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Comparative Analysis of Growth, Seed Yield, Essential Oil and Fatty Acid Composition of Two Tunisian Caraway (*Carum carvi* L.) Ecotypes

Bochra Laribi[1*] • Karima Kouki[1] • Taoufik Bettaieb[1] • Abdelaziz Mougou[1] • Brahim Marzouk[2]

[1] Institut National Agronomique de Tunisie. 43, Av. Charles Nicolle-1082, Tunis, Tunisia
[2] Laboratoire des Substances Bioactives, Centre de Biotechnologie à la technopole de Borj-Cédria (CBC), BP 901, 2050, Hammam-Lif, Tunisia

Corresponding author: * **bochra_laribi@yahoo.fr**

ABSTRACT

In this study, two Tunisian caraway ecotypes originating from the regions of Menzel Temime and Souassi (Tunisia) were investigated regarding their seed yield and its components as well as the composition of their essential oils and fatty acids. For this purpose, the two ecotypes were cultivated, harvested and processed under the same conditions whereas the seed essential oil was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). On one hand, results concerning the biomass production, seed yield and yield components (number of umbels/plant, number of umbellets/umbel and 1000-seed weight) were higher for Menzel Temime ecotype in comparison to Souassi one. On the other hand, total fatty acid (TFA) content was 2.95 and 5.68% in Menzel Temime and Souassi ecotypes, respectively. Petroselinic acid (C18:1n-12) was the major fatty acid in both ecotypes, with a higher proportion being found in Menzel Temime ecotype (38.36% TFA) than in Souassi one (37.39% TFA). In addition, the seed essential oil yields differed significantly between the two ecotypes: 0.82% and 1.20% for Menzel Temime and Souassi ecotypes, respectively. Forty one volatile compounds were identified in the two essential oil samples where carvone and limonene constituted the main components but with significant different proportions. However, the two ecotypes displayed the same chemotype, namely carvone. Since the environmental and technical parameters effects were considered negligible, the observed differences concerning the seed yield and its components as well as the essential oil and fatty acid composition seem likely to result from genetic variation.

Keywords: *Carum carvi* L., essential oil, fatty acids, growth, seeds, yield

INTRODUCTION

In recent years, aromatic and medicinal plant research has being increasing all over the world. People have expressed a desire to reduce the use of synthetic drugs in conventional medicine or chemicals in food preservation and recognize that natural products, predominantly those derived from plants, may exhibit health benefits (Burt 2004; Chandrasekaran *et al*. 2010). For many aromatic and medicinal plants, numerous investigations have been, therefore, performed mainly in the areas of horticulture, phytochemistry, and pharmacognosy (Briskin 2000).

Accordingly, there has been a considerable interest in bioactive compounds from aromatic and medicinal plants of current interest. Although these bioactive substances can have a variety of functions in plants; it is likely that their ecological function may have some bearing on potential medicinal effects for humans (Briskin 2000; Jagetia *et al*. 2004). In this context, essential oils are among the bioactive substances produced within the various organs of these plants. They have been reported to exhibit a wide spectrum of biological activities as well as they tend to have low human toxicity, less environmental effects and wide acceptance by consumers (Briskin 2000; Bakkali *et al*. 2008; Chandrasekaran *et al*. 2010). Due to their great nutraceutical and economic importance, essential oils had formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Milner 1999; Di Pasqua *et al*. 2005; Kroll and Cordes 2006).

Species of current interest include the caraway (*Carum carvi* L.), which is widely known for its wide range of healing properties. It is generally used as spice in food due to its pleasant flavour. Besides, caraway essential oil is reputed as antioxidant (Wojdylo *et al*. 2007), antibacterial (Iacobellis *et al*. 2005; Shan *et al*. 2007), fungicidal (Soliman and Badeaa 2002), insecticidal (Lopez *et al*. 2008), acaricidal (El-Zemity *et al*. 2006), larvicidal (Pitasawat *et al*. 2007) and molluscicidal (Kumar and Singh 2006). Different studies have proven its efficacy as diuretic (Lahlou *et al*. 2007), hypoglycaemic (Eddouks *et al*. 2004; Ene *et al*. 2007; Tahraoui *et al*. 2007), hypocholesterol (Lemhadri *et al*. 2006) and anti-cancerous (Naderi-Kalali *et al*. 2005; Kamaleeswari *et al*. 2006).

