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Effect of environmental variables on phytonutrients of *Origanum vulgare* L. in the sub-humid region of the northwestern Himalayas

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Abstract Ecological and soil physiochemical parameters impact the crop quality and development. In spite of the huge commercial prospective, the phytonutrient and chemometric profiles of Himalayan oregano (*Origanum vulgare* L.) have not been evaluated, and their relationships with ecological parameters are still lacking. The objective of this research study was to evaluate the disparity in the phytonutrient profiles of different ecotypes of *O. vulgare* in wild and cultivated populations and determine whether such variation was related to the diverse climatic and edaphic conditions prevailing in the northwestern Himalayas. Micrometeorological, atomic absorption spectroscopy for micro-elemental analysis was determined for soil. HPLC was used to determine the disparity in phytonutrient (quercetin, betacarotene, ascorbic acid, and catechin) and phytochemical (arbutin) levels. Cultivated populations had lower phytonutrient levels than wild populations. The habitat exhibiting pH values ranging from 6 to 7 elevated organic carbon (2.42%), nitrogen (97.41 kg ha^{-1}), and manganese (10–12 μg g^{-1}) and zinc contents (0.39–0.50%) show luxirant growth of *Origanum vulgare*l. The phytonutrient (quercetin, betacarotene, ascorbic acid, arbutin, and catechin) levels had a direct relationship with UV-B flux ($r^2 = 0.82$) and potassium ($r^2 = 0.97$). Wild accessions predominantly contained catechin and ascorbic acid, with maximum values of 163.8 and 46.88 μg g^{-1}, respectively, while the cultivated accessions had the highest level of arbutin (53.42 μg g^{-1}). Maximum variation was observed in quercetin (114.61%) followed by β-carotene (87.53%). Cultivated accessions had less quercetin (0.04–1.25 μg g^{-1}) than wild accessions (1.25–2.87 μg g^{-1}). Wild accessions had higher phytonutrient values for catechin, β-carotene, and ascorbic acid while cultivated accessions had maximum values for arbutin. The correlation of environmental variables with phytonutrient levels paves the way for metabolomic-guided enhancement of agricultural practices for better herb quality.

Keywords Soil · Phytonutrient · Catechin · Quercetin · Betacarotene · Ascorbic acid

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Introduction

Origanum vulgare (L.) is a medicinal, perennial herb, locally known as Jungali Tulsi, oregano, or Himalayan marjoram (Verma et al. 2010). It is found in temperate to

subalpine regions of the Himalayas from Kashmir to Sikkim at an altitude of 1500–3600 m (Bisht et al. 2009). *Origanum vulgare* L. is an underutilized herb that has scope as a nutraceutical (Rodríguez-Meizoso et al. 2006). Oregano benefits human health in the provision of high phytonutrient constituents—for instance, β-carotene, catechin, ascorbic acid, quercetin, and essential nutrients—which confer resistance against many diseases and can detoxify free radicals (Conforti et al. 2011). Ascorbic acid and catechin are the most predominant phytonutrients in oregano. Ascorbic acid is responsible for the sour flavor, and catechin offers its distinct aroma. Phytonutrients are essential for the final nutritional quality and commercial value of this herb. Various reports in vegetable and fruits have identified a significant correlation between climatic variables and phytonutrients such as ascorbic acid, catechin, and β-carotene (Hamzah et al. 2013; Ahmed et al. 2014a; Mattos et al. 2014) but there are no such reports for herbs. Epidemiological research has demonstrated the health benefits of consuming aromatic herbs rich in phenolics and flavonoids which protect humans against cancer, cardiovascular disease, and age-associated diseases, such as Alzheimer's disease (Lagouri and Alexandri 2013; Sarikurkcu et al. 2015; Amir Aslani and Ghobadi 2016).

The northwestern Himalayan region is characterized by high UV flux, low temperatures, and dense snow cover for about 5 months in the lead up to spring. Edaphic factors such as physiochemical properties and macro and micronutrient availability in the soil are determinants for phytonutrient accumulation in herbs. These environmental variables affect yield plus product value.

However, data is lacking on the impact of ecological variables and their correlation with agricultural practices in reference to phytonutrient value. It is well known that high UV flux and low temperatures increase the proclivity of plants to accumulate phenolics and flavonoids (Leyva et al. 2013). UV flux and low temperatures induce the accretion of ascorbic acid, catechins, and β-carotene which help plants to grow under extreme conditions. The accretion of phytonutrients in wild herbs can be beneficial for both growers and consumers (Nurmi et al. 2009). One study demonstrated that tomato plants grown under high light conditions accumulated twice as many phenolics, such as rutin and chlorogenic acid, as those under low light intensity (Wilkens et al. 1996).

There are contradictory reports pertaining to ascorbic acid content under extreme environmental conditions, with some increasing in response to high solar radiation flux (Ghasemzadeh et al. 2016; Jan et al. 2016) and others decreasing (Kumari and Agrawal 2010; Rosales et al. 2010; Schreiner et al. 2012; Leyva et al. 2013). These studies described the effect of environmental variables on productivity and phytonutrient availability in crops. Hence, it is important to collate climatic factors with land use responses to maximize the benefits for farmers as well as for consumers of high-quality products (Thompson et al. 2011). The choice of landscape will determine the climatic parameters that influence crop quality via reallocation in organic and anthropogenic of agro-ecosystem that affect consumer preference and food security (Ahmed and Stepp 2016). For mass production, *O. vulgare* L. is cultivated under field conditions with uniform agricultural practices.

Keeping in the view the previous research reports, we hypothesized that there exists direct correlation between environmental variables, the phytonutrient aspects, and aroma of wild and cultivated accessions of *O. vulgare* L. So, we intended to explore the influence of ecological parameters like (UV flux, PAR), chemometric parameters, and physiochemical aspects of soil on the phytonutrient content of ecotypes of *O. vulgare*.

Material and methods

Survey and collection of material

Wild and cultivated populations of *Origanum vulgare* L. were collected from diversemicroclimatic zones of the Kashmir Himalayas (Table 1). The collection sites were in areas where *O. vulgare* L. thrives well. In the northwestern Himalayan area, seven wild populations of Kashmiri oregano (Table 1) were located in four microclimatic zones (Dhar and Dhar 2000). The ecological characterization of the studied populations and their microclimatic factors, bio-geographic coordinates, altitude, photon flux density (PPFD), and UV are listed in Table 1.

