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### Essential Oil Composition of *Mentha longifolia* from Wild Populations Growing in Tajikistan

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Keywords: Carvone, *cis*-piperitone epoxide, horse mint, menthone, piperitenone oxide, pulegone, thymol, wild mint.

#### ABSTRACT

Mentha longifolia selections, collected from three different sites in south-central Tajikistan, were analyzed to determine essential oil constituency. Essential oils were extracted by hydrodistillation of the plants and subsequently analyzed by gas chromatography - mass spectrometry. A total of 82 compounds were identified, representing 84.5-99.0% of total oil composition. Although qualitatively similar, the Tajikistan M. longifolia samples did show quantitative differences. The major components and their percentage of the oil were *cis*-piperitone epoxide (7.8-77.6%), piperitenone oxide (1.5-49.1%), carvone (0.0-21.5%), pulegone (0.3-5.4%), menthone (0.0-16.6%), thymol (1.5-4.2%),  $\beta$ -thujone (0.2-3.2%), carvacrol (0.0-2.7%), and (E)-caryophyllene (0.9-2.5%).

#### **INTRODUCTION**

The mints, *Mentha* species belonging to the family Labiatae (Lamiaceae), are widely distributed in Eurasia, Australia, and South and North Africa (Gulluce *et al.*, 2007; Lange and Croteau, 1999). Various species of *Mentha* have been used as folk remedies for treatment of bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints, due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharral activities (Al-Bayati, 2009; Džamić *et al.*, 2010; Gulluce *et al.*, 2007; Hajlaoui *et al.*, 2010; Hussain, 2009; Mimica-Dukić

*et al.*, 1991; Mkaddem *et al.*, 2009; Oyedeji and Afolayan, 2006; Rasooli and Rezaei, 2002; Viljoen *et al.*, 2006). The active virtues of the mints depend on the abundant volatile oils that contain a wide variety of terpenes and terpenoids.

The mint species, *Mentha longifolia* (L.) Huds., has been commonly used as a kitchen and medicinal plant for centuries. Known as wild mint and horse mint, the plant can reach to 1.5 m high in favorable conditions. The plant has a strong aroma.

The objectives of this study were to analyze the composition of the oil of *Mentha longifolia* growing wild in different areas of Tajikistan. In this report, the essential oil compositions of five samples collected at three sites in south-central Tajikistan. To our knowledge, no previous reports on *M. longifolia* essential oil in this area have been made.

#### MATERIALS AND METHODS

**Plant material.** Aerial parts of *M. longifolia* were collected from the three regions of Tajikistan. Samples numbered 1, 2, and 4 were gathered in the area of Korvon village, Dushanbe (38.506044N, 68.751535E, 800 m above sea level), on 25 April 2010. Sample number 3 was gathered in the area of Khonaobod village, Muminobod region (38.107547 N, 69.966431 E, 1200 m above sea level), on 7 May 2010, and sample number 5 was gathered in the Chormaghzak village area, Yovon region, (38.417502 N, 69.172175 E, 1300 m above sea level), on 25 July 2010. The plants were identified by F. S. Sharopov,

and a voucher specimens (TJ2010-031) have been deposited in the herbarium of the Chemistry Institute of the Tajikistan Academy of Sciences.

From the collected plant samples, 300 g of each were air dried, crushed into smaller pices, and hydrodistilled for 3 h, producing yellow colored essential oils at a yield of 0.5-0.9%. The oils were dried over sulfuric acid and subsamples were taken for analysis of the oil constituents.

**Essential oil analysis**. The essential oils were analyzed using an Agilent 6890 gas chromatograph connected to an Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/s). A fused silica capillary column (HP-5 ms, 30 m × 0.25 mm) coated with 5% phenyl-polymethylsiloxane (0.25 µm phase thickness) was used in the gas chromatography, the carrier gas was helium with a flow rate of 1 mL/min, and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 min at 40°C before ramping to 200°C at 3°C/min, and then to 220°C at 2°C/min. The interface temperature was 280°C.