One of the most important factors of interest in caraway seed oil is its high amount of an unusual monounsaturated fatty acid: petroselinic acid. This can be oxidatively cleaved to produce a mixture of lauric acid – a useful compound in the production of detergents – and adipic acid – a C_6 dicarboxylic acid which can be used in nylon polymer synthesis (Murphy *et al*. 1994).

The present study was undertaken to compare the growth, seed yield and its components of two Tunisian caraway ecotypes. We also investigated the essential oil and fatty acid composition of caraway seeds for potential applications. Such information is valuable to strengthen the valorisation of the Tunisian caraway as an important raw material for maintenance of optimal health and also for developing functional products.

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MATERIALS AND METHODS

Plant material and growth conditions

Caraway seeds were collected from cultivated plants in the regions Menzel Temime (North eastern Tunisia; latitude 36° 46′ 56″ N; longitude 10° 59′ 15″ E; altitude 38 m) and Souassi (South of Tunisia; latitude 35° 34′ 12″ N; longitude 10° 16′ 6″ E; altitude 41 m) on July 2004. The two ecotypes were cultivated under the same pedoclimatic and cultural conditions. The field experiment was carried out in the experimental farm of the National Agronomic Institute of Tunisia in Mornag (20 Km south of Tunis, Tunisia) at latitude 36° 41′ N and longitude 10° 18′ W. The site was characterized by a semi-arid climate with a mean annual precipitation of 500 mm (mainly during the winter) and an average temperature of 18°C. The soil had a clayey-loamy texture with pH 8.4.

The experiment was carried out using complete random blocs with three replications. Each ecotype sown area was of 50 m² (12.5 m × 4 m). Seeds were sown directly in the field on November 23, 2004 with row spacing of 0.25 m and by respecting a density of 250 plants m^{-2}. Fertilization consisted of 250, 200 and 100 kg ha^{-1} of P_2O_5, K_2O and N, respectively, incorporated uniformly to the soil before sowing, and supplemented by 100 kg ha^{-1} of N brought twice during the crop cycle. Pre-irrigation was done immediately after sowing for uniform emergence and establishment of seedlings. Irrigation was done by submersion one to twice frequencies per week. In addition, weeds were controlled by hand when needed. Harvest was on June 10 and 21, 2005. Seeds harvested were air-dried and stored at 4°C until use for further analysis.

Growth, seed yield and its components parameters

Measurements of plant height, fresh and dry matter weights were evaluated by destructive harvests of ten randomly selected plants from the centre rows. Plants were harvested at the soil surface and immediately weighed (fresh matter weight). After that, plants were wrapped in a clean paper bags, labelled and then oven-dried at 65°C for 48 h to constant weight and reweighted (dry matter weight). Their dry matter contents were computed using the following equation:

$$DM\ (\%) = \frac{DMW}{FMW} \times 100$$

where DM: dry matter, FMW: fresh matter weight and DMW: dry matter weight.

Seed yield and yield components such as the main branches number/plant, umbel number/plant and umbellets (umbellules)/umbel, seed yield and 1000-seed weight were determined at the end of the experiment.

Total lipids extraction

Triplicate subsamples of 1 g were extracted using the modified method of Bligh and Dyer (1959).Samples were first kept in boiling water for 5 min to inactivate phospholipase (Douce 1964) and then ground manually using a mortar and pestle. A chloroform/methanol/hexane (LabScan Ltd) mixture (1: 2:1 v/v/v) was used for total lipid extraction. After washing with water and decantation over 24 h at + 4°C, the organic layer containing total lipids was recovered and dried under a stream of nitrogen. The residue was dissolved in a known volume of toluene/ethanol (4: 1 v/v) at -20°C prior to analysis.

Fatty acid methylation and analysis

Fatty acids were converted to Fatty Acids Methyl Esters (FAMEs) using 3% sodium methylate (Sigma, Aldrich) in methanol according to the method described by Cecchi *et al*. (1985). For quantification of FAMEs, a known volume of heptadecanoic acid methyl ester (C17:0) used as an internal standard was added during methylation. FAMEs obtained were analyzed by gas chromatography using a Hewlett-Packard HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. They were separated on a RT-2560 capillary column (100 m length × 0.25 mm i.d., 0.2 µm film thickness). The oven temperature was kept at 170°C for 2 min, followed by a 3 °C min^{-1} ramp to 240°C and finally held there for an additional 15 min period. The flow of the carrier gas (N_2, U) was 1.2 ml min^{-1}. The injector and detector temperatures were maintained at 225°C.

