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# **To cite this version:**

Kaoutar Aboukhalid, Chaouki Al Faiz, Mohamed Bakha, Ahmed Douaik, Abdeslam Lamiri, et al.. Agronomic evaluation and essential oil composition characterization of Origanum compactum accessions cultivated in Morocco. Journal of Essential Oil Bearing Plants, 2023, 26 (3), pp.705 - 722.  $10.1080/0972060\mathrm{x}.2023.2234401$  .  $\,$  hal-04211162

# **HAL Id: hal-04211162 <https://hal.science/hal-04211162>**

Submitted on 19 Sep 2023

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# **Agronomic evaluation and essential oil composition characterization of**  *Origanum compactum* **accessions cultivated in Morocco**

# **Kaoutar Aboukhalid1\*, Chaouki Al Faiz<sup>2</sup> , Mohamed Bakha<sup>3</sup> , Ahmed Douaik<sup>2</sup> , Abdeslam Lamiri<sup>4</sup> , Marc Gibernau<sup>5</sup> and Félix Tomi<sup>5</sup>**

- <sup>1</sup> Regional Center of Agricultural Research of Oujda, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco
- <sup>2</sup> Regional Center of Agricultural Research of Rabat, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco
- <sup>3</sup> Laboratory of Plant Bio- technology and Sustainable Development of Natural Resources, Polydisciplinary Faculty of Beni Mellal, Sultan Moulay Slimane University, P.B 592, 23000 Beni Mellal, Morocco

<sup>4</sup> Laboratory of Applied Chemistry and Environment, Faculty

of Sciences and Techniques, Hassan I University, B.P 577, 26000 Settat, Morocco

<sup>5</sup> Laboratoire Sciences Pour l'Environnement, Université de Corse-CNRS, UMR 6134 SPE, Route dessanguinaires, 20000 Ajaccio, France

**Corresponding Author** Kaoutar Aboukhalid *[kaoutar.aboukhalid@inra.ma](mailto:kaoutar.aboukhalid@inra.ma)*

#### **Abstract**

*Origanum compactum* is one of the most important aromatic and medicinal species, widely used in Morocco for various therapeutic and culinary purposes. The present study was carried out to evaluate the agronomic behavior of the species over two years of cultivation (2015-2016) and to characterize the chemical compositions of cultivated accessions under Moroccan pedoclimatic conditions. The 88 investigated *O. compactum* accessions, are originating from different bioclimate and geographical zones, representing all areas where the species is growing in Morocco. Agronomic performances varied significantly according to genotypes and cropping year. The accessions showed satisfactory adaptation capacity to cropping. Higher essential oil (EO) yields were observed in the second crop year, and plants belonging to carvacrol and thymol chemotypes were the richest in oil. A chemical variation of cultivated plants was detected for samples dominated by *p*-cymene and/or γ-terpinene, where the percentages of these components decreased in the second year of cultivation against a significant increase in carvacrol and thymol content. However, most of the EOs compositions of plants rich in carvacrol, thymol and carvacryl methyl oxide conserved their composition after cultivation. The different associations found between chemical and agronomic characters would help breeders to develop improved *O. compactum* cultivars with desirable agronomic and chemical traits.

*O. compactum* has great cultivation ability and therefore, it could be a promising alternative to ensure a sustainable utilization of this threatened medicinal and aromatic species.

#### **Keywords**

*Origanum compactum*; Agronomic parameters; Chemical characterization; Essential oils; Domestication

#### **Introduction**

The genus *Origanum* belongs to the Lamiaceae family and includes 43 species and 9 natural hybrids<sup>1-6</sup>, mostly distributed in the Mediterranean area but also in Asia, Middle East and North Africa<sup>1,7</sup>. Oregano is one of the most famous medicinal plants and its use as a therapeutic agent goes back to the ancient Romans and Greeks.

The market's interest in aromatic and medicinal plants is growing dramatically due to the new needs of cosmetic and pharmaceutical sectors. Oregano is among the most important of them, since several species of the genus *Origanum* have been reported to possess numerous pharmacological and biological activities: antioxidant, antibacterial, antifungal, anti- anticancer properties  $etc<sup>8-13</sup>$ .

*Origanum compactum* Benth. is the most widely distributed oregano species in Morocco. It grows spontaneously mainly in the Central and North-Eastern part of the country<sup>14</sup>. It is a diploid species having chromosome number of 2n = 30<sup>15</sup> . *O. compactum* has been used in Moroccan popular medicine as a powder or infusion to treat different ailments including dysentery, colitis, bronco- pulmonary and gastro-intestinal diseases $16,17$ .

Most of the oregano production is concentrated in Turkey, Greece, Mexico and Italy<sup>18</sup>, but many other Mediterranean countries, Morocco included, have a significant production, represented mainly by the species *O. compactum*. Wild populations of Moroccan *O. compactum* have declined to such an extent that they are at risk of extinction, due essentially to overharvesting, drought and climatic change<sup>19</sup>. One promising solution for the conservation of the species is to exploit its cultivation and *ex situ* conservation. However, the cultivation of wild medicinal species requires a good understanding of the effect of domestication on their agronomic performances and essential oil content and composition, in order to develop new cultivars with desirable chemical and agronomic traits.

Previous studies about *O. compactum* have focused mainly on biological activities and chemical variability of essential oils<sup>10,11,20-22</sup>, while and to the best of our knowledge, there are no comprehensive research undertaken regarding the agronomic characterization and the effect of cultivation on essential oil yield and composition. Therefore, the present study was performed to evaluate the agronomic and chemical responses of *O. compactum* through two years of field experiments. These informations are of great importance for selection of superior genotypes based on productivity, yield and chemical composition of essential oilsfor further domestication and improvement programs.