A 1% w/v solution of each oil was prepared in  $CH_2Cl_2$  was prepared, and 1 µL of the samples was used in the analysis. The sample was injected into the gas chromatograph using a splitless injection. Identification of the oil components was based on their retention indices as determined by reference to a homologous series of *n*-alkanes (C<sub>9</sub>-C<sub>30</sub>), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007), and stored on the MS library [NIST database (G1036A revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080)]. The percentages of each essential oil component are reported as raw percentages based on total ion current without standardization.

**Numerical cluster analysis**. For comparison of the essential oil constituency, the 47 samples of *Mentha longifolia* were treated as operational taxonomic units (OTUs). The percentage of the 26 major essential oil components (carvone, piperitenone oxide, menthofuran, menthone, pulegone, *cis*-piperitone epoxide, 1,8cineole, *trans*-piperitone epoxide, *cis*-carveol, menthol, limonene, piperitone, (*E*)-caryophyllene,  $\beta$ pinene, *trans*-dihydrocarvone, isomenthone, diosphenol, germacrene D, borneol, myrcene,  $\alpha$ pinene, piperitenone, rotundifolone, thymol, *cis*dihydrocarvone, and menthyl acetate) were used to determine the chemical relationship between the different *M. longifolia* essential oil samples by cluster analysis using the NTSYSpc software, version 2.2 (Rohlf, 2005). Correlation was selected as a measure of similarity, and the unweighted pairgroup method with arithmetic average (UPGMA) was used for cluster definition.

#### RESULTS

A total of 82 compounds were identified in the essential oils extracted from *M. longifolia* plants collected in Tajikistan (Table 1). The identified oil compounds represented 84.5-99.0% of the total oil compositions. The major components of Tajikistan *M. longifolia* oil were *cis*-piperitone epoxide (7.8-77.6%), piperitenone oxide (1.5-49.1%), carvone (0.0-21.5%), menthone (0.0-16.6%), thymol (1.5-4.2%), pulegone (0.3-5.4%),  $\beta$ -thujone (0.2-3.2%), (*E*)-caryophyllene (0.9-2.5%), myrcene (0.3-2.5%), carvacrol (0.0-2.7%), borneol (0.9-1.8%), and *p*-cymene (0.2-1.9%).

Although qualitatively similar, the Tajikistan *M. longifolia* oils showed notable quantitative differences. For example, *cis*-piperitone epoxide was relatively abundant in all samples, ranging from a low of 7.8% in sample #1 from Dushanbe to 77.6% in sample #5 from Yovon. Similarly, piperitenone oxide had the lowest concentration in the Yovon sample (1.5%), but highest in Dushanbe #1 (49.1%). Neither carvone nor menthone were detected in Yovon #5, but were both present in the other oil samples.

#### DISCUSSION

*M. longifolia* essential oils from other geographical locations have been extensively studied. The species has demonstrated a great degree of morphological diversity (Gobert *et al.*, 2002), and the Missouri Botanical Garden lists some 276 subspecies, varieties, and forms (Missouri Botanical Garden, 2011).

