Acta Botanica Hungarica 62(3–4), pp. 205–215, 2020
DOI: 10.1556/034.62.2020.3-4.1

CHEMICAL ANALYSIS OF POPULATION VARIABILITY IN *PEGANUM HARMALA* (VAR. *HARMALA*)

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(Received: 14 November 2019; Accepted: 25 February 2020)

Both the primary and secondary metabolisms of higher plants are influenced by environmental conditions. *Peganum harmala* L. synthesise amazing diversity of secondary metabolites, such as alkaloids, volatile oils, triterpenes or sterols. We have no detailed information on genetic chemical contents and particularly the fatty acid contents of this plant species in Iran. Therefore, population-based fatty acid and alkaloid analyses were conducted in four geographical populations. Seeds fatty acid analysis revealed linoleic acid, oleic acid and palmitic acid were the main components in seeds oil. The highest fatty acid component was the linoleic acid in both mature and premature seeds of four studied populations. Low levels of 18:3/18:2 ratio in mature seeds (≤ 0.01) in comparison to premature seeds (> 0.04) were found. Grouping of the populations by WARD clustering, separated mature seeds from premature seeds. CCA plot of *P. harmala* populations based on fatty acid contents, revealing the influence of geographical features on population differentiation. The main alkaloid contents in premature and mature seeds were harmine and harmaline. PCoA plots of the studied populations based on alkaloid contents for both premature and mature seeds separated the populations from each other.

Key words: alkaloid, fatty acid, *Peganum harmala*, premature and matured seeds

INTRODUCTION

Peganum harmala L., a perennial herbaceous plant, is widely distributed in the landscape of steppe, semidesert, and desert territories in southern Europe, northern Africa, and southwestern Asia. It forms many local geographical populations in Iran. It is extensively used in the Iranian traditional medicine, due to the antimicrobial compounds found in its seeds and roots (El-Bakatoushi and Aseel Ahmed 2018, Goudarzi and Azimi 2016).

Plant secondary metabolites provide the plant with specific adaptations to changing environmental conditions and, therefore, they are an important part of plant defence system against pathogenic attacks and environmental stresses. In addition, some plants made use of secondary metabolites as signals for communication between plants and symbiotic microorganisms, as well as served to attract pollinators and seed dispersers (Rasmussen *et al.* 2012, Yang *et al.* 2018).

Various genetic, ontogenic, morphogenetic and environmental factors can influence the biosynthesis and accumulation of primary and secondary metabolites. Metabolomics is an interesting approach that has been used in the plant sciences, especially in ecological studies, in investigating the effects of environmental factors on plant metabolism (Riedl *et al.* 2012, Veldhoen *et al.* 2012). One of the most useful terms is the recently introduced term 'Ecological metabolomics', which was first defined by Macel *et al.* (2010) and refers to the application of metabolomics to ecological issues. Metabolomic profile data have been utilised to compare different species from the same family, or individuals from populations from the same species growing under different environmental conditions, or changes in metabolite production of individuals within the same population at different seasons (Jones *et al.* 2013). Investigations that aim to characterise plants at the biochemical level, allow a search for underlying genes and associations with higher level complex traits, such as yield and nutritional value. Efficient and reliable methods to characterise metabolic variation in economically important species are considered of high value to the evaluation and prioritisation of germplasm and breeding lines (Cao *et al.* 2017).

In spite of the medicinal importance of *Peganum harmala* L. in Iran, we have no detailed information on genetic–chemical contents and particularly the fatty acid contents of this plant species in the country. Therefore, population-based alkaloid and fatty acid content analyses were conducted in four geographical populations of this valuable plant by using HPLC and GC-Mass gas chromatography, in both matured versus premature seeds.

MATERIAL AND METHODS

Plant materials

Plant seeds were randomly collected in four localities within the North Khorasan province of Iran. Geographical details of the studied *Peganum harmala* L. (var. *harmala*) populations are provided in Table 1. The seeds were ground by a domestic coffee grinder just before extraction.

Fatty acid analyses

Extraction and methylation – Soxhlet n-hexane-dichloromethane extraction was carried in triplicate for 5 g of ground seed of *P. harmala* by 160 ml of n-hexane-dichloromethane for 8 h. A rotary evaporator was used for the efficient removal of solvent from samples by evaporation at 60 °C. The extracts were stored in glass jars in the deep freezer (−20 °C) pending for further analysis.