Essential oil isolation

A total of 50 g air-dried seeds were submitted to hydrodistillation for 90 min after a kinetic survey for 30, 60, 90 and 120 min. The obtained distillate was extracted from the distillate using diethylether as the solvent (v/v) and dried over anhydrous sodium sulphate (Na_2SO_4). The obtained essential oil was stored at -20°C prior to analysis. All experiments were done in triplicates and results were expressed on the basis of DMW.

GC and GC MS analysis

Gas chromatography analyses were carried out on a Hewlett-Packard 6890 gas chromatograph series II (Agilent Palo Alto, CA, USA) equipped with HP Innowax (PEG) and HP-5 (30 m × 0.25 mm, 0.25 µm film thickness) capillary column. The oven temperature was initially held for 10 min at 35°C, ramped at 3°C min^{-1} up to 205°C, and held isothermal for 10 min. Injector and detector FID temperatures were held at 250 and 300°C, respectively. The carrier gas (N_2) was 1.6 ml min^{-1} and the split ratio was 1:60.

The GC-MS analyses were performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, California, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m × 0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed to rise from 40 to 280°C at a rate of 5°C min^{-1}. The carrier gas was helium, with a flow rate of 1.2 ml min^{-1}. The scan time and mass range were 1 s and 50-550 *m/z*, respectively. The injected volume was 1 µl, and the total run time was approximately 63 min.

Compounds identification

The volatile components were identified by comparison of their retention index (RI) relative to (C_7-C_{20}) *n*-alkanes with those of literature and/or with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded spectra with those stored in Wiley 275.L library of the GC-MS data system and other published mass spectra (Adams 2001). FAMEs were identified by comparison of their retention times of with those of pure reference standards (Labscan Ltd.) analyzed in the same conditions. Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak areas without the use of correction factor.

Statistical analysis

Results were expressed as means of three determinations. One way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare means at the significance level $P < 0.05$. All analyses were performed by the "STAISTICA, version 5.1" software (Statsoft 1998).

RESULTS AND DISCUSSION

Growth, seed yield and its components

As can be seen in **Table 1**, the two ecotypes displayed significantly similar plant height (83 cm) and number of branches per plant (6.5 branches). These results are similar to that obtained by Seidler-Łożykowska and Bocianowski (2012) who reported that the plant height ranged from 71.5 to 107.8 cm whereas the number of branches on the main stem varied from 5.3 to 10 in 23 selected caraway genotypes.

However, the biomass production was significantly higher in the ecotype Menzel Temime in comparison to

Table 1 Plant growth parameters of two Tunisian caraway (*Carum carvi* L.) seed ecotypes.

Ecotype	Height (cm)	Branches/plant (n.plant^{-1})	Fresh matter weight (g)	Dry matter weight (g)	Dry matter (%)
Menzel Temime	83.35^{a} ± 4.57	6.84^{a} ± 1.09	46.77^{a} ± 0.94	11.82^{a} ± 1.05	25.29^{a} ± 2.30
Souassi	83.54^{a} ± 4.07	6.33^{a} ± 0.98	18.38^{b} ± 0.76	3.73^{b} ± 0.15	20.30^{b} ± 1.03

Values are expressed as mean ± SD of triplicates. Values within raw followed by the same letter did not share significant differences at P<0.05% (DMRT).

Table 2 Seed yield and its components of two Tunisian caraway (*Carum carvi* L.) seed ecotypes.

Ecotype	Number of umbels.plant^{-1}	Number of umbellets.umbel^{-1}	Seed yield per plant (g)	1000 seed weight (g)
Menzel Temime	25.33^{a} ± 3.44	12.55^{a} ± 1.25	6.46^{a} ± 0.89	4.13^{a} ± 0.35
Souassi	10.20^{b} ± 2.85	12.04^{a} ± 1.59	2.86^{b} ± 0.74	3.46^{b} ± 0.14

Values are expressed as mean ± SD of triplicates. Values within raw followed by the same letter did not share significant differences at P<0.05% (DMRT).

Souassi one. Indeed, the fresh and dry matter weights of the ecotype Menzel Temime were 46.77 g and 11.82 g, respectively. Moreover, this ecotype exhibited a higher dry matter than the Souassi one (25.29% for Menzel Temime *versus* 20.30% for Souassi).

