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Essential oil profile of oregano (*Origanum vulgare* L.) populations grown under similar soil and climate conditions

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ABSTRACT

Oregano (*Origanum vulgare* L.) is one of the most commercially important herbs in the Lamiaceae family. It is a rich natural source of bioactive components including phenolic glucosides, flavonoids, tannins, sterols, triterpenes, resins and essential oil. In the present study, the variation of the essential oil compositions among seven populations of Iranian oregano, originating from different bioclimate and geographical zones was investigated over two harvest years (2014 and 2015), and under controlled soil and climate conditions. The essential oil content showed a wide variability, ranging from 0.12% to 1.76% (v/w), correlated to the chemical profile. GC-FID and GC–MS analyses of the essential oils characterized a total of forty-two constituents in oregano populations. Carvacrol (0.3–46.8%), linalyl acetate (0.2–44.3%), (*Z*)-α-bisabolene (0.0–40.3%), (*E*)-*β*-caryophyllene (0.0–24.0%), and caryophyllene oxide (0.1–21.3%) were identified as the main components of the essential oils, depending on the population and harvest year. The highest amounts of these components were recognized in the essential oil of Baneh, Rasht, Gilan, Kaleybar and Ardabil populations, respectively. According to cluster and principal component analyses (PCA), the studied populations were grouped into four main chemotypes: *i.e.*, chemotype I (carvacrol), chemotype II ((*Z*)-α-bisabolene), chemotype III (linalyl acetate), chemotype IV (caryophyllene oxide/germacrene D/(*E*)-*β*-caryophyllene). Variability of essential oil constituents in oregano populations studied can primarily be explained by differences in efficiency and/or activity of the methylerythritol 4-phosphate (MEP) and mevalonate pathways. Intraspecific chemical variability in Iranian oregano provides possibility of selection of those batches with specific aromas and chemical profiles for industrial intentions. The results also provided new insight for development of effective conservation strategies, domestication and breeding programs in Iranian *O. vulgare* germplasm.

1. Introduction

Origanum vulgare L., commonly known as oregano, is a flavoring herb widely used throughout the world to flavor various foods and processed meats, such as salads, pizza and sausages (Charles, 2012; Azizi et al., 2009; Olivier, 1997). The plant has been used for centuries as a medicinal herb in ethnopharmacological preparations to treat various ailments such as convulsive coughs, digestive disorders, menstrual problems, bronchitis and asthma and frequently as a carminative, diaphoretic, expectorant, stimulant, anti-oxidant, anti-inflammatory and anti-microbial agent among other medical applications (Sarikurkcu et al., 2015; Charles, 2012; Gulluce et al., 2012; Mozaffarian, 2013; Ocana-Fuentes et al., 2010; Bakkali et al., 2008; Kulisic et al., 2004). Oregano has a great economic importance as a natural source of active compounds and is among the most traded and consumed spice plants in the world (Lukas et al., 2015; Chishti et al., 2013; Verma et al., 2010).

O. vulgare is a bushy, herbaceous perennial plant native to Europe and central Asia, and contains a wide range of biologically active components including vitamins, tannins, resins, sterols, flavonoids, phenolic glucosides and essential oil (Lukas et al., 2015; Charles, 2012; Chou et al., 2011; Liu et al., 2011). The flavoring properties of oregano is mainly associated with its aromatic substances, especially the essential oil (Yan et al., 2016; Skoula and Harborne, 2002). Oregano is an extremely variable species and, according to The Plant List database (www.theplantlist.org), has been classified into five variable subspecies including subsp. *glandulosum*, subsp. *gracile*, subsp. *hirtum*, subsp. *virens*

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Table 1
Origin, geographical characteristics and voucher numbers of studied *Origanum vulgare* populations.

Voucher number	Habitat	Altitude (m)	Latitude (N)	Longitude (E)	Subspecies	Area of sampling collection	Populations
6440	Woody-semihumid	20	36°39′	51°22′	virence	Mazandaran province − Chalus	Mazandaran
6439	Mountain-semihumid	177	36°51′	49°29′	virence	Gilan province − Roudbar	Gilan
6436	Mountain-semiarid	1320	38°20′	48°15′	virence	Ardebil province-Ardabil	Ardabil
6435	Mountain-semiarid	1370	38°24′	48°30′	virence	Ardebil province-Aliabad	Namin
6438	Mountain-semiarid	1360	38°54′	47°08′	viridulum	East Azerbaijan province-Kaleybar	Kaleybar
6437	Woody-semiarid	950	38°57′	46°53′	viridulum	East Azerbaijan province- Arasbaran	Arasbaran
6434	Mountain-semihumid	1750	36°06	45°59′	gracile	Kordistan province-Baneh	Baneh

and subsp. *viridulum* (Morshedloo et al., 2017a; Kokkini, 1996; Ietswaart and Ietswaart, 1980). The first three subspecies are rich in essential oil, whereas the remaining ones are essential oil poor plants (Padulosi, 1997). In Iran, the *Origanum* genus is represented by three species including *O. vulgare*, *O. strobilaceum* and *O. laevigatum* (Jamzad, 2012). In this country *O. vulgare* occurs with three subspecies (subsp. *viridulum,* subsp. *viride* and subsp. *gracile*) (Jamzad, 2012; Rechinger, 1982).