#### **Materials and methods**

#### *Plant material, experimental design, and growing conditions*

Rooted plants of 88 *O. compactum* populations were collected from different regions of Morocco. The plants were identified by the botanist Prof. Mohamed Ibn Tattou and voucher specimens were deposited in the Herbarium of the National Institute of Agricultural Research of Rabat, Morocco. The collection sites of accessions and their origin are listed in [table 1.](#page-12-0) The 88 investigated accessions are originating from different bioclimate and geographical zones, representing all areas where the species is growing in Morocco. They were transplanted in the green house and then planted in the experimental field, when they were with a well expanded root system. *O. compactum* plants were raised under similar climatic conditions following normal agricultural practices. The *ex-situ* experiments were carried out in the experimental field of the National Institute of Agricultural Research (INRA), in the beginning of May and during two consecutive years (2015 and 2016). The geographical coordinates of the experimental station, located near Sefrou city in Central Morocco, are 33°57'38.4''N and 4°10'37.4''W, its elevation is 1330 m above mean sea level and it has a climate with hot summers and cold winters. The average minimum and maximum temperatures are respectively 7°C and 19°C with an annual average rainfall of 550 mm. The soil characteristics were the following: clay- loamy soil, pH (7.7), electrical conductivity (1.38 ms/cm), Organic Matter (4.94%), Na (1.84 meq/100g), N (0.06%), P<sub>2</sub>O<sub>5</sub> (35.1 ppm), K<sub>2</sub>O

(1018.23 ppm) and  $CaCO<sub>3</sub>$  (35.1%). The experimental design was replicated five times and consisted of the installation of plants within rows. The spacing between plants within a row was 0.3 m. Different rows were separated from each other by 1.0 m. Plants were grown on biodegradable plastic mulch with an irrigation drip automated system. From the second year from planting, mechanical weed control was performed among rows, while manual weeding was carried out on the rows. No inorganic fertilizer and systemic pesticide were used during the experiment. A period of one year was left after transplantation in order to allow plants to adapt to the new field conditions.

#### *Data observations*

For each accession, agronomic observations and measurements were made on a sample plot of 5 to 10 plants. Harvesting of accessions was carried out when at least 70% of the plants were in full flowering stage for both years. The observations were recorded on different agronomic parameters:

*Biomass (t/ha)*: The fresh herb yield was weighted for each accession and the resultant weights were extrapolated into tonnes per hectare.

*Dry matter (t/ha):* Dry weight measurements were carried out after drying samplesin a shaded and well aerated environment and the resultant weights were extrapolated into tonnes per hectare. *Plant vigor:* A value scale from 1 to 5 was provided whereby 1 being the least vigorous accession and 5 being the most vigorous one<sup>23</sup>.

*Precocity:* The accessions were subdivided into the following precocity classes: very early, early, medium late, and late maturity.

*Diseases incidence:* We measured *O. compactum* disease incidence severity according to a scale of attack severity: 1 no symptoms; 2 very little disease incidence; 3 medium disease incidence; 4 high disease incidence; 5 extensive disease incidence.

*Seeds yield (Kg/ha):* At seed maturity stage, the seeds were harvested and weighted and the resultant yields were extrapolated into Kg per hectare.

*Leaves/stems weight ratio:* At flowering stage and after being dried in a in shaded and well aerated environment until constant weight, the leaves were separated from stems and then weighted separately to determine leaves/stems weight ratio.

# *Effect of cultivation on essential oil yield and quality Collection of cultivated plants*

From a germplasm collection of nearly 800 individual plants and 88 accessions collected from the wild and planted under uniform cultivation conditions, we selected 50 individual plants to use in this study according to their chemical composition. We selected 50 representative individual plants representing 5 different chemotypes. The selection was done according to the results of a previous study about wild *O. compactum*<sup>21</sup>. To avoid variability in the EO yield and composition due to season, climate, and growing conditions, the plants were transplanted and raised under similar climatic conditions. Aerial parts from each cultivated individual plant were harvested during flowering stage after the first and second year of cultivation. Thus, 100 EO samples were obtained over two years on the same individuals.

# *Extraction of EOs*

At flowering stage, the aerial parts from each genotype were dried at room temperature to constant weight and submitted to extraction of the EOs by hydrodistillation using a Clevenger- type apparatus. The determined EO yield was based on dry weight basis. The obtained EOs from all the above samples were characterized by GC-FID and GC/MS analysis.

# *GC-FID analysis*

The essential oil samples were analyzed using a Clarus 500 Perkin-Elmer apparatus, equipped with two flame ionization detectors (FID) and a fused silica capillary column (50 m x 0.22 mm i.d., film thickness 0.25 μm): apolar BP-1 (polydimethylsiloxane). The oven temperature was programmed to rise from 60°C to 220°C at a rate of 2°C/min and then held isothermal at 220°C for 20 min; injector temperature was 250°C; detector temperature, 250°C ; Helium was used as the carrier gas at a flow rate of 0.8 ml/min; split ratio was 1/60. Retention indices were determined relative to the retention times of a series of n-alkanes with linear interpolation using the software Target Compounds from PerkinElmer.