The extract samples were methylated by KOH–MeOH methylation according to the method of Kramer *et al.* (1997). 20 μL of seed oil was placed into 10 mL centrifugal tubes to which 2 mL of KOH–MeOH solution (0.5 M) and 7 ml n-hexane was added. The mixture was heated at 60 °C for 45 min. The extracts were used for GC analysis.

Table 1
Geographical features of the studied *Peganum harmala* populations

No.	Population	Latitude	Longitude	Altitude (m)	Soil EC
1	Salehabad	35° 16′ 53″	61° 66′ 32″	739	1.26
2	Fariman	36° 16′ 26″	59° 99′ 77″	1,066	5.15
3	Hosseinabad	36° 35′ 35″	59° 76′ 40″	950	6.62
4	Ferdosi	34° 49′ 06″	59° 53′ 26″	1,025	5.85

GC/MS analyses

GC/MS were carried out using a Trace DSQ GC System (Thermo Finnigan) equipped with a DB-5 capillary column (30 m, 0.25 μm × 0.25 μm) with a mass spectrometer, operated in electron-impact ionisation mode (70 eV). GC/MS analyses were carried out in split mode (split ratio 1:25), using helium as the carrier gas (1 mL/min flow rate). The injector temperature was fixed at 250 °C. The sample volume injected was 1 μL. Oven temperature was held at 50 °C for 2 min and then programmed at 7 °C/min to a final temperature of 250 °C, where it was maintained for 15 min.

Alkaloid analyses

Extraction and sample preparation – According to the literature (Kartal *et al.* 2003), dried seeds of Syrian rue (1 g) were ground and macerated three times with 25 mL methanol for 1 h. The extract was then filtered and evaporated in a rotary evaporator under vacuum at a temperature of 45 °C. The residue was then treated with 25 mL hydrochloric acid solution (2% v/v), filtered and extracted three times with 10 mL petroleum ether to remove colorant impurities. The aqueous acid layer was then basified with ammonia to reach pH 10 and extracted three times with 25 mL chloroform. The organic solvent was then evaporated. The obtained residue was dissolved in 10 mL methanol and then filtered through a 0.45 μm polypropylene filter. A 20 μL aliquot was injected onto HPLC column.

HPLC analyses – HPLC analyses were performed using Agilent–infinit II equipped with a UV absorbance detector to detect Cyclomaltooctadecaose (CD18), cyclic oligosaccharides composed of 18 D-glucose units. The mobile phase consisted of potassium phosphate buffer (10 mM, pH 7) and acetonitrile (50:50 v/v) with a flow rate of 1.5 mL/min at room temperature (25 °C). The eluents were monitored at 330 nm. All the calculations concerning the quantitative analysis were performed with external standardisation by measurement of peak areas.

Individual stock solutions of harmine, harmaline and harmane (Sigma, USA) were prepared at four concentrations of 100–1000 μg/ml in methanol and used to draw a standard curve.

Data analyses

Comparison between groups was made by one-way ANOVA, followed by Duncan test ($p \leq 0.05$). Grouping of the populations based on chemical contents was done by WARD clustering. The Mantel test between geographical features and chemical contents as well as CCA plot of the studied populations based on chemical contents of the studied

populations were performed (Podani 2000). Grouping of the populations based on alkaloid contents was done by PCoA plots. PAST version 2.17 was used for multivariate analysis.

RESULTS

Fatty acid analyses

Twelve fatty acid components were determined in each population. Linoleic acid, oleic acid and palmitic acid were the main components in seed oil. The highest fatty acid component was the linoleic acid in both mature and premature seeds of four studied populations. The percentages of linoleic acid and oleic acid in mature seeds were significantly ($p \leq 0.05$) more than the premature seeds, while the contents of palmitic acid and linolenic acid are more in premature seeds, in comparison of mature seeds. Among the defined fatty acids, stearic acid did not show significant differences in all studied samples (Table 2).

We also found low levels of laurinate (12:0) (< 0.1%), in seeds. This is a very rare fatty acid component in the plant seed lipids. Other fatty acids identified included myrisitic acid, myristoleic acid, palmitoleic acid, margaric acid, arachidic acid, behenic acid. Palmitoleic acids were lower in mature seeds than premature seeds (Table 3).