				ole nur				5			Sam	ple nu	mhor	
RI	Oil constituent <sup>1</sup>	1	2	3	4	5		RI	Oil constituent	1	2	3	4	5
RI NI	On constituent	1		-		5		NI	Oli constituent	1	_	-	-	5
054	(25) Havenal			of total		0.1	-	1242	Comucia	0.0		of total		<u> </u>
-	(2E)-Hexenal		0.1			0.1	_		Carvone	0.8	1.1	10.2	21.5	
-	(2E)-Hexenol		0.1				-		<i>cis</i> -Piperitone epoxide	7.8	27.1	25.0	23.1	77.6
-	Santolina triene		0.1	0.1	0.1		-		cis-Chrysanthenyl acetate				0.5	
-	α-Thujene		tr <sup>3</sup>	0.1		tr	-		Unidentified	0.7				
	α-Pinene	0.1	0.2	0.1	0.2	0.4	-		trans-Carvone oxide			0.1		
	Camphene		0.1	0.1		0.2			Bornyl acetate	0.1	0.2	0.2		0.4
	Benzaldehyde		tr	tr					Dihydroedulan I	0.1			0.2	
	Sabinene		0.1	0.1	0.3	0.3	-		Isothymol	0.4		0.3		
	β-Pinene		0.1	0.1	0.2	0.6			Thymol	4.2	1.5	3.1	3.5	3.0
981	1-Octen-3-ol		tr	0.1			-		Diosphenol				0.1	0.7
992	Myrcene	0.8	2.2	1.9	2.5	0.3			Unidentified	0.6				
996	3-Octanol	0.2	0.6	0.5	0.7	0.9			6-Hydroxy-6-isopropyl-3- methylcyclohex-2-enone		0.7	0.9		3.2
1004	α-Phellandrene	0.2	0.3	0.2	0.3	0.1		1305	Carvacrol	2.7	0.8	2.5	1.7	
1016	α-Terpinene	0.1	0.1	0.1	0.1	0.1	ſ	1340	Piperitenone	0.6	0.3	0.3	0.4	
1024	<i>p</i> -Cymene	0.6	1.6	1.8	1.9	0.2	Ī	1368	Piperitenone oxide	49.1	29.4	28.2	20.4	1.5
1028	Limonene	0.1	0.3	0.2	0.6	0.9	Ē	1386	β-Bourbonene		0.1	0.2	0.2	
1030	1,8-Cineole	0.2	0.4	0.4	0.5	0.2	Ē	1388	Unidentified	1.3				
	Santolina alcohol	0.3	0.3	0.5	0.5		Ē	1392	4a-α,7-β,7a-α-Nepetalactone		0.1	0.2	0.1	
-	(Z)-β-Ocimene					0.1	-		Unidentified	0.7				
	Phenylacetaldehyde		tr			0.1			β-Elemene		0.1			
	( <i>E</i> )-β-Ocimene		tr	tr			_		(Z)-Jasmone		0.1	0.1		
	γ-Terpinene	0.3	0.4	0.4	0.6	0.2	-		(E)-Caryophyllene	1.4	2.1	1.7	2.5	0.9
-	cis-Sabinene hydrate			0.4			-		α- <i>trans</i> -Bergamotene		0.1			
	Terpinolene					0.1			Unidentified	6.4	0.3			
	3-Nonanol		tr			0.1			α-Humulene	0.2	0.2	0.2	0.2	
	trans-Sabinene hydrate			0.1					Unidentified	0.2			0.2	1.5
	Linalool	0.1	0.1	0.1	0.1	0.4			( <i>E</i> )-β-Farnesene	0.1	0.1	0.2	0.2	
	α-Thujone	0.1	0.1	1.1	0.1	0.4			Unidentified	0.1				
	β-Thujone	1.5	2.6	3.2	2.5	0.3			Unidentified	0.3	0.2	0.1	0.2	
							-							
-	<i>cis-p</i> -Menth-2-en-1-ol		tr	0.1			-		Germacrene D	0.3	0.6	0.3	0.7	0.1
	3-Octyl acetate		0.2	0.1	0.1				(E)-β-lonone			0.1		
-	Camphor		0.1	0.1		0.2			Bicyclogermacrene		0.1			
	Menthone	2.8	16.6	4.1	2.1		-		β-Bisabolene	0.3	0.3	0.5	0.1	
	Pinocarvone					0.1	-		δ-Cadinene			0.1		
	Borneol	1.6	1.8	1.0	0.9	1.4	-		(E)-Nerolidol	0.1	0.1			
	Menthol	0.2	0.1	0.1			-		Unidentified			0.6		
	cis/trans-Isopulegone		0.4	0.4	0.7		-		Spathulenol	0.9	0.5	0.5	0.3	
1176	Terpinen-4-ol	0.2				0.2			Caryophyllene oxide	1.6	0.9	1.4	0.7	
	<i>p</i> -Cymen-8-ol	0.3	0.1	0.4	0.5			1610	Unidentified		0.3	0.9		
1189	α-Terpineol	0.3	0.2	0.2	0.2	0.2		1635	Isospathulenol	0.2	0.1	0.1		
1195	Myrtenal	0.1	0.1			0.1	ſ	1642	(2 <i>S</i> ,5 <i>E</i> )-Caryophyll-5-en-12-al	0.2				
1195	cis-Dihydrocarvone			0.3	0.6		ſ	1654	α-Cadinol	0.1				
1220	Coahuilensol methyl ether	0.3	0.1	0.2	0.2	0.1	ſ	1669	14-Hydroxy-9- <i>epi</i> -( <i>E</i> )-caryophyllene	0.2				
1224	Citronellol	0.5					ľ	1685	Germacra-4(15),5,10(14)-trien-1-α-ol	0.1				
	Unidentified		0.1	0.2	0.5	1.4	-		14-Hydroxy-α-Muurolene	0.1				
-	Thymol methyl ether	0.1					-		Manool oxide	0.1				
-	Pulegone	1.6	1.6	2.6	5.4	0.3	╞		Total constituents identified	84.5	98.2	97.0	99.0	95.6
1	-						1		$392 - 4a_{\alpha} 7_{\beta} 87a_{\alpha}$ Nepet					55.0