# *GC/MS analysis*

GC/MS analyses were performed using a Perkin- Elmer TurboMass quadrupole detector, coupled to a Perkin-Elmer Autosystem XL, equipped with a Rtx-1 (polydimethylsiloxane) fused-silica capillary column (60 m x 0.22 mm i.d., film thickness 0.25 μm). The oven temperature was programmed to rise from 60 to 220°C at a rate of 2°/min and then held isothermal at 220°C for 20 min; injector temperature was 250°C; ion-source temperature, 150°C; carrier gas, He (1 ml/min); split ratio, 1/80; injection volume, 0.2 ml; and ionization energy, 70 eV. The electron ionization (EI) mass spectra were acquired over the mass range 35-350 Da.

*Identification and quantification of components* Identification of the individual components was achieved on the basis of (i) comparison of the GC retention indices (RIs) on apolar columns, determined relative to the retention times of a series of n-alkanes with linear interpolation ('Target Compounds' software of Perkin- Elmer), and with those of authentic compounds.

(ii) Computer matching against commercial mass spectral libraries<sup>24,25</sup>, and comparison of the spectra with literature data<sup>26,27</sup>. The relative contents of the oil components were expressed as percentage obtained by peak area normalization.

# *Statistical analysis*

The Pearson correlation coefficient was calculated to determine the relationships between the studied agronomic traits. Relationships among accessions were investigated by principal component analysis (PCA). The Gower distances<sup>28</sup> were calculated and a cluster analysis of the accessions using Ward's method<sup>29</sup> was applied on the distance matrix. Agronomic data were also subjected to one-way ANOVA analysis in order to evaluate the effect of accession and cropping year on the main productive parameters. The level of significance was tested with F values ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ ). Statistical analysis of the agronomic data was performed using SAS software. Concerning chemical data, the significance of differences in essential oils yield and composition was tested by analysis of variance (ANOVA) and the differences between measurements were considered to be significant at  $p < 0.05$ . Correlation analysis between agronomic and chemical compositions was performed using multiple regression analysis to identify association between them using SAS software.

# **Results and discussion**

# *Agronomic characterization*

The mean, minimum and maximum values, standard deviation, and coefficients of variation (CV) of the agronomic variables in studied populations are summarized in [table 2.](#page-14-0) Significant differences were observed between minima and maxima for all studied agronomic traits. Coefficient of variation was the lowest for dry matter productivity ( $1<sup>st</sup>$  year) (CV = 25.1%), while it was the highest for seeds yield (CV  $= 70.2\%$ ). The average seed yield was 201 Kg/ ha. However, there was a substantial variation among the accessions, ranging from 5 to 945.1 Kg/ha. There are many factors that influence seed yield such as planting date, density, soil fertility, climatic conditions, irrigation, cultivation methods and farm management practices. For example, planting date and density affected seeds yield of *O. vulgare*  cultivated in Iran<sup>30</sup>. The early planting date and the highest density induced a significantly higher seed yield. Indeed, early planting date provide longer period of main and lateral stems and inflorescence development and longer growth season, less heat stress and therefore high seed yield, while the higher seed yield at higher densities may be due to the quick formation of plant canopy, the increase in leaf area index and better utilization of solar radiation and other resources<sup>31</sup>. In our study, *O. compactum* genotypes were cultivated under the same environmental conditions and growing conditions. Therefore, the seed yield variability may be due to genetic factors.

The biomass and dry matter productivity achieved in the second year were on average significantly higher than those obtained in the first year. Also, the growth and branching of the stems are more important. After one year of plantation, the developed root system is short and superficial, which exposes the plants to different biotic and abiotic stresses, expressed by a relatively low productivity. Thus, it seems that a fairly developed root system of *O. compactum* accessions contributes to the improvement of agronomic performances through the improvement of the absorption of water and nutrients. Our results are in agreement with those obtained by other authors, who found that biomass and dry matter productivity of *O. vulgare* recorded during the second year of cultivation was greater than that obtained in the first year $32,33$ .

Selection of performant genotypes would be more appropriate by combining several agronomic parameters and identifying their combinations. Principal component analysis (PCA) was conducted to reduce the number of factors, understand relationships between characteristics as well as between populations and to determine the most suitable traits for classification [\(Fig. 1,](#page-17-0) [Fig. 2\)](#page-17-1). PCA reduced the variables to four principal components explaining 73% of the total variance. The first component which explained 32% of the total variation is positively correlated with biomass ( $1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) and dry matter productivity ( $1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) [\(Table 3\)](#page-14-1). The second components (PC2) representing 18.8% of total variance is defined by the positive correlation between plants vigor (2<sup>nd</sup> year), diseases incidence and flowering OUAZ<sub>55</sub>, OUAZ<sub>56</sub>, OUAZ<sub>58</sub>, OUAZ<sub>60</sub>, OUAZ<sub>61</sub> and OUL<sub>88</sub> are the most vigorous (2 year), early flowering  $(1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) and showing very little disease incidence. However, accessions  $BENS_7$ ,  $BENS_8$ ,  $BENS_9$ ,  $BENS_{10}$ ,  $BENS_{11}$ ,  $BENS_{12}$ ,  $ROM_{78}$ ,  $ROM_{79}$  and  $OUT_{86}$  showed an opposite trend [\(Fig.](#page-17-1) 2). Regarding PC3, earliness ( $1<sup>st</sup>$  and  $2<sup>nd</sup>$  year). The third component accessions BENS  $_{10}$ ,  $BENS_{11}$ ,  $OUAZ_{43}$ ,  $OUAZ_{54}$ , (PC3), describing 12.8% of the total variance, is positively correlated with plants vigor (1<sup>st</sup> year) and seeds yield, while the last factor (PC4), explaining 9.4% of the data variability, is positively related to leaves/stems weight ratio and negatively correlated with seeds yield.