In all studied populations, maturation of seeds induces an important decrease in the content of palmitic acid and increase the oleic acid and linoleic content. We also found low levels of 18:3/18:2 ratio in mature seeds (≤ 0.01) in compare to premature seed (> 0.04).

In the premature and matured seeds of all studied populations, the amounts of total saturated fatty acids were lower than total unsaturated fatty acids. Grouping of the populations by WARD clustering, based on major fatty acids contents (Fig. 1), separated mature seeds from premature seeds when each of these two major clusters, the studied populations, was placed intermixed. Therefore, the fatty acid contents of the mature seeds versus premature seeds are different in quantity, but the populations studied cannot be differentiated on these accounts.

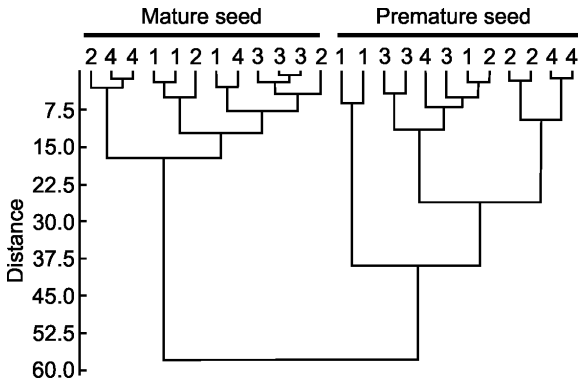

Fig. 1. WARD clustering based on fatty acid contents in *Peganum harmala* populations. 1–4 are four populations based on Table 1

However, CCA plot of the studied populations (Fig. 2), based on fatty acid

Table 2

Main fatty acids in premature and mature seeds of *Peganum harmala* populations. Data are means of three replicates with standard errors (mean ± SE). Different letters indicate significant differences between treatments (P ≤ 0.05)

Seed	Population	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	18:3/18:2
Premature	Salehabad	14.5±1.08(c)	3.05±0.32(a)	17.5±1.42(cd)	44.53±6.11(c)	3.2±1.32(a)	0.072
	Fariman	18.5±1.29(b)	5.26±1.69(a)	17.3±1.12(cd)	47.43± 3.28(bc)	2.09± .02(ab)	0.044
	Hosseinabad	14.7±0.72(c)	3.80±0.55(a)	15±1.15(d)	57±0.95(ab)	3.07±0.92(a)	0.054
	Ferdosi	24.8±2.37(a)	4.60±0.25(a)	16.43±0.43(d)	46.93± 2.58(bc)	1.96±0.12(ab)	0.042
Mature	Salehabad	8.13±0.14(d)	3.90±0.28(a)	22.26±1.09(b)	62.23±0.70(a)	0.8±0.05(b)	0.0128
	Fariman	11.5±0.89(cd)	3.53±0.63(a)	21.13± 2.66(b)	60.91±2.47(a)	0.3±0.25(b)	0.005
	Hosseinabad	10.8±0.08(d)	3.43±0.18(a)	23.43±0.21(b)	59.16±0.61(a)	0.2±0.11(b)	0.004
	Ferdosi	9.11± 0.67(d)	4.10±0.20(a)	27.5±0.35(a)	55.4±1.15(ab)	0.75±0.17(b)	0.013

Table 3

Minor fatty acids in premature and mature seeds of *Peganum harmala* populations. Data are means of three replicates with standard errors (mean±SE). Different letters indicate significant differences between treatments (P ≤ 0.05)

