Elsevier Editorial System(tm) for Industrial Crops and Products

Manuscript Draft

Manuscript Number: INDCRO-D-18-03276R2

Title: Essential Oil Chemical Diversity of Tunisian Mentha spp. collection

Article Type: Research Paper

Section/Category: Bio-based Materials from Crops

Keywords: chemical diversity; cluster analysis; Essential oil; GC/FID; GC/MS; Mentha spp.

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Abstract: Mints are perennial herbs that are cultivated for medicinal and aromatic purposes. They are highly polymorphic and their taxonomy is difficult. Sixty mint accessions, representing seven Mentha species (M. aquatica L., M. longifolia L., M. piperita L., M. pulegium., M. rotundifolia L., M. spicata L. and M. spicata var. crispa 'moroccan'), were collected at full flowering from 51 Tunisian localities. Essential oil yields were found to vary from 0.45 to 2.5 %, (w/w). Analyses of these oils by GC/FID and GC/MS and their subsequent classification by statistical analysis resulted in six clusters with significant variations in their terpenoid compositions: i) pulegone/isomenthone/menthone; ii) isomenthone/pulegone; iii) menthone/pulegone ; iv) piperitenone oxide; v) linalool/linalyl acetate/1,8 cineol/myrcene; and vi) carvone/limonene/1.8 cineol. M. pulegium accessions grouped two chemotypes: one rich in pulegone and the second rich in isomenthone. M. longifolia grouped one chemotype rich in pulegone and a second rich in menthone. M. spicata grouped one chemotypes characterized by a moderate to high carvone content and the second pulegone-rich. M. rotoundifolia accessions were piperitone oxide-rich. M. aquatica and M. piperita have linalool and linalyl acetate as major compounds. These results clearly indicate that there were a large biochemical diversity among the investigated Tunisian Mentha spp. accessions. Genetic and ecological diversities may explain this chemical diversity.

Dear Editor-in-chief of *Industrial Crops and Products*

Date: 29/08/2018

Dear Editor

We are submitting a paper entitled Essential Oil Chemical Diversity of Tunisian *Mentha* spp. collection.

To start with, *Mentha* systematic is complicated and often questionable due to natural interspecific hybridation in section *Mentha*. Despite the importance of genus *Mentha* as a medicinal and aromatic plant with industrial potentials, research regarding this genus in Tunisia is still limited and few studies have addressed its genetic diversity.

The aim of this study was to analyze chemical composition of hydro distilled essential oils of 60 mint accessions, representing seven *Mentha* species (*M. aquatica* L., *M. longifolia* L., *M. piperita* L., *M. pulegium*., *M. rotundifolia* L., *M. spicata* L. and *M. spicata* var. *crispa* 'moroccan'), to determine their essential oil components and further to classify these accessions based on their phytochemical traits.

High polymorphism and great diversity was found in essentials oils compositions and several chemotypes are observed in species from various locations

We this manuscript, we expect to contribute to this field and hope it will be considered for publication on Industrial Crops and Products.

Yours faithfully,

Zaineb Soilhi

Response to Reviewers

We would like to thank the reviewer for careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript. Our response follows.

1) Highlight 2, replace 60 by Sixty

Reply: The correction has been made.

2) l. 42 northern

Reply: The correction has been made.

3) et al., not in Italics, correct all

Reply: The correction has been made.

4)l. 238-244 Do not start sentences with abbreviations l. 237 Principal Component 1, Spell out PC1,PC2, PC3 and PC 4 or rewrite the sentences

Reply: The correction has been made.

5) l 238-244 r must be Italics

Reply: The correction has been made.

6) l. 238-244 chemical compounds are written in small case piperitone oxide, bornyl, menthol, isomenthone, ... Correct all names

Reply: The correction has been made.

7) l. 246 Fig. 3

Reply: The correction has been made.

8. Follow check list instructions to format Tables 2,3,4,5. No horizontal or vertical lines allowed except for top , bottom and below header lines, delete all other lines

Reply: The correction has been made.

9) Table 1 add space between value and m for elevations, replace comma for period for decimal

Reply: The correction has been made.

10) I do not think Table 4 is necessary. PCA analysis are usually shown in Figures a Table like this is impossible to visualize . Make figures for PCA's combinations in biplots, show first 3 PCAs only . in the biplots for PCAs show the important correlations and parameters

grouped together. Usually only 2 or 4 components are significantly correlated with each PC. This needs major work. Do not repeat everything in the figures and tales in text. Only focus in what is important for each PC.

Reply: The correction has been made.

11) Fig. 2 and 3 are not clear , Make a table for data on Figure 2 , it would be much more useful to see the content of each accession make table with several columns dividing accessions

Reply: The correction has been made.

12) Fig 3 cluster analysis is not clear, make a better figure.

Reply: The correction has been made.

Research Highlights

Essential Oil Chemical Diversity of Tunisian *Mentha* spp. collection

Soilhi et al.

- Essential oil diversity in Mentha spp from Tunisia was exhibited.
- Sixty mint accessions were analysed via GC-FID and GC-MS.
- Cluster analysis classified these accessions into six chemical groups.
- High polymorphism and great diversity was found in essential oil composition.
- Several chemotypes was observed in *Mentha* species from various locations.

Essential Oil Chemical Diversity of Tunisian *Mentha* spp. collection

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Abstract

Mints are perennial herbs that are cultivated for medicinal and aromatic purposes. They are highly polymorphic and their taxonomy is difficult. Sixty mint accessions, representing seven *Mentha* species (*M. aquatica* L., *M. longifolia* L., *M. piperita* L., *M. pulegium*., *M. rotundifolia* L., *M. spicata* L. and *M. spicata* var. *crispa* 'moroccan'), were collected at full flowering from 51 Tunisian localities. Essential oil yields were found to vary from 0.45 to 2.5 %, (w/w). Analyses of these oils by GC/FID and GC/MS and their subsequent classification by statistical analysis resulted in six clusters with significant variations in their terpenoid compositions: i) pulegone/isomenthone/menthone; ii) isomenthone/pulegone; iii) menthone/pulegone ; iv) piperitenone oxide; v) linalool/linalyl acetate/1,8 cineol/myrcene; and vi) carvone/limonene/1.8 cineol. *M. pulegium* accessions

grouped two chemotypes: one rich in pulegone and the second rich in isomenthone. *M. longifolia* grouped one chemotype rich in pulegone and a second rich in menthone. *M. spicata* grouped one chemotypes characterized by a moderate to high carvone content and the second pulegone-rich. *M. rotoundifolia* accessions were piperitone oxide-rich. *M. aquatica* and *M. piperita* have linalool and linalyl acetate as major compounds. These results clearly indicate that there were a large biochemical diversity among the investigated Tunisian *Mentha* spp. accessions. Genetic and ecological diversities may explain this chemical diversity.

Keywords: chemical diversity; cluster analysis; Essential oil; GC/FID; GC/MS; *Mentha spp*.

Introduction

Mentha is an aromatic perennial herb that is widespread throughout the temperate zones of the northern hemisphere, although a few are found in the southern hemisphere too (Harley and Brighton, 1977; Mkaddem et al., 2007). They are fast growing and generally tolerate a wide range of agro-climatic conditions, with a global distribution across Africa, Asia, Australia, Europe, and North America (Chambers, 1992; Mkaddem et al., 2007). Mints an important medicinal and aromatic plants, comprise a groups of 25-30 species of the genus *Mentha* (Gupta et al., 2017). In Tunisia, the genus *Mentha* is represented by the species *Mentha rotundifolia* L., *Mentha longifolia* L. Huds., *Mentha spicata* (*Mentha viridis*) L., *Mentha aquatica* L., and *Mentha pulegium* L. (Pottier-Alapetite, 1981). Many naturalized species (*Mentha piperita*, *Mentha spicata*, *Mentha longifolia*) are cultivated for family usages or for small commerce (Mkaddem et al., 2007).