Table 1. Chemical composition of Mentha longifolia essential oils from Tajikistan.

<sup>1</sup>Size of text in RI 1303 = 6-Hydroxy-6-isopropyl-3-methylcyclohex-2-enone,  $1392 = 4a - \alpha, 7 - \beta, 7a - \alpha$ -Nepetalactone, 1642 = (2S, 5E)-Caryophyll-5-en-12-al, 1669 = 14-Hydroxy-9-*epi*-(*E*)-caryophyllene, and 1685 = Germacra-4(15), 5, 10(14)-trien-1- $\alpha$ -ol was reduced to fit table. Constituent identification based on RI & MS matching using Adams (2007) and NIST database. tr = trace (<0.01%).

With the extent of morphological diversity in M. longifolia, a great degree of chemical variation in the species might be expected as well. Indeed, results from previous studies on several wild and cultivated *M. longifolia* have produced a number of chemotypes (Table 2). Identified chemotypes of M. longifolia include those dominated by piperitenone oxide (Baser et al., 1999; Gulluce et al., 2007; Hussain, 2009; Maffei, 1988; Mastelic and Jerkovic, 2002; Rezaei et al., 2000; Sharipova et al., 1983; Venskutonis, 1996; Viljoen et al., 2006), piperitone epoxide (Baser et al., 1999; Fleisher and Fleisher, 1998; Fraisse et al., 1985; Hussain, 2009; Karousou et al., 1998; Kokkini and Papageorgiou, 1988; Vidal et al., 1985; Viljoen et al., 2006), carvone (Banthorpe et al., 1980; Fraisse et al., 1985; Kokkini et al., 1995; Lawrence, 1978; Lawrence, 2007; Mastelic and Jerkovic, 2002; Monfared et al., 2002; Vidal et al., 1985; Younis and Beshir, 2004), menthone (Fraisse et al., 1985; Hajlaoui et al., 2010; Mimica-Dukić et al., 2003; Oyedeji and Afolayan, 2006; Vidal et al., 1985), pulegone (Fleisher and Fleisher, 1991; Gulluce et al., 2007; Hajlaoui et al., 2010; Mkaddem et al., 2009; Oyedeji and Afolayan, 2006), piperitone (Džamić et al., 2010; Ghoulami et al., 2000; Rasooli and Rezaei, 2002; Rezaei et al., 2000), trans-dihydrocarvone (Džamić et al., 2010; Matovic and Lavadinovic, 1999; Mimica-Dukić et al., 1991), isomenthone (Mimica-Dukić et al., 1991; Mimica-Dukić et al., 2003; Mkaddem et al., 2009), menthofuran (Mimica-Dukić et al., 1991; Viljoen et al., 2006), menthol (Al-Bayati, 2009; Hajlaoui et al., 2010), 1,8-cineole (Fleisher and Fleisher, 1998; Oyedeji and Afolayan, 2006), isopiperitenone (Rezaei et al., 2000), piperitenone (Ghoulami et al., 2000), and borneol (Hussain, 2009).

Comparing the results obtained for our *M*. longifolia plant samples with those reported for the same species from other locations of the world reveals profound differences in essential oil composition. A cluster analysis (Figure 1) illustrated the numerous different chemotypes of *M*. longifolia and showed that the essential oil of Tajikistan samples labeled #1, #2, #4 (Dushanbe region), and #3 (Muminobod region) form a cluster (rich in piperitenone oxide and *cis*-piperitone epoxide) distinct from other *M. longifolia* samples. The essential oil of Tajikistan sample #5 (Yovon region), dominated by *cis*-piperitone epoxide, is separate from the cluster formed by the other samples from Tajikistan.