Seed	Population	Myrisitic acid (14:0)	Myristoleic acid (14:1)	Palmitoleic acid (16:1)	Margaric acid (17:0)	Arachidic acid (20:0)	Behenic acid (22:0)
Premature	Salehabad	0.65±0.39(ab)	0.46±0.26(a)	0.29±0.16(ab)	0.51±0.16(bc)	1.47±0.06(a)	0.66±0.08(a)
	Fariman	0.66±0.05(ab)	0.30±0.30(a)	0.35±0.13(ab)	0.62±0.16(ab)	1.12±0.28(ab)	0.26±0.26(ab)
	Hosseinabad	0.41±0.20(ab)	0.52±0.26(a)	0.22±0.13(ab)	0.38±0.19(abc)	1.17±0.16(ab)	0.02±0.02(c)
	Ferdosi	0.80±0.17(a)	0.60±0.23(a)	0.60±0.17(a)	1.00± 0.15(a)	0.5±0.05(c)	0.0±0.0(c)
Mature	Salehabad	0.15±0.02(b)	0.94±0.35(a)	0.06±0.03(c)	0.10±0.05(cd)	0.8±0.05(bc)	0.1±0.05(b)
	Fariman	0.20±0.05(b)	0.57±0.03(a)	0.03±0.03(c)	0.03±0.03(d)	1.17±0.31(ab)	0.21±0.11(ab)
	Hosseinabad	0.23±0.12(b)	0.66±0.12(a)	0.10±0.05(c)	0.16±0.08(cd)	0.83±0.03(bc)	0.53±0.31(ab)
	Ferdosi	0.41±0.07(ab)	0.23±0.04(a)	0.09±0.04(c)	0.21±0.08(abc)	0.84±0.2(bc)	0.23±0.17(ab)

Table 4
Alkaloid contents in premature and mature seeds of *Peganum harmala* populations. Data are means of three replicates with standard errors (mean±SE). Different letters indicate significant differences between treatments ($P \leq 0.05$)

Seed	Population	Harmaline	Harmine	Harman
Premature	Salehabad	39.58±9.56(bc)	68.28±22.63(bc)	16.92±4.719(bc)
	Fariman	94.74±34.79(ab)	111.71±28.74(b)	21.14±6.28(ab)
	Hosseinabad	28.87±2.633(bc)	82.24±1.96(bc)	18.267±3.77(bc)
	Ferdosi	18.46±2.99(c)	37.01±4.75(c)	4.467±0.41(c)
Mature	Salehabad	124.78±35.53(a)	89.52±13.24(bc)	10.87±2.614(bc)
	Fariman	59.14±18.26(bc)	110.89±32.047(b)	11.83±5.267(bc)
	Hosseinabad	133.17±17.10(a)	181.43±6.35(a)	32.23±6.61(a)
	Ferdosi	17.74±2.63(c)	49.29±12.49(c)	9.97±0.75(bc)

contents, separated population 1 (Salehabad) from the other populations under the influence of longitude. The rest of populations were grouped closer to each other, under effect of latitude and altitude.

Alkaloid analyses

Details of alkaloid contents in the studied populations are provided in Table 4. The highest value for harmaline, harmine, and harman alkaloids in the mature seeds occurred in population Hosseinabad, while the highest value for the same parameters in premature seeds was observed in populations Fariman, and Hosseinabad, respectively. Mature and premature seeds of Ferdosi population had the minimum levels of these alkaloids (Table 4).

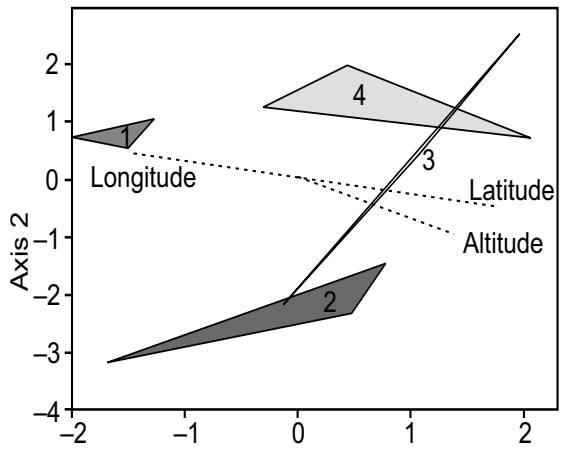

Fig. 2. CCA plot of *Peganum harmala* L. populations based on fatty acid contents, revealing the influence of geographical features on population differentiation. 1–4 are four populations based on Table 1

ANOVA produced significant differences for alkaloid contents among the studied populations. Therefore, *P. harmala* populations can produce different quantities of alkaloids, too.