The systematics of the *Mentha* genus is very complicated and still unclear. This is mostly due to the existence of variation in basic chromosome number, frequent interspecific

hybridization, cytomixis, polymorphism in morphology and essential oil composition under different environmental conditions, colonial mutant propagation, as well as the occurrence of polyploidy, aneuploidy and nothomorphs (Gobert et al., 2002; Tucker and Chambers, 2002; Tyagi, 2003; Denslow and Poindexter, 2009; Tucker, 2012; Jabeen et al., 2012 and Jedrzejczyk et al., 2018).

Mints are cultivated for their essential oils and herbage yields. They have several applications in pharmaceutical, perfumery, food, confectionery, and cosmetic industries (Gobert et al 2002., Zeinali et al., 2004; Kumar et al., 2015). This makes many *Mentha* species very priceless for industry, as mint oil is a supplement for wide spectrum of products (Jedrzejczyk et al., 2018). *Mentha canadensis* (Japanese mint), *Mentha cardiaca* (Spearmint), *Mentha spicata* (Native spearmint), *Mentha pulegium* (Corn mint), *Mentha* × *gracilis* (Scotch spearmint) and *Mentha* × *piperita* are the most significant species for the industry (Smolik et al., 2007; Hua et al., 2011; Rodrigues et al., 2013).

The essential oil composition of *Mentha* species has received considerable study due to its commercial importance. The EO components can be categorized by compound class such as hydrocarbon, alcohol, ester, aldehyde, ketone, or miscellaneous compounds (Bahl et al., 2000; Voirin et al., 1999). *Mentha* aerial parts bears essential oils containing a large number of aroma chemicals like menthol, menthone, isomenthone, menthyl acetate and menthofuran (Sangwan et al., 2001; Simões and Spitzer, 2001). Different chemotypes are characterized by distinct smells and bioactivities, indicating different uses in aromatic and medicinal industries (Karousou et al., 2007). On the basis of biosynthetic pathway followed in different species of *Mentha*, subjected to varying geographical conditions, mints were classified based on the dominant monoterpene compound prevailing in essential oils; linalool and linalyl acetate are produced by linalool pathway; menthol, menthone and menthofuran result from menthol pathway; and carvone, dihydrocarvone and carveol outcome from

carvone pathway (Mahmoud and Croteau, 2001; Lawrence, 2007; Šarić-Kundalic´ et al., 2009).

Mentha L. accessions from different geographical population generally explain numerous variations in the essential oil properties (Orav et al., 2004; Oyedeji and Afolayan, 2006; Hajlaoui et al., 2008). The existence of different chemotypes, based on qualitative differences within a taxon, is a common feature in most *Mentha* species and hybrids (Kokkini and Vokou, 1989). Several chemotypes was observed in *Mentha* L. from various locations. Nine chemotypes have been reported for five *Mentha* species including *Mentha arvensis*, *Mentha aquatic*, *Mentha longifolia*, *Mentha spicata*, *Mentha suaveolens* (Kokkini, 1992). A series of chemotypes of *Mentha spicata* have been described in previous studies, such as pulegone, carvone, linalool, piperitone, piperitone oxide, menthone/isomenthone, pulegone/menthone/isomenthone and pulegone/piperitone (Baser et al., 1999; Telci et al., 2004, 2010). Two chemotypes of *Mentha longifolia* (menthofuran, and cis-piperitone oxide/piperitenone oxide chemotypes) are reported from Southern Africa (Viljoen et al., 2006). Studies showed three chemotypes of *Mentha pulegium* L. with the following major oil components: (1) pulegone, (2) piperitenone and/or piperitone and (3) isomenthone/ neoisomenthol (Topalov and Dimitrov, 1969; Cook et al., 2007). Different chemotypes was observed for the species *Mentha rotundifolia* growing in various parts of the world (Kokkini and Papageorgiou, 1988; El Arch et al., 2003). In addition to chemotypic and genetic factors, essential oils may be linked to numerous intrinsic as well as extrinsic factors including development stage (Rodriguesa et al., 2013; Maffei et al., 1989), growth conditions (Maffei and Scannerini, 1999; Karray-Bouraoui et al., 2009) and climate conditions (Catherine et al., 2007). The interaction of different genotype with environment has great interest in many aspects of genomic and breeding research (Patel et al., 2015).

The evolution of chemical complexity has been a major driver of plant diversification, with novel compounds serving as key innovations (Boachon et al., 2018). However, to the best of our knowledge no systematic chemical investigations have been carried out on essential oil composition of *Mentha* spp in Tunisia. Therefore, aim of the present study was to investigate the chemical diversity among the *Mentha* spp growing in Tunisia and to characterize the chemotypes of this genus. This study will contribute to the knowledge of chemical diversity of a *Mentha* that could improve the use of Tunisia *Mentha* spp. Our study may be useful to mint evolution study and future breeding programs.

2. Materials and methods

2.1. Plant material

Sixty accessions of *Mentha* spp. were collected from natural habitats and cultivated fields during the flowering stage from 51 Tunisian localities (Fig. 1, Table 1). Mint accessions represent most of the geographic range covered by the species in Tunisia. GPS was used to locate the origins of these accessions. Their taxonomic identities were attributed in a previous work with morphological descriptors and dichotomous keys. Thirty three morphological descriptors were considered from *Mentha* standardized descriptors (Upov, 2007), several flora volumes (Tutin et al., 1972; Pottier-Alapetite, 1981; Cullen et al., 1984-2000), and several publications (Tucker and Naczi, 2006, Lawrence 2006 and Šarić-Kundalić et al., 2009). This morphological study was carried from May to August during three seasons from 2015 to 2017.

2.2. Essential oil Extraction

Aerial parts of 60 mint accessions were collected in the flowering stage. Leaves were air dried for one month in the absence of light at room temperature and then stored in sealed

paper bags. Dried aerial parts were subjected to steam distillation for 3 h using a Clevenger-type apparatus. Plant material (50 g) was distilled in 500 ml dH_2O in a 1000 ml flask. The oil phase was separated and dried over anhydrous sodium sulfate and kept in brown glass bottle at 4 °C for further analysis. Three replicates were performed for each plant material.

2.3. Essential oil analysis

Essential oils were diluted 1/10 in n-hexane (v/v, VWR, Leuven, Belgium) prior to analysis. Gas chromatography analysis of volatile components was carried out using an Agilent 6890N GC equipped with a flam ionization detector (FID, Interscience Louvain-La Neuve, Belgium) equipped with an Optima 5 MS (Macherey-Nagel, Düren, Germany) capillary column (30 m × 0.25 mm I.D., 0.25 µm film). GC-FID was performed using splitless injection, with injector set at 280°C. The oven temperature program was initiated at 40°C, held for 2 min then raised at 8°C/min to 280°C and then maintained at 280°C for 5 min. Helium (He) was used as a carrier gas at a flow rate of 35 ml/min. Volume injected: 1 µL (diluted in hexane). Retention indices (RI) were determined by co-injection of a series of a mixture of aliphatic hydrocarbons alkanes (C_7-$C_{30\ at\ 10ng/}$ µL in n-hexane, sigma, Bornem, Belgium). The retention indices of all components were determined according to the Van Den Dool's method (Van Den Dool et al., 1963).

Gas chromatography with mass spectrometry (GC-MS, Agilent 6890-USA) was done for essential oil qualitative and quantitative analysis using the electron impact ionization (70 eV) method and mass spectra. GC-MS conditions were the same as in the GC-FID analysis. One µl of essential oil solutions was injected in the splitless mode.

2.4. Component identification

Identification of the components was made by determination of their retention indices (KI) relative to those of a homologous series of n-alkanes (C7-C30) and by matching their recorded mass spectra with those stored in the spectrometer database from the Wiley 275L and the literature values (Adams, 2001). Whenever possible, the identification were confirmed by confirmed by comparison the recorded retention data with those of pure available standard compounds injected in the same conditions. Most of the non-identified components are present as traces with relative abundances of less than 0.01%. The identified constituents are listed in the order of their elution.

2.5. Data Analysis

Essential oil components percentage content was treated by multivariate statistical procedures. A cluster analysis was performed using Euclidean distance coefficient functions based on the dissimilarity between pairs. Hierarchical cluster analysis (HCA) was performed using the Ward's method with square Euclidean distance measure was used to construct the dendrogram. The Principal Components Analysis (PCA) and the cluster analysis were carried out with SPSS Version 20.