Kokkini and co-workers (Karousou et al., 1998; Kokkini et al., 1995) observed analogous chemical differences between samples from western Crete compared with those from the eastern end of the island. Other notable clusters in this analysis include a piperitenone oxide cluster (India and S. Africa #9), a trans-piperitone epoxide cluster (Crete #5 and #6), a menthone/menthol cluster (Tunisia samples #4, #5, #6, and #7), a *cis*-carveol cluster (Iran samples #3, #4, and #5), a menthofuran cluster (S. African samples #5, #6, #7, #10, #11, and #12), a carvone cluster (Iran #1, Sudan, Crete #1, Crete #2, and Greece #1), a menthone/pulegone/1,8-cineole cluster (S. Africa #1, #2, and #3) as well as a pulegone/menthone/1,8-cineole cluster (Tunisia #2 and #3).

Viljoen and co-workers (2006) had reported the clustering of their South African samples (#5, #6, #7, #10, #11, and #12), distinct from two other samples in their study (#8 and #9), and these are all chemically distinct from other samples from South Africa reported by Asekun and co-workers (2007). Seasonal variation in essential oil does occur (Hussain, et al., 2010) and geographical location and environmental factors (climate/weather, soil/nutrition, herbivory/disease) undoubtedly play a large role in the morphological and chemical differentiation of *Mentha longifolia*.

	Main accordial all accorditations.	Defense
Country	Main essential oil constituents	Reference
Crete	Carvone (56-66%), 1,8-cineole (2-13%), limonene (3-11%), trans-dihydrocarvone (1-33%)	(Kokkini et al., 1995)
Croatia	Carvone, piperitenone oxide, limonene and β-caryophyllene	(Mastelic and Jerkovic, 2002)
France	Chemotype I:Menthone (60%), pulegone (10%) and 1,8-cineole (9%);Chemotype II:Piperitone oxide isomer (60%), piperitenone oxide (15%), $\alpha$ -muurolol (6%) and 1,8-cineole (3%);Chemotype III:Carvone (57%), 1,8-cineole (13%) and limonene (7%)	(Fraisse et al., 1985; Vidal et al., 1985)
Greece	Piperitone oxide	(Kokkini and Papageorgiou, 1988)
Greece	Chemotype 1: Carvone (55%), limonene (20%) Chemotype 2: cis-Piperitone epoxide (33%), 1,8-cineole (25%), trans- piperitone epoxide (17%)	(Koliopoulos et al., 2010)
India	Piperitenone oxide (54%), trans-piperitone epoxide (20%)	(Singh et al., 2008)
Israel	1,8-cineole (29%), <i>cis</i> -piperitone oxide (15%) and piperitone (14%)	(Fleisher and Fleisher, 1998)
Iran	Piperitone (68%), 1,8-cineole (12%)	(Jaimand and Rezaei, 2002)
Iran	Carvone (62%), limonene (19%)	(Monfared et al., 2002)
Iran	Piperitone (44%), limonene (14%) and <i>trans</i> -piperitol (13%)	(Rasooli and Rezaei, 2002)
Iran	Isopiperitenone (12-58%), piperitenone oxide (20-34%), piperitone (8-44%)	(Rezaei <i>et al.</i> , 2000)
Iraq	(-) Menthol	(Al-Bayati, 2009)
Italy	Piperitenone oxide (77%)	(Maffei, 1988)
Jordan	Pulegone (70%)	(Fleisher and Fleisher, 1991)
Kazakhstan	Piperitenone oxide (52%)	(Sharipova et al., 1983)
Lithuania	Piperitenone oxide (44-57%), 1, 8-cineole (8-15%), myrcene (6-10%)	(Venskutonis, 1996)
Morocco	Piperitenone and piperitone	(Ghoulami et al., 2000)
Netherlands	Carvone (66%)	(Lawrence, 1978)
Pakistan	Piperitenone oxide (40-65%), piperitone (2-16%), and borneol (2-13%)	(Hussain, 2009)
Serbia	<i>trans</i> -Dihydrocarvone (24%), piperitone (17%), <i>cis</i> -dihydrocarvone (16%)	(Džamić et al., 2010)
Serbia	trans-Dihydrocarvone (16%-31%)	(Matovic and Lavadinovic, 1999)
Serbia	Chemotype A: <i>trans</i> -Dihydrocarvone (18%), isomenthone (12%), piperitone (8%) Chemotype B: Isomenthone (42%), methone (12%) Chemotype C: Menthofuran (38%), 1,8-cineole (10%), ( <i>E</i> )-caryophyllene (11%)	(Mimica-Dukić <i>et al.</i> , 1991)
Serbia	Menthone and isomenthone	(Mimica-Dukić et al., 2003)
South Africa	Menthone (31-48%), pulegone (18-35%), 1,8-cineole (13-17%)	(Asekun et al., 2007)
South Africa	Menthone (51%), pulegone (19%),1,8-cineole (12%)	(Oyedeji and Afaloayan, 2006)
South Africa	Menthofuran (51-62%), <i>cis</i> -piperitone oxide (15-36%), piperitenone oxide (15-66%)	(Viljoen <i>et al.</i> , 2006)
Sudan	Carvone (77%)	(Banthorpe et al., 1980)
Sudan	Carvone (67%), limonene (14%)	(Younis and Beshir, 2004)
Tunisia	Pulegone (54%), isomenthone (12%)	(Mkaddem et al., 2009)
Tunisia	Menthol (33%), menthone (21%), pulegone (18%)	(Hajlaoui <i>et al.</i> , 2010)
Turkey	Piperitone oxide (65%), piperitenone oxide (12%),	(Baser et al., 1999)