With regard to PC1, accessions  $TT_2, TT_3, SK_5, SK_6$ ,  $BENS_{14}$ ,  $BENS_{15}$ ,  $TA_{24}$ ,  $TA_{27}$ ,  $TA_{28}$  and  $CHEF_{37}$ were clustered at the positive part of axis 1. These accessions were characterized by their important biomass (1<sup>st</sup> and 2<sup>nd</sup> year) and dry matter (1<sup>st</sup> and 2<sup>nd</sup> year) productivity. In contrast, accessions TA<sub>16</sub>, TA<sub>17</sub>,  $TA_{18}$ ,  $TA_{19}$ ,  $OUAZ_{67}$ ,  $KH_{75}$ ,  $ROM_{76}$ ,  $MDZ_{81}$ ,  $MDZ_{82}$  and  $OUT_{84}$  were the least productive in biomass and dry matter. With respect to PC2, accessions  $TT_1$ ,  $SK_5$ ,  $TA_{23}$ ,  $TA_{26}$ ,  $OUAZ_{39}$ ,  $OUAZ_{44}$ ,  $OUAZ_{45}$ ,  $\text{OUAZ}_{51}$ ,  $\text{OUAZ}_{52}$ ,  $\text{OUAZ}_{55}$ ,  $\text{OUAZ}_{56}$ ,  $\text{OUAZ}_{60}$ ,  $\text{AZ}_{68}$ ,  $\text{BM}_{71}$  and  $\text{OUL}_{88}$  were the most productive in seeds and the most vigorous (1<sup>st</sup> year). Concerning PC4, populations  $TA_{18}$ ,  $OUAZ_{39}$ ,  $OUAZ_{41}$ ,  $OUAZ_{44}$ ,  $\text{OUAZ}_{67}$ , MDZ<sub>81</sub> and  $\text{OUL}_{84}$  are those showing the highest leaves to stems weight ratio and the lowest seeds yield [\(Fig.](#page-17-1) 2).

Cluster analysis(CA) was performed to classify and differentiate the analyzed *O. compactum* accessions according to their agronomic characteristics. [Fig.](#page-18-0) 3 presents the corresponding dendrogram using Ward's method. Considering the 11 studied variables, three major groups were defined, whose composition is summarized in [table 4.](#page-15-0) The resulting dendrogram reported in [fig. 3](#page-18-0) showed the existence of a relatively high intraregional similarity of cultivated *O. compactum*. The following groups have been defined:

*Group A:* consisted mainly on populations originating from Northern regions (Tangier- Tetouan, Chefchaouen, Taounate and Ouazzane). Accessions belonging to this group are distinguished by: very high biomass productivity (5.7 t/ha  $\pm$  1.2 in the 1<sup>st</sup> year and 6.4 t/ha  $\pm$  1.3 in the 2<sup>nd</sup> year); very high dry matter productivity (2.6 t/ha  $\pm$  0.5 in the 1<sup>st</sup> year and 3.4 t/ha  $\pm$  0.5 in the 2<sup>nd</sup> year); low seeds yield (159.8 Kg/ha  $\pm$  90.5); very low diseases incidence; late flowering in the 1<sup>st</sup> year and moderately late flowering in 2<sup>nd</sup> year.

*Group B:* included mainly accessions from Ouazzane and Sidi Kacem. Accessions belonging to this group are characterized by: high biomass productivity  $(4.7 \text{ t/ha} \pm 1.2 \text{ in the } 1^{\text{st}}$  year and 5.3 t/ha  $\pm$  1.3 in

the 2<sup>nd</sup> year); high dry matter productivity (2.4 t/ha  $\pm$  0.5 in the 1<sup>st</sup> year and 3.4 t/ha  $\pm$  0.5 in the 2<sup>nd</sup> year); very high seeds yield (238.0 Kg/ha  $\pm$  201.7); low diseases incidence; moderately early flowering in the 1st year and early flowering in the 2nd year.

*Group C*: consisted mainly on accessions from Benslimane, Moulay Driss Zeroun and Oulmes regions. This group is characterized by: low biomass productivity  $(3.0 t/ha \pm 1.3 t)$  in the 1<sup>st</sup> year and 4.1 t/ha  $\pm 1.8 t$ the 2<sup>nd</sup> year); low dry matter productivity (1.9 t/ha  $\pm$  0.5 in the 1<sup>st</sup> year and 2.2 t/ha  $\pm$  0.6 in the 2<sup>nd</sup> year); high seeds yield (220.1 Kg/ha  $\pm$  110.5); extensive disease incidence and very early flowering in the 1<sup>st</sup> and 2<sup>nd</sup> years.

Biomass and dry matter parameters in *O. compactum* genotypes cultivated under the same environmental conditions revealed a significant genotypic variability. In general, and except few accessions, the transplantation of samples originating from humid zones in an experimental field with climatic conditions more or less similar to those of the natural habitat (irrigation), has a positive effect on biomass and dry matter productivity. Conversely, samples collected from semi-arid zones, very different from the cultivation site (sub-humid climate), penalized biomass and dry matter yield. Thus, any domestication program of *O. compactum* performant genotypes should be undertaken at the local level to maintain the productivity of plants. It is obvious that biomass and plant growth are much more important in the second year of cultivation. The high productivity in biomass and dry matter in cultivated plants is a positive response of plants to more favorable environmental conditions (soil, irrigation, etc.) reflecting their adaptation and interaction with their new environment.