3. Results and discussion

Chemical characterization of mints accessions illustrated a diversity of their essential oil yields (0.45 - 2.5 %, w/w based on dry weight) (Figure 2, Table 2). There is little correlation between the changes of essential oil yield and the geographic positions (longitude, latitude and altitude) of collection sites of these accessions. The average essential oil yield of each species is in accordance with some reported oil yields at full flowering for wild *M. pulegium* (1.2%, Hassanpouraghdam et al., 2011), *M. arvensis*, *M. piperita*, *M. spicata* and *M. longifolia* (1.7%, 1.2%, 1.2%, 1.0%, respectively, Hussain et al., 2010) but some studies

178 reported much higher yields in *M. pulegium* (3.8%, Kokkini et al., 2004; 3.9%, Cook et al.,
179 2007). These results clearly indicate the existence of huge variation between and within the
180 species. These variations may be linked to numerous intrinsic as well as extrinsic factors.
181 Several studies suggest that oil yield is correlated with species, climatic conditions; etc.
182 (Voirin et al., 1990; Kokkini et al., 2004). Variations in essential oil yield can be attributed to
183 genetic, environmental and ontogeny factors, as well as analytical methods (Lawrence, 2002;
184 Heywood, 2002; Ghasemi et al., 2016). The quantitative composition of the essential oils of
185 many aromatic plants is greatly influenced by the genotype and agronomic conditions, such as
186 harvesting time, plant age and crop density (Marotti et al., 1994). Results from selected
187 studies revealed that the amount of essential oil content in the leaves is strongly ontogeny
188 dependent, and therefore, harvest time may have significant consequences to essential oil
189 quantity and quality (Mrlianova et al., 2001). The environmental factors during the growth
190 period such as the amount of available water are very important in the yield of dried material
191 and essential oil (Farahani et al., 2009). Essential oil content in fresh materials is slightly
192 higher than that of dried one (Patora et al., 2003).

193 Essential oils extracted and analyzed from the sixty mint accessions include 63
194 chemical constituents, representing 87-100 % of total oils. These oil components are listed in
195 order of their elution on the DB-1 column (S1: Supplementary Data). There was a significant
196 variation among the accessions for qualitative and quantitative major constituents. The
197 number of identified compounds for each essential oil ranged from 15 to 46.

198 Considerable variations were observed at chemical level among the investigated
199 accessions of '*pulegium* group', which revealed the existence of two distinct chemotypes/
200 sub-groups (Table 3). Accessions Acc 5, Acc10, Acc 20, Acc 24, Acc26, Acc 27, Acc31,
201 Acc57, and Acc59 were characterized by the presence of higher amounts of pulegone (39.95-
202 77.17%), menthone (0.57–28.44%) and isomenthone (0.99 -28.9%). Further, accessions Acc3,

Acc7, and Acc40 were dominated by isomenthone (59.43-77.73%), menthone (1.46%-27.55%), pulegone (0.16-28.45%) and cis-isopulegone (0.44-6.54%).

A total of six investigated accessions of '*longifolia* group*'* could be classified in to two chemotypes. Accessions Acc30, Acc46, Acc49 and Acc52 were found to be rich in pulegone (59.98-67.42%), menthone (7.15%-15.38%), isomenthone (5.03% to 9.16%) and 1.8-cineole (5.39- 8.11%). Further, accession Acc34 and Acc36 were dominated by menthone (31.08-40.20%), pulegone (29.23-35.2%), 1.8–cineol (6.36-10.41%) and menthol (4.93-5.70%).

The '*rotoundifolia* group' (Acc2, Acc8, Acc11 and Acc 29) represented by only one chemotype, which was characterized by the presence of piperitone oxide (45.79-74.25%) followed by menthol (0-4.49%), limonene (0-4.48%) and viridiflorol (0.62-4.56%).

Essential oil of accession Acc1, belongs to '*piperita* group' was found to be rich in linalool (45. 86%), linalyl acetate (14. 29%), 1.8-cinéole (9.311%) and β-myrcene (8.75%)**.**

On the other hand, Accessions belonging to '*aquatica* group' (Acc6, Acc17, Acc44 and Acc50) could be arranged as only one chemotype and were rich in linalool (27.29%-47.2%) and linalyl acetate (13.90-18.4%).

Accessions belonging to '*spicata* group' could be arranged as two distinct chemotypes. Among these, Acc4, Acc9, Acc12, Acc13,Acc14, Acc16, Acc18, Acc23, Acc32, Acc33, Acc35, Acc37, Acc39, Acc41, Acc42, Acc43, Acc59, Acc45, Acc53, Acc51, Acc54, Acc55, Acc56 and Acc 60) were rich in carvone (39.21-62.51%), 1.8-cineole (7.24-12.49%), limonene (6.07-18.45%), and dihydro-carveol cis (1.17-6.56%). However, accessions Acc38 are dominated by pulegone (38.74%), menthone (28.56%), 1.8-cineole (10.413%), and menthol (5.64%).

The *'spicata* var. crispa 'moroccan' group' (Acc15, Acc19, Acc21, Acc28 and Acc47) represented by only one chemotype, which was dominated by carvone (62.69-75.53%) followed by limonene (10.52-17.94%), transcarveol (0-5.22%) and dihydro-carveol cis (0.39-

3.62%). Finally, the two accessions (Acc22 and Acc 25) belonging the 'unidentified group' represented by only one chemotype, which was characterized by carvone (57-65.54%), 1,8-cineole (9.61-10.18%), limonene (6.63-8.72%), and dihydro-carveol cis (2.28-3.77%) as main constituents.

Above chemotypic differentiation of the *Mentha* accessions was also validated statitistically. To evaluate whether the identified essential oil constituents may be useful in reflecting similarity and differences within the investigated accessions. Principal component analysis (PCA) is one of the multivariate methods that were applied to determine the most significant characteristics in the data set. The same data set (60 accessions × 18 components) was used in this section. The first three PCs revealed the highest variation. The other PCs are shown in table S2 (Supplementary Data) for more information. The first PC (PC1) explained 24.18% of total variation and possessed positive correlation with pulegone and menthone and high negative correlation with L-carvone, limonene and 1.8-cineol. The second PC2 showed 19.52% of total variance and had positive correlation with linalool, linalyl acetate, β-myrcene and α-terpinyl acetate and the third PC3 explained piperitenone oxide, bornyl acetate and viridiflorol as positive component accounted for 14.61% of the total variance. Since PC1 and PC2 possessed a great share in respect to compounds, so scatter plot of PC1 and PC2 were applied to determine phytochemical distance (Fig. 3a-3b). According to PCA analysis the studied accessions were divided into four groups. As depicted in (Fig. 3a-3b) the presence and amount of some essential oil chemical compounds are useful for the identification of groups. For example, the '*aquatica* group' (Acc6, Acc17, Acc44 and Acc50) and '*piperita* group' (Acc1) were characterized by linalool, linalyl acetate, β-myrcene, and α-terpinyl acetate and were situated in the top right quadrant of the plot. The '*spicata* group' was characterized by carvone, 1.8-cineole, limonene, and dihydro-carveol cis and was situated in the low right quadrant of the plot.

A dendrogram constructed from the statistical analysis of the identified constituents, was displayes in Fig 4, Essential oils composition showed remarkable differences among *Mentha* spp. accessions. Dendrograms show that the 60 *Mentha* species can be clustered into six clusters. The existence of chemical diversity within the species and similarity with the accessions of other species could easily be seen from the distribution pattern of the *Mentha* accessions in different clusters. The twelve accessions of '*Pulegium* group' were distributed in two clusters (I: 09 accessions and II: 03). Further, six accession of '*longifolia* group' were found to be divided in two clusters (I:4 and III: 2). However, four accession of '*rotoundifolia* group' were found placed in cluster IV due to similar chemical characters. Accessions belonging the '*piperita* group' and '*aquatica* group' were placed in the same cluster V. Except for one accession of '*spicata* group', placed on the cluster III, the other accessions of this group were grouped in cluster VI with accessions of *M. spicata* var. crispa '*moroccan*' and the two unidentified accessions.