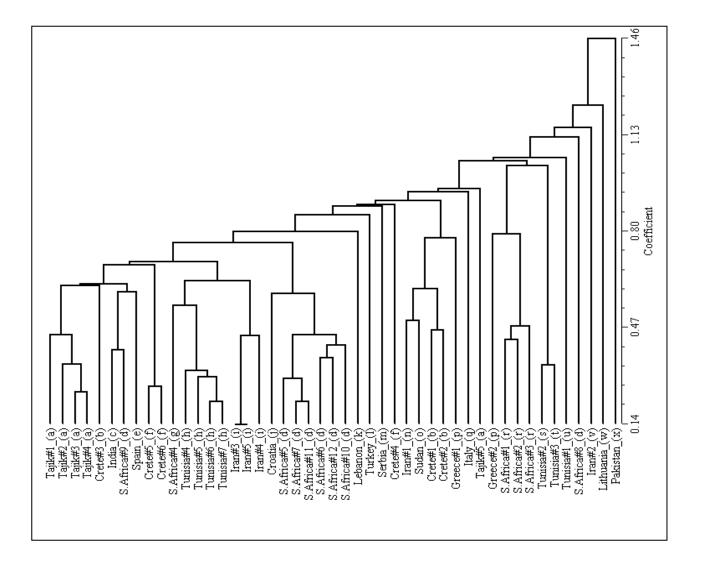


Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *Mentha longifolia* samples. Results are based on correlation and use of the unweighted pair-group method with arithmetic average (UPGMA). Data from: (a) current study; (b) Kokkini *et al.*, 1995; (c) Singh *et al.*, 2008; (d) Petkar, 2006; (e) Pérez Raya *et al.*, 1990; (f) Karousou *et al.*, 1998; (g) Oyedeji and Afolayan, 2006; (h) Hajlaoui *et al.*, 2008; (i) Zeinali *et al.*, 2005; (j) Mastelic and Jerkovic, 2002; (k) Hilan *et al.*, 2006; (l) Gulluce *et al.*, 2007; (m) Džamić *et al.*, 2010; (n) Monfared *et al.*, 2002; (o) Younis and Beshir, 2004; (p) Koliopoulos *et al.*, 2010; (q) Maffei, 1988; (r) Asekun *et al.*, 2007; (s) Snoussi *et al.*, 2008; (t) Hajlaoui *et al.*, 2009; (u) Mkaddem *et al.*, 2009; (v) Jaimand and Rezaei, 2002; (w) Venskutonis, 1996; (x) Hussain, 2009.

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