Essential Oil Content and Constituents of Black Zira (*Bunium persicum* [Boiss.] B. Fedtsch.) from Iran During Field Cultivation (Domestication)

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Abstract

Bunium persicum fruit oils from wild type (WT), first (CY1) and second year (CY2) cultivars (fourth and fifth year plants) were analyzed by GC and GC/MS. The essential oil content of the WT (9.1% v/w) was higher than the oil content of the CY1 (6.2% v/w) and CY2 (5.1% v/w). No significant differences were found with respect to the oil constituents. The main constituents were γ-terpinene (WT: 44.2%, CY1: 40.8%, and CY2: 36.8%), associated with cuminaldehyde (WT: 16.9, CY1: 14.1, and CY2: 11.8%), and γ-terpinen-7-al (WT: 10.5, CY1: 10.6, and CY2: 18.7%). In total 35 components could be identified covering 95.4% (WT), 94.9% (CY1) and 96.3% (CY2) of the oil content. We also studied the phenology of the plants during the period 1999–2004.

Key Word Index

Bunium persicum, Apiaceae, essential oil composition, γ-terpinene, p-cuminaldehyde, γ-terpinen-7-al (p-mentha-1,4-dien-7-al).

Introduction

(*Bunium persicum* [Boiss.], Fedtsch), Black Zira (Zireh e Irani, Zireh kuhi) is an important aromatic plant that belongs to the Apiaceae family. It originates from central Asia to North India. According to the literature fruits of *B. persicum* contain nearly 9% essential oil (1). The seeds are consumed widely as a condiment. In the indigenous system of medicines, seeds are regarded as stimulants, carminatives and found to be useful in diarrhea and dyspepsia (2). In addition the plant is used for culinary purposes and flavoring foods and beverages (3).

It is a perennial plant producing a tuberous root at 10 cm depth of the soil. After flowering, the fruits from wild collected plants (WT) as well as first (CY1) and second year (CY2) cultivars were harvested and the oils contents and constituents determined by hydrodistillation and GC/MS analysis, respectively.

The aim of this study was to cope with the fact that after recent relentless extraction of seeds obtained from wild, the plant has been forced into the endangered category. The prime cause of depletion has been found to be the thoughtless and unscientific commercial collection of its seeds (a.o. in Iran) for rapid financial gains. The competition for its seeds is so severe that, instead of collecting the ripe seed, the entire plant is removed even when the seeds are immature.

Medicinal plants are a living resource, exhaustible if overused and sustainable if used with care and wisdom. At present 95% of the collection of medicinal plants is from wild habitats. Current practices of harvesting are unsustainable and many studies have highlighted depletion of the resource base. Several medicinal plants have been assessed as endangered, vulnerable and threatened due to over harvesting or unskillful harvesting in the wild. The other main source of medicinal plants is from cultivation. Cultivated material is infinitely more appropriate for use in the production of drugs. Indeed, standardization whether for pure products, extracts or crude drugs are critical and while become increasingly so, as quality requirements continue to become more stringent. Excessive collection from the wild has resulted in significant erosion of the plant. Field cultivation is necessary for inhibition of this process.

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Table I. Physicochemical properties of the soil of the cultivation site of *Bunium persicum* (Feiz Abad)

K (ppm)	P (ppm)	N (ppm)	OC (%)	EC (dS/m)	pH	Soil texture
147	9.3	300	0.62	1.3	7.7	Sandy loam

OC = Organic content; EC = Electrical conductivity; dS/m = Desi simence/meter (the dimension of electrical conductivity of soil).

Table II. Environmental conditions of wild population of *Bunium persicum* and of its cultivation site

Place	Name of site	Latitude (North degree)	Altitude m	Min temp (°C/year)	Max temp (°C/year)	Precipitation (mm/year)
Wild population site	Kalat-e-Nader Mountains	34°3'–37°5'	2850	-20	+20	320
Cultivation site	Feiz Abad	34°4'–35°8'	1000	-12	+40	185

In this research we conducted a field experiment to find out the possible field production and establishment of the plant in Iran and to study the change in content and chemical composition of its oils during field propagation. In parallel we investigated the most suitable time for harvesting and phenology of the plant during the period 1999–2004.

Experimental

Plant material: The wild sample material of *Bunium persicum* [Boiss.], Fedtsch (Apiaceae) was collected in June 1999 from the Kalat-e-Nader Mountain in the Khorasan province of Iran and dried in the shade at about 25°C. The cultivated seeds were collected after 4 years (2003, CY1) and 5 years (2004, CY2) from Feiz Abad. Voucher specimens have been deposited (#24018¨FUMH¨) at the Herbarium of the Ferdowsi University of Mashhad.