These results clearly showed that the certain taxonomically different species were grouped in same cluster because of the presence of similar major chemical constituents. Further, the existence of different chemotypes, based on qualitative differences within a taxon, is a common feature in most *Mentha* species and hybrids (Kokkini and Vokou, 1989). Although many species and hundreds of subspecies and/or varieties were described by previous researchers, the species make up the *Mentha* genus is not clearly distinct in taxonomy, and numerous chemotypes are widely distributed around the world. Moreover, one chemotype usually could be found in several species (Kokkini et al., 1995; Telci et al., 2004). *Mentha* accessions used in the present study represent a widely variation in the content of these constituents.

277 Compositional analysis of the essential oils of *Mentha* taxa has revealed a comprehensive
278 diversity in the oil components, and the different chemovarieties have been reported from
279 various regions of the world. There is a huge variation in the chemical composition of *M.*
280 *pulegium*, Literature data suggests that *M. pulegium* is a chemical polymorph species in both
281 qualitative and/or quantitative composition (Kokkini et al., 2004). Studies showed three
282 chemotypes of *M. pulegium* L. with the following major oil components: (1) pulegone, (2)
283 piperitenone and/or piperitone and (3) isomenthone/ neoisomenthol (Topalov and Dimitrov,
284 1969; Cook et al., 2007). Pulegone was found to be one of the main constituents of *M.*
285 *pulegium* oils followed by menthone also in other studies; Lawrence 1978, Sivropoulou et al.,
286 1995, Beghidja et al., 2007, Bekhechi 2008, Baser et al.,1999; Lorenzo et al., 2002; Agnihotri
287 et al., 2005; El-Ghorab, 2006; Diaz-Maroto et al., 2007; Mata et al., 2007; Hajlaoui et al.,
288 2009; Boukhebti et al., 2011; Ait-Ouazzou et al., 2012, and Cherrat et al., 2014. Possible
289 exaplanation for such differences could be associated with habitats, environmental growing
290 conditions, collecting periods, etc. (Boukhebti et al., 2011). In present study, pulegone (Acc 5,
291 Acc10, Acc 20, Acc 24, Acc26, Acc 27, Acc31, Acc57, and Acc5) and isomenthone (Acc3,
292 Acc7, and Acc40) rich accessions have been identified for *M. pulegium*. Further, present
293 study revealed the existence of a new essential oils/chemotypes (isomenthone) of the specie
294 *M.* longifolia (Acc3, Acc7, and Acc40) which were not described before from in this region.
295 The present study showed that pulegone should not be considered as a specific
296 chemosystematic marker for *M. pulegium* because pulegone was found also in the oils of *M.*
297 *longifolia*, whose chemosystematics is most complicated. In present study, pulegone (Acc30,
298 Acc46, Acc49 and Acc52) and isomenthone (Acc34 and Acc36) rich accessions have been
299 identified for *M. Longifolia.* With the extent of morphological diversity in *M. longifolia*, a
300 great degree of chemical variation in the species might be expected as well. Indeed, results
301 from previous studies on several wild and cultivated *M. longifolia* have produced a number of

chemotypes. Identified chemotypes of *M. longifolia* include those dominated by menthone (Fraisse et al., 1985; Vidal et al., 1985; Mimica-Dukić et al., 2003; Oyedeji and Afolayan, 2006; Hajlaoui et al., 2010), pulegone (Fleisher and Fleisher, 1991; Oyedeji and Afolayan, 2006; Gulluce et al., 2007; Mkaddem et al., 2009; Hajlaoui et al., 2010), piperitenone oxide (Maffei, 1988; Baser et al., 1999; Venskutonis, 1996; Rezaei et al., 2000; Mastelic and Jerkovic, 2002; Viljoen et al., 2006; Gulluce et al., 2007; Hussain, 2009), piperitone oxide (Fraisse et al., 1985; Vidal et al., 1985; Kokkini and Papageorgiou, 1988; Fleisher and Fleisher, 1998; Karousou et al., 1998; Baser et al., 1999; Viljoen et al., 2006; Hussain, 2009), carvone (Lawrence, 1978; Fraisse et al., 1985; Vidal et al., 1985; Kokkini et al., 1995; Mastelic and Jerkovic, 2002; Monfared et al., 2002; Younis and Beshir, 2004; Lawrence, 2007), piperitone (Ghoulami et al., 2000; Rasooli and Rezaei, 2002; Rezaei et al., 2000; Džamić et al., 2010), trans-dihydrocarvone (Mimica-Dukić et al., 1991; Džamić et al., 2010), isomenthone (Mimica-Dukić et al., 1991; Mimica-Dukić et al., 2003; Mkaddem et al., 2009), menthofuran (MimicaDukić et al., 1991; Viljoen et al., 2006), menthol (AlBayati, 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).

Piperitone oxide is mentioned as the main constituent of *M. rotoundifolia* oil also by many other authors (Miyazawa et al., 1998; Benayad 2004; Brada et al., 2007; Lawrence 2007; Hajlaoui et al., 2009). Piperitone oxide is the typical component of *M. rotoundifolia* oils and can be used as chemical markers. In contrast with our results the study of Riahi et al., (2013) shows that essential oils from Beja locality were most complex and present 45 compounds representing 96.83% of the total oil composition. The major components of these oils are caryophyllene (26.67%), germacrene D (12.31%) and carveol (7.38%). Essential oils

from Bizerte include 40 components, representing 95.81% of total oils. Those oils are dominated by Pulegone (32.09%), Piperitenone oxide (17.28%) and 5-Acetyl Thiazole (11.26%). Our results confirmed previous reports which cite different chemotypes for *M. rotundifolia* growing in various parts of the world (Kokkini and Papageorgiou, 1988; El Arch et al., 2003). Two chemotypes have been found in the essential oils of *M. rotundifolia* (L.) growing in Greece, which are characterized by piperitone oxide and menthyl acetate, respectively as the main compounds (Kokkini and Papageorgiou, 1988). In others reports, the major compound of *M. rotundifolia* essential oils is Pulegone (85%, El Arch et al., 2003). According to Lorenzo et al. (2002), Piperitenoneoxide and (z)-sabinene hydrate were the major components in *M. rotundifolia*.

M×piperita L (Acc1) did not contain menthol and menthone as major compounds commonly found in peppermint essential oil (İşcan et al., 2002; Mimicam Dukić et al., 2003; Hussain et al., 2010). Instead they were found to be rich in linalool (45,86%), linalyl acetate (14,29%), 1.8-cinéole (9.311%) and β-myrcene (8.75%) as decribed by Mimica-Dukic et al., 2003; Gracindo et al. 2006 and Garlet et al., 2013

Although the wild growing *M. aquatica* seems to be the only species of the genus characterized by a specific essential oil composition with menthofuran as a main compound (Malingré et al., 1974; Kokkini 1992; Mimica-Dukic et al., 2003) plants rich in linalool and/or linalyl acetate are also known from cultivation (Harley 1977 and Kokkini 1992). Moreover, there are results of the changes in monoterpene composition of *M. aquatica* essential oil produced by gene substitution from the other mint species (Hefendehl 1972). Furthermore, it is well known that the chemical composition of the essential oils depends on various complex factors, both endogenous and exogenous such as chemotypes, geographical and climatic conditions, collection time, drying conditions, mode of distillation, etc. (Bozin et al., 2006).