In addition, results concerning the seed yield and yield components such as the number of umbels per plant, the number of umbellets per umbel and the 1000 seed weight are summarized in **Table 2**. As shown in this table, Menzel Temime ecotype was characterized by the highest number of umbels per plant (25.33 umbels/plant) compared to Souassi one (10.20 umbels/plant). In the same context, Németh and Székely (2000) found that the number of umbels varied from 5 to 31 in 8 annual caraway populations in different years. They explained these results by the influence of the intraspecific taxon or cultivar and the plant culture conditions. Our results are also in agreement with those of Petraitylė *et al*. (2001) who noted that the number of umbels/plant ranged from 11 to 35 in the evaluated populations of caraway. In contrast, Seidler-Łożykowska and Bocianowski (2012), who studied 23 selected caraway genotypes, reported a much higher number of umbels/plant varying from 91.4 to 251.9.

In the same way, the number of umbellets/umbel of Menzel Temime ecotype was significantly similar to that of Souassi one and exceeds 12 umbellets/umbel. These values were higher than those found by Sedláková *et al*. (2003) who showed that the number of umbellets/umbel ranged from 8.07 to 11.10 in annual caraway during the experimented years. These authors reported morphological differences between the experimented varieties of annual caraway (Sedláková *et al*. 2003).

Concerning the seed yield, this parameter was significantly different and remarkably higher for Menzel Temime ecotype (6.46 g/plant) in comparison to Souassi one (2.86 g/plant). These results were different to those of Sedláková *et al*. (2003) who reported that the achene weight/plant in the three studied annual caraway varieties ranged from 2.0 to 5.2 g/plant. In the contrary, a very higher fruit yield (14.2-48.5 g) was reported by Seidler-Łożykowska and Bocianowski (2012) in 23 selected caraway genotypes.

In summary, the best performance of Menzel Temime ecotype regarding the yield components parameters was perhaps attributable to a better flowering and fructification of plants. Accordingly, seed yield was higher. It is has been supposed that pollination was better in Menzel Temime ecotype and could be explained by a higher floral nectar production and pollen attractive to flies and bees (Langenberger and Davis 2002a). Indeed, good pollination (by insects and wind) was a prerequisite for a high seed yield which seemed to be determined mainly by the weather conditions during flowering and the genotype (Bouwmeester *et al*. 1994; Bouwmeester and Smid 1995).

In fact, Langenberger and Davis (2002b) highlighted a noteworthy genotypic influence on nectar yield in annual caraway. On the other hand, Bouwmeester and Smid (1995) reported that assimilate availability and not pollination limits caraway seed yield. The quality of seed evaluated through the 1000 seed weight was of 4.13 g in Menzel Temime ecotype and was consequently higher in comparison to Souassi one (3.46 g). Similar results were obtained by Bailer *et al*. (2001) who found that the 1000-seed weight in 4 annual caraway varieties ranged from 3.1 to 3.5 g, with an average of 3.3 g. Additionally, Seidler-Łożykowska and Bocianowski (2012) noted that the 1000 seed weight varied from 1.81 to 3.31 g in 23 selected caraway genotypes.

However, the 1000-seed weight was described as one of the more stable parameter in *C. carvi* which varied slightly with ecological conditions (Petraitylė 2003). In addition, this parameter was determined by assimilate availability during the whole seed-filling period (Bouwmeester *et al*. 1995).

In fact, the growth of caraway and its biomass production has been shown to be greatly influenced by the impact of abiotic conditions such as water availability. Moreover, seed yield and its components are severely affected by drought (Laribi *et al*. 2009, 2011).

Total fatty acids content

The Souassi ecotype exhibited significantly higher TFA content (5.68% DMW) than the Menzel Temime ecotype (2.95% DMW). Our results are much higher than those of Baysal and Starmans (1999) and Chemat *et al*. (2004) who found that total oil content of caraway seeds was 2.09 and 1.84% DMW, respectively.

In contrast, previous studies on other aromatic plants belonging to the Apiaceae family and which originated from Tunisia reported that *Coriandrum sativum* L. (Sriti *et al*. 2009) and *Cuminum cyminum* L. (Bettaieb *et al*. 2011) seed oil proportion was 15.40 and 22.65%, respectively.

In this study, the two caraway seed ecotypes were cultivated under the same environmental conditions, so the observed differences in their TFA content were closely related to genotype as reported by Bettaieb *et al*. (2011) in Tunisian cumin seeds. In an earlier study, Angelini *et al*. (1997) attributed the variations in total oil content of coriander accessions from different geographic origins, to genetic factors.