Wild collected seeds with eliminated weak and unripe seeds were selected for cultivation in the open field (1 Dec) in rows of 50 cm apart and in 2 cm depth of the soil and irrigated. The seeding density was 3 g/m^2. The physicochemical properties of the field soil (Feiz Abad) and the environmental conditions are shown in Table I and in Table II.

Essential oil: *Isolation procedure:* The oil sample was isolated from 16.0 g of freshly ground (1 min in an IKA mill M20) seed material by hydrodistillation for 4 h in 300 mL water, according to the determination of the oil content in vegetable drugs, using the apparatus described in the Nederlandse Farmacopee, 6th edition, 2nd printing (4). Xylene (100 µL) was used as the collection liquid, and the oil was stored at -20°C until analyzed. The oil was diluted 50 times with cyclohexane prior to GC and GC/MS analysis.

In addition, the oil was separated into two fractions with hydrocarbons and oxygen-containing compounds, respectively, by eluting 250 µL of oil on a Bakerbond SPE column, filled with 1 g of silica gel (#7086-07, J.T. Baker, Deventer, The Netherlands), with subsequently 5 mL hexane and 5 mL diethyl ether. After gentle evaporation of the solvents of both fractions, 50 µL of each residue were diluted with 950 µL cyclohexane and submitted to GC and GC/MS analysis.

Gas chromatography: GC analysis was performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a 7673 injector and a Hewlett Packard 3365 Series II Chemstation, under the following conditions: column, WCOT fused-silica (J&W) DB-5 (30 m x 0.26 mm; film thickness 0.25 µm); oven temperature program, 60–290°C at 3°C/min; injector temperature, 250°C; detector (FID) temperature, 300°C; carrier gas, He; inlet pressure, 18 psi; linear gas velocity, 31.8 cm/s; split ratio, 56:1; injected volume, 1.0 µL.

Gas chromatography-mass spectrometry: A Shimadzu GC/MS QP5000 system was used equipped with a GC-17A gas chromatograph, an AOC-20i auto injector, and GC/MS solution version 1.10 software. The GC conditions were: column, WCOT fused-silica (J & W) DB-5 (30 m x 0.26 mm; film thickness 0.25 µm); oven temperature program, 60–240°C at 3°C/min; injector temperature, 275°C; carrier gas, He; inlet pressure, 75 pKa; linear gas velocity, 81.4 cm/s; column flow, 2.5 mL/min; total flow, 56.7 mL/min; split ratio, 21:1; injected volume, 1.0 µL. MS conditions: ionization energy, 70 eV; ion source temperature, 250°C; interface temperature, 250°C; scan speed, 3 scans/s; mass range, 34–350 u.

The identity of the components was assigned by comparison of their retention indices, relative to C_9-C_{16} n-alkanes, mass spectral databases and from the literature (5–7). The percentages of the components were calculated from the GC peak areas, using the normalization method.

Results and Discussion

The oil content of the WT (9.1% v/w) was higher than the oil content of the CY1 (6.2% v/w), and CY2 (5.1% v/w) cultivated plants of *Bunium persicum*. The percentage monoterpene hydrocarbon fraction of all three investigated samples was comparable, with 95.4% (WT), 94.9% (CY1) and 55.7% (CY2) of the total oil. γ-Terpinene was the main monoterpene

Table III. Composition of the fruit oils of *Bunium persicum* wild type (WT), first (CY1) and second year (CY2) cultivation

Name	RI[a]	WT(%)	CY1(%)	CY2(%)
α-thujene	925	0.4	0.5	0.5
α-pinene	932	1.0	1.5	1.3
camphene	946	0.1	0.2	0.1
sabinene	970	1.2	1.2	1.1
β-pinene	975	1.6	2.2	2.1
myrcene	990	1.0	1.2	1.1
δ-3-carene	1002	tr[b]	tr	tr
isosylvestrene	1013	0.3	0.3	0.2
p-cymene	1019	8.0	9.5	9.4
limonene	1025	2.0	2.5	2.4
1.8-cineole	1026	2.9	5.3	4.9
(Z)-β-ocimene	1037	0.1	tr	0.1
γ-terpinene	1055	4.2	0.8	6.8
3-methylbenzaldehyde	1059	tr	tr	tr
cis-sabinene hydrate	1061	tr	tr	tr
terpinolene	1085	0.7	0.9	0.6
linalool	1093	0.1	0.1	0.1
trans-sabinene hydrate	1095	0.1	0.1	0.1
borneol	1162	0.1	tr	0.1
terpinen-4-ol	1170	0.4	0.1	0.4
α–terpineol	1189	tr	tr	tr
m-cuminol	1217	tr	tr	tr
p-cuminaldehyde	1231	16.9	14.1	11.8
trans-o-menth-2-en-7-ol	1261	0.2	0.1	0.1
perillaldehyde	1266	0.2	0.1	0.1
bornyl acetate	1281	2.9	2.1	3.3
α-terpinen-7-al	1280	0.4	0.7	0.5
γ-terpinen-7-al	1287	10.5	10.6	18.7
thymol	1289	0.1	0.1	0.1
9-epi-β-caryophyllene	1413	tr	tr	tr
germacrene D	1474	0.1	0.2	0.1
ar-curcumene	1476	tr	0.1	0.1
zingiberene	1490	tr	0.2	0.1
(EE)-α-farnesene	1503	tr	0.2	0.1
β-sesquiphellandrene	1518	0.1	0.1	0.1
Identified (%)		95.6	95.0	96.4
Grouped components				
Monoterpene hydrocarbons		60.6	60.8	55.7
Oxygen-containing monoterpenes		34.6	33.4	35.3
Sesquiterpene hydrocarbons		0.2	0.8	0.5
Oil yield (% v/w)		9.1	6.2	5.1