Edaphic analysis

Soil from each studied population was characterized using a composite sample collected from the 15–20 cm soil layer at three points randomly distributed in

Table 1 Collection and procurement of material from naturalized and cultivated *Origanum vulgare* L. populations across the northwestern Himalayan region

	Place	Longitude and latitude	Altitude (feet)	Photon flux density (μmol m^{-2} s^{-1})	Aug–Sep max/min temperatures (°C)	UV (μmol m^{-2} s^{-1})
Cultivated populations	Tangmarg (Yarikhah)	34° 03′ N 74° 25′ E	8400	1243.1	18/11	78
	Pulwama (Bonera)	33° 88′ N 74° 92′ E	5400	1045	24/13	83
	Sanatnagar	17° 29′ N 78° 25′ E	5426	1123	28/14	92
Wild populations	Churwan (Gurez)	34° 63′ N 74° 83′ E	8027	1682.1	15/8	123.1
	Kanzalwan (Gurez)	34° 63′ N 74° 83′ E	8000	1600	17/10	117
	Markoot (Gurez)	34° 63′ N 74° 83′ E	7945	1496	18/9	107
	Izmarg (Gurez)	34° 63′ N 74° 83′ E	7875	1015.7	20/12	109.7
	Dachigam	34° 81′ N 75° 21′ E	9500	1012	12/6	104
	Yusmurg	33° 83′ N 75° 30′ E	7861	1298	20/13	95
	Uri	34° 50′ N 74° 20′ E	4472	948	17/9	73

each oregano population. The pH and electrical conductivity (EC) of the soil were determined in 1:1 (*v*/*v*) deionized water via symphony SB80PC conductivity/pH meter (VWR Scientific Products, St Paul, MN, USA) (Table 2a). Physicochemical analyses were performed according to standardized analytical soil techniques (Ryan et al. 2007) using Kjeldahl steam distillation for total nitrogen (N), the Olsen and Bray method for soluble phosphorus (P), and flame photometry for potassium (Table 2c).

Micro-elemental analysis

Available micronutrients—iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) were determined by atomic absorption using the DTPA extraction method (Table 2c). Atomic absorption measurements were performed via Varian Spectra with setting correction and hollow cathode lamps. The air-acetylene flame was employed for estimation of all elements.

Macro-elemental analysis

Twenty plants were harvested indiscriminately from each population and washed with double-distilled water. The plant parts were dissected to stem, leaves, and roots and investigated for nitrogen (N) and sulfur (S) composition. Following dissection, samples were dried in oven at 65 °C for 48 h. The dried samples were milled finely and sieved via 72 μm mesh screen. The powder was tagged and stored in polyethylene bags for subsequent investigation.

PAR and UV-B flux measurements

Using the spectroradiometer, we have selected the estimated reflectance of the randomly selected 15 plants by exposing branches and leaves in numerous sheets on black plane covering. Moreover, we have employed UV quantum sensors for determination of photon flux density (PPFD) and photosynthetically active radiations (PAR) plus the spectral reflectance in reference to previously established reference standards (Lydon et al. 2009; Jan et al. 2016). These instruments detect 252 spectral bands between 390 and 1100 nm for the sensor of visible radiation and between 207 and 407 nm for UV radiation sensor. When data was recorded, the sun was at solar pinnacle angle lower than 45 °C. The spectral determination was calculated with spectroradiometer directed perpendicularly descending (nadir) from a height of 1 m by mounting it on a boom. Four scans were averaged for each measurement.

Table 2 Edaphic analysis to check soil status of collection sites

(a) Physicochemical analysis of collection sites

Place	pH	Electrical conductivity (dS m^{-1})	Soil texture
Tangmarg (Yarikhah)	5.96 ± 0.08	1.59 ± 0.583	Loam
Pulwama (Bonera)	6.14 ± 0.075	1.65 ± 0.583	–
Sanatnagar	5.64 ± 0.103	1.59 ± 0.583	–
Churwan (Gurez)	7.54 ± 0.133	1.90 ± 0.843	–
Kanzalwan (Gurez)	7.04 ± 0.144	1.86 ± 0.843	–
Markoot (Gurez)	7.76 ± 0.093	1.85 ± 1.091	–
Izmarg (Gurez)	7.08 ± 0.208	1.82 ± 1.28	–
Dachigam	7.36 ± 0.136	1.97 ± 0.975	–
Yusmurg	7.14 ± 0.103	1.54 ± 1.068	–
Uri	6.76 ± 0.286	1.76 ± 0.927	–

(b) Total CNS analysis per gram dry weight by CHNS analyzer

Place	Carbon (%)	Sulfur (%)	Nitrogen (%)	C/N ratio
Tangmarg (Yarikhah)	4.33 ± 0.017	0.085 ± 0.001	0.224 ± 0.001	19.13 ± 0.011
Pulwama (Bonera)	4.42 ± 0.016	0.121 ± 0.001	0.213 ± 0.004	20.68 ± 0.015
Sanatnagar	3.83 ± 0.011	0.230 ± 0.002	0.193 ± 0.004	19.74 ± 0.010
Churwan (Gurez)	6.43 ± 0.016	0.166 ± 0.004	0.263 ± 0.002	24.13 ± 0.10
Kanzalwan (Gurez)	7.84 ± 0.012	0.174 ± 0.006	0.414 ± 0.006	19.69 ± 0.009
Markoot (Gurez)	7.59 ± 0.010	0.018 ± 0.009	0.451 ± 0.007	16.71 ± 0.008
Izmarg (Gurez)	5.99 ± 0.012	0.166 ± 0.04	0.325 ± 0.003	18.43 ± 0.014
Dachigam	5.54 ± 0.016	0.005 ± 0.001	0.275 ± 0.008	18.84 ± 0.016
Yusmurg	6.05 ± 0.014	0.144 ± 0.003	0.217 ± 0.003	21.650 ± 0.011
Uri	4.78 ± 0.016	0.064 ± 0.001	0.367 ± 0.001	13.03 ± 0.014

(c) Micronutrient and macronutrient analysis of soil (per gram dry weight)