PCoA plots of the studied populations based on alkaloid contents for both pre-

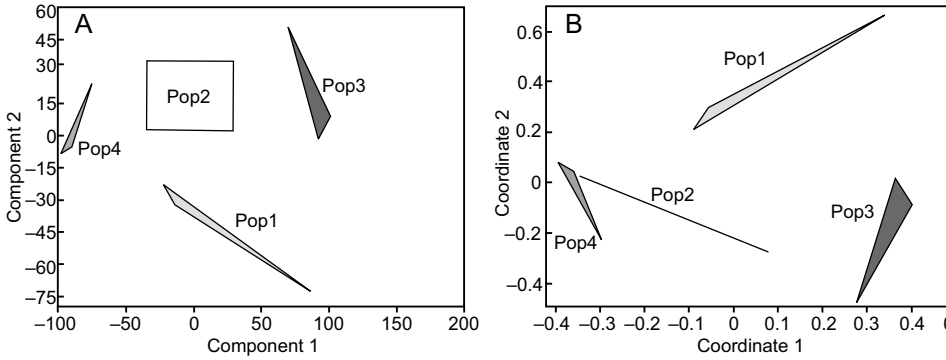

Fig. 3. PCoA plot of the studied *Peganum harmala* populations based on alkaloid contents in premature seeds (A) and mature seeds (B). Pops 1–4 are four populations based on Table 1

mature and mature seeds (Fig. 3) separated the populations from each other. In cases of premature seeds and mature seeds, the populations Salehabad (Pop 1) and Hosseinabad (Pop 3) are placed far from the other studied populations and far from each other. This indicates that these two populations differ greatly in the alkaloids of their premature and mature seeds (Fig. 3).

The Mantel test performed between chemical contents and genetic data of the same populations by using SRAP molecular markers (unpublished data) also did not produce significant associations. But these populations were also separated based on molecular data (unpublished data). Therefore, the studied *P. harmala* populations are differentiated based on biochemical content as well as genetic structure.

DISCUSSION

Both omega-3 (linolenic acid) and omega-6 (oleic acid) fatty acids are important components of cell membranes. Wastes from processing medicinal plants are cheap sources of such compounds. The oil content of the harmal seeds can be considered as good source for lipids.

In this study saturated fatty acids (SFA – 12:0, 14:0, 16:0, 17:0, 18:0, 20:0, 22:0) and unsaturated fatty acids (USFA – 14:1, 16:1, 18:1, 18:2, 18:3) were defined in immature and mature seeds. Total content of USFA was more than SFA in all the studied seeds. It can be said that fatty acid amounts of *P. harmala* in general were similar in point of both SFA and USFA. In the same way, oleic acid, linoleic and palmitic acids were the main components in seed oil. This phenomenon was reported by other researchers (Bagci *et al.* 2001). Similar investigation on *P. harmala* reported the occurrence of palmitic acid as the most abundant compound in the essential oils of *P. harmala* (Almaghrabi and Moussa 2016, Li *et al.* 2018).

Results revealed that premature versus matured seeds in *P. harmala* differed in fatty acid contents. A similar situation has been noticed in the other plant species (Peiretti *et al.* 2004, Özcan 2013). Investigation on developing fruit of *Pistacia lentiscus* L. showed changing profile of fatty acids during maturation, which had been marked mainly by an increase in oleic acid content paralleling a decrease in linoleic acid content (Trabelsi *et al.* 2012).

It is reported that the fatty acid composition and the ratio of (18:3/18:2) can serve as taxonomic markers in some family members (Albishri *et al.* 2013, Marin *et al.* 1991). In this study the ratio of 18:3/18:2 fatty acid components was found lower in premature seeds than mature ones, but no significant difference was found in this ratio between the mature or premature seeds of different populations.

CCA plot of the studied populations, based on fatty acid contents, separated population 1 (Salehabad) from the other populations under the influence of longitude. The rest of the populations was grouped closer to each other, under the effect of longitude and altitude.

Variation in fatty acid quantities was also reported from different geographical areas in various plant species, such as *Quercus* (Özcan 2007). One of the possible reasons might be the climatic conditions prevailing in local populations (Carvalho *et al.* 2006). However, in the Lamarque and Guzmán (1997) study on *Prosopis chilensis* it was stated that fatty acids had no significant correlation with geographic parameters analysed, some unsaturated fatty acids correlated significantly with altitude and the variability observed might indicate that there is sufficient intraspecific difference to permit improvement by selection and breeding.