[a]Retention index relative to C_9-C_{16} n-alkanes on the DB-5 column; [b]trace (< 0.05%)

hydrocarbon with 44.2% (WT), 40.8% (CY1) and 36.8% (CY2). The p-cymene content was 8.0% (WT), 9.5% (CY1) and 9.4% (CY2).

Also the oxygen-containing monoterpene content was comparable with 34.8%, 33.4%, and 35.3% for the WT, CY1, and CY2, respectively. Cuminaldehyde in the cultivated plants decreased from 14.1% in the first year, to 11.8% in the second year. The WT contained 16.9% cuminaldehyde. γ-Terpinen-7-al (p-mentha-1,4-dien-7-al) was present in the highest amount in CY2 (18.7%), where WT and CT1 contained lower amounts: 10.5% and 10.6%, respectively.

One unknown oxygen-containing monoterpene with a retention index (RI) of 1187 was detected in all samples, but could not be identified. Based on a comparison of the mass fragmentation pattern of this component with perillaldehyde (RI 1266), the unknown compound is likely to be an isomer. This unknown component was present with 1.9% (WT), 1.3% (CY1) and 0.6% (CY2). The amount of perillaldehyde present was much lower, with 0.2% (WT) and 0.1% (CY1 and CY2). The mass fragmentation pattern of the unknown aldehyde was: *m/z* (rel. int.): 152[M] + (32), 136(3), 121(30), 109(79), 91(37), 81(87), 79(85), 67(74), 39(58), 43(76), 41(100). The sesquiterpene hydrocarbon content in all investigated material was less than 1.0%, whereas no oxygenated-sesquiterpenes hydrocarbons could be detected. A complete overview of the identified constituents is given in Table III.

In the literature a number of studies on the essential oil

content and constituents of *B. persicum* have been reported.

Thappa et al. (8) found almost the same amounts of γ-terpinene (25.6–42.9%), and significant higher amounts of p-cymene (24.0–27.8%) in the wild source of *Bunium persicum* and less aldehydes compared to our results. In the seeds from cultivated sources, they found high amounts of cuminaldehyde (27.3–34.1%), p-mentha-1,3-dien-7-al and p-mentha-1,4-dien-7-al (29.6–36.8%). Our investigations showed a higher amount of γ-terpinene (44.2%) but much lower p-cymene content (8.0%) in the wild type. The cuminaldehyde content in our investigated cultivars CY1 and CY2 were much lower, with 14.1% and 11.8%, respectively. Also our α-terpinen-7-al (p-mentha-1,3-dien-7-al, 0.5%–0.7%) and γ-terpinen-7-al (p-mentha-1,4-dien-7-al, 10.6–18.7%) content is much lower than in the study of Thappa et al. (8).

Sadykov et al. (9) investigated the essential oil (yield 2.5%) and described p-cymene (19.2%) and cuminaldehyde (40.7%) as the main components. Minor components were α- and β-pinene, limonene, camphor, acetic, propionic, butyric, oleic, and benzoic acid. The amount of the main components is significantly higher (8.0–9.4% for p-cymene, and 11.8–16.9% for cuminaldehyde) than in our study.

Fruits of Pakistani origin *Bunium persicum* yielded between 5.3% and 7.1% oil (10). The main components were γ-terpinene (19.8–28.9%), cuminaldehyde (14.8–22.5%), p-cymene (12.4–32.8%), p-mentha-1,3-dien-7-al (4.8–7.2%), and p-mentha-1,4-dien-7-al (3.5–11.2%). One chemotype did not contain any aldehydes, but p-cymene (41.1%), γ-terpinene (24%), linalool (16%), dillapiol (4%), and limonene (3.6%) were the main components (10).