#### *Correlations between agronomic parameters*

In this study, a significant positive correlation was observed between biomass, dry matter productivity  $(1<sup>st</sup>$  and  $2<sup>nd</sup>$  years) and plants vigor [\(Table 5\)](#page-15-1). These parameters are the most important criteria for the production of dry leaves to ensure an increased economic profit. Furthermore, a significant positive correlation has been established between flowering earliness and seeds yield. In this context, it has been reported that genes controlling the flowering of plants influence flowering date and seeds productivity through their effects on the beginning and duration of the reproductive season, the number of branches and the number of flowers per node. Genotypes that have escaped terminal drought through early flowering provide high seeds productivity<sup>34,35</sup>. In this study, flowering earliness is a component that could be used as selection criterion for improving seeds yield of *O. compactum*.

In addition, there was a significant negative correlation between biomass and dry matter productivity  $(1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) on one hand and flowering earliness  $(1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) on the other hand [\(Table 5\)](#page-15-1). Therefore, it seems that flowering earliness is associated with a short period of accumulation of favorable reserves for the development of biomass, leading to reduced biomass productivity. Furthermore, a significant negative correlation was noted between seeds yield and leaves/stems weight ratio, suggesting that leaves weight may be a good selection criterion to improve seed yields of *O. compactum* accessions.

#### *Chemical characterization EOs yield*

Statistical analysis revealed significant differences in the EO content  $(P < 0.05)$  between the two years. Our findings revealed that in average, the yield increased in the second year of cultivation. The higher essential oil yield in the second year of the study could be explained by the higher yield of aerial parts biomass in the second year compared to the first year<sup>36,37</sup>. The yield based on dry weight ranged from 1.22% to 4.50%, and an important variability was observed among samples. The EO yields achieved in this study are higher than those obtained by other authors, who showed that EOs yield of cultivated *O.* compactum using seed germination and cutting ranged from 1.33% to 2.80%<sup>38</sup>. This finding might be ascribed to the variations of climatic conditions between different Moroccan accessions, genetic factors, experimental design and growing conditions. Previous study about wild *O. compactum* revealed that EO yield ranged from 0.67 to 2.88%<sup>21</sup>. Increased yield after cultivation may be related to soil optimization, increased and continuous irrigation, and moderate environmental conditions. Further- more, significant differences in the EOs yield were found among chemotypes  $(P < 0.05)$ . [Fig.](#page-18-1) [4](#page-18-1) shows the variation of the EOs yield (% on dry weight basis) for each chemotype. EO yields were higher for plants belonging to carvacrol and thymol chemotypes. Hence, carvacrol and thymol rich plants exhibit the highest values of oil content, showing the highest potential to be selected for maximizing the oregano essential oil yield. Yields were obtained from *O. compactum* plants grown under the same edapho-climatic and cultural conditions, thus, variability in their EO production attributable to environmental parameters was considered negligible. In all chemical groups, the lowest yields have been registered in the first year: 1.9%±0.2 to 3.6%±0.6 in average, while higher yields were registered in the second year: 2.5%±0.3 to 3.9%±0.7. These values are comparable to those reported for other *Origanum* species like *O. syriacum*  collected from the wild and cultivated in Turkey  $(2.9 - 3.5%)^{39}$ , and higher than those of *O. vulgare* ssp.  $viridulum$  cultivated in Italy  $(0.09-0.75\%)$ <sup>40</sup>.

#### *Chemical variability of EOs*

The chemical diversity of plants is of great interest when the potential use of a species is related to its compounds. However, it is well known that yield and EO components of *Origanum* species are determined by various factors including plant genetic background, growth stage, climate, edaphic factors, altitude, topography, etc. or by any of their interactions<sup>22,41-43</sup>. Therefore, all chemotaxonomic statements should be based on comparable material, grown and harvested under comparable circumstances. In total, 30 components were identified representing more than 95% of the essential oils. The components present in appreciable content were carvacrol, thymol, *p*-cymene, γ-terpinene, carvacryl methyl oxide, and αterpineol. It could be noticed that in cultivated *O compactum*, the oxygenated monoterpenes were the most abundant (48.8- 93.6%) followed by monoterpene hydrocarbons (1.3-48.7%). The sesquiterpene fraction occurred only in small proportions, ranging from 0.1 to 5.9% [\(Table 6\)](#page-19-0).

For wild plants rich in carvacrol/*p*-cymene/γ- terpinene, a significant quantitative variation of some compounds (P<0.05) was detected after cultivation. The amount of oxygenated monoterpenes increased largely, due to the increase in carvacrol content, whereas the content of monoterpene hydrocarbons decreased drastically due to an important decrease in the content of *p*-cymene and γ-terpinene. In fact, after one year of cultivation, EOs were characterized by the dominance of carvacrol (66.0% $\pm$ 14.8), and only a slight decrease in the content of this component was observed after the second year of cultivation (M=63.4% $\pm$ 6.1) [\(Table](#page-19-0) 6). Differences between chemical traits after plantation in this study demonstrate the effect of the environment on the expression of some chemical compounds. It confirms the ability of the plant to modify trait expression as a response of environmental changes.