Fatty acid composition

The fatty acids composition of *C. carvi* L. seeds is presented in **Table 3**. The Souassi ecotype showed a significantly higher saturated fatty acids (SFA) proportion (12.94% TFA) than the Menzel Temime one (10.36% TFA). This could be explained by the presence of a higher proportion of myristic acid (C14:0) in the Souassi ecotype (6.80% TFA). Although the proportion of palmitic acid (C16:0) was slightly higher (5.21% TFA) in Menzel Temime ecotype than in Souassi ecotype (4.79% TFA), no significant differences were observed between the two ecotypes for stearic (C18:0) acid proportion. However, Menzel Temime ecotype contained a higher unsaturated fatty acid proportion (89.64% TFA) than the Souassi ecotype (87.06% TFA), making it more sensitive to oxidation.

In addition, monounsaturated fatty acid (MUFA) proportions of the two ecotypes were mainly represented by petroselinic acid (C18:2n-12) which proportion was 38.36 and 37.39% in Menzel Temime and Souassi ecotypes, respectively. In fact, a high percentage of this fatty acid was reported in coriander (81.20% TFA) and parsley (81.90% TFA) seeds (Gunstone 1991) while in Tunisian cumin seeds, its proportion was 55.90% TFA (Bettaieb *et al*. 2011).

Table 3 Total fatty acid composition (%TFA) of two Tunisian caraway (*Carum carvi* L.) seed ecotypes.

Fatty acid	Menzel Temime	Souassi
Myristic acid (C14:0)	$3.67^{b} \pm 0.89$	$6.80^{a} \pm 1.92$
Palmitic acid (C16:0)	$5.21^{a} \pm 0.10$	$4.79^{b} \pm 0.05$
Stearic acid (C18:0)	$1.48^{a} \pm 0.30$	$1.35^{a} \pm 0.06$
Oleic acid (C18:1n-9)	$19.62^{a} \pm 4.54$	$18.58^{a} \pm 4.92$
Petroselinic acid (C18:1n-12)	$38.36^{a} \pm 3.82$	$37.39^{a} \pm 3.52$
Linoleic acid (C18:2)	$31.18^{a} \pm 0.50$	$30.76^{a} \pm 0.39$
Linolenic acid (C18:3)	$0.47^{a} \pm 0.05$	$0.33^{b} \pm 0.02$
SFA	$10.36^{b} \pm 0.43$	$12.94^{a} \pm 0.68$
MUFA	$57.99^{a} \pm 4.18$	$55.97^{a} \pm 4.22$
PUFA	$31.65^{a} \pm 0.28$	$31.09^{a} \pm 0.20$

Values are expressed as mean ± SD of triplicates. Values within raw followed by the same letter did not share significant differences at $P<0.05\%$ (DMRT). TFA, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Petroselinic acid is characteristic of Apiaceae seeds, where it usually accumulates as the major (up to 80% TFA) storage fatty acid (Kleiman and Spencer 1982). The other representative MUFA was oleic acid (C18:1n-9), whose proportion accounted for 19.62 and 18.58% TFA, in Menzel Temime and Souassi ecotypes, respectively.

Furthermore, it was observed in both ecotypes that the proportion of MUFA was higher than that of polyunsaturated fatty acids (PUFA). This last was mainly represented by linoleic acid (C18:2n-6) whereas linolenic acid (C18:3) was weakly represented and even below 0.5% of TFA.

Our results showed that, in the two caraway seed ecotypes, 10.36-12.94% of the fatty acids present were saturated, 55.97-57.99% were monounsaturated and 31.09-31.65% were polyunsaturated. Consequently, Tunisian caraway seeds were characterized by high amounts of total unsaturated fatty acids compared to saturated fatty acids. Similar results were found in Tunisian coriander (Sriti *et al*. 2009) and cumin (Bettaieb *et al*. 2011) seeds.

Results suggested that Tunisian caraway seed oil could be a valuable source of essential unsaturated fatty acids which are well known for their nutritional value and pharmaceutical industrial uses (Yu *et al*. 2005; Yetim *et al*. 2008).

Essential oil yield

The hydrodistillation of air-dried *C. carvi* seeds yielded yellowish oils with aromatic spicy odour. As shown in **Table 4**, the essential oil yield (based on dry matter weight) was significantly higher for Souassi ecotype (1.20%) than for Menzel Temime one (0.82%). Since the two caraway ecotypes were cultivated under the same pedoclimatic and cultural conditions and these essential oils isolated and analysed under the same operating conditions, the influence of the environmental and technical parameters was considered negligible and the main source of variance could be related to genotype (Galambosi and Peura 1996; Hosni *et al*. 2010; Bettaieb *et al*. 2011).