Genetic diversity may also be associated with fatty acid content difference as also indicated in *Echium* species (Guil-Guerrero *et al.* 2001). However, the present investigation did not reveal association between SRAP molecular markers and fatty acid contents of the studied *P. harmala* populations.

The most abundant alkaloids in premature and mature seeds were hamine and harmaline. This result was in accordance with many researches (Herraiz *et al.* 2010, Moloudizargari *et al.* 2013, Shao *et al.* 2013).

Results showed that the studied populations produce different quantities of alkaloids. PCoA plots of the studied populations based on alkaloid contents for both premature and mature seeds revealed that the populations Salehabad and Hosseinabad are placed far from the other studied populations and far from each other. We found the soils EC of these regions are different. Although the prediction of the effect of each environmental condition on the synthesis of secondary metabolites is difficult because the environmental factors do not seem to exert a constant effect on the entire metabolism of the plant (Gargallo-Garriga *et al.* 2014, Sampaio 2016, Yang *et al.* 2018), so each respective plant part responds differently to changes in environmental con-

ditions (such as soil/sediment organic matter content, pH and temperature) (Kumar and Kumari 2018).

Alkaloid variation has also been reported in populations of plant species, like white lupin (*Lupinus albus* L.) (Kamel *et al.* 2016, Kroc *et al.* 2017), *Senecio jacobaea* (Stastny *et al.* 2005, Willis *et al.* 2000). The discovery of biochemical content variation among the plant populations offers opportunities to probe into the genetic basis of the variation, and provides a valuable resource to gain insight into biochemical functions and to relate its variation with higher level traits in the species (Fernie and Klee 2011, Święcicki *et al.* 2015).

CONCLUSIONS

Fatty acid content depends on seed maturity stages. The comparison of the results obtained from the analysis of alkaloids revealed that the immature and mature seeds of four populations did not show similar differences from the viewpoint of quantity and quality of alkaloids. The populations based on alkaloid and fatty acid contents for both premature and mature seeds separated from each other. We found the quality and quantity of fatty acid and alkaloid content is partly related to geographical features of the studied populations and may be under the influence of genetic differentiation.

REFERENCES

Albishri, H. M., Almaghrabi, O. A. and Moussa, T. A. A. (2013): Characterization and chemical composition of fatty acids content of watermelon and muskmelon cultivars in Saudi Arabia using gas chromatography/mass spectroscopy. – *Pharmacogn. Mag.* **9**(33): 56–64. https://doi.org/10.4103/0973-1296.108142

Almaghrabi, O. A. and Moussa, T. A. A. (2016): Fatty acid constituents of Peganum harmala plant using gas chromatography-mass spectroscopy. – *Saudi J. Biol. Sci.* **23**(3): 397–403. https://doi.org/10.1016/j.sjbs.2015.04.013

Bagci, E., Genç, H. and Sahun, A. (2001): Fatty acid composition of four Lathyrus aphaca L. varieties, a chemotaxonomic approach. – *Pak. J. Biol. Sci.* **4**(7): 872–874. https://doi.org/10.3923/pjbs.2001.872.874

Cao, M., Fraser, K., Jones, C. H., Stewart, A., Lyons, T., Faville, M. and Barrett, B. (2017): Untargeted metabotyping Lolium perenne reveals population-level variation in plant flavonoids and alkaloids. – *Front. Plant. Sci.* **8**: 133. https://doi.org/10.3389/fpls.2017.00133

Carvalho, P. O., Arrebola, M. B., Sawaya, A. Ch. F., Cunha, I. B. S., Bastos, D. H. M. and Eberlin, M. N. (2006): Comparative study of lipids in mature seeds of six Cordia species (family Boraginaceae) collected in different regions. – *Lipids* **41**: 813–817. https://doi.org/10.1007/s11745-006-5035-4

El-Bakatoushi, R. and Aseel Ahmed, D. G. (2018): Evaluation of genetic diversity in wild populations of Peganum harmala L., a medicinal plant. – *J. Genet. Eng. Biotechnol.* **16**(1): 143–151. https://doi.org/10.1016/j.jgeb.2017.11.007

Fernie, A. R. and Klee, H. J. (2011): *The use of natural genetic diversity in the understanding of metabolic organization and regulation*. – Front. Plant Sci., Vol. 2, Article 59. https://doi.org/10.3389/fpls.2011.00059

