaf width was observed in Gelyard in 2015 (8.8 mm) and the minimum of this trait was in Dehdar in 2014 (4.4 mm). Leaf petiole length had a higher average in the 2015 year (5.53 mm) in comparing with the 2014 year (4.51 mm). The average of 1000 seeds weight was 16.09 and 15.20 g in 2014 and 2015 year, respectively. Also, the average weight of 100 fresh fruits in first and second years was 14.34 and 15.40 g, respectively. The highest and lowest amount of fruit moisture was observed in Fashandak in 2015 (4.97 mm) and Gooran in 2014 (8.47 mm) respectively.

**Table 2.** Results of means comparisons for fruit traits among studied *Hippophae rhamnoides* populations during 2014 and 2015 harvesting season.

| | | Fruit traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Populations** | **Year** | **Plant height (cm)** | **Canopy width (cm)** | **Trunk diameter (cm)** | **Fruit length (mm)** | **Fruit width (mm)** | **Seed length (mm)** | **Seed width (mm)** | **Fruit peduncle length (mm)** | **Leaf length (mm)** | **Leaf width (mm)** |
| **Bozaj** | **2014** | 322 | 208$^{e}$ | 8.39$^{bc}$ | 6.57$^{b}$ | 4.83 | 4.53$^{b}$ | 3.44 | 4.93$^{a}$ | 58.9$^{bc}$ | 6.2$^{a}$ |
| | **2015** | 333 | 234$^{e}$ | 8.40$^{bc}$ | 5.95$^{b}$ | 4.46 | 4.51$^{bc}$ | 3.52$^{ab}$ | 5.58 | 67.0 | 7.3$^{abc}$ |
| | **Mean** | 328$^{b}$ | 221$^{e}$ | 8.40$^{cd}$ | 6.26$^{b}$ | 4.65 | 4.52$^{bc}$ | 3.48$^{ab}$ | 5.26$^{a}$ | 63$^{abc}$ | 6.7$^{abc}$ |
| **Parachan** | **2014** | 394 | 343$^{abc}$ | 11.64$^{ab}$ | 5.40$^{b}$ | 4.05 | 4.46$^{b}$ | 3.32 | 3.79$^{abc}$ | 64.5$^{ab}$ | 6.0$^{a}$ |
| | **2015** | 410 | 365$^{abc}$ | 11.67$^{ab}$ | 5.74$^{b}$ | 3.70 | 4.63$^{bc}$ | 3.01$^{ab}$ | 4.12 | 60.4 | 7.9$^{ab}$ |
| | **Mean** | 402$^{ab}$ | 354$^{bc}$ | 11.65$^{b}$ | 5.57$^{c}$ | 3.88 | 4.55$^{bc}$ | 3.17$^{abc}$ | 3.96$^{bcd}$ | 62.5$^{ab}$ | 7.0$^{abc}$ |
| **Jostan** | **2014** | 446 | 440$^{a}$ | 10.39$^{bc}$ | 5.58$^{b}$ | 4.49 | 4.88$^{b}$ | 3.33 | 4.21$^{abc}$ | 62.3$^{ab}$ | 5.6$^{a}$ |
| | **2015** | 457 | 454$^{a}$ | 10.42$^{bc}$ | 6.24$^{b}$ | 4.51 | 5.17$^{b}$ | 3.58$^{a}$ | 4.60 | 66.8 | 6.6$^{abc}$ |
| | **Mean** | 452$^{a}$ | 447$^{a}$ | 10.40$^{bc}$ | 5.91$^{bc}$ | 4.50 | 5.02$^{b}$ | 3.46$^{ab}$ | 4.40$^{abc}$ | 64.5$^{ab}$ | 6.1$^{bc}$ |
| **Khodkavand** | **2014** | 382 | 280$^{cde}$ | 6.62$^{c}$ | 5.81$^{b}$ | 4.45 | 4.29$^{b}$ | 2.90 | 4.43$^{ab}$ | 60.3$^{abc}$ | 5.9$^{a}$ |
| | **2015** | 400 | 307$^{cde}$ | 6.66$^{c}$ | 5.47$^{b}$ | 4.51 | 4.43$^{bc}$ | 3.02$^{ab}$ | 4.71 | 66.2 | 6.9$^{abc}$ |
| | **Mean** | 391$^{ab}$ | 294$^{cd}$ | 6.64$^{d}$ | 5.64$^{bc}$ | 4.48 | 4.36$^{bc}$ | 2.96$^{abc}$ | 4.57$^{ab}$ | 63.2$^{abc}$ | 6.4$^{bc}$ |
| **Dehdar** | **2014** | 422 | 394$^{ab}$ | 7.68$^{bc}$ | 6.16$^{b}$ | 4.44 | 4.26$^{b}$ | 2.90 | 3.40$^{bc}$ | 54.8$^{c}$ | 4.4$^{b}$ |
| | **2015** | 430 | 414$^{ab}$ | 7.71$^{bc}$ | 5.60$^{b}$ | 4.16 | 4.10$^{bc}$ | 2.96$^{b}$ | 3.86 | 60.5 | 5.1$^{c}$ |
| | **Mean** | 426$^{ab}$ | 404$^{ab}$ | 7.70$^{cd}$ | 5.88$^{bc}$ | 4.30 | 4.18$^{c}$ | 2.93$^{bc}$ | 3.63$^{bcd}$ | 57.6$^{c}$ | 4.8$^{d}$ |
| **Shahrak** | **2014** | 455 | 295$^{cde}$ | 11.39$^{ab}$ | 6.15$^{b}$ | 4.70 | 4.30$^{b}$ | 3.22 | 3.11$^{c}$ | 63.8$^{ab}$ | 6.6$^{a}$ |
| | **2015** | 479 | 311$^{cde}$ | 11.41$^{ab}$ | 6.03$^{b}$ | 4.54 | 4.49$^{bc}$ | 3.52$^{ab}$ | 3.74 | 69.0 | 7.6$^{ab}$ |
| | **Mean** | 467$^{a}$ | 303$^{cd}$ | 11.40$^{b}$ | 6.09$^{bc}$ | 4.62 | 4.39$^{bc}$ | 3.37$^{abc}$ | 3.42$^{d}$ | 66.4$^{ab}$ | 7.1$^{ab}$ |
| **Fashandak** | **2014** | 336 | 233$^{de}$ | 10.17$^{bc}$ | 5.90$^{b}$ | 4.45 | 4.23$^{b}$ | 3.16 | 3.30$^{bc}$ | 60.2$^{abc}$ | 5.7$^{a}$ |
| | **2015** | 349 | 246$^{de}$ | 10.19$^{bc}$ | 4.97$^{b}$ | 3.92 | 4.11$^{bc}$ | 2.96$^{ab}$ | 3.67 | 58.5 | 5.2$^{c}$ |
| | **Mean** | 343$^{b}$ | 240$^{de}$ | 10.18$^{bc}$ | 5.43$^{bc}$ | 4.18 | 4.17$^{c}$ | 3.06$^{abc}$ | 3.49$^{cd}$ | 59.4$^{bc}$ | 5.4$^{cd}$ |
| **Gelyard** | **2014** | 387 | 329$^{bcd}$ | 15.25$^{a}$ | 6.40$^{b}$ | 4.58 | 4.51$^{b}$ | 3.08 | 4.06$^{abc}$ | 64.7$^{ab}$ | 6.9$^{a}$ |
| | **2015** | 398 | 343$^{bcd}$ | 15.28$^{a}$ | 6.12$^{b}$ | 4.94 | 4.63$^{bc}$ | 3.56$^{a}$ | 4.83 | 73.3 | 8.8$^{a}$ |
| | **Mean** | 392$^{ab}$ | 336$^{c}$ | 15.27$^{a}$ | 6.26$^{b}$ | 4.76 | 4.57$^{bc}$ | 3.32$^{abc}$ | 4.44$^{abc}$ | 69$^{a}$ | 7.9$^{a}$ |
| **Gooran** | **2014** | 343 | 263$^{cde}$ | 7.79$^{bc}$ | 8.47$^{a}$ | 4.99 | 6.01$^{a}$ | 2.57 | 3.78$^{abc}$ | 66.9$^{a}$ | 5.8$^{a}$ |
| | **2015** | 361 | 278$^{cde}$ | 7.83$^{bc}$ | 8.06$^{a}$ | 4.87 | 6.42$^{a}$ | 3.06$^{ab}$ | 4.24 | 70.7 | 5.2$^{c}$ |
| | **Mean** | 352$^{b}$ | 271$^{de}$ | 7.81$^{cd}$ | 8.27$^{a}$ | 4.93 | 6.22$^{a}$ | 2.81$^{c}$ | 4.01$^{bcd}$ | 68.8$^{a}$ | 5.5$^{cd}$ |
| **Nesa** | **2014** | 444 | 401$^{ab}$ | 10.55$^{bc}$ | 5.79$^{b}$ | 4.41 | 4.31$^{b}$ | 3.57 | 4.24$^{abc}$ | 62.0$^{ab}$ | 6.5$^{a}$ |
| | **2015** | 468 | 416$^{ab}$ | 10.67$^{bc}$ | 5.11$^{b}$ | 4.02 | 3.99$^{c}$ | 3.47$^{ab}$ | 4.90 | 65.6 | 8.2$^{a}$ |
| | **Mean** | 456$^{a}$ | 409$^{ab}$ | 10.59$^{bc}$ | 5.45$^{bc}$ | 4.22 | 4.15$^{c}$ | 3.52$^{a}$ | 4.57$^{ab}$ | 63.8$^{abc}$ | 7.4$^{ab}$ |
| **Mean** | **2014** | 393 | 319 | 9.99 | 6.22$^{a}$ | 4.54 | 4.58 | 3.15 | 3.93$^{b}$ | 61.8$^{b}$ | 6.0$^{b}$ |
| | **2015** | 409 | 337 | 10.02 | 5.93$^{b}$ | 4.36 | 4.65 | 3.27 | 4.43$^{a}$ | 65.8$^{a}$ | 6.9$^{a}$ |
| | **Mean** | 401 | 328 | 10.01 | 6.08 | 4.45 | 4.61 | 3.21 | 4.18 | 63.8 | 6.4 |