There is a huge variation in the chemical composition of *M. spicata*, wild as well as cultivated, around the world. A series of chemotypes of the species have been described in previous studies, such as pulegone, carvone, linalool, piperitone, piperitone oxide, menthone/isomenthone, pulegone/menthone/isomenthone and pulegone/piperitone (Baser et al., 1999; Telci et al., 2004, 2010). Carvone is mentioned as the main constituent of *M. spicata* oil also by many other authors (Adam et al., 1998; Mkaddem et al., 2009; Zhao et al., 2013; Brahmi et al., 2016). Four chemotypes of *M. spicata* are found in Greece, characterized by the dominant occurrence of linalool, carvone/dihydrocarvone, piperitone oxide/piperitetone oxide, and menthone/isomenthone/pulegone, respectively (Kofidis et al., 2004). Carvone is mentioned as the main constituent of *M. spicata* oil also by many other authors (Adam et al., 1998; Chauhan et al., 2009; Mkaddem et al., 2009; Zhao et al., 2013; Brahmi et al., 2016). However, in the present study, an Except for one accession of '*spicata* group', all the rest of accession were grouped together into a single chemotype carvone. This supports that *M. spicata* populations in Tunisia show certain stability in essential oils. An alternative hypothesis, which was widely accepted, is that interspecific and intraspecific hybridization occurring naturally may contribute to the variable chemical composition. This is supported by the present investigation too. *M. spicata* Acc 38 were collected at the place where *M. longifolia* (Acc 34) was growing naturally, implying that natural gene exchange may occur among these plants. As already described, these were incorporated into *M. longifolia* group. Further, present study revealed the existence of a new essential oils/chemotypes (pulegone) of the specie *M.* spicata which were not described before from this region.

The taxonomy of Mentha is complex due to interspecific hybridization and polyploidy of the species in the genus (Denslow and Poindexter, 2009; Tucker, 2012; Jabeen et al., 2012 and Jedrzejczyk, et al., 2018). Further, the occurrence of huge chemical variations among

Mentha accessions collected from diverse localities seems to be due to the divergent climatological and geographical conditions; existing variations in oil content and composition may be attributed to factors related to ecotype and the environment including temperature, relative humidity, irradiance and photoperiod (Chauhan et al., 2009). Similarly, chemotype of the plants, cultivation practices and method of extraction also leads to variation in oil content and chemical composition (Pavela, 2009). Other factors affecting essential oil composition, relates to agronomic and genotype conditions, such as harvesting time, plant age and crop density (Telci et al., 2010; Marotti et al., 1994). Similarly, different photoperiodic treatment was also shown to be influencing concentrations of oil components in *Mentha* species (Fahlen et al., 1997).

4. Conclusion

The results of present study clearly indicated that there were large amount of biochemical diversity among the investigated populations of genus *Mentha*. Accessions with similar chemotypic characters differed considerably in their taxonomic rang. Therefore, chemical constituents could not be necessarily correlated with their taxonomical category. The existence of different chemotypes, based on qualitative differences within a taxon, is a common feature in most *Mentha* species and hybrids (Kokkini and Vokou, 1989). These chemotypes offers the great opportunity for production of *Mentha* to meet the market supplies of specific essential oils or individual compounds. Further, present study revealed the existence of a new essential oils/chemotypes (isomenthone) of the species *M.* longifolia (Acc3, Acc7, and Acc40) which were not described before from this region. As well, the existence of a new essential oils/chemotypes (pulegone) of the specie *M.* spicata which were not described before from this region. Further, the characterization of essential oil variation is of commercial importance as well as helpful in the development of *Mentha* resources for perfume and pharmaceutical industries.

Acknowledgment

The authors gratefully acknowledge Prof.Georges C.Lognay (University of Liège-GxABT) for his analytical support". We are very thankful to Prof. Claude Grasland and Etienne Toureille from the Research Federation CIST (http:/www.gris-cist.fr) for their kindly assistance and support.

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729 **Figure Captions**

730

731 **Fig 1.** Distribution map of the studied accessions of *Mentha* spp

732

733 **Fig 2.** Essential oil yield of different accessions of *Mentha* spp.

734 **Fig 3.** a) PCA Score plot for main variation of essential oil compositions among Tunisian

735 *Mentha* spp accessions. b) Loading plot for volatile constituents explaining 44.29% of the

736 variation on PC1 and PC2 axes.

737 **Fig 4.** Dendrogram generated by cluster analysis of the essential oil composition of sixty.

738 using the HCA by Ward's method and Euclidean distances. i) pulegone; ii) isomenthone; iii)

739 menthone/pulegone ; iv) piperitenone oxide; v) linalool; vi) carvone.

740 **Tables**

741

742 **Table 1.** Latin names, collecting sites, habitats of a sixty Tunisian accessions of *Mentha* spp.
743

Accession	*Mentha* spp.	Locality	longitude Latitude and altitude	Habitat
Acc3		Al Ayaida-Beja	36°46'01.66"N/ 9°01'47"E/ 568 m	Riverside
Acc5		Maagoula-Beja	36°42'29.78" N/ 9°11'57.09"E/ 151 m	Riverside
Acc7		Tabarka- Jandouba	36°55'41.19"N/ 8°44'13.96"E/ 820 m	Riverside
Acc10		Ain El Awafi- El Kef	35°58'50.41"N/ 9°05'44.40"E/ 740 m	Riverside
Acc20		Zaghouan	36°24'00.48"N/ 10°08'48.61"E/ 423 m	Riverside
Acc24		Ain Kamech-Nabeul	36°27'03.72"N/ 10°44'10.38"E/ 270 m	Riverside
Acc26		El agiula- Gafsa	34.41130N/ 008.7409 E/ 252 m	Riverside
Acc27		El kasar - Gafsa	34.39501 N/ 008.80772 E/ 283 m	Riverside
Acc31		Mornag-Ben Arous	36°33'43.32"N/ 10°18'48.66"E/ 155.26 m	Cultivated field
Acc40		Cherfech-Ariana	36°56’04.28"N/ 10°02’21.547"/ E 58.27 m	Riverside
Acc57		Silaian	36°04’06.53"N/ 9°22'42.4"E/ 5.98 m	Riverside
Acc59	***M. pulegium* L.**	Sejnane-Bizerte	36°57’ 62.53"N/ 9°32’53.305"E / 105 m	Pasture
Acc 30		Mornag-Ben Arous	36°33'43.32"N/ 10°18'48.66"E/ 155.26 m	Cultivated field
Acc 35		Bouchama-Gabes	33°45'23.93"N/ 10°03'59.72"E/ 1.88 m	Cultivated field
Acc36		Dgech- Tozeur	33°51'28’’N/ 007°57'29"E/ 38 m	Cultivated field
Acc46		Borj twil-Ariana	10°09’55.81"N/ 36°55’46.840"E / 100.8 m	Cultivated field
Acc49		El karia-Gassrine	34°55'52.45"N/ 8°52'50.31"E/ 9.14 m	Cultivated field
Acc52	***M. longifolia* L.**	Bir hfay-Sidi Bouzid	34°55'54.23"N/ 9°10'57.55"/ 871 m	Cultivated field
Acc1	***M*×*piperita***	Ain El Goussa -Beja	36°46'11.68" N/ 9°01'53.41" E/ 568 m	Cultivated field
Acc6		Maagoula-Beja	36°55'57.09" N/ 9°11'57.09"E/ 207 m	Cultivated field
Acc17		Tebolba-Monastir	35°38'56.91"N/ 10°65'08.69"E/ 20 m	Cultivated field
Acc44		Borj twil-Ariana	36°55’46.84"E/ 10°09’55.814"N/ 100.84 m	Cultivated field
Acc50	***M.aquatica* L.**	Chott Mariem-Sousse	35°55'20.76"N/ 10°34'08.95" E	Cultivated field
Acc2		Al Ayaida- Beja	36°46' 06.41" N/9°01' 51.20"E/ 568 m	Riverside
Acc8		Tabarka- Jandouba	36°35'31.56"N/ 8°44'14.06"E/ 243 m	Riverside
Acc11		in El Less- El Kef	35°57'21.67"N/ 9°05'47.27"N/ 740 m	Riverside
Acc29	***M. rotundifolia* L.**	Chebika- Kairouan	35°34'01.34"N/ 9°58'52.31"E/ 400 m	Cultivated field
Acc15		El Jam-Mahdia	35°17'49.32"N/ 10°42'29.79"E/ 238 m	Cultivated field
Acc19		Tebolba-Monastir	35°38'56.91"N/ 10°65'08.69"E/ 665 m	Cultivated field
Acc21		Zaghouan	36°24'06.31"N/ 10°08'49.67"E/ 289 m	Cultivated field
Acc28		Chebika- Kairouan	35°37'29.22"N/ 10°02'08.94"E/ 3,37 m	Cultivated field
Acc47	***M. spicata* var. crispa 'moroccan'.**	Chott Mariem-Sousse	35° 55'20.76"N/ 10°34'08.95" E	Cultivated field
Acc4		Medjez-el bab-Beja	36°38'59.47"N/ 9°37' 05.29" E/ 568 m	Cultivated field
Acc9		Bou salem Jandouba	36° 36'33.53"N/ 8°85'82.07"E/ 243 m	Cultivated field
Acc12		Essers- El Kef	35°29'53.8"N/ 10°36'34.4"E/ 33 m	Cultivated field
Acc13		Bir Salah- Sfax	35°12'43.27"N/ 10°42'37.19"E/ 114 m	Cultivated field
Acc14		Thyna- Sfax	34°40'23.14"N/ 10°40'58.23"E/ 400 m	Cultivated field
Acc16		El Jam-Mahdia	35°17'49.32"N/ 10°42'29.79"E/ 238 m	Cultivated field
Acc18		Tebolba-Monastir	35°38'56.91"N/ 10°65'08.69"E/ 665 m	Cultivated field
Acc23		Nabeul	36°27'03.72"N/ 10°44'10.38"E/ 270 m	Cultivated field
Acc32		Mornag-Ben Arous	36°33'43.32"N/ 10°18'48.66"E/ 155.26 m	Cultivated field
Acc33		Chott Salem-Gabes	33°53'44.05"N/ 10°6'38.88"E/1.69 m	Cultivated field
Acc35		Bouchama-Gabes	33°45'23.93"N/ 10°03'59.72"E/ 1.88 m	Cultivated field
Acc37		Dgech- Tozeur	33° 51'28’’N/ 007°57'29"E/ 38 m	Cultivated field
Acc38		Dgech- Tozeur	33°59'80"N/ 007°57'10"E/ 31 m	Cultivated field
Acc39		Zir tenbib -Kebili	008°89’ 659"E/ 33.70'644"N/ 17 m	Cultivated field
Acc41		Sidi makhlouf-Medenine	33°22’42.229"E/ 10°42’42.229"N/ 36.4 m	Cultivated field
Acc42		Darghoulia-Medenine	10°38’36.496"E/ 33°23’ 32.870"N/ 61.9 m	Cultivated field
Acc43		Darghoulia-Medenine	33°23’32.870" E/ 10°39’19.535"N/ 32.7 m	Cultivated field
Acc60		Sejnane-Bizerte	9°32’53.305"E/ 36°57’ 62.53"N/ 105 m	Pasture
Acc42		Darghoulia-Medenine	10°38’36.496"E/ 33°23’ 32.870"N/ 61.9 m	Cultivated field
Acc43		Darghoulia-Medenine	33°23’32.870" E/ 10°39’19.535"N/ 32.7 m	Cultivated field
Acc45		Borj twil-Ariana	36°55’46.84"E/ 10°09’55.81"N/ 100.84 m	Cultivated field
Acc53		Edhraa-Sidi Bouzid	35° 00'10.87"N/ 9°22'36.90"/ 9.27 m	Cultivated field
Acc51		El karia-Gassrine	34°55'52.45"N/ 8°52'50.31"E/ 9.14 m	Cultivated field
Acc54		Bir hfay-Sidi Bouzid	34°55'54.23"N/ 9°10'57.55"/ 871 m	Cultivated field
Acc55		Siliana	36°04,06.53"N/ 9°22'42.4"E/ 5.98 m	Cultivated field
Acc 56	***M. spicata* L.**	Siliana	36°04,06.53"N/ 9°22'42.4"E/ 5.98 m	Cultivated field
Acc 22		Zaghouan	36°24'06.31"N/ 10°08'49.67"E/ 289 m	Cultivated field
Acc25	***Unidentified***	Zaghouan	36°24'06.31"N/ 10°08'49.67"E/ 289 m	Cultivated field