According to the extensive phytochemical studies on the essential oil composition of *O. vulgare* and other related species, a wide chemical diversity with a considerable intraspecific qualitative and quantitative variation in constituents is found (Mechergui et al., 2016; Lukas et al., 2015; Moradi et al., 2014; Béjaoui et al., 2013; Lukas et al., 2013; Crocoll et al., 2010; Verma et al., 2010; Azizi et al., 2009; de Barros et al., 2009; Mockute et al., 2001; D'Antuono et al., 2000; Vokou et al., 1993). This variability could be related to the effect of variables such as genetic factors, geographical distribution, plant part studied, collection time, methods of extraction, environmental conditions, etc. (Morshedloo et al., 2015a, 2015b; Padulosi, 1997). According to previous studies, individuals rich in essential oil usually accumulate large amounts of phenolic monoterpenes such as carvacrol, thymol and their biosynthetic precursors γ-terpinene and *p*-cymene, whereas the plants with poor essential oil content are often characterized by high amounts of sesquiterpenes (such as germacrene D, (*E*)-*β*-caryophyllene, γ-muurolene and caryophyllene oxide), acyclic monoterpenoids (such as linalool and/or linalyl acetate, *β*-ocimene or myrcene) and/or bicyclic 'sabinyl'-type monoterpenoids (mainly sabinene and *cis-/trans*-sabinene hydrate) (Lukas et al., 2015; Azizi et al., 2009; Skoula and Harborne, 2002; Padulosi, 1997).

Although there are some reports on the volatile constituents of *O. vulgare* growing wild in Iran (Moradi et al., 2014; Andi et al., 2012), to the best of our knowledge there are no systematic and comprehensive studies undertaken to explore the essential oil variability in Iranian oregano populations. Therefore, the present study was performed to characterize the chemical compositions of seven Iranian *O. vulgare* populations, originating from different bioclimate and geographical zones. The plants were grown in similar climatic conditions in order to avoid the effects of environmental factors and growing conditions on the production of secondary metabolites. Cluster analysis (CA) and principal component analyses (PCA) was performed to characterize the chemotypes based on their main volatile components. As most of oregano from Iran was collected from wild sources without focusing on the specific subspecies and chemotype, the presented study, aimed at detecting interesting chemotypes (with genetically differences from others) for industrial use, breeding programs and development of effective conservation strategies.

2. Materials and methods

2.1. Plant and soil materials

To diminish the effects of seasons, climate, and growing conditions on essential oil yield and composition, seven populations of *O. vulgare* were gathered from different bioclimatic zones of Iran, and seeded in a controlled glass greenhouse at the College of Agriculture and Natural Resources, University of Tehran, Iran, over 2014 to 2015. The seeds were obtained from the gene bank of the Forest and Rangeland Research Institute, Tehran, Iran, and germinated in a coco peat: perlite mixture, (70:30, w: w), in a plastic germination tray.

In this study, twenty uniformly sized seedlings from each population, were transplanted from seedling bed into seven L pots (two plant per pot) thirty-two days after seeding. The soil mixture used in this experiment was a clay soil. The soil material was taken from the layer of 0–20 cm, sieved, homogenized and mixed with sand and leaf mold (soil: sand: leaf mold = 2:1:1w/w). Each pot was supplemented with 6.9 mg P, 1.4 mg N, and 12.8 mg K to warrant the optimal nutrient supply for plant growth. The mixed soil was adjusted to a pH 7.2 and electrical conductivity (EC) of 1.2 dS m^{-1}. The pots containing the seedlings were placed in a glass greenhouse (longitude 50°59′51" E, latitude 35°48°20" N, altitude 1343 m a.s.l.). During the experiment, the average minimum and maximum temperatures inside the greenhouse were 16.5 °C and 32.5 °C, respectively, under natural light conditions. Two harvests were performed at the full flowering stage in 2014 and 2015 (the whole plants were used for extraction of essential oil). To warrant a good comparison, the stems of the experimental oregano plants were uniformly cut at five cm above the soil just before the beginning of the second growing season. Voucher specimens were collected at flowering stage, and deposited in the herbarium of the College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. The origin, geographical characteristics and voucher numbers of studied populations are presented in Table 1.

2.2. Essential oil isolation

Plant materials were air dried in the shade for one week after harvesting at flowering stage. To isolate the essential oil, 20 g of dried leaves and inflorescences were hydro-distilled for 3 h using a Clevenger-type apparatus according to the method reported in the European Pharmacopoeia (Council of Europe (COE), 2007). Isolated essential oils were dried over anhydrous sodium sulphate and kept at −20 °C until analysis. Essential oil content was measured as relative percentage units (volume in ml of oil for 100 g of dry weight of plant). For GC-FID and GC–MS analysis, the essential oils were transferred to the Mass Spectrometry Center, University of Massachusetts, Amherst, USA.

2.3. GC-FID and GC/MS analyses

Gas chromatography analysis was performed using a Shimadzu GC-FID Model 2014 (Japan), equipped with a Supelco fused-silica gel capillary RTX-5 column (30 m length, 0.25 mm diameter, and 0.25 µm film thickness). The oven temperature was programmed at 60 °C for 5 min, then from 60 to 210 °C at 3 °C/min, and held at 210 °C for 10 min. Both injector and detector temperatures were 230 °C. Helium was used as carrier gas with a flow rate of 1 mL/min. The samples were injected using the split sampling technique, with a ratio of 1:30. The percentage composition of the essential oils was computed by the normalisation method from the GC peak areas, without using correction factors (Venditti et al., 2015). The GC–MS unit consisted of an Agilent