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**Table 2.** Results of means comparisons for fruit traits among studied *Hippophae rhamnoides* populations during 2014 and 2015 harvesting season (Continued).

| Populations | Year | Fruit traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Petiole length (mm) | 1000 seed wt.(g) | 100 fruit fresh weight (g) | Fruit moisture (%) | Leaf total phenol (mg/g) | Fruit total phenol (mg/g) | Leaf tannin (mg/g) | Fruit tannin (mg/g) | Leaf glucose (mg/g) | Fruit glucose (mg/g) |
| **Bozaj** | **2014** | 4.92 | 18.30$^{a}$ | 13.73$^{cde}$ | 70.81$^{bcd}$ | 43.27$^{efg}$ | 48.55$^{a}$ | 23.22$^{e}$ | 6.52$^{a}$ | 43.07$^{f}$ | 64.93$^{b}$ |
| | **2015** | 6.15 | 15.89$^{a}$ | 15.45$^{bc}$ | 66.61$^{b}$ | 23.10$^{f}$ | 25.09$^{cd}$ | 11.46$^{d}$ | 4.13$^{bcde}$ | 53.20$^{cde}$ | 100.27$^{b}$ |
| | **Mean** | 5.54 | 17.10$^{a}$ | 14.59$^{c}$ | 68.71$^{c}$ | 33.18$^{f}$ | 36.82$^{a}$ | 17.34$^{fg}$ | 5.32$^{a}$ | 48.13$^{f}$ | 82.60$^{c}$ |
| **Parachan** | **2014** | 4.66 | 13.75$^{e}$ | 15.54$^{bc}$ | 65.47$^{efg}$ | 41.86$^{fg}$ | 20.50$^{cde}$ | 23.44$^{e}$ | 2.03$^{c}$ | 59.96$^{d}$ | 30.78$^{d}$ |
| | **2015** | 4.76 | 13.26$^{c}$ | 14.73$^{c}$ | 60.48$^{c}$ | 32.07$^{de}$ | 24.26$^{cd}$ | 10.13$^{d}$ | 6.85$^{a}$ | 51.48$^{de}$ | 57.07$^{d}$ |
| | **Mean** | 4.71 | 13.51$^{f}$ | 15.13$^{c}$ | 62.98$^{d}$ | 36.96$^{ef}$ | 22.38$^{d}$ | 16.78$^{fg}$ | 4.44$^{ab}$ | 55.72$^{d}$ | 43.92$^{f}$ |
| **Jostan** | **2014** | 4.03 | 16.81$^{abcd}$ | 12.25$^{e}$ | 64.64$^{fg}$ | 57.63$^{bc}$ | 18.45$^{e}$ | 30.03$^{cd}$ | 1.40$^{c}$ | 51.56$^{e}$ | 48.22$^{c}$ |
| | **2015** | 6.30 | 16.12$^{ab}$ | 13.14$^{de}$ | 60.22$^{c}$ | 61.36$^{b}$ | 16.02$^{e}$ | 36.33$^{a}$ | 2.71$^{efg}$ | 50.86$^{de}$ | 43.43$^{ef}$ |
| | **Mean** | 5.17 | 16.47$^{abc}$ | 12.70$^{d}$ | 62.43$^{d}$ | 59.50$^{b}$ | 17.23$^{e}$ | 33.18$^{b}$ | 2.05$^{cd}$ | 51.21$^{ef}$ | 45.82$^{f}$ |
| **Khodkavand** | **2014** | 4.84 | 15.83$^{bcde}$ | 14.68$^{bcd}$ | 68.38$^{def}$ | 73.43$^{a}$ | 22.75$^{de}$ | 40.56$^{a}$ | 3.53$^{b}$ | 79.66$^{b}$ | 51.47$^{c}$ |
| | **2015** | 5.09 | 16.21$^{a}$ | 16.44$^{bc}$ | 68.71$^{b}$ | 83.77$^{a}$ | 36.22$^{c}$ | 39.89$^{a}$ | 4.96$^{bc}$ | 99.01$^{a}$ | 80.39$^{c}$ |
| | **Mean** | 4.97 | 16.02$^{cd}$ | 15.56$^{bc}$ | 68.55$^{c}$ | 78.60$^{a}$ | 29.48$^{c}$ | 40.22$^{a}$ | 4.25$^{b}$ | 89.34$^{a}$ | 65.93$^{d}$ |
| **Dehdar** | **2014** | 4.34 | 15.06$^{de}$ | 14.49$^{bcde}$ | 74.32$^{ab}$ | 53.67$^{cd}$ | 20.40$^{cde}$ | 30.77$^{c}$ | 2.03$^{c}$ | 60.51$^{d}$ | 62.22$^{b}$ |
| | **2015** | 4.27 | 13.98$^{bc}$ | 15.34$^{bc}$ | 69.30$^{ab}$ | 54.78$^{bc}$ | 36.02$^{c}$ | 27.49$^{b}$ | 3.62$^{cde}$ | 49.58$^{e}$ | 53.10$^{de}$ |