744

745 **Table 2.** Essential oil yields of 60 Tunisian accessions of *Mentha* spp.

746

	***M. spicata* L.**											
Accessions	Acc4	Acc9	Acc12	Acc13	Acc14	Acc16	Acc18	Acc23	Acc32	Acc33	Acc35	Acc37
Oil yields	1.02	0.77	1.22	1.35	1.30	1.37	1.12	0.78	0.87	1.08	0.62	0.80

747

748 **Table 2.** (continued)

	***M. spicata* L.**											
Accessions	Acc 41	Acc 42	Acc 43	Acc 60	Acc 45	Acc 53	Acc 51	Acc 54	Acc 55	Acc 56	Acc 38	Acc 39
Oil yields	1.07	1.02	0.68	0.73	1.10	0.52	0.77	0.45	0.59	0.68	1.08	0.62

749

750 **Table 2.** (continued)

	***M. spicata* var. crispa 'moroccan'**						***M. rotundifolia* L.**				***M×piperita***
Accessions	Acc 15	Acc 19	Acc 21	Acc 28	Acc 47	Acc 29	Acc 11	Acc 8	Acc 2	Acc 50	Acc 1
Oil yields	1.55	1.47	1.22	0.7	1.18	1.18	1.01	1.25	1.25	2.07	1.70

751

752 **Table 2.** (continued)

	***M. aquatica* L.**						***M. longifolia* L.**						***Unidentified***
Accessions	Acc 50	Acc 44	Acc 17	Acc 6	Acc 52	Acc 49	Acc 46	Acc 36	Acc 35	Acc 30	Acc 59	Acc 22	Acc 25
Oil yields	2.07	1.88	1.75	1.77	1.10	1.65	1.78	1.70	0.62	1.35	1.87	1.40	1.10

753

754 **Table 2.** (continued)

	***M. pulegium* L**											
Accessions	Acc 10	Acc 20	Acc 24	Acc 26	Acc 27	Acc 31	Acc 40	Acc 57	Acc 59	Acc 3	Acc 5	Acc 7
Oil yields	2.47	1.13	2.17	1.48	1.78	1.82	1.68	2.08	1.87	2.07	2.13	1.88

755

Table 3. Important essential oil components (%) of *Mentha* accesions and chemotypes

	Pulegium group		***Longifoliagroup***		***Rotoundifolia group***	***Piperita* group**	***Aquatica group***	***Spicata group***		***Spicata* var. crispa 'moroccan' group**	***Unidentified group***
	Chemotype 1	Chemotype 2	Chemotypes1	Chemotypes 2				Chemotypes 1	Chemotypes 2		
β-myrcene	0-0.17	0-0.28	0.24-0.43	0.29-0.41	0.12-0.98	8.75	7.72-7.84	0.76-1.21	0.417	0.5-1.21	3-3.329
Limonene	0.26-1.94	1.50-3.88	0.49-0.81	0.79-1.39	0-4.48	1.2	1.12 -5.61	6.07-18.45	0.783	10.52-17.94	6.63-8.72
1.8-cineole	0-0.72	0-0.21	5.31-8.11	6.63-10.34	0-0.22	9.31	3.98 - 8.31	7.24-12.49	10.413	0-5.4	9.61-10.18
Linalool	0-0.09	0-0.22	0-0.068	0-0.05	0-0.76	45.87	27.22-47.02	0-1.57	0	0.17-0.28	0.03-0.26
Menthone	0.57-28.44	1.46-27.54	7.14-15.38	31.08-40.20	0-1.88	0	0-0.7	0-6.12	28.562	0-0.32	0-0.163
Isomenthone	0.99-28.90	59.43-77.73	5.03-9.16	1.33-1.76	0-1.50	0	0	0-0.37	1.906	0-1.21	0.45-0.52
Cis-isopulegone	0.82-1.53	0.40-6.54	1.35-1.74	0-0.61	0	0	0-0.630	0-0.023	0	0.28-0.59	0.42-1.56
Menthol	0-0.22	0	0.61-1.40	4.93-5.7	0-4.49	0	0	0.152-0.54	5.646	0	0
Dihydro-carveol cis	0-0.07	0	0	0-0.21	0	0	0	1.17-6.56	0	0.39-3.62	2.28-3.77
Trans carveol	0-0.05	0	0-0.27	0-0.17	0	0	0	0.04-0.35	0	0-5.22	0.28-0.48
Pulegone	39.95-77.17	0.16-28.45	59.98-67.42	29.23-35.2	0-10.43	0	0.09 - 3.72	0.49-3.42	38.74	0-3.43	1.67-2.01
L-carvone	0-3.93	0-0.86	0.21-0.82	0.40-2.61	0-12.20	0.08	0 - 1.4	39,21-62,51	0.549	62.69-75.53	57-65.54
Linalyl acetate	-	0-0.84	0-0.44	0	0-0.58	14.29	13.91-18.39	0	0	0	0
Bornyl acetate	0-0.68	0	0-0.31	0-0.09	0.35-0.78	0	0	0.021-0.083	0	0	0-0.19
Piperitenone	0.135-0.85	0-0.07	1.08-3.56	0.47-1.26	0-0.031	0	0	0.01-0.41	1.034	0-0.11	0.04-0.11
Alpha-terpinyl acetate	-	0	0	0	0	0	0 - 7.7	0 .04-0.61	0	0-0.07	0-0.04
Piperitenone oxide	0-1.8	0-0.29	0-0.61	0	45.79-74.25	0	0	0.02-0.1	0	0-0.12	0-0.15
Viridiflorol	0-0.18	0-0.37	0	0	0.62-4.56	0	0 - 0.06	0-0.06	0	0	0-0.02