The EO compositions of plants belonging to carvacrol chemotype remained essentially identical to the original plants in the wild. EOs obtained from the first and second year were characterized by high contents of carvacrol (71.2% $\pm$ 9.2 and 76.9% $\pm$ 5.4 respectively). All the remaining components varied only to a small extent (less than 4%).

Concerning plants rich in carvacrol, carvacryl methyl oxide, *p*-cymene and γ-terpinene, the percentages of γ-terpinene in cultivated plants decreased by 7.2% and 6.7% after the first and second year respectively. Furthermore, the content of *p*-cymene decreased by 12.5% and 16.8% after the first and second year respectively. Except carvacrol, which increased significantly after the cultivation in detriment of *p*-cymene and γ-terpinene, the other components presented more or less comparable amounts.

With regard to thymol, carvacrol and *p*-cymene rich plants, the content of *p*-cymene decreased after cultivation of 10.4% and 11.0% in average, at the first and second harvest respectively, while the amount of γ-terpinene decreased from 11.5% to 4.1% and 7.3% in average, at the first and second year respectively. The influence of growing conditions may account for these variations in the EOs compositions.

For thymol chemotype, plants cultivated under identical environmental conditions displayed no major effect on the EO composition. Thymol remained the major compound after cultivation. However, this component increased after the second year of cultivation of  $15.1\%$  to reach  $69.3\% \pm 5.7$ . A close examination of the other EO components revealed very similar chemical profiles for wild and cultivated *O. compactum* plants with less than 8% quantitative variation for all constituents, even after the second year of cultivation. Briefly, the patterns of the EOs chemical composition have largely remained the same after two years of cultivation under new growing conditions.

From these results, it appears that the *ex situ* plantation induced some changes in *O. compactum* EO composition. Indeed, cultivation favored the conversion of *p*-cymene and γ-terpinene to carvacrol and/or thymol, since the monoterpenes γ-terpinene and *p*-cymene are considered the biogenetic precursors, via enzymatic hydroxylation, of the phenolic monoterpenes carvacrol and thymol<sup>44,45</sup>. Previous studies have demonstrated that several factors affect the oregano secondary metabolites such as environmental conditions, season of collection, age of plants, and/or genetic factors $32,33,41,42,46-48$ . However, the obtained results in our study showed that chemical compositions of *O. compactum* EOs are influenced by genetic factors as the plants were grown under similar edapho-climatic conditions.

#### *Correlations between phytochemical and agro- nomic traits*

Yield and chemical composition of EOs showed statistically significant correlation with some agronomic traits, especially variables related to biomass and dry matter productivity, leaf/stem weight ratio and seeds yield. Indeed, a negative correlation was found between EO yield and biomass and dry matter productivity [\(Table 7\)](#page-16-0). As a result, any increase in EO content induces decreased biomass productivity. The same observation has been reported on *O. vulgare ssp. hirtum* genotypes cultivated in Greece<sup>49</sup>. Furthermore, a positive correlation has been registered between biomass productivity  $(1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) on one hand, and the content of thymol (0.33\* and 0.34\*\* respectively), α-terpineol (0.42\*\*\* and 0.44\*\* respectively) and carvacryl methyl oxide (0.26\* and 0.31\* respectively) on the other hand. However, biomass productivity ( $1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) showed a negative correlation with carvacrol content (-0.29\* and -0.31\* respectively). Moreover, a positive correlation has been established between dry matter productivity (1<sup>st</sup> and 2<sup>nd</sup> year) and thymol (0.33<sup>\*</sup> and 0.44<sup>\*\*\*</sup> respectively),  $\alpha$ -terpineol (0.40<sup>\*\*</sup> and 0.45\*\*\* respectively) and carvacryl methyl oxide content (0.35\* and 0.28\* respectively). However, the dry matter yield ( $1<sup>st</sup>$  and  $2<sup>nd</sup>$  year) is negatively related to carvacrol content (-0.31\* and -0.40\*\* respectively). These results suggested that accessions rich in thymol, α-terpineol and carvacryl methyl oxide exhibit higher biomass and dry matter productivity. On the other hand, accessions with high amounts of carvacrol are the least productive. It has been reported in a previous study about *O. compactum*  that accessions growing in regions with humid climate showed high thymol, α-terpineol, and carvacryl methyl oxide content and accessions belonging to semi- arid climate were characterized by high carvacrol content<sup>41</sup>. Therefore, the transplantation of plants rich in thymol,  $\alpha$ -terpineol and carvacryl methyl oxide, which are essentially originated from humid zones, in a site with climatic conditions more or less similar to those of the natural habitat (sub-humid climate, irrigation), has a positive effect on biomass and dry matter productivity. In contrast, samples rich in carvacrol, collected mainly from semi-arid areas, very different from the cultivation site, penalized biomass and dry matter productivity. Thus, any domestication program must be undertaken at local scale in order to maintain plant productivity.

Overall, high genetic diversity was observed among the evaluated accessions, which belong to different geographical origins, for all the parameters studied. In particular, large variations were shown for some economically important traits, such as seed yield, essential oil content, flowering earliness, diseases incidence, etc. This would be potentially useful for breeding program by selecting the most performant, homogeneous, and stable accessions regarding a specific trait.