Places	Micro-elemental analysis (ppm)				Macro-elemental analysis			
	Manganese	Iron	Zinc	Copper	Organic carbon (%)	Phosphorus (kg ha^{-1})	Nitrogen (kg ha^{-1})	Potassium (kg ha^{-1})
Tangmarg (Yarikhah)	4.2 ± 0.012	1.6 ± 0.013	0.20 ± 0.013	0.125 ± 0.019	0.7 ± 0.09	14.2 ± 0.37	72.12 ± 2.11	254.32 ± 22.23
Pulwama (Bonera)	3.5 ± 0.009	2.4 ± 0.017	0.17 ± 0.014	0.132 ± 0.023	0.8 ± 0.013	26.27 ± 0.45	56.44 ± 3.23	223.12 ± 30.13
Sanatnagar	2.9 ± 0.013	1.7 ± 0.019	0.12 ± 0.017	0.10 ± 0.017	2.23 ± 0.017	97.82 ± 2.76	87.80 ± 2.19	542.97 ± 34.67
Churwan (Gurez)	11.5 ± 0.015	2.5 ± 0.021	0.54 ± 0.019	0.199 ± 0.013	1.8 ± 0.019	94.49 ± 2.82	109.76 ± 12.34	533.79 ± 45.98
Kanzalwan (Gurez)	12.3 ± 0.011	1.5 ± 0.010	0.43 ± 0.021	0.224 ± 0.015	1.05 ± 0.020	82.76 ± 4.011	81.53 ± 13.34	502.99 ± 12.98
Izmarg (Gurez)	4.5 ± 0.017	3.1 ± 0.045	0.38 ± 0.023	0.220 ± 0.011	2.38 ± 0.018	72.67 ± 2.011	97.21 ± 25.46	359.29 ± 13.89
Markoot (Gurez)	5.1 ± 0.019	2.6 ± 0.056	0.42 ± 0.016	0.15 ± 0.017	2.10 ± 0.014	76.29 ± 2.43	84.67 ± 6.78	543.53 ± 37.11
Dachigam	12.4 ± 0.20	1.8 ± 0.023	0.25 ± 0.09	0.110 ± 0.013	2.42 ± 0.019	72.67 ± 3.02	97.41 ± 5.09	359.29 ± 36.34
Uri	11.3 ± 0.014	1.46 ± 0.017	0.14 ± 0.08	0.45 ± 0.09	1.94 ± 0.009	70.1 ± 1.90	81.67 ± 4.19	312.12 ± 34.09
Yusmarg	11.8 ± 0.010	1.29 ± 0.011	0.5 ± 0.019	0.36 ± 0.07	2.03 ± 0.012	74.91 ± 1.87	94.67 ± 12.80	295.92 ± 27.89

Morphological evaluation of quantitative traits

The evaluation of morphological traits associated with agronomic potential was procured by harvesting randomly 20 plants from each population as represented in (Table 3). The agronomic traits determined included plant height (PH, cm), number of branches (NB), branch length (BL, cm), stem diameter (SD, mm), number of nodes per stem (NN), distance between internodes (DI, cm), number of leaves per node (NL), leaf length (LL, cm), leaf width (LW, cm), and dry mass (DM, g plant^{-1}) (Azizi 2010).

HPLC analysis of phytonutrients

Leaves and shoots from randomly harvested plants at each location were evaluated following the method of Huang et al. (2004). Harvested biomass was oven dried at 55 °C for 16 h and then ground to a powder with a Wiley Mill (Model No. 4276, Thomas Scientific, USA). Dried powder was extracted with petroleum ether using Soxhlet apparatus for 48 h, and the ether extract was concentrated. For HPLC analyses, 3 g of powdered plant material was extracted with 80 ml petroleum ether for 2 h to recover the extraction solvent using a Supelco-soxhlet extraction apparatus with a thermoregulator. The resulting extract was concentrated and diluted with 10 ml of methanol and then filtered through 45 μm pore size before ultra-sonication for 60 min. Quantification was obtained using the corresponding standards for calibration curve preparation.

HPLC instrumentation

HPLC analysis was carried out on Shimadzu chromatographic apparatus (Kyoto, Japan) using a Symmetry C18 column. The mobile phase was selected variably at a flow rate of 1 ml min^{-1}. Other variables for method establishment such as mobile phase, UV detection wavelength, injection volume, and standard range for a calibration curve for each standard are detailed in Supplementary Table 1.

Statistical analysis

The data were subjected to analysis by statistical software OPSTAT to determine the critical difference (CD) and coefficient of variance. Correlations of climatic and chemometric parameters with phytonutrients among

Table 3 Phenotypic data on 10 quantitative traits related to the agronomic and morphological characters and averaged across 10 individual plants of each accession acquired from different naturalized populations

Place	PH	NB	BL	SD	NN	DI	NL	LL	LW	DM
Tangmarg (Yarikhah)	72.8 ± 1.06	24.4 ± 1.43	53.5 ± 0.01	4.68 ± 0.04	25.2 ± 1.65	2.5 ± 0.007	7.0 ± 0.316	33.69 ± 0.019	17.82 ± 0.02	77.14 ± 0.025
Pulwama (Bonera)	75.8 ± 0.021	23.2 ± 1.06	66.3 ± 0.01	4.12 ± 0.06	20.8 ± 1.06	3.48 ± 0.022	5.8 ± 0.374	35.89 ± 0.012	18.94 ± 0.024	59.58 ± 0.038
Sanatnagar	80.16 ± 0.05	19.80 ± 1.77	52.1 ± 0.007	3.86 ± 0.024	30.4 ± 1.72	3.14 ± 0.018	6.0 ± 0.316	36.68 ± 0.015	24.34 ± 0.024	36.74 ± 0.025
Churwan (Gurez)	50.28 ± 0.01	25..60 ± 0.67	46.2 ± 0.03	3.89 ± 0.037	18.4 ± 1.41	3.99 ± 0.01	17.0 ± 1.34	29.32 ± 0.016	20.30 ± 0.042	23.40 ± 0.032
Kanzalwan (Gurez)	56.14 ± 0.11	31.80 ± 1.28	47.1 ± 0.04	4.6 ± 0.05	15.6 ± 1.07	2.59 ± 0.02	17.6 ± 0.812	23.29 ± 0.015	16.30 ± 0.05	37.94 ± 0.025
Markoot (Gurez)	59.74 ± 0.024	37.20 ± 0.86	48.3 ± 0.05	5.2 ± 0.02	20.6 ± 1.70	3.89 ± 0.05	6.80 ± 0.374	24.0.5 ± 0.016	18.46 ± 0.024	36.18 ± 0.031
Izmarg (Gurez)	39.22 ± 0.03	24.60 ± 0.51	35.7 ± 0.03	3.68 ± 0.06	20 ± 1.73	2.29 ± 0.03	15.80 ± 0.583	23.40 ± 0.018	19.88 ± 0.020	22.42 ± 0.037
Dachigam	63.44 ± 0.02	24.80 ± 0.86	57.3 ± 0.02	3.88 ± 0.02	17.8 ± 1.02	3.09 ± 0.01	6.40 ± 0.510	21.72 ± 0.018	22.78 ± 0.020	34.98 ± 0.020
Yusmurg	69.52 ± 0.02	21.40 ± 1.07	39.4 ± 0.01	2.48 ± 0.03	12.40 ± 0.51	2.61 ± 0.04	6.0 ± 0.316	30.86 ± 0.012	21.32 ± 0.020	30.06 ± 0.024
Uri	67.380 ± 0.390	22.80 ± 1.02	31.6 ± 0.03	2.62 ± 0.04	18.0 ± 0.70	2.38 ± 0.03	5.60 ± 0.40	31.21 ± 0.019	23.28 ± 0.050	38.12 ± 0.020

PH, plant height (cm); NB, number of branches; BL, branch length; SD, stem diameter (mm); NN, number of nodes per stem; DI, distance between internodes (cm); NL, number of leaves per node; LL, leaf length (mm); LW, leaf width (mm); DM, dry mass (g plant^{-1})

wild and cultivated accessions were calculated using the SPSS statistical package. Principal component, a cluster analysis using SAS, was undertaken to determine the contribution of each environmental variable in the correlation matrix by eigenvalue.