| | **Mean** | 4.31 | 14.52$^{ef}$ | 14.92$^{c}$ | 71.81$^{b}$ | 54.23$^{c}$ | 28.21$^{bc}$ | 29.13$^{c}$ | 2.82$^{c}$ | 55.05$^{de}$ | 57.66$^{e}$ |
| **Shahrak** | **2014** | 4.43 | 13.92$^{e}$ | 13.32$^{cde}$ | 71.87$^{bcd}$ | 49.24$^{de}$ | 22.91$^{bc}$ | 28.04$^{cd}$ | 2.26$^{c}$ | 89.87$^{a}$ | 95.84$^{a}$ |
| | **2015** | 5.09 | 15.43$^{a}$ | 16.72$^{bc}$ | 66.60$^{b}$ | 27.72$^{ef}$ | 28.18$^{c}$ | 14.25$^{d}$ | 1.71$^{fg}$ | 74.64$^{b}$ | 125.57$^{a}$ |
| | **Mean** | 4.76 | 14.67$^{de}$ | 15.02$^{c}$ | 69.24$^{bc}$ | 38.48$^{e}$ | 25.54$^{c}$ | 21.14$^{e}$ | 1.99$^{cd}$ | 82.26$^{a}$ | 110.70$^{a}$ |
| **Fashandak** | **2014** | 4.57 | 17.54$^{abc}$ | 12.69$^{de}$ | 73.44$^{abc}$ | 47.96$^{def}$ | 25.83$^{b}$ | 27.09$^{d}$ | 4.53$^{b}$ | 36.92$^{g}$ | 51.20$^{c}$ |
| | **2015** | 5.22 | 16.18$^{a}$ | 13.25$^{cd}$ | 70.45$^{ab}$ | 49.85$^{c}$ | 47.64$^{a}$ | 22.23$^{c}$ | 5.32$^{b}$ | 42.90$^{f}$ | 58.61$^{d}$ |
| | **Mean** | 4.90 | 16.56$^{ab}$ | 12.97$^{d}$ | 71.95$^{b}$ | 48.91$^{d}$ | 36.73$^{a}$ | 24.66$^{d}$ | 4.93$^{ab}$ | 40.91$^{g}$ | 54.90$^{e}$ |
| **Gelyard** | **2014** | 4.19 | 17.92$^{ab}$ | 16.06$^{b}$ | 69.46$^{cde}$ | 64.36$^{b}$ | 19.93$^{cde}$ | 34.79$^{b}$ | 1.47$^{c}$ | 71.35$^{c}$ | 24.81$^{d}$ |
| | **2015** | 5.57 | 15.56$^{a}$ | 17.34$^{b}$ | 66.17$^{b}$ | 61.65$^{b}$ | 24.49$^{cd}$ | 36.45$^{a}$ | 1.22$^{g}$ | 95.22$^{a}$ | 46.38$^{ef}$ |
| | **Mean** | 4.88 | 16.74$^{ab}$ | 16.70$^{b}$ | 67.82$^{c}$ | 63.01$^{b}$ | 22.21$^{d}$ | 35.62$^{b}$ | 1.35$^{d}$ | 83.28$^{b}$ | 35.60$^{g}$ |
| **Gooran** | **2014** | 4.16 | 16.14$^{bcd}$ | 18.19$^{a}$ | 77.20$^{a}$ | 38.78$^{g}$ | 22.16$^{cd}$ | 16.26$^{f}$ | 2.03$^{c}$ | 71.44$^{c}$ | 90.23$^{a}$ |
| | **2015** | 4.77 | 15.37$^{a}$ | 19.60$^{a}$ | 73.24$^{a}$ | 29.82$^{ef}$ | 40.03$^{c}$ | 14.49$^{d}$ | 3.11$^{def}$ | 57.80$^{c}$ | 93.67$^{b}$ |
| | **Mean** | 4.47 | 15.76$^{bcd}$ | 18.90$^{a}$ | 75.22$^{a}$ | 34.30$^{ef}$ | 31.09$^{b}$ | 15.37$^{g}$ | 2.55$^{c}$ | 65.12$^{c}$ | 91.95$^{b}$ |
| **Nesa** | **2014** | 4.92 | 15.64$^{cde}$ | 12.49$^{de}$ | 62.56$^{g}$ | 39.09$^{g}$ | 28.16$^{cd}$ | 16.44$^{f}$ | 3.67$^{b}$ | 46.32$^{f}$ | 46.32$^{c}$ |
| | **2015** | 8.03 | 13.99$^{bc}$ | 11.94$^{e}$ | 66.84$^{b}$ | 38.41$^{d}$ | 21.41$^{de}$ | 21.74$^{c}$ | 4.51$^{bcd}$ | 57.90$^{cd}$ | 42.34$^{f}$ |
| | **Mean** | 6.47 | 14.81$^{de}$ | 12.22$^{d}$ | 64.70$^{cd}$ | 38.75$^{e}$ | 24.78$^{d}$ | 19.09$^{ef}$ | 4.09$^{b}$ | 52.11$^{de}$ | 44.33$^{f}$ |
| **Mean** | **2014** | 4.51$^{b}$ | 16.09 | 14.34 | 69.82$^{b}$ | 50.93$^{a}$ | 24.96$^{b}$ | 27.06$^{a}$ | 2.95$^{b}$ | 61.07 | 56.60$^{b}$ |
| | **2015** | 5.53$^{a}$ | 15.20 | 15.40 | 66.86$^{a}$ | 46.25$^{b}$ | 29.94$^{a}$ | 23.45$^{b}$ | 3.81$^{a}$ | 63.26 | 70.08$^{a}$ |
| | **Mean** | 5.02 | 15.65 | 14.87 | 68.34 | 48.59 | 27.45 | 25.26 | 3.38 | 62.16 | 63.34 |