Essential oil composition

The identified oil components of Souassi and Menzel Temime ecotypes, representing 98.34 and 99.93% of the total essential oils, respectively are listed in **Table 4**. Forty one volatile compounds were detected in these seed essential oils. These lasts were constituted of a complex mixture of monoterpene hydrocarbons, aldehydes, ketones, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, phenols, esters and aliphatic hydrocarbons. However, caraway seed essential oil was characterized by the predominance of ketones (76.23 and 80.43% in Menzel Temime and Souassi ecotypes, respectively) which were represented by carvone, *trans*-dihydrocarvone and *cis*-dihydrocarvone. Also, we noted that the monoterpene hydrocarbons (12.01 and 18.6% in Souassi and Menzel Temime ecotypes, respectively) formed the second major class and contained limonene as the main constituent. The remaining fractions, such as aldehydes, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, esters and aliphatic hydrocarbons were found as the minor essential oil chemical classes of caraway seeds in the two studied ecotypes. However, Souassi seed essential oil presented more oxygenated monoterpenes (1.01%), sesquiterpene hydrocarbons (5.20%) and phenols (0.92%) than the Menzel Temime one. On the other hand, the two ecotypes did not share significant differences for the proportion of aldehydes (0.25 and 0.29% in Menzel Temime and Souassi ecotypes, respectively), esters (0.04% in both ecotypes) and aliphatic hydrocarbons (0.02% in both ecotypes).

Although the same main compounds were present in both ecotypes, there were differences in their proportions. Seed essential oil of Souassi ecotype was characterized by a higher carvone percentage (80.53%) than that of Menzel Temime (76.78%). Conversely, the latter was characterized by a higher limonene percentage (20.29%) than the seed essential oil of Souassi ecotype (13.05%).

When compared with literature data, our results on caraway seed essential oil partly agree with those previously reported for the same species. In fact, carvone and limonene were found as the major essential oil constituents of different caraway seed varieties (Bailer *et al*. 2001; Sedláková *et al*. 2003; Chemat *et al*. 2004, 2005; Iacobellis *et al*. 2005). Besides, it is important to note that the aforementioned studies reported very lower proportions of these two compounds and particularly those of carvone in comparison to those obtained herein. In contrast, previous investigations on the caraway seed essential oils reported that cuminaldehyde (22.08%), γ-terpinene (17.86%), γ-terpinene-7-al (15.41%) and *p*-cymene (7.99%) were the chief components (Razzaghi-Abyanech *et al*. 2009). Furthermore, report from Iran showed that γ-terpinene (24.40%), 2-methyl-3-phenylpropanal (13.20%) and 2, 4(10)-thujadien (14.02%) were the major constituents of caraway seed essential oil (Jelali-Heravi *et al*. 2007).

Since the environmental and technical parameters effects were considered negligible, the observed differences concerning the essential composition seems likely to be closely related to genotype. It is worth mentioning that the existence of this genetic variation was also been found in many aromatic plants such as cumin (Bettaieb *et al*. 2011), thyme (Echeverrigary *et al*. 2001; Ložienė and Venskutonis 2005), basil (Chalchat and Özcan 2008) and myrtle (Aidi Wannes *et al*. 2009).

Besides, others minor monoterpene hydrocarbons compounds i.e., β-myrcene, (E)-β-ocimene and *p*-cymene were identified in the two caraway essential oils. However, their proportions were significantly higher in Menzel Temime ecotype in comparison to the Souassi one. The latter showed more oxygenated monoterpenic components mainly represented by dihydrocarveol (1.45%) and citronellol (0.11%) than Menzel Temime ecotype. The same tendency was also observed for sesquiterpene hydrocarbons compounds with β-selinene (0.76%), α-selinene (0.44%) and δ-cadinene (0.17%) being present in higher percentages in Souassi ecotype in comparison to Menzel Temime one. Additionally, germacrene-D was present in trace amount in the latter while in Souassi ecotype it was 0.04%. Moreover, the percentages of phenol components and particularly those of thymol (0.22%) and carvacrol (0.68%) were relatively higher in Souassi ecotype than in Menzel Temime one.