Results

The wild and cultivated accessions of *O. vulgare* L. exhibited significant variation in phytonutrient levels (ascorbic acid, catechin, quercetin, β-carotene) and the phytochemical arbutin. There were also strong correlations between the measured phytonutrients and climatic and soil variables (Supplementary Tables 1–4). All phytonutrients were environmentally responsive. Wild accessions of *O. vulgare* L. had higher levels of catechin and ascorbic acid than cultivated accessions (Table 4) while the reverse was true for arbutin.

Description of environmental variables and their effects on phytonutrients

The cultivated habitats of *O. vulgare* L. had higher maximum and minimum air temperatures (28/14 °C) than those in the wild habitats (18/8 °C) (Table 1). Minimum temperatures in the wild habitats varied little during harvest but the cultivated habitats were almost 3 °C higher than the wild populations.

The wild habitats of *O. vulgare* L. varied in their photon flux density and UB-flux values—and recorded the highest values (1682.1 μmol photons m^{-2} s^{-1} and 123.1 μmol m^{-2} s^{-1}, respectively)—while the cultivated habitats had lower and more uniform photon flux density and UV flux values (Table 1). In the wild populations, UV flux increased with increasing altitude, recording a minimum UV flux of 73 μmol $m^{-2}s^{-1}$at an altitude of 4472 ft and a maximum UV flux of 123.1 μmol $m^{-2}s^{-1}$at an altitude of 15,420 ft (Table 1).

Physicochemical soil parameters

Soil analyses prior to sampling were carried out to determine the baseline nutrient availability for that season's crop growth. The pH of soil samples from cultivated habitats ranged from 5.96 to 6.14, being very slightly alkaline, while those from wild habitats ranged from 6.76 to 7.54, being strongly alkaline.

Electrical conductivity represents the soluble salts present in the soil. The electrical conductivity of soil samples from cultivated habitats ranged from 1.59 to 1.69 dS while those from wild habitats ranged from 1.54 to 1.97 dS (Table 2a). Soil texture was determined as loamy for both cultivated and wild habitats.

Table 4 HPLC analysis of phytonutrients and phytochemicals in *Origanum vulgare* L. populations

	Place	Arbutin (μg $g^{-1}dw^{-1}$)	β-Carotene (μg $g^{-1}dw^{-1}$)	Ascorbic acid (μg g^{-1} dw^{-1})	Quercetin (μg g^{-1} dw^{-1})	Catechin (μg g^{-1} dw^{-1})
Cultivated populations	Tangmarg (Yarikhah)	53.42 ± 0.436	0.73 ± 0.012	14.46 ± 0.016	0.34 ± 0.014	60.18 ± 0.019
	Pulwama (Bonera)	53.14 ± 0.448	0.47 ± 0.018	13.46 ± 0.013	0.21 ± 0.012	52.45 ± 0.017
	Sanatnagar	51.10 ± 0.510	0.34 ± 0.010	12.56 ± 0.012	0.04 ± 0.009	41.78 ± 0.015
Wild populations	Churwan (Gurez)	31.10 ± 0.382	11.04 ± 0.013	24.72 ± 0.017	1.25 ± 0.018	98.22 ± 1.01
	Kanzalwan (Gurez)	29.38 ± 0.595	6.68 ± 0.015	46.88 ± 0.016	2.17 ± 0.015	163.8 ± 2.09
	Markoot (Gurez)	38.32 ± 0.02	5.15 ± 0.021	30.55 ± 0.015	1.77 ± 0.019	72.82 ± 0.010
	Izmarg (Gurez)	40.10 ± 0.510	7.07 ± 0.017	31.38 ± 0.011	7.79 ± 0.012	92.34 ± 0.012
	Dachigam	16.36 ± 0.232	3.62 ± 0.019	28.88 ± 0.015	1.45 ± 0.018	78.68 ± 0.011
	Yusmarg	47.06 ± 0.403	1.612 ± 0.024	23.45 ± 0.014	1.38 ± 0.02	67.58 ± 0.009
	Uri	48.38 ± 0.753	2.24 ± 0.023	20.86 ± 0.012	2.87 ± 0.06	71.34 ± 0.018
CD		1.355	0.039	0.034	0.047	0.054
CV		27.88	87.53	41.15	114.61	41.40

Macro and micronutrient analysis of soil

Total and available soil macroelements were determined prior to harvest. Soil samples from wild habitats had significantly more total carbon (4.78–7.84%) than those from cultivated habitats (3.83–4.33%). Soil samples from wild habitats had relatively low sulfur contents (0.005–0.174%) compared with those from cultivated habitats (0.08–0.23%). Soil samples collected from wild habitats had more nitrogen (0.21–0.45%) than those from cultivated habitats (0.19–0.22%). There was no distinct trend for C/N ratio in soil samples collected from cultivated and wild habitats (Table 2b).

Soil samples from cultivated and wild habitats exhibited significant variation in macronutrients. Organic carbon ranged from 1.05 to 2.42% in soil samples from wild habitats and 0.7 to 2.23% in soil samples from cultivated habitats. The maximum critical difference (10.032 and 12.45) was observed in available phosphorus and potassium, respectively, among soil samples collected from wild and cultivated habitats. Available nitrogen varied little in soil samples collected from cultivated and wild habitats (Table 2c).

Among the micronutrients, zinc had limited accumulation in both cultivated and wild habitats. Iron was at optimum levels ranging from 1.8 to 2.5 μg g^{-1} and 1.7 to 2.4 μg g^{-1} in soil samples from wild and cultivated habitats, respectively. Manganese ranged from 2.9 to 4.2 μg g^{-1} and 5.1 to 12.4 μg g^{-1}in soil samples from cultivated and wild habitats, respectively (Table 2c). Copper levels were below threshold values in both cultivated and wild soil samples but higher in the wild samples (0.36–0.45 μg g^{-1}).

Morphological evaluation of quantitative traits from wild and cultivated accessions

Since the wild and cultivated populations were growing at different altitudes, we maximized the sample number ($n = 10$) to procure average values for each trait. The plants from cultivated populations had more vigorous growth than the wild ecotypes. Of the investigated quantitative traits, plant dry mass, leaf width, and plant height varied among wild and cultivated ecotypes while stem diameter and distance between internodes had more consistent values (Table 3).