756

Figure


N
Bizerte
Ariana
Tunis
Nabeul
Jendouba
Beja
Zaghouan
Le Kef
Siliana
Sousse
Monastir
Kairouan
Mahdia
Kasserine
SidiBouzid
Sfax
Gafsa
Tozeur
Gabes
Kebili
Medenine
Tataouine
Legend
Cultivated accession
Wild accession
50km

Figure

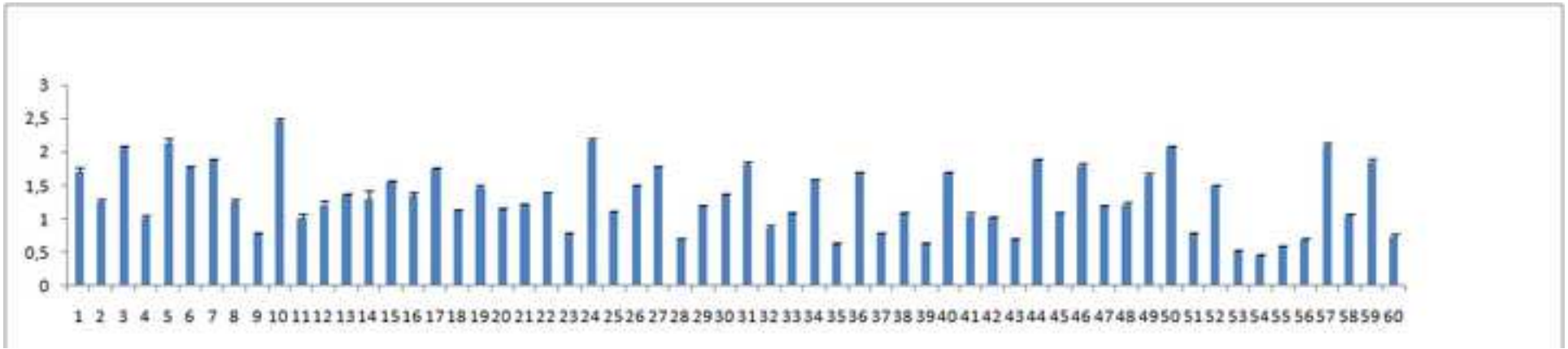
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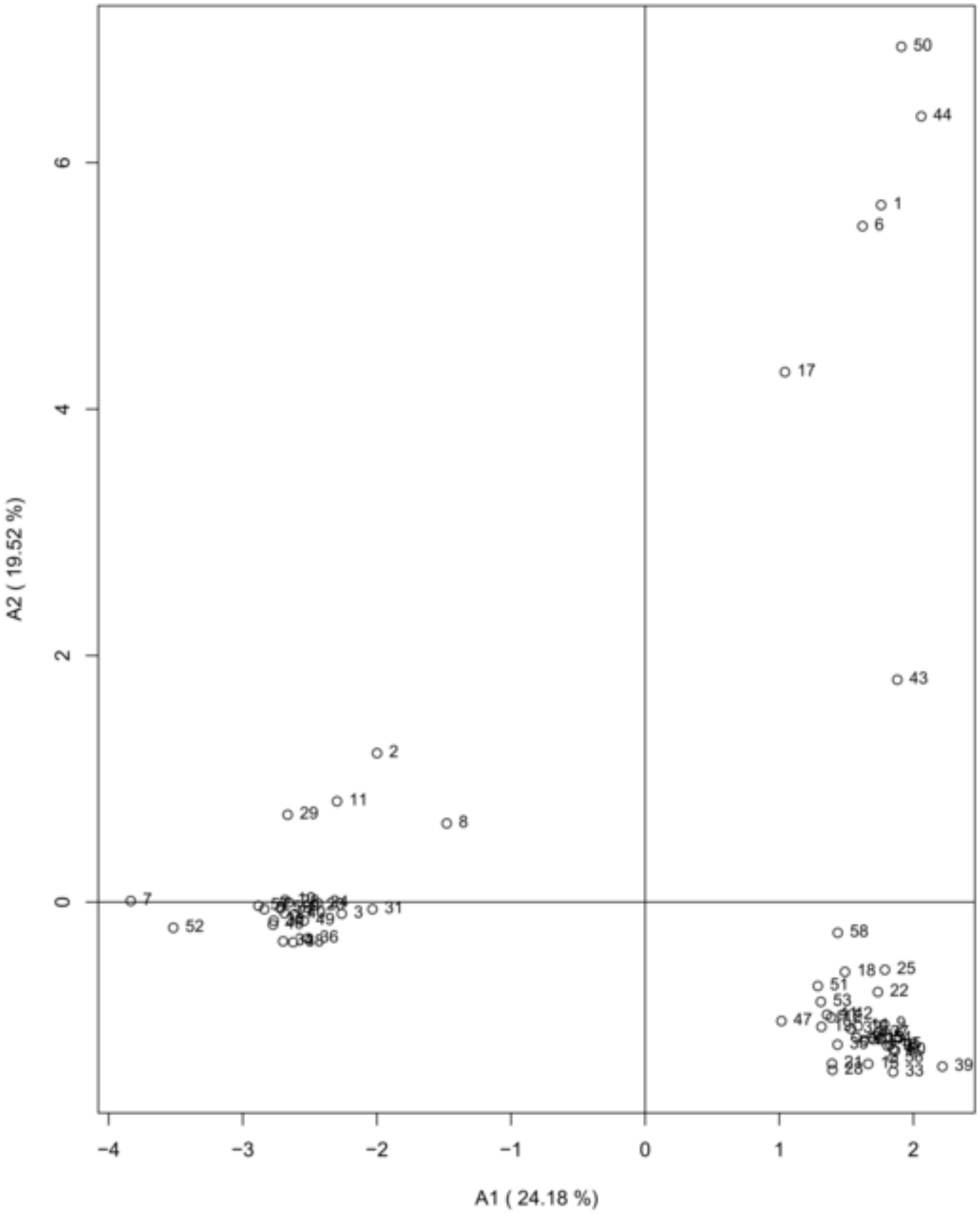
Figure


