# Chemical Composition of Essential Oils from *Rosmarinus officinalis* L and Acridicide Activity on *Dociostaurus maroccanus* Thunberg, 1815 in Morocco

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**Abstract:** This work aims to study the chemical composition of essential oils from *Rosmarinus officinalis* L. samples collected in spring in Moyen-Atlas (Morocco) and to test their insecticide activity on survival of the pest locust *Dociostaurus maroccanus*. These essential oils are characterized by the presence of 1,8-cineole (42,24%), α-pinene (16,31%), camphor (10.81%), sabinene (8,64%) and myrtenol (5,01%) as major compounds. Comparative analyses among plants collected in different countries evidenced 4 well defined chemotypes, associated to more or less extended territories. Our extract was