Chromatographic analysis of phytonutrients from wild and cultivated accessions

Wild accessions of *O. vulgare* L. contained more catechin (67.58–163.8 μg g^{-1}) and ascorbic acid (20.86–46.88 μg g^{-1}) than cultivated accessions (41.78–60.18 and 12.56–24.72 μg g^{-1}, respectively). For *O. vulgare* L., most of the phenolics are catechin and epicatechin, with catechin the main phenolics resource. Hence, it was surprising that the variation in catechin levels in wild accessions was considerably greater than cultivated accessions, about 41.40%, with wild accessions having about 2.7-fold more catechins than cultivated accessions (Table 4). In contrast, cultivated accessions had several-fold more arbutin than wild accessions. The relative proportions of arbutin varied among wild accessions, with only 16% in Dachigam but 30–41% in Gurez. Unlike the phenolics, quercetin followed no clear trend between wild and cultivated accessions (Table 4) and had the maximum variation (114.61%) among the tested accessions.

Discussion

This study describes the influence of environmental variables on the phytonutrient profiles of *O. vulgare* L. thriving in diverse cultivated and wild habitats.

Environmental variables modulate phytonutrient accumulation

Earlier research illustrated how phytochemical and phytonutrient profiles of crops change on the basis of environmental/ecophysiological parameters (Björkman et al. 2011; Ahmed et al. 2014a, b) and management strategies (Ahmed et al. 2013; Barański et al. 2014). Variations in average rainfall, high temperature, carbon dioxide concentration, soil composition, herbivory, and agro-ecological parameters are contributing factors to the secondary metabolite and phytonutrient levels in plants (Tharayil et al. 2011; Myers et al. 2014). Climate variables such as high temperature, rainfall, humidity, solar radiation, and altitude affect crop quality, which can be determined by variation in secondary metabolite levels that modulates sensory properties and nutritional aspects for human consumers (Varshney et al. 2011; Waha et al. 2013). Quality parameters may augment or decline in response to diverse climatic variables. The

sampling sites selected in this study were based on an extensive survey and information provided by the Center for Biodiversity and Taxonomy regarding the distribution of the major wild oregano locations in the valley of Kashmir. The majority of these sites were in hilly or mountainous sites with sloping, principally limestone-rich soil. Our results identified variation in altitude, PPFD, and UV flux among cultivated and wild populations of *O. vulgare* L. The differences in climatic parameters among the regions studied affected vegetative growth and phytonutrient values in *O. vulgare* L., with a distinct trend at the regional level.

Climatic and edaphic factors for crop quality

The significant and positive relationships observed between phytonutrients such as β-carotene, ascorbic acid, arbutin, catechin, and soil variables including pH, nitrogen, manganese, zinc, and organic carbon suggest that higher pH, high organic carbon, elevated levels of UV-B, and increased iron contents favor *O. vulgare* L. growth. In this study, pH values ranged from 6.3 to7.2, and the highest phytonutrient levels were associated with higher pH values (7.5 and 7.7). Variations in soil composition may influence crop quality in aromatic species (Azizi et al. 2009; Dudai and Belanger 2016). In oregano, Dudai (2006) cited macronutrient levels as the main soil factor affecting crop quality, mainly in the biosynthesis of phytonutrients. Across all the collection sites at different altitudes, *O. vulgare* plants had some similar morphological features including the number of branches, stem diameter, and the distance between internodes. Plants grown at higher altitudes tend to have wider, thicker, and more rounded leaf blades (Arzac et al. 2016; Lyu et al. 2017) and we noted wider leaves in wild populations thriving above 9500 ft. The increasing altitude together with higher UV flux leads to a progressive decline in plant height. Oregano plants growing at higher elevations (7861–9000 ft) were shorter than those at relatively lower elevations (4470–5000 ft), which is related to shorter growing seasons, lower temperatures, and higher UV-B flux (Larcher 1995). Reduced plant height and increased leaf width at higher elevations is an adaptive approach to avert mechanical damage due to intense winds (Körner 2003). In general, wild oregano plants growing in conditions of high nitrogen, alkaline pH, and high manganese and zinc levels had higher phytonutrient values. These observations agree with reports on Mexican oregano and other aromatic species, suggesting that plants link their phytonutrient value to climatic factors such as the macro- and micro-elemental composition of soil (Tuttolomondo et al. 2013). Remarkably, these climatic factors modulate phytonutrient profiles and the accumulation of secondary metabolites leading to improved aroma (Mikkelsen 2005). However, the variation observed among wild populations within a given bioclimatic region suggests that each population represents specific microclimatic and edaphic conditions that influence the secondary metabolism of individual plants.

Correlation in phytonutrient accumulation among wild and cultivated accessions

The HPLC analysis of phytonutrients showed distinct differences between the cultivated and wild populations of *O. vulgare* L. thriving under diverse environmental conditions. All cultivated accessions had a positive correlation between β-carotene and arbutin ($r^2 = 0.98$); as arbutin increased from 22 to 29 μg g^{-1}, β-carotene reached its maximum value (Fig. 1a). In contrast, wild accessions had a weaker relationship between β-carotene and arbutin ($r^2 = 0.61$); β-carotene increased as arbutin increased from 40 to 46 μg g^{-1}, but then declined with further increases in arbutin. The elemental stoichiometry of soil in diversemicroclimatic zones affects the plant metabolome, particularly under harsh conditions, thereby modifying its phytonutrient profile and maintaining an organism's flexibility to preserve optimal fitness (Lester 2006; Sardans et al. 2012).

As arbutin increased in cultivated accessions, ascorbic acid declined, while the reverse was true for the wild accessions—as arbutin declined from 31 to 29.38 μg g^{-1}, ascorbic acid increased from 12.56 to 46.88 μg g^{-1} (Fig. 1b). As arbutin accumulated to its maximum value of 48.38 μg g^{-1}in wild accessions, ascorbic acid declined to 20.86 μg g^{-1}. In cultivated accessions, there was a meager shift; as ascorbic acid accumulated to 14.46 μg g^{-1}, arbutin reached a maximum value of 53.42 μg g^{-1}. Elemental stoichiometry resolves the competence of an organism to produce phytonutrients and thus to contour metabolomic responses. Hence, for C/N/P/K biomass stoichiometry, seasonal and climatic variations in ecological ratios of macronutrient availability determine the metabolomic response in high altitude herbs (Sardans et al. 2011a, b).