A2 (19.52 %)
A1 (24.18 %)
6
4
2
0
−4
−3
−2
−1
0
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50
44
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17
43
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11
29
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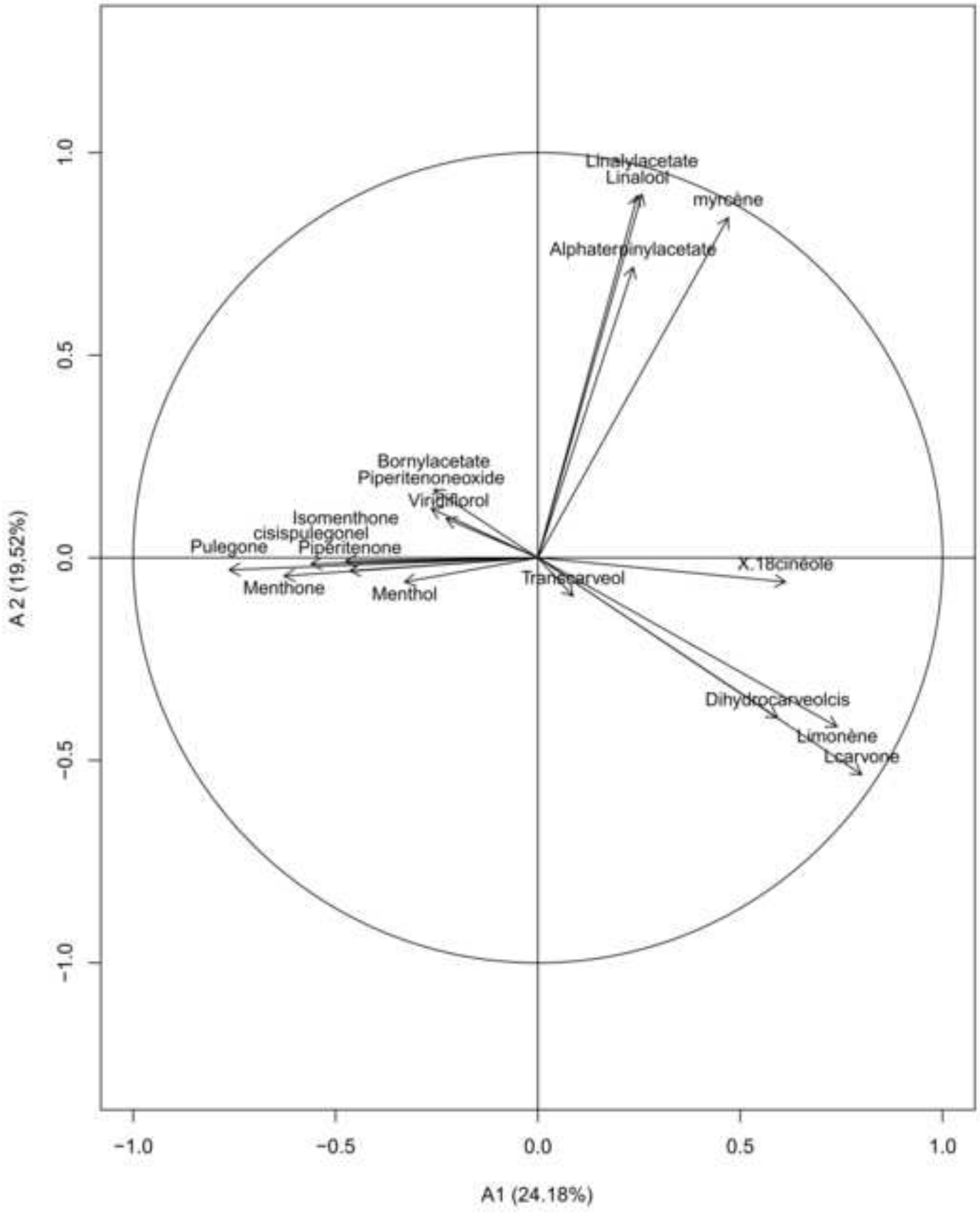
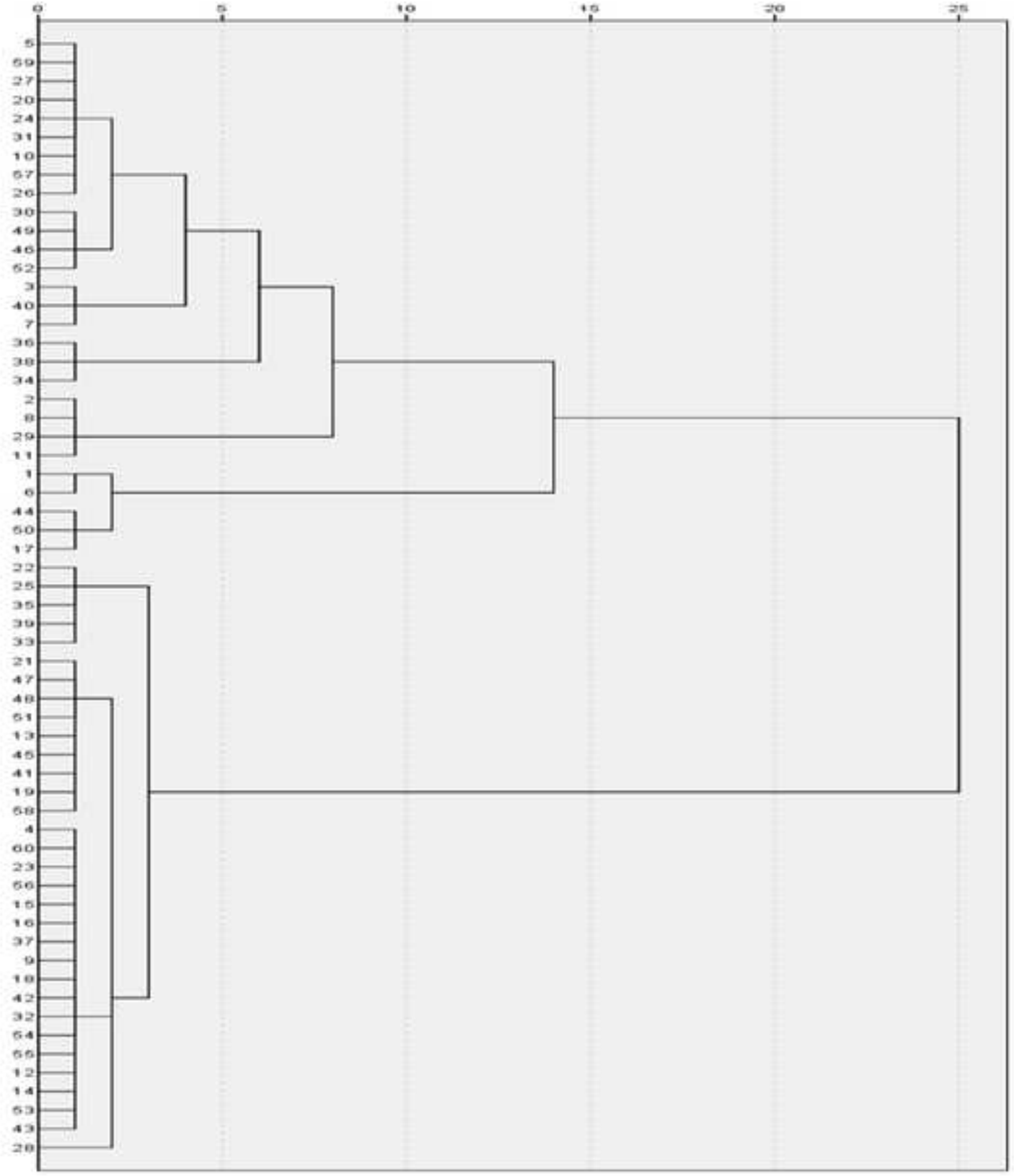
Figure


Linalylacetate
Linalool
myrcène
Alphaterpinylacetate
Bornylacetate
Piperitenoneoxide
Viridiflorol
Isomenthone
cisispulegonel
Pulegone
Piperitenone
Menthone
Menthol
X.18cinéole
Transcarveol
Dihydrocarveolcis
Limonène
Lcarvone
A 2 (19,52%)
A1 (24.18%)
1.0
0.5
0.0
−0.5
−1.0
−1.0
−0.5
0.0
0.5
1.0

Figure


0
5
10
15
20
25
5
59
27
20
24
31
10
57
26
30
49
46
62
3
40
7
36
38
34
2
8
29
11
1
6
44
50
17
22
25
35
39
33
21
47
48
51
13
45
41
19
58
4
60
23
56
15
16
37
9
18
42
32
54
55
12
14
53
43
28

Supplementary Interactive Plot Data (CSV)

Click here to download Supplementary Interactive Plot Data (CSV): Table S1. Supplementary Data.docx

Supplementary Interactive Plot Data (CSV)

Click here to download Supplementary Interactive Plot Data (CSV): Table S2. Supplementary Data.docx