Gargallo-Garriga, A., Gargallo, G., Sardans, J., Pérez-Trujillo, M., Rivas-Ubach, A., Oravec, M., Vecerova, K., Urban, O., Jentsch, A., Kreyling, J., Beierkuhnlein, C., Parella, T. and Peñuelas, J. (2014): Opposite metabolic responses of shoots and roots to drought. – *Sci. Rep.* **4**: 6829. https://doi.org/10.1038/srep06829

Goudarzi, M. and Azimi, H. (2016): Antimicrobial activity of *Peganum harmala* against methicillin-resistant Staphylococcus aureus strains and assessment of its cytotoxicity effect on HEK-293 cells. – *Int. J. Infect.* **4**(4): e15592. https://doi.org/10.5812/iji.15592

Guil-Guerrero, J. L., Gómez-Mercado, F., Rodríguez-García, I., Campra-Madrid, P. and García-Maroto, F. (2001): Occurrence and characterization of oils rich in γ-linolenic acid (III): the taxonomical value of the fatty acids in Echium (Boraginaceae). – *Phytochemistry* **58**(1): 117–120. https://doi.org/10.1016/s0031-9422(01)00184-4

Herraiz, T., González, D., Ancín-Azpilicueta, C., Arán, V. J. and Guillén, H. (2010): β-Carboline alkaloids in Peganum harmala and inhibition of human monoamine oxidase (MAO). – *Food Chem. Toxicol.* **48**: 839–845. https://doi.org/10.1016/j.fct.2009.12.019

Jones, O. A. H., Maguire, M. L., Griffin, J. L., Dias, D. A., Spurgeon, D. J. and Svendsen, C. (2013): Metabolomics and its use in ecology. – *Austral. Ecol.* **38**(6): 713–720. https://doi.org/10.1111/aec.12019

Kamel, K. A., Swiecicki, W., Kaczmarek, Z. and Barzyk, P. (2016): Quantitative and qualitative content of alkaloids in seeds of a narrow-leafed lupin (Lupinus angustifolius L.) collection. – *Genetic Resources and Crop Evolution* **63**: 711–719. https://doi.org/10.1007/s10722-015-0278-7

Kartal, M., Altun, M. L. and Kurucu, S. (2003): HPLC method for the analysis of harmol, harmalol, harmine and harmaline in the seeds of Peganum harmala. – *J. Pharm. Biomed. Anal.* **31**: 263–269. https://doi.org/10.1016/S0731-7085(02)00568-X

Kramer, J. K. G., Fellner, V., Dugan, M. E. R., Sauer, F. D., Mossoba, M. M. and Yurawecz, M. P. (1997): Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. – *Lipids* **32**(11): 1219–1228. https://doi.org/10.1007/s11745-997-0156-3

Kroc, M., Rybiński, W., Wilczura, P., Kamel, K., Kaczmarek, Z., Barzyk, P. and Święcicki, W. (2017): Quantitative and qualitative analysis of alkaloids composition in the seeds of a white lupin (Lupinus albus L.) collection. – *Genet. Resour. Crop. Evol.* **64**(8): 1853–1860. https://doi.org/10.1007/s10722-016-0473-1

Kumar, R. and Kumari, M. (2018): Adaptive mechanisms of medicinal plants along altitude gradient: contribution of proteomics. – *Biologia plantarum* **62**: 630–640. https://doi.org/10.1007/s10535-018-0817-0

Lamarque, A. L. and Guzmán, C. A. (1997): Seed chemical variation in Prosopis chilensis from Argentina. – *Genet. Resour. Crop. Evol*. **44**(6): 495–498.

Li, Y., He, Q., Du, S., Guo, S., Geng, Z. and Deng, Z. (2018): Study of methanol extracts from different parts of Peganum harmala L. using 1H-NMR plant metabolomics. – *J. Anal. Methods Chem.* **20**: 1–9. https://doi.org/10.1155/2018/6532789

Macel, M., van Dam, N. M. and Keurentjes, J. J. B. (2010): Metabolomics: the chemistry between ecology and genetics. – *Mol. Ecol. Resour.* **10**(4): 583–593. https://doi.org/10.1111/j.1755-0998.2010.02854.x