In cultivated accessions of *O. vulgare* L., as arbutin increased, quercetin decreased ($r^2 = 0.99$), but there was no

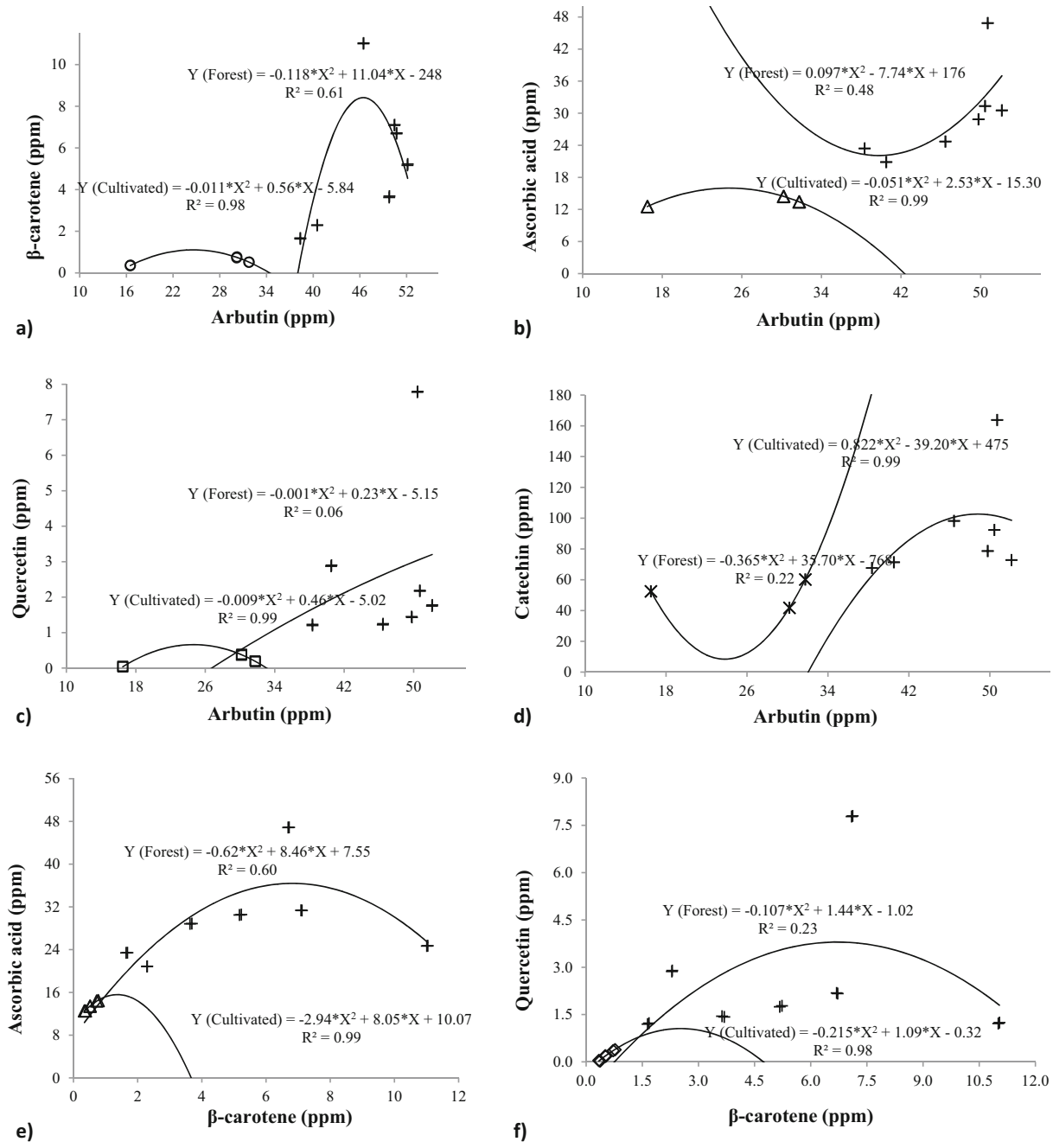


Fig. 1 Relationship between phytonutrient concentrations in *Origanum vulgare* L. as influenced by dissimilar land use in the northwest Himalayan region

association in wild accessions ($r^2 = 0.06$) (Fig. 1c). Nevertheless, there was a steep increase in arbutin as quercetin increased. Increase in quercetin is related to altitude as UV flux and PPFD (Neugart et al. 2012; Barnes et al. 2015; Nenadis et al. 2015). The UV flux/PPFD ratio has a significant effect on escalating metabolites, which has a potential role in plant defenses under intense sunlight (Schreiner et al. 2012; Shimizu 2016). In contrast, at higher altitudes, the dense forest cover at Dachigam interferes with PPFD and UV flux thereby reducing arbutin. The radical shifts in the elemental stoichiometry of soil in cultivated and wild accessions may reallocate the