### **Conclusion**

The results of this study are of great importance to initiate the cultivation of the species at large scale by selecting genotypes with superior agronomic characteristics and improved EO composition. The accession  $SK<sub>5</sub>$  could be recommended as the best cultivar for herb production, due to its high biomass and dry matter productivity, vigor, flowering earliness and free disease incidence, it is an example of performant genotype at different levels. Despite some chemical variability, our findings showed that cultivation of *O. compactum* improved the EO yield and composition, and therefore the economic production of *O. compactum* EOs. Therefore, domestication may constitute an alternative solution to *ex- situ* conservation of this threatened medicinal species. The high agronomic and chemical differences within and among *O. compactum* accessions could provide useful information for *ex-situ* conservation of this endangered species as well as for selection of performant cross-parents in breeding programs to develop improved cultivars.

# **Acknowledgments**

We acknowledge the financial support of the Moroccan-French cooperative program (PRAD).

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<b>Region</b>	<b>Accessions</b>	<b>Collection site</b>	<b>Climate</b>
Tangier-Tetouan	$TT_1$	Ain Lahcen	Humid
	TT,	Khmiss Anjra	Humid
	TT <sub>3</sub>	Khmiss Anjra	Humid
	$TT_{4}$	Melloussa	Humid
Sidi Kacem	$SK_{\varsigma}$	Zeggota	Semi-arid
	$SK_{6}$	<b>Tnine Srafeh</b>	Semi-arid
Benslimane	$BENS_7$	Bouznika towards Benslimane	Semi-arid
	BENS <sub>8</sub>	Krama forest	Semi-arid
	$BENS_{\rm o}$	<b>Benslimane</b> forest	Sub-humid
	$BENS_{10}$	<b>Oued Cherrat</b>	Semi-arid
	$BENS_{11}$	Ain dakhla	Sub-humid
	$BENS_{12}$	Khatouat	Semi-arid
	$BENS_{13}$	Benslimane towards Sidi Yahya Zaer	Semi-arid
	$BENS_{14}$	Benslimane towards Rommani	Semi-arid
	$BENS_{15}$	Benslimane towards Rommani	Semi-arid
Taounate	$TA_{16}$	Ouartzagh	Sub-humid
	$TA_{17}$	Oudka	Humid
	$TA_{18}$	Tabouda	Humid
	$TA_{19}$	Kissane	Sub-humid
	$TA_{20}$	Sidi Mokhfi	Humid
	$TA_{21}$	Zrizer	Humid
	$TA_{22}$	Timezgana	Humid
	$TA_{23}$	Galaz	Sub-humid
	$TA_{24}$	Ghafsai	Humid
	$TA_{25}$	Beni zeroual	Sub-humid
	$TA_{26}$	Beni zeroual	Humid
	$TA_{27}$	Bibane	Humid
	$TA_{28}$	Tafrant	Sub-humid
Chefchaouen	$CHEF_{29}$	Talassemtane	Humid
	$CHEF$ <sub>30</sub>	Akchour	Humid
	$CHEF_{31}$	Talambote	Humid
	$CHEF_{32}$	Akchour	Humid
	$CHEF_{33}$	Jbel Meggou	Humid
	$CHEF_{34}$	Jbel Tissouka	Per-humid
	$CHEF_{35}$	Beni bouhlou	Humid
	CHEF <sub>36</sub>	Assifane	Humid
	$CHEF_{37}$	Beni Ahmed	Humid
<b>Ouazzane</b>	$OUAZ_{38}$	Ain beida	Humid
	$OUAZ_{39}$	<b>Brikcha</b>	Sub-humid
	$OUAZ_{40}$	<b>Brikcha</b>	Sub-humid
	$OUAZ_{41}$	<b>Oued loukouss</b>	Humid
	$OUAZ_{42}$	Mokrisset	Sub-humid
	$OUAZ_{43}$	Mokrisset	Humid

<span id="page-12-0"></span>**Table 1.** Origin, sampling location and climate of studied *O. compactum* populations



<b>Agronomic parameters</b>	Mean	<b>Minimum</b>	Maximum	<b>Standard</b> deviation	Coefficient of variation%
Biomass productivity(t/ha) $(1st year)$	4.2	0.8	9.2	1.5	35.9
Biomass productivity(t/ha) $(2nd$ year)	5.7	1.8	10.2	1.8	31.0
Dry matter (t/ha) $(1st year)$	2.4	1.1	4.3	0.6	25.1
Dry matter (t/ha) $(2nd$ year)	3.0	1.3	5.0	0.9	27.9
Leaves/stems weight ratio	2.0	0.9	3.6	0.6	29.6
Seeds yield (Kg/ha)	201.0	5.0	945.1	141 2	70.2

<span id="page-14-0"></span>**Table 2.** Descriptive statistics for quantitative agronomic traits among studied *O. compactum* populations

<span id="page-14-1"></span>**Table 3.** Cumulative variance of the four major factors obtained from principal component analysis (PCA) based on the main agronomic traits of *O. compactum* accessions

<b>Principal Components</b>							
<b>Agronomic traits</b>	Axe <sub>1</sub>	Axe <sub>2</sub>	Axe3	Axe 4			
Biomass productivity $(1st$ year)	0.91	0.09	$-0.24$	0.06			
Biomass productivity $(2nd$ year)	0.88	0.17	0.03	0.02			
Dry matter productivity $(1st year)$	0.91	0.09	$-0.02$	0.04			
Dry matter productivity $(2nd$ year)	0.89	0.21	$-0.05$	$-0.06$			
Leaves/stems weight ratio	0.46	$-0.01$	$-0.02$	0.98			
Seeds yield	$-0.11$	$-0.02$	0.65	$-0.56$			
Vigor $(1st$ year)	0.08	0.02	0.85	0.08			
Vigor $(2nd$ year)	0.45	0.58	0.10	$-0.22$			
Diseases incidence	0.20	0.64	0.43	$-0.06$			
Flowering earliness $(1st$ year)	0.19	0.71	$-0.25$	$-0.03$			
Flowering earliness $(2nd$ year)	0.01	0.85	0.02	0.11			
% of variance	32.0	18.8	12.8	9.4			
Cumulative % of variance	32.0	50.8	63.6	73.0			