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**Table 2.** Results of means comparisons for fruit traits among studied *Hippophae rhamnoides* populations during 2014 and 2015 harvesting season (Continued).

| Populations | Year | Fruit traits | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Leaf carotenoid (mg/g) | Fruit carotenoid (mg/g) | Leaf lycopene (mg/g) | Fruit lycopene (mg/g) | Leaf *β*-carotene (mg/g) | Fruit *β*-carotene (mg/g) | Leaf flavonoid (mg/g) | Fruit flavonoid (mg/g) | Fruit TSS brix (%) | Fruit vitamin C (mg/g) | Seed oil (%) |
| **Bozaj** | **2014** | 2.24$^{a}$ | 1.49$^{a}$ | 0.53$^{a}$ | 0.36$^{a}$ | 0.99$^{a}$ | 0.43$^{a}$ | 3.14$^{b}$ | 1.45$^{b}$ | 19.3$^{c}$ | 5.52$^{b}$ | 5.94$^{bc}$ |
| | **2015** | 1.63$^{a}$ | 1.15$^{b}$ | 0.32$^{ab}$ | 0.19$^{a}$ | 0.71$^{a}$ | 0.27$^{bc}$ | 2.27$^{bc}$ | 1.82$^{bc}$ | 27.3$^{b}$ | 4.01$^{bc}$ | 6.59$^{bc}$ |
| | **Mean** | 1.94$^{a}$ | 1.22$^{cd}$ | 0.43$^{a}$ | 0.28$^{a}$ | 0.85$^{a}$ | 0.35$^{ab}$ | 2.70$^{cd}$ | 1.64$^{cd}$ | 23.3$^{b}$ | 4.77$^{c}$ | 6.26$^{bc}$ |
| **Parachan** | **2014** | 0.66$^{d}$ | 1.55$^{a}$ | 0.27$^{d}$ | 0.29$^{a}$ | 0.35$^{b}$ | 0.38$^{ab}$ | 4.45$^{a}$ | 1.24$^{bc}$ | 8.6$^{f}$ | 1.39$^{e}$ | 5.28$^{bc}$ |
| | **2015** | 0.70$^{bc}$ | 1.79$^{a}$ | 0.33$^{ab}$ | 0.20$^{a}$ | 0.26$^{b}$ | 0.46$^{a}$ | 3.81$^{a}$ | 1.39$^{de}$ | 15.1$^{de}$ | 1.55$^{d}$ | 7.38$^{ab}$ |
| | **Mean** | 0.68$^{cd}$ | 1.67$^{a}$ | 0.31$^{bc}$ | 0.25$^{ab}$ | 0.31$^{c}$ | 0.43$^{a}$ | 4.13$^{a}$ | 1.31$^{ef}$ | 11.9$^{ef}$ | 1.47$^{f}$ | 6.33$^{bc}$ |
| **Jostan** | **2014** | 0.95$^{cd}$ | 1.61$^{a}$ | 0.33$^{cd}$ | 0.34$^{a}$ | 0.47$^{b}$ | 0.36$^{ab}$ | 3.33$^{b}$ | 1.84$^{a}$ | 14.2$^{de}$ | 2.90$^{cde}$ | 8.98$^{a}$ |
| | **2015** | 0.84$^{bc}$ | 1.07$^{bc}$ | 0.18$^{cde}$ | 0.18$^{ab}$ | 0.33$^{b}$ | 0.23$^{bcd}$ | 3.70$^{a}$ | 1.78$^{bcd}$ | 16.5$^{de}$ | 3.78$^{bc}$ | 8.47$^{a}$ |
| | **Mean** | 0.90$^{bc}$ | 1.34$^{ab}$ | 0.26$^{cd}$ | 0.26$^{ab}$ | 0.40$^{bc}$ | 0.30$^{bc}$ | 3.51$^{b}$ | 1.81$^{bc}$ | 15.4$^{cd}$ | 3.34$^{de}$ | 8.72$^{a}$ |
| **Khodkavand** | **2014** | 1.83$^{a}$ | 1.32$^{b}$ | 0.50$^{ab}$ | 0.25$^{b}$ | 0.78$^{a}$ | 0.34$^{bcd}$ | 2.70$^{bc}$ | 1.10$^{c}$ | 13.7$^{de}$ | 4.01$^{bc}$ | 5.27$^{bc}$ |
| | **2015** | 1.96$^{a}$ | 0.92$^{cd}$ | 0.38$^{a}$ | 0.15$^{bc}$ | 0.89$^{a}$ | 0.24$^{bc}$ | 2.87$^{b}$ | 1.96$^{bc}$ | 20.6$^{c}$ | 2.62$^{cd}$ | 5.78$^{c}$ |
| | **Mean** | 1.90$^{a}$ | 1.12$^{d}$ | 0.44$^{a}$ | 0.20$^{cd}$ | 0.83$^{a}$ | 0.29$^{bc}$ | 2.79$^{c}$ | 1.53$^{de}$ | 17.2$^{c}$ | 3.32$^{de}$ | 5.52$^{cd}$ |
| **Dehdar** | **2014** | 0.49$^{d}$ | 1.24$^{b}$ | 0.23$^{d}$ | 0.22$^{b}$ | 0.35$^{b}$ | 0.21$^{e}$ | 2.80$^{bc}$ | 1.04$^{c}$ | 16.6$^{cd}$ | 4.52$^{bc}$ | 4.69$^{c}$ |
| | **2015** | 0.37$^{c}$ | 0.77$^{de}$ | 0.11$^{e}$ | 0.13$^{cd}$ | 0.24$^{b}$ | 0.17$^{cd}$ | 2.07$^{c}$ | 0.92$^{f}$ | 15.7$^{de}$ | 4.43$^{b}$ | 5.52$^{c}$ |