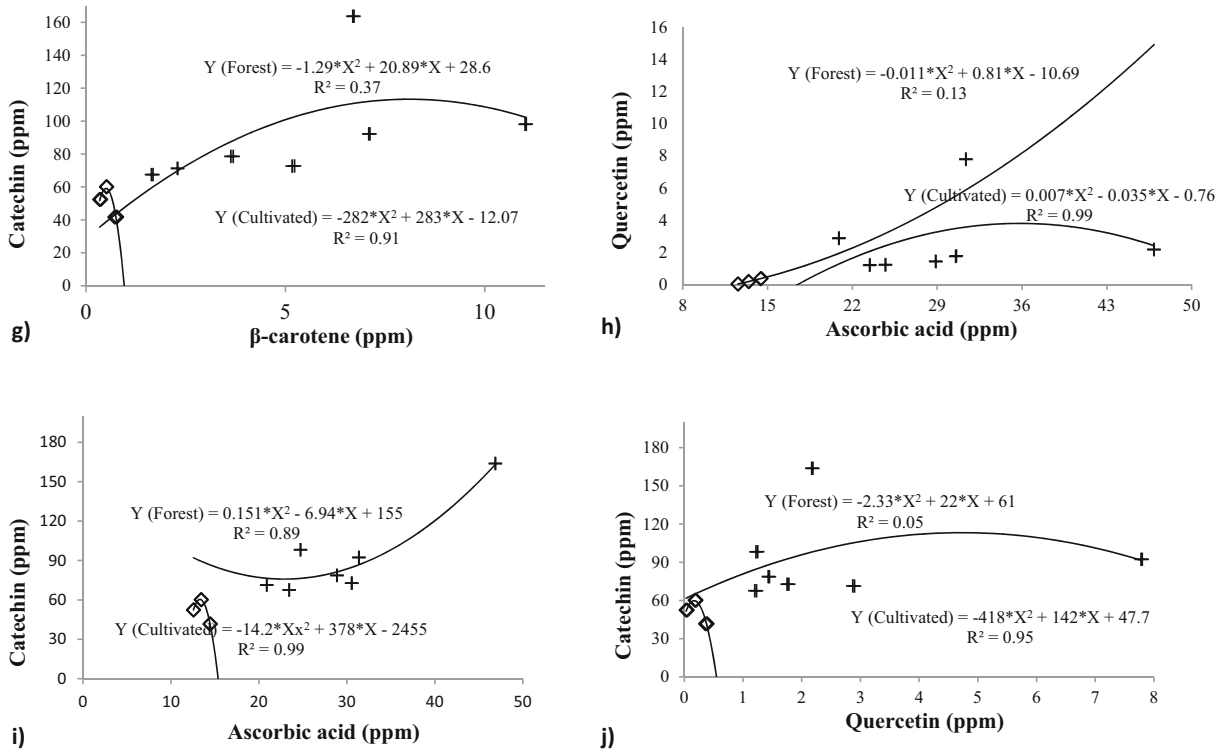


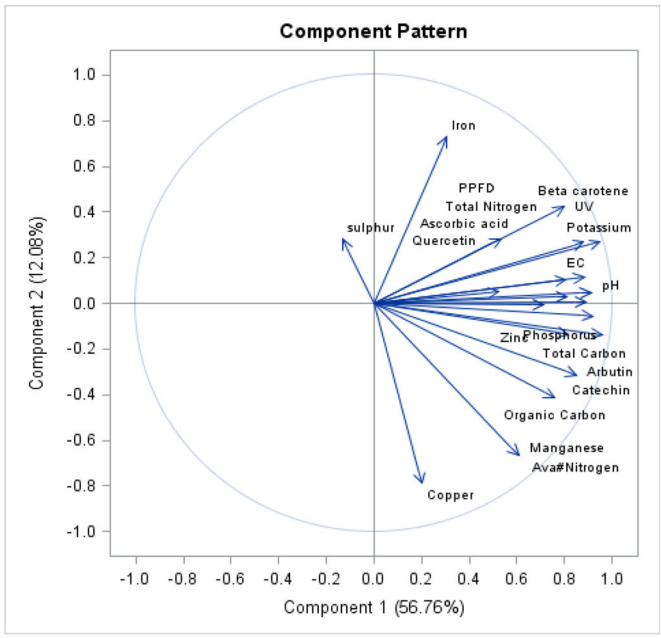
Fig. 1 (continued)

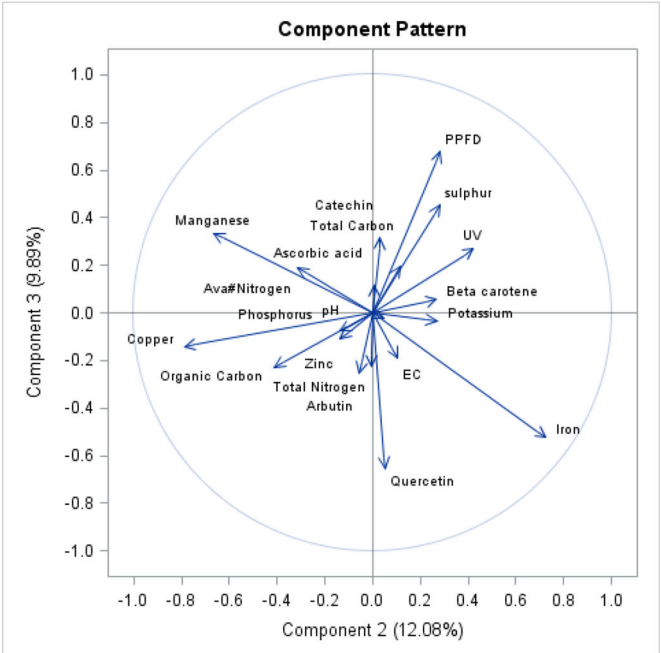
metabolome in response to diverse microclimatic variations such as high UV flux and low temperature (Sardans et al. 2011a, b, 2012). Our results confirm our hypothesis that a strong correlation exists between elemental stoichiometry and phytonutrient profiles. The integration of stoichiometry with ecometabolomics can advance our perceptive of developmentally and ecologically associated shifts in C/N/P/K levels to acquire an optimal distribution for growth and other functions for example storage, defense, reproduction, or resistance to stress (Gargallo-Garriga et al. 2015). This integration improves our preceptive of the impacts of stoichemetry on the lifecycle of the organisms and the constitution, role, and development of the ecosystems (Rivas-Ubach et al. 2013; Gargallo-Garriga et al. 2016).

Catechin and arbutin were strongly correlated (r^2 = 0.99) in cultivated accessions of *O. vulgare*, but much less so in wild accessions (r^2 = 0.22). In wild accessions, as catechin reached a maximum value of 163.8 μg g^{-1}, arbutin dropped to 29.38 μg g^{-1}, after that there was a concomitant trend among two metabolites (Fig. 1d). Catechins tended to be higher in soils with more organic carbon such as calcareous soils in the case of Kanzalwan (Gurez) while the highest arbutin level occurred at Uri with high UV flux. Catechins are basic phenolics that accumulate in response to abiotic stress, as is the case with arbutin (Kirakosyan et al. 2004; Chobot et al. 2009).

Ascorbic acid and β-carotene increased concurrently and were strongly correlated (r^2 = 0.99) in cultivated accessions of *O. vulgare*. In wild accessions, as β-carotene increased from 4 to 7 μg g^{-1}, ascorbic acid increased to 46.88 before immediately dropping to 20.86 μg g^{-1} (Fig. 1e). Ascorbic acid and β-carotene are phytonutrients that accumulate in response to the macro- and micro-elemental composition of soil (Singh et al. 2012; Landi et al. 2013). Ascorbic acid decreases with increasing temperature while carotene increases with temperature increases from low to medium but decreases as temperatures rise (Wildi and Lutz 1996; Dumas et al. 2003).