<b>Agronomic traits</b>	<b>Group A</b>	<b>Group B</b>	<b>Group C</b>
Biomass productivity $(1st year)$ (t/ha)	$5.7 \pm 1.2$	$4.7 \pm 1.2$	$3.0 \pm 1.3$
Biomass productivity $(2nd$ year) (t/ha)	$6.4 \pm 1.3$	$5.3 \pm 1.3$	$4.1 \pm 1.8$
Dry matter productivity $(1st year)$ (t/ha)	$2.6 \pm 0.5$	$2.4 \pm 0.5$	$1.9 \pm 0.5$
Dry matter productivity $(2nd year)$ (t/ha)	$3.4 \pm 0.5$	$3.4 \pm 0.5$	$2.2 \pm 0.6$
Seeds yield (Kg/ha)	$159.8 \pm 90.5$	$238.0 \pm 201.7$	$220.1 \pm 110.5$
Diseases incidence	Good resistance to diseases	Good resistance to diseases	Sensibility to diseases
Flowering eraliness $(1st$ year)	Late flowering	Moderately late flowering	Very precoce flowering
Flowering eraliness $(2nd$ year)	Moderately late flowering	Precoce flowering	Very precoce flowering

<span id="page-15-0"></span>**Table 4.** Mean percentages and standard deviations of discriminant agronomic traits of studied Moroccan *O. compactum* accessions

<span id="page-15-1"></span>**Table 5.** Combined analysis of variance for agronomic traits in *O. compactum* genotypes

	<b>B1</b>	B <sub>2</sub>	DM1	DM <sub>2</sub>	<b>LSR</b>	<b>SY</b>		FE1	FE <sub>2</sub>
B1									
B <sub>2</sub>	$0.76***$								
DM1	$0.85***$	$0.70***$							
DM <sub>2</sub>	$0.75***$	$0.79***$	$0.80***$						
<b>LSR</b>	0.08	0.09	0.07	0.02					
$\mathbf{S}\mathbf{Y}$	$-0.10$	$-0.04$	$-0.08$	$-0.05$	$-0.18*$	1			
V	$0.18*$	$0.17*$	$0.16*$	$0.17*$	0.03	0.11			
FE1	$-0.25***$	$-0.24***$	$-0.26***$	$-0.28***$	0.05	$0.21**$	$-0.11$		
FE <sub>2</sub>	$-0.27***$	$-0.26***$	$-0.29***$	$-0.26***$	0.03	$0.24**$	$-0.10$	$0.25**$	
B1: Biomass (1 <sup>st</sup> year); B2: Biomass (2 <sup>nd</sup> year); DM1: Dry matter (1 <sup>st</sup> year); DM2: Dry matter (2 <sup>nd</sup> year); LSR: Leaves/									
stems weight ratio; SY: Seeds yield; V: Vigor; FE1: Flowering eraliness (1st year); FE2: Flowering eraliness (2 <sup>nd</sup> year)									

<b>Parameters</b>	<b>Biomass</b> (1 <sup>st</sup> year)	<b>Biomass</b> (2 <sup>nd</sup> year)	(1 <sup>st</sup> year)	Dry matter Dry matter (2 <sup>nd</sup> year)	PF/PT	<b>Seeds</b> yield
Thymol	$0.33*$	$0.34**$	$0.33*$	$0.44***$	0.10	$-0.03$
$p$ -Cymene	0.09	0.06	0.20	0.15	$-0.22$	$-0.35**$
$\gamma$ -Terpinene	0.08	$-0.07$	0.04	0.11	$-0.25$	$-0.17$
Carvacrol	$-0.29*$	$-0.31*$	$-0.31*$	$-0.40**$	$-0.08$	0.14
$\alpha$ -Terpineol	$0.42***$	$0.44***$	$0.40**$	$0.45***$	0.17	$-0.17$
Carvacryl methyl oxide	$0.26*$	$0.31*$	$0.35*$	$0.28*$	$0.31*$	$-0.21$
EO yield	$-0.24*$	$-0.29*$	$-0.25*$	$-0.27*$	0.11	$-0.09$

<span id="page-16-0"></span>**Table 7.** Pearson's correlation coefficient (r) between chemical and quantitative agronomic parameters



<span id="page-17-0"></span>**Figure 1.** Principal Component Analysis (PCA) of *O. compactum* genotypes, based on all agronomic traits, according to the first two principal components (PC1 and PC2)



<span id="page-17-1"></span>**Figure 2.** Principal Component Analysis (PCA) of *O. compactum* genotypes, based on all agronomic traits, according to the principal components PC3 and PC4



<span id="page-18-0"></span>**Accessions Figure 3.** Dendrogram of the Hierarchical Cluster Analysis (HCA) representing agronomic traits similarity relationships among *O. compactum* accessions



<span id="page-18-1"></span>**Figure 4.** Yields (% on dry weight basis) of *O. compactum* EOs for each chemotype

<span id="page-19-0"></span>

RT: Retention Time; RI: Retention index determined on the apolar column (BP-1); RI lit: retention index from literature ; tr: Traces (<0.01)