Therefore, it seems that caraway seed essential oil of the two ecotypes preserves the same qualitative composition with the prevalence of carvone but showed quantitative differences in its constituents. Obviously, these findings indicate that the essential oils from the two caraway ecotypes displayed the same chemotype, namely carvone. These two ecotypes could be hence considered as potential sources of carvone. Interest in this natural bioactive compound as health promoting agent has expanded in recent

Table 4 Essential oil yield and composition (w/w %) of two Tunisian caraway (*Carum carvi* L.) seed ecotypes.

Essential oil yield (% on the basis of dry matter weight)				Menzel Temime	Souassi
				$0.82^{b} \pm 0.03$	$1.20^{a} \pm 0.06$
Volatile compound*	**RI [a]**	**RI [b]**	**Identification [c]**	**% Composition [d]**	
Monoterpene hydrocarbons					
α-Pinene	934	1032	RI, MS	$0.08^{a} \pm 0.04$	$0.09^{a} \pm 0.06$
Camphene	951	1086	RI, Co-GC	$0.03^{a} \pm 0.01$	$0.02^{a} \pm 0.00$
β-Pinene	980	1123	RI, Co-GC	$0.02^{a} \pm 0.00$	$0.01^{a} \pm 0.00$
β-Myrcene	991	1166	MS	$0.17^{a} \pm 0.00$	$0.10^{b} \pm 0.04$
Limonene	1030	1206	MS	$20.29^{a} \pm 0.00$	$13.05^{b} \pm 3.52$
γ-Terpinene	1062	1255	RI, MS, Co-GC	$0.01^{b} \pm 0.06$	$1.20^{a} \pm 0.00$
(*E*)-β-Ocimene	1050	1266	RI, MS, Co-GC	$0.04^{a} \pm 0.00$	$0.02^{b} \pm 0.01$
p-Cymene	1026	1280	RI, MS, Co-GC	$0.02^{a} \pm 0.00$	$0.01^{a} \pm 0.00$
Terpinolene	1092	1290	RI, MS, Co-GC	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$
Aldehydes					
Z-3-Hexenol	855	1370	RI, MS, Co-GC	$0.02^{b} \pm 0.01$	$0.09^{a} \pm 0.04$
trans-limonene oxide	1136	1463	RI, MS	$0.07^{a} \pm 0.00$	$0.04^{b} \pm 0.03$
Cuminaldhyde	1238	1785	RI, MS, Co-GC	$0.03^{a} \pm 0.00$	$0.04^{a} \pm 0.00$
Perilla-aldehyde	1272	1789	RI, MS, Co-GC	$0.12^{a} \pm 0.00$	$0.12^{a} \pm 0.00$
Ketones					
trans-dihydrocarvone	1204	1627	MS	$0.16^{a} \pm 0.01$	$0.14^{b} \pm 0.04$
cis-dihydrocarvone	1197	1645	MS	$0.14^{a} \pm 0.00$	$0.13^{a} \pm 0.01$
Carvone	1241	1740	MS	$76.78^{b} \pm 0.08$	$80.53^{a} \pm 3.00$
Oxygenated monoterpenes					
Camphor	1143	1532	GC-MS, Co-GC	$0.02^{a} \pm 0.00$	$0.02^{a} \pm 0.01$
Linalool	1100	1545	GC-MS, Co-GC	$0.03^{b} \pm 0.00$	$0.05^{a} \pm 0.01$
Terpinene-4-ol	1178	1611	RI, MS, Co-GC	$0.03^{a} \pm 0.00$	$0.01^{b} \pm 0.01$
α-Terpineol	1189	1700	RI, MS, Co-GC	$0.03^{b} \pm 0.00$	$0.04^{a} \pm 0.03$
Dihydrocarveol	1253	1720	MS	$0.20^{b} \pm 0.08$	$1.45^{a} \pm 1.36$
Citronellol	1229	1766	RI, MS, Co-GC	$0.04^{b} \pm 0.00$	$0.11^{a} \pm 0.07$
Nerol	1228	1797	GC-MS, Co-GC	$0.02^{a} \pm 0.00$	$0.02^{a} \pm 0.00$
trans-carveol	1218	1841	MS	$0.08^{a} \pm 0.03$	$0.05^{b} \pm 0.02$
cis-carveol	1230	1869	MS	$0.04^{a} \pm 0.00$	$0.04^{a} \pm 0.01$
Perilla-alcool	1296	2001	RI, MS, Co-GC	$0.02^{a} \pm 0.00$	$0.02^{a} \pm 0.00$
Sesquiterpene hydrocarbons					
β-Elemene	1594	1600	RI, MS, Co-GC	$0.02^{b} \pm 0.01$	$0.04^{a} \pm 0.02$
β-Caryophyllene	1419	1612	RI, MS, Co-GC	$0.06^{a} \pm 0.00$	$0.06^{a} \pm 0.00$
Allo-aromadendrene	1474	1661	RI, MS, Co-GC	$0.10^{a} \pm 0.00$	$0.06^{b} \pm 0.04$
Germacrene-D	1480	1719	RI, MS, Co-GC	tr	$0.04^{a} \pm 0.03$
β-Selinene	1481	1742	RI, MS, Co-GC	$0.20^{b} \pm 0.01$	0.76a ± 0.59
α-Selinene	1485	1745	RI, MS, Co-GC	0.18b ± 0.02	0.44 ± 0.27
α-Farnesene	1508	1755	RI, MS, Co-GC	$0.29^{a} \pm 0.00$	$0.17^{b} \pm 0.15$
δ-Cadinene	1517	1772	RI, MS, Co-GC	$0.12^{b} \pm 0.00$	$0.17^{a} \pm 0.04$
γ-Cadinene	1511	1776	RI, MS, Co-GC	$0.32^{a} \pm 0.00$	$0.30^{a} \pm 0.01$
Oxygenated sesquiterpenes					
Spathulenol	1575	2125	RI, MS, Co-GC	$0.04^{a} \pm 0.01$	$0.01^{b} \pm 0.00$
Phenols					
Eugenol	1356	2192	RI, MS, Co-GC	$0.03^{a} \pm 0.00$	$0.02^{b} \pm 0.01$
Thymol	1290	2198	RI, MS, Co-GC	$0.09^{b} \pm 0.04$	$0.22^{a} \pm 0.12$
Carvacrol	1296	2215	RI, MS, Co-GC	$0.03^{b} \pm 0.00$	$0.68^{a} \pm 0.33$
Esters					
Linalyl acetate	1257	1556	RI, MS, Co-GC	$0.04^{a} \pm 0.00$	$0.04^{a} \pm 0.01$
Aliphatic hydrocarbons					
Nonadecane	1900	1900	RI, MS, Co-GC	$0.02^{a} \pm 0.00$	$0.02^{a} \pm 0.00$
Total				$98.34^{a} \pm 0.66$	$99.93^{a} \pm 0.75$