Quercetin increased with increasing β-carotene in cultivated accessions of *O. vulgare*. In wild accessions, as β-carotene increased from 6 to 7 μg g^{-1}, quercetin accumulated to a maximum of 7.79 μg g^{-1} and then declined (Fig. 1f). Quercetin is a secondary metabolite that accumulates in response to low temperature and high light intensity as described by high UV flux and





Eigenvalues of the Correlation Matrix				
	Eigenvalue	Difference	Proportion	Cumulative
1	11.3520935	8.9370280	0.5676	0.5676
2	2.4150655	0.4371092	0.1208	0.6884
3	1.9779563	0.3781301	0.0989	0.7873
4	1.5998261	0.4584134	0.0800	0.8672
5	1.1414127	0.3980092	0.0571	0.9243
6	0.7434035	0.2400797	0.0372	0.9615
7	0.5033238	0.3389591	0.0252	0.9867
8	0.1643647	0.0618108	0.0082	0.9949
9	0.1025539	0.1025539	0.0051	1.0000
10	0.0000000	0.0000000	0.0000	1.0000
11	0.0000000	0.0000000	0.0000	1.0000
12	0.0000000	0.0000000	0.0000	1.0000
13	0.0000000	0.0000000	0.0000	1.0000
14	0.0000000	0.0000000	0.0000	1.0000
15	0.0000000		0.0000	1.0000

Fig. 2 Principal component analysis of environmental variables and phytonutrient concentrations in *Origanum vulgare* L. as influenced by dissimilar land use in the northwest Himalayan region

PPFD (Sardans et al. 2011b), while β-carotene is a primary phytonutrient that increases in response to a richer soil environment (Lester 2008; Rosales et al. 2010). Catechin increased with increasing β-carotene in cultivated accessions (Fig. 1g). In wild accessions, β-carotene increased to 7 μg g^{-1} with a corresponding increase in catechin, after which catechin levels declined. The cultivated populations of *O. vulgare* had lower values of quercetin and ascorbic acid, with a strong correlation between the two ($r^2 = 0.99$). In wild accessions, quercetin increased with increasing ascorbic acid to 30–32 μg g^{-1}, after which it declined abruptly

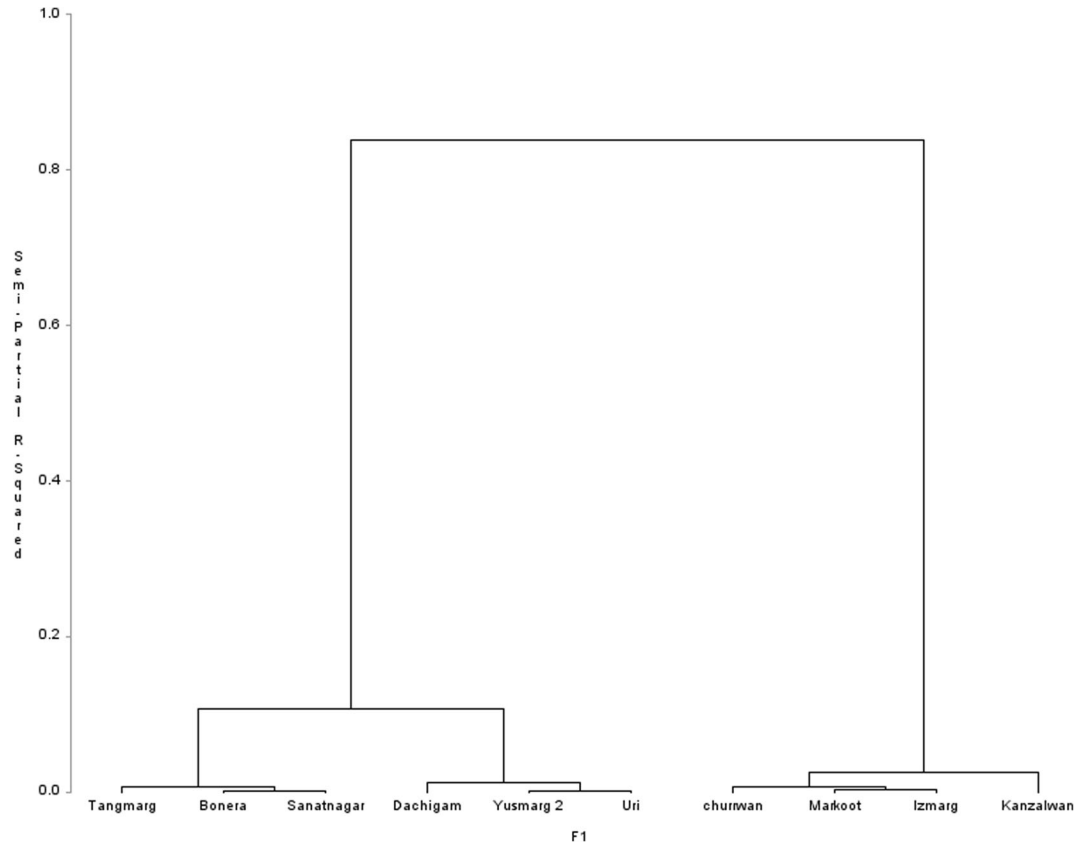


Fig. 3 Cluster analysis of environmental variables and phytonutrient concentrations in *Origanum vulgare* L. as influenced by dissimilar land use in the northwest Himalayan region

(Fig. 1h). Wild accessions had a strong correlation between catechin and ascorbic acid ($r^2 = 0.89$), increasing to a maximum before gradually dropping (Fig. 1i). A similar trend was observed in cultivated populations. In cultivated accessions, catechin decreased as quercetin decreased, while in wild accessions, catechin and quercetin reach a maximum together and then declined gradually (Fig. 1j). Correlation among environmental variables and phytonutrients is described in supplementary Tables 2, 3 and 4.

The PCA factor loadings, percentage of variance explained, and cumulative variance for the extracted PCs are given in Fig. 2. PCA using wild and cultivated populations and different environmental variables and phytonutrients showed that the first two components explained 68.84% of the total variation. First principal component (PC1) accounted for 56.67% of the total variation and the second PC (PC2) explained 12.08% of the total variation and (PC3) contributes only (9.89%) variation. Hierarchical cluster analysis grouped populations into two clusters based on variation in environmental variables (Fig. 3). Cluster 1 consisted of six populations with two groups one having three cultivated populations (Tangmarg, Bonera, and Sanatnagar) and three wild populations (Dachigam, Yusmarg, and Uri) while cluster 2 grouped into two having one group containing Churwan, Markoot, and Izmarg and another having Kanzalwan wild population. Hence, study demonstrates the population from Gurez highly exclusive with respect to variation among environmental variables.

Conclusion

Considerable geographic variation in phytonutrient profiles predominantly quercetin, β-carotene, catechin, and ascorbic acid of Himalayan oregano was observed across bioclimatic regions, as well as between populations and individuals. On the contrary, arbutin exhibited least variation within different ecotypes of *O. vulgare*. Populations of *O. vulgare* located in microclimatic regions with high organic carbon had, on average, higher

phytonutrient values. This study showed that edaphic variables help to explain the variability observed in phytonutrient profiles. Plants established on soils with higher pH and higher nitrogen and iron contents presented higher phytonutrient levels and more vigorous vegetative growth. The fact that much of the variation was explained among individuals within populations suggests that micro-environmental and genetic factors should be considered when explaining the variability observed in *O. vulgare* in phytonutrient profiles.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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