Ripe fruits, collected in Tajikistan (7.3% oil) contained cuminaldehyde (40.7%) and p-cymene (19.2%) as the main components. Fruits from Kulob (Tajikistan) yielded 3.3% oil. Here p-mentha-1,4-dien-7-al (29.0%), γ-terpinene (27.7%), β-pinene (15.6%), cuminaldehyde (11.7%) and p-mentha-1,3-dien-7-al (5.1%) were the main components (2).

The results from our studies thus differ substantially from these earlier studies reported in the literature.

According to Foroumadi, fruits from wild sources, collected from Siriz to Zarand (Kerman province, Iran) yielded 3.1% oil. In this oil 25 components were identified. The main components were cuminaldehyde (27.0%), γ-terpinene (25.8%), p-cymene (12.1%) and cumin alcohol (6.0%). Our results are more in agreement with this study, although the oil content we found was considerably higher (9.1%). In addition, γ-terpinen-7-al was not detected (11).

The most recent study on *B. persicum* from Iran is the paper by Pourmortazavi et al. (3). They investigated the differences in oil constituents obtained by solid phase extraction (SPE) and hydrodistillation. Remarkable differences were found between the quantitative amounts of the components. α-Methyl-benzenemethonol was present in these samples, but we could not detect this component in our experiments. Most of the components are present in a higher amount in the hydrodistillation sample. The amount of γ-terpinene (45.7–44.2% (WT)) and cuminaldehyde (12.7–16.9% (WT)) was more in agreement with our results. Remarkable is the absence of α-terpinen-7-al as well as γ-terpinen-7-al in both samples where we found 10.5% in the WT.

Seed oil (7.3%) from India contained α-pinene (1.5%), β-pinene, a combination of p-cymene, limonene, phellandrene and cineole (34.0%), γ-terpinene (43.6%), cuminaldehyde (9.4%), and a combination of 1,3-p-menthadiene-7-al and 1,4-p-menthadienal (8.7%) (12). These results are in agreement with our results.

The oil composition and pharmacological activity of the oil were investigated by Khaidarov et al. Thirteen components were identified. p-Cymene and cuminaldehyde were isolated. Other identified components were lactic acid, acetic acid, benzoic acid, oleic acid, camphor, α-pinene, thymol, citronellol, β-pinene, and limonene (13). No concentrations for these identified components are given.

The main components of *B. persicum* oil investigated in 1994 (14) were myrtenal (24.2%), cuminaldehyde (29.8%) and safranal (22.3%). Other components identified were p-cymene (2.8%), β-pinene (0.2%), limonene (0.9%), α-pinene (10.4%), thymol (1.1%), perilla alcohol (5.0%), terpinene-4-ol (1.0%) and γ-cadinene (1.4%). Remarkable is the difference in composition of this oil in comparison with our investigations and other results from the literature. The authors may have investigated another *Bunium* species or another chemotype.

The antioxidative activity of four oils from the Umbelliferae family from Pakistan has been investigated without any remarks concerning the composition of these oils. The authors investigated among others *B. cylindricum* and *B. persicum* (15). The seeds of *B. cylindricum* are mainly used as an adulterant of *B. persicum* (15). The main components of the white seed oil of *B. cylindricum* (1.4%) were limonene (13.7%), myristicin (67.2%) and cuminaldehyde (2.1%). The main components of the black seed oil of *B. cylindricum* (1.8%) were β-selinene (10.9%), cadinene (13.4%), dillapiol (11.0%), elemicin (39.3%) and myristicin (4.1%) (16). The differences between these oils and the *B. persicum* oil can be a marker for adulterants.

From our study and after comparison with previously conducted research on the oil composition of *B. persicum* fruit oil, it can be concluded that big differences appear to exist indicating the possible occurence of chemotypes in this species. Differences may be ascribed both to genetical variation (existence of chemotypes) and to environmentally determined fluctuation (soil, climate). These results are of importance not only for the taste but also for the use as spice. Further investigations should be undertaken to obtain a spice with a constant high quality. A monograph on *Bunium persicum* fruits and fruit oil, in which quality parameters are defined, is to be compiled. Till now, there is no monograph about *B. persicum* in Iran.

Phenology

From the wild population the tuberous roots start to grow as soon as the snow is melting in March, and the fruits are collected after flowering in the same year.

Stratification of the seeds was necessary for successful germination. After seeding in the field in December, weak cotyledon leaves were produced after 62 days. Detection of

the seedling from weeds is very difficult. In early June the seedling dries and disappears. It switches over to dormancy till November (during the dry period of the year) but it forms a very small tuberous root (0.1–1 g) in the ground surface (10 cm depth), After that period it re-grows in the middle of November and produced a true leaf in March and tuberous roots growth in the soil. Finally it can produce flowering stems 4–5 years after seed cultivation. As a result we showed that after seed germination in early March, in four years a tuberous root can grow to a diameter of 4 cm and can produce flowering stems.

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