*Components are listed in order of elution in apolar column (HP-5); RI [a], RI [b]: Retention indices calculated using respectively an apolar column (HP-5) and polar column (HP-Innowax); [c]: RI: retention indices relative to (C_7-C_{20}) *n*-alkanes on the HP-Innowax, MS = mass spectrometry, Co-GC = co-injection with authentic compound; [d]: The percentage composition was calculated from the chromatograms obtained on the HP-Innowax column; tr: trace; values within raw followed by the same letter are not significantly different at $P<0.05\%$ (DMRT).

years. Indeed, carvone had a wide variety of applications, as fragrance and flavour, potato sprouting inhibitor, antimicrobial agent mainly in the food and the pharmaceutical industries. The cosmetic and perfume industries are other outlets of this bioactive compound (de Carvalho and da Fonseca 2006).

CONCLUSION

The results reported here for the growth, seed yield and its components as well as the essential oil and fatty acid composition of two Tunisian caraway seed ecotypes originating from the regions of Menzel Temime and Souassi revealed a great variability according to the genotype and hence seems likely to result from genetic variation. Indeed, the biomass production, seed yield and yield components were higher for Menzel Temime ecotype in comparison to Souassi one. However, total fatty acid (TFA) content was 2.95 and 5.68% in Menzel Temime and Souassi ecotypes, respectively. Besides, the high proportion of petroselinic acid content in seed oil suggests the exploitation of Tunisian caraway seeds as a valuable source of essential unsaturated fatty acids which are well known for their nutritional value and pharmaceutical industrial uses. Furthermore, the seed essential oil yields differed significantly between the two ecotypes (0.82% and 1.20% for Menzel Temime and Souassi ecotypes, respectively). Although there were significant differences in the two main volatile components (limonene

and carvone) proportions of seed essential oil, the two ecotypes displayed the same chemotype, namely carvone. Additionally, they could be considered as potential sources of bioactive components mainly carvone which had a wide variety of applications, as fragrance and flavour, potato sprouting inhibitor, antimicrobial agent mainly in the food and the pharmaceutical industries.

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