Marin, P. D., Sajdl, V., Kapor, S., Tatić, B. and Petkovic, B. (1991): Fatty acids of the Satureioideae, Ajugoideae and Scutellarioideae (Lamiaceae). – *Phytochemistry* **30**: 2979–2982. https://doi.org/10.1016/s0031-9422(00)98235-9

Moloudizargari, M., Mikaili, P., Aghajanshakeri, S., Asghari, M. H. and Shayegh, J. (2013): Pharmacological and therapeutic effects of Peganum harmala and its main alkaloids. – *Pharmacogn. Rev.* **7**(14): 199–212. https://doi.org/10.4103/0973-7847.120524

Özcan, T. (2007): Characterization of Turkish Quercus L. taxa based on fatty acid compositions of the acorns. – *J. Amer. Oil. Chem. Soc.* **84**(7): 653–662. https://doi.org/10.1007/s11746-007-1087-8

Özcan, T. (2013): Accumulation patterns of some seed oil components from wild sources of Turkey. – *Nat. Prod. Res.* **27**(1): 54–60. https://doi.org/10.1080/14786419.2011.650639

Peiretti, P. G., Palmegiano, G. B. and Salamano, G. (2004): Evaluation of the quality and fatty acid content of borage (Borago officinalis L.) during the growth cycle Italian. – *J. Food Sci.* **16**: 177–184.

Podani, J. (2000): *Introduction to the exploration of multivariate data*. – Backhuys, Leiden, 407 pp.

Riedl, J., Kluender, C., Sans-Piché, F., Heilmeier, H., Altenburger, R. and Schmitt-Jansen, M. (2012): Spatial and temporal variation in metabolic fingerprints of field-growing Myriophyllum spicatum. – *Aquat. Bot.* **102**: 34–43. https://doi.org/10.1016/j.aquabot.2012.03.017

Rasmussen, M. K., Zamaratskaia, G., Andersen, B. and Ekstrand, B. (2012): Dried chicory root modifies the activity and expression of porcine hepatic CYP3A but not 2C- effect of in vitro and in vivo exposure. – *Food Chem. Toxicol.* **50**: 4175–4179. https://doi.org/10.1016/j.fct.2012.08.024

Sampaio, B. L., Edrada-Ebel, R. and Da Costa, F. B. (2016): Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: a model for environmental metabolomics of plants. – *Sci. Rep.* **6**(1): 29265. https://doi.org/10.1038/srep29265

Shao, H., Huang, X., Zhang, Y. and Zhang, C. (2013): Main alkaloids of Peganum harmala L. and their different effects on dicot and monocot crops. – *Molecules* **18**: 2623–2634. https://doi.org/10.3390/molecules18032623

Stastny, M., Schaffner, U. and Elle, E. (2005): Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness? – *J. Ecol.* **93**: 27–37. https://doi.org/10.1111/j.1365-2745.2004.00962.x

Święcicki, W., Kroc, M. and Kamel, K. A. (2015): *Lupins (Lupinus spp.). In:* de Ron, A. M. (ed.): Grain legumes. Series Handbook of Plant Breeding. – Springer, New York. https://doi.org/10.1007/978-1-4939-2797-5_6

Trabelsi, H., Cherif, O. A., Sakouhi, F., Villeneuve, P., Renaud, J., Barouh, N., Boukhchina, S. and Mayer, P. (2012): Total lipid content, fatty acids and 4-desmethylsterols accumulation in developing fruit of Pistacia lentiscus L. growing wild in Tunisia. – *Food Chem.* **131**: 434–440. https://doi.org/10.1016/j.foodchem.2011.08.083

Veldhoen, N., Ikonomou, M. G. and Helbing, C. C. (2012): Molecular profiling of marine fauna: integration of omics with environmental assessment of the world's oceans. – *Ecotoxicol. Environ. Safety* **76**: 23–38. https://doi.org/10.1016/j.ecoenv.2011.10.005

Willis, A. J., Memmott, J. and Forrester, R. I. (2000): Is there evidence for the post-invasion evolution of increased size among invasive plant species? – *Ecology Letters* **3**: 275–283. https://doi.org/10.1046/j.1461-0248.2000.00149.x

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F. and Wang, Q. (2018): Response of plant secondary metabolites to environmental factors. – *Molecules* **23**(4): 762. https://doi.org/10.3390/molecules23040762