| | **Mean** | 0.43$^{d}$ | 1.01$^{e}$ | 0.17$^{e}$ | 0.17$^{e}$ | 0.29$^{c}$ | 0.19$^{d}$ | 2.43$^{cd}$ | 0.98$^{g}$ | 16.2$^{c}$ | 4.47$^{cd}$ | 5.11$^{d}$ |
| **Shahrak** | **2014** | 1.21$^{c}$ | 1.57$^{a}$ | 0.37$^{c}$ | 0.29$^{a}$ | 0.27$^{b}$ | 0.28$^{de}$ | 2.34$^{c}$ | 1.82$^{a}$ | 27.8$^{a}$ | 10.00$^{a}$ | 6.75$^{ab}$ |
| | **2015** | 1.16$^{b}$ | 1.75$^{a}$ | 0.44$^{a}$ | 0.16$^{bc}$ | 0.35$^{b}$ | 0.14$^{d}$ | 2.31$^{bc}$ | 2.46$^{a}$ | 35.2$^{a}$ | 7.92$^{a}$ | 7.05$^{b}$ |
| | **Mean** | 1.19$^{b}$ | 1.66$^{a}$ | 0.30$^{bc}$ | 0.22$^{d}$ | 0.31$^{bc}$ | 0.26$^{c}$ | 2.32$^{d}$ | 2.14$^{a}$ | 31.5$^{a}$ | 8.96$^{a}$ | 6.89$^{b}$ |
| **Fashandak** | **2014** | 0.89$^{cd}$ | 1.65$^{a}$ | 0.31$^{cd}$ | 0.30$^{a}$ | 0.43$^{b}$ | 0.45$^{a}$ | 3.11$^{b}$ | 1.51$^{b}$ | 16.7$^{cd}$ | 3.78$^{bcd}$ | 5.91$^{bc}$ |
| | **2015** | 0.78$^{bc}$ | 0.95$^{bcd}$ | 0.16$^{cde}$ | 0.16$^{bc}$ | 0.29$^{b}$ | 0.30$^{b}$ | 3.48$^{a}$ | 2.02$^{bc}$ | 28.8$^{b}$ | 2.74$^{cd}$ | 6.51$^{bc}$ |
| | **Mean** | 0.83$^{c}$ | 1.30$^{abc}$ | 0.23$^{cde}$ | 0.23$^{bc}$ | 0.36$^{bc}$ | 0.38$^{ab}$ | 3.29$^{b}$ | 1.77$^{bcd}$ | 22.8$^{bc}$ | 3.26$^{de}$ | 6.21$^{bc}$ |
| **Gelyard** | **2014** | 1.28$^{bc}$ | 1.23$^{b}$ | 0.41$^{bc}$ | 0.24$^{b}$ | 0.60$^{b}$ | 0.33$^{cd}$ | 2.83$^{bc}$ | 1.95$^{a}$ | 7.8$^{f}$ | 1.37$^{e}$ | 5.09$^{c}$ |
| | **2015** | 1.13$^{b}$ | 0.71$^{e}$ | 0.25$^{bc}$ | 0.12$^{d}$ | 0.45$^{b}$ | 0.23$^{bcd}$ | 2.36$^{bc}$ | 1.64$^{cde}$ | 12.8$^{e}$ | 2.03$^{d}$ | 7.70$^{ab}$ |
| | **Mean** | 1.20$^{b}$ | 0.97$^{ef}$ | 0.33$^{b}$ | 0.18$^{de}$ | 0.53$^{b}$ | 0.28$^{bc}$ | 2.60$^{cd}$ | 1.80$^{bc}$ | 10.3$^{f}$ | 1.70$^{f}$ | 6.39$^{bc}$ |
| **Gooran** | **2014** | 1.74$^{ab}$ | 1.53$^{a}$ | 0.49$^{ab}$ | 0.31$^{a}$ | 0.96$^{a}$ | 0.36$^{ab}$ | 4.32$^{a}$ | 1.87$^{a}$ | 23.7$^{b}$ | 8.55$^{a}$ | 5.40$^{bc}$ |
| | **2015** | 1.97$^{a}$ | 0.90$^{cd}$ | 0.41$^{a}$ | 0.15$^{bc}$ | 0.88$^{a}$ | 0.17$^{cd}$ | 3.56$^{a}$ | 2.13$^{ab}$ | 14.3$^{e}$ | 6.78$^{a}$ | 6.61$^{bc}$ |
| | **Mean** | 1.86$^{a}$ | 1.21$^{bcd}$ | 0.45$^{a}$ | 0.24$^{abc}$ | 0.92$^{a}$ | 0.26$^{c}$ | 3.94$^{a}$ | 2.00$^{ab}$ | 24.0$^{b}$ | 7.67$^{b}$ | 6.00$^{bc}$ |
| **Nesa** | **2014** | 0.67$^{d}$ | 1.20$^{b}$ | 0.26$^{d}$ | 0.25$^{b}$ | 0.40$^{b}$ | 0.31$^{d}$ | 2.97$^{bc}$ | 1.24$^{bc}$ | 12.4$^{e}$ | 1.75$^{de}$ | 7.52$^{ab}$ |
| | **2015** | 0.58$^{c}$ | 0.81$^{cde}$ | 0.12$^{e}$ | 0.14$^{cd}$ | 0.23$^{b}$ | 0.13$^{d}$ | 2.20$^{bc}$ | 1.27$^{ef}$ | 14.5$^{e}$ | 2.95$^{cd}$ | 8.28$^{a}$ |
| | **Mean** | 0.63$^{cd}$ | 0.90$^{f}$ | 0.19$^{de}$ | 0.19$^{ef}$ | 0.32$^{bc}$ | 0.22$^{cd}$ | 2.58$^{cd}$ | 1.25$^{f}$ | 13.5$^{de}$ | 2.35$^{ef}$ | 7.90$^{a}$ |
| **Mean** | **2014** | 1.20 | 1.46$^{a}$ | 0.37 | 0.28$^{a}$ | 0.56 | 0.35$^{a}$ | 3.20 | 1.51$^{b}$ | 16.1 | 4.38$^{a}$ | 6.08$^{b}$ |
| | **2015** | 1.11 | 1.09$^{b}$ | 0.27 | 0.16$^{b}$ | 0.46 | 0.23$^{b}$ | 2.86 | 1.74$^{a}$ | 20.1 | 3.88$^{b}$ | 7.01$^{a}$ |
| | **Mean** | 1.15 | 1.26 | 0.32 | 0.22 | 0.51 | 0.29 | 3.03 | 1.62 | 18.1 | 4.13 | 6.54 |



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PCA indicated four components with explaining 84.8% of the total variance. The first three components (PC1-PC3) explained 76.18% of the total variation (Table 3). The remaining component (PC4) explained less variability (8.63% of total variance) and included other variables. In the first component (PC1) morpho-physiological traits such as fruit length, 100 fresh fruit weight, fruit moisture, seed length and width, fruit width, petiole length, plant height and canopy width showed the highest variance, respectively. Also, in PC2, leaf length and width, fruit peduncle length, fruit width and seed width showed the highest variance, respectively. While, in PC3, the highest variance was observed for plant height, 1000 seed weight, canopy width and fruit peduncle length, respectively.

Morpho-physiological cluster analysis based on Wards method at similarity coefficient of 10, divided populations into three main groups with high morpho-physiological diversity (Fig. 1). The first main group was divided into four populations, consisted of populations from Khodkavand, Fashandak, Dehdar and Bozaj with similar characteristics such as the lower amount of trunk diameter. The second group was comprised of Shahrak, Gelyard, Jostan, Nesa and Parachan populations. At last, third group included Gooran population. Some of the prominent characteristics of this population are fruit and seed length and 100 fruit fresh weight which made them distinct from the other populations.

**Table 3.** Eigenvectors of the first three principal component axes from PCA analysis of morpho-physiological variables in studied *H. rhamnoides* populations.

| Character | Component | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Plant height | -0.60** | -0.05 | 0.65** |
| Canopy width | -0.56** | -0.11 | 0.55** |
| Trunk diameter | -0.38 | 0.46 | 0.40 |
| Fruit length | 0.86** | 0.32 | 0.24 |
| Fruit width | 0.66** | 0.60** | 0.03 |
| Seed length | 0.73** | 0.32 | 0.31 |
| Seed width | -0.71** | 0.59** | -0.10 |
| Fruit peduncle length | -0.14 | 0.65** | -0.50** |
| Leaf length | 0.32 | 0.83** | 0.42 |
| Leaf width | -0.47 | 0.74** | 0.14 |
| Petiole length | -0.62** | 0.45 | -0.38 |
| 1000 seed weight | 0.28 | 0.44 | -0.65** |
| 100 fruit fresh weight | 0.83** | 0.19 | 0.33 |
| Fruit moisture | 0.83** | -0.26 | -0.16 |
| Eigen value | 5.20 | 3.28 | 2.18 |
| % of variance | 37.14 | 23.44 | 15.60 |
| Cumulative % | 37.14 | 60.58 | 76.18 |

** Eigenvalues are significant ≥ 0.50

### *3.2. Phytochemical traits*

The maximum amount of leaf total phenol was founded in Khodkavand in 2015 (83.77 mg/g) and minimum content of this trait was in Bozaj (23.10 mg/g) in the same year. However, the highest content of fruit total phenol was measured in Bozaj in 2014 (48.55 mg/g) and the lowest content was in Jostan in 2015 (16.02 mg/g). Mean comparison results showed the average leaf tannin content (27.06 mg/g) in the first year was higher than the second year (23.45 mg/g). The highest and lowest value of fruit tannin content was related to Parachan (6.85 mg/g) and Gelyard (1.22 mg/g) in 2015, respectively.

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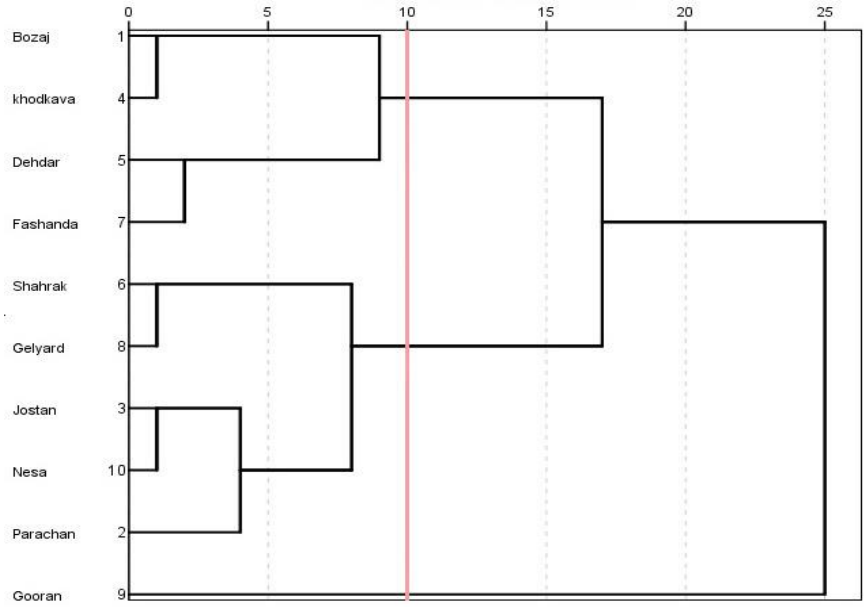



**Fig. 1.** Wards cluster analysis of *H. rhamnoides* populations based on morpho-physiological traits.

The average content of leaf glucose in first and second years was 61.07 and 63.26 mg/g, respectively. However, the maximum content of fruit glucose was reported from Shahrak (125.57 mg/g) and minimum content of this trait was in Gelyard (24.81 mg/g). The leaf carotenoid content varied between 0.43 mg/g (Dehdar) and 1.94 (Bozaj) in the mean of two studied years. The highest content of fruit carotenoid was in Fashandak in 2014 (1.65 mg/g) and lowest content of this trait was in Gelyard in 2015 (0.71 mg/g).

Leaf lycopene content ranged from 0.17 (Dehdar) and 0.45 mg/g (Gooran) in the mean result of two studied years. The maximum and minimum content of fruit lycopene reported from Bozaj in 2014 (0.36 mg/g) and Gelyard in 2015 (0.12 mg/g), respectively. Mean result comparison showed the *β*-carotene content of leaf varied between Dehdar (0.29 mg/g) to Gooran (0.92 mg/g) in the mean of two studied years. The highest and lowest amount of *β*-carotene content in fruit observed from Parachan in 2015 (0.46 mg/g) and Nesa in 2015 (0.13 mg/g). The average content of leaf flavonoid in first and second years was 3.20 and 2.86 mg/g, respectively. The maximum and minimum content of fruit flavonoid were reported from Shahrak (2.46 mg/g) and Dehdar (0.92 mg/g) in 2015 year, respectively. The highest and lowest amount of fruit total soluble solids was observed in Shahrak in 2015 (35.2 %) and Gelyard in 2014 (7.8 %), respectively. The maximum and minimum level of vitamin C were registered from Shahrak in 2014 (10 mg/g) and Gelyard in 2014 (1.37 mg/g). Seed oil content had the highest content in Jostan population in 2014 (8.98 %) and lowest content in Dehdar in 2014 (4.69 %).

To determine the degree of phytochemical variations, a principal component analysis (PCA) was performed using a correlation matrix of all seventeen major phytochemical compounds (Table 4). The first three components of the PCA accounted 69.60% of the total variation. The first component (PC1) contributed to 31.99% of the variance and phytochemical characters included fruit glucose, leaf lycopene, fruit TSS, fruit vitamin C, leaf carotenoid, fruit flavonoid, fruit



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lycopene, fruit carotenoid, leaf tannin, leaf *β*-carotene and fruit phenol showed the highest variance, respectively. While PC2 contributed to 23.20% of total variance and the highest variance were observed for leaf glucose, fruit *β*-carotene, leaf tannin, fruit lycopene, leaf phenol, leaf flavonoid and fruit carotenoid, respectively.

The dendrogram obtained from phytochemical compounds using Wards method revealed high variation between studied samples and grouped them into four main groups (Fig. 2). Group one was divided two populations from Dehdar and Nesa with similar characteristics according to mean comparisons, such as in leaf carotenoid and lycopene and fruit β-carotene and flavonoid. Group two was comprised of Parachan, Fashandak and Jostan populations and had similar characteristics such as in leaf flavonoid. Group three consisted of two populations from Khodkavand and Gelyard with similar characteristics in some traits such as leaf phenol, tannin and glucose. Also, group four had populations from Bozaj, Gooran and Shahrak with similar characteristics such as in fruit glucose, TSS and vitamin C.

**Table 4.** Eigen values and cumulative variance for three major factors obtained from principal component analysis (PCA) based on the main phytochemical compositions for *H. rhamnoides* populations.

| Character | Component | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Leaf phenol | -0.53** | 0.60** | 0.16 |
| Fruit phenol | 0.51** | 0.07 | 0.63** |
| Leaf tannin | -0.54** | 0.62** | 0.04 |
| Fruit tannin | 0.21 | -0.46 | 0.73** |
| Leaf glucose | 0.06 | 0.80** | -0.19 |
| Fruit glucose | 0.86** | 0.29 | -0.22 |
| Leaf carotenoid | 0.68** | 0.49 | 0.37 |
| Fruit carotenoid | 0.61** | -0.50** | -0.34 |
| Leaf lycopene | 0.78** | 0.43 | 0.15 |
| Fruit lycopene | 0.63** | -0.60** | -0.03 |
| Leaf *β*-carotene | 0.52** | 0.44 | 0.55** |
| Fruit *β*-carotene | 0.38 | -0.63** | 0.26 |
| Leaf flavonoid | 0.18 | -0.55** | 0.15 |
| Fruit flavonoid | 0.64** | 0.24 | -0.41 |
| Fruit TSS | 0.76** | 0.12 | -0.28 |
| Fruit vitamin C | 0.73** | 0.32 | -0.42 |
| Seed oil | -0.12 | -0.40 | -0.57** |
| Eigenvalue | 5.44 | 3.95 | 2.45 |
| % of variance | 31.99 | 23.20 | 14.41 |
| Cumulative % | 31.99 | 55.19 | 6bar.60 |

** Eigenvalues are significant ≥ 0.50

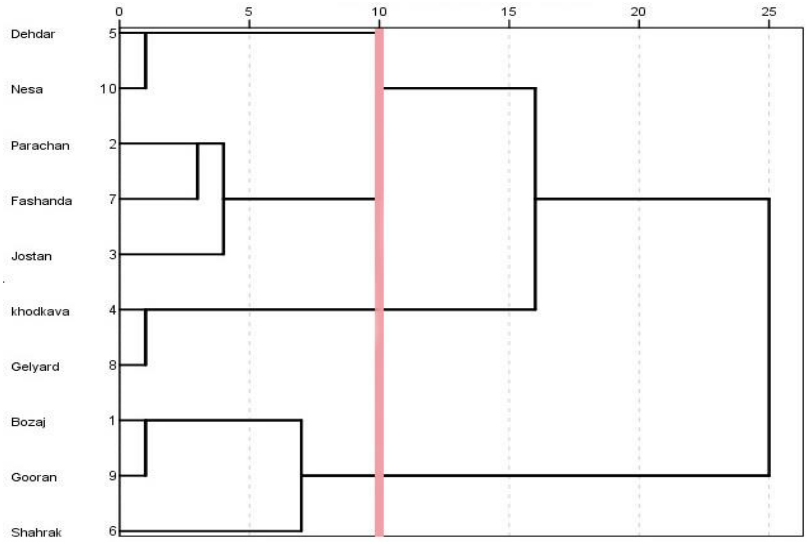



**Fig. 2**. Wards cluster analysis of *H. rhamnoides* populations based on phytochemical traits.



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## 4. Discussion

The present study revealed wide variability in morpho-physiological and phytochemical traits among sea buckthorn populations in Taleghan region. In similar research projects, there are consistent and controversial reports on our studied traits in several regions. For example in a study in Pakistan [23], plant height ranged from 75.6-110 cm among the populations compared, which this lower plant height may be due to the high altitude (over 3000 m ASL) of the northern regions of Pakistan. Near to our results, according to Aras et al results about sea buckthorn collected from different regions of Turkey, fruit size ranges between 6.17 - 9.87 mm in length and 4.16 - 7.86 mm in width and also seed size ranges between 2.86 - 6.77 mm in length and 1.13 - 3.54 mm in width [24]. In another study in different regions of India, the average fruit length ranged from a minimum value of 5.78 mm to a maximum of 7.92 mm and fruit width ranged 5.51 to 7.24 mm in all the locations. In related to weight of 100 fresh fruit, this investigation showed that this trait varied from 11.53 to 18.87 g [11] which is same to our results. In accordance to our work in a study, total phenolic content in dry leaves of sea buckthorn was determined with the Folin-Ciocalteu reagent method and samples had in average 56.28 ± 2.30 mg/g total phenolic contents [25]. In Korekar et al. study [26], in relation to seventeen natural population of sea buckthorn from Trans-Himalaya, the fruits were found to be rich in the total phenolic content ranging from 9.64 to 107.04 mg/g. Total phenolic contents in these studies were more than our study, may be due to their study locations.

In a study in Sweden, fruits of sea buckthorn cultivars comprised from 0.12 to 1.42 mg/g of total carotenoids [27] and In another study between six Romanian sea buckthorn varieties, total carotenoid content varied between 0.53 and 0.97 mg/g dry weight in berries and between 0.035 and 0.042 mg/g DW in leaves [6], this variation may be depending on cultivar, region climate, harvest time, and year. In accordance to our work, previous studies have reported a typical variation of 2 - 1000 mg/100 g of vitamin C content in sea buckthorn berries [22, 28].

In this study same to other similar studies, Factor analysis was used based on principal components to provide a reduced dimension model indicating differences measured among groups. PCA allows to evaluate multicollinear data and to determine the traits most suitable for classification [29]. Same to our results, according to the result of sea buckthorn populations clustering based on morpho-physiological and phytochemical traits in some studies, researchers observed large diversity exists in naturally growing populations [such as 11, 30]. In our work clustering results showed that studied traits couldn't be influenced by altitude, latitude or any definite environmental pattern. The existence of a wide range of variation across the populations can be exploited for selection of suitable genotypes to improvement and commercial exploitation of this plant. However, such variation may be due to population specificity and the adaptation of plants in different environmental or soil conditions.

## 5. Conclusion

There was a wide variability in morpho-physiological and phytochemical traits of different *H. rhamnoides* populations in Taleghan region of Alborz province (Iran). So that, the highest value of some more important traits in fruit were observed in Parachan population for fruit tannin and *β*-carotene, Bozaj population for fruit total phenol and lycopene, Shahrak for fruit glucose, flavonoid, TSS and vitamin C,



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Fashandak for fruit carotenoid and Jostan for seed oil percentage. The first component of factor analysis was contributed by some traits (especially fruit length) with 37.14% of the total variation for morpho-physiological traits, some traits (especially fruit glucose) with 31.99% of the total variation for phytochemical traits. Hierarchical cluster analysis divided populations into 3 and 4 main groups for morpho-physiological and phytochemical traits, respectively. Wide range of variation across the sea buckthorn populations can be exploited for selection of suitable genotypes to improvement and commercial exploitation of this plant.

## Author contributions

The first author carried out the experiment and collected available literature and prepared the first draft of the manuscript with support from the second and third authors. The second author analyzed the statistical data and verified the accuracy of the tests. The third author designed the model and the computational framework and he was also responsible for the correspondence. The fourth author edited the manuscript as a plant science consultant.

## Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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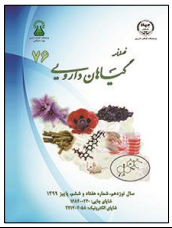
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مقاله تحقیقاتی

# تنوع فیتوشیمیایی و مورفوفیزیولوژیکی جمعیت‌های سنجدتلخ در منطقه طالقان، ایران

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**اطلاعات مقاله**

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**چکیده**

**مقدمه:** سنجدتلخ درختچه‌ای خاردار متعلق به خانواده سنجدیان با توانایی تثبیت نیتروژن در خاک می‌باشد. **هدف:** در این مطالعه، خصوصیات فیتوشیمیایی و مورفوفیزیولوژیکی در جمعیت‌های وحشی سنجدتلخ طی دو سال متوالی در منطقه طالقان در ایران مورد بررسی قرار گرفت. **روش بررسی:** برخی از خصوصیات مورفوفیزیولوژیکی در برخی از اندام‌های مختلف بوته‌ها اندازه‌گیری شد. همچنین، برخی از آنالیزهای فیتوشیمیایی برای پالپ میوه (با استفاده از روش اسپکتروفتومتری) و محتوای روغن دانه انجام شد. **نتایج:** نتایج نشان داد که جمعیت‌های مختلف سنجدتلخ در بسیاری از خصوصیات اصلی مورفوفیزیولوژیکی و تمام خصوصیات فیتوشیمیایی برگ و میوه‌های گیاهان در دو سال مورد مطالعه طی فصول رشد در سطوح آماری یک و پنج درصد تفاوت معنی‌داری داشتند. بیشترین و کمترین مقدار برخی از خصوصیات مهم میوه از ۱۶/۰۲ تا ۴۸/۵۵ میلی‌گرم برگرم فنل کل (جوستان-بزج)، ۰/۷۱ تا ۱/۶۵ میلی‌گرم برگرم کاروتنوئید (گیلیارد-فشاندک)، ۰/۹۲ تا ۲/۴۶ میلی‌گرم برگرم فلاونوئید (دهدار-شهرک) و ۱/۳۷ تا ۱۰ میلی‌گرم برگرم ویتامین ث (گلیارد-شهرک) محاسبه شد. تجزیه و تحلیل عاملی بر اساس تجزیه به مولفه‌های اصلی نشان داد که سه مولفه اول حدود ۷۶ درصد از کل تنوع در صفات فیتوشیمیایی و مورفوفیزیولوژیکی جمعیت‌ها را به خود اختصاص داده است. مؤلفه اول توسط اثربخشی مهمترین صفات شامل گلوکز میوه، کل مواد جامد محلول، ویتامین ث و لیکوپن برگ برای ممیزی تنوع جمعیت‌ها مورد استفاده قرار گرفتند. **نتیجه‌گیری:** طیف گسترده تنوع جمعیت‌های سنجدتلخ در این منطقه را می‌توان برای انتخاب ژنوتیپ‌های مناسب جهت بهبود و بهره‌برداری دارویی از این گیاه درصنایع دارویی ایران استفاده کرد.

*مخففها:* PCA؛ تجزیه به مولفه‌های اصلی، TSS؛ مواد جامد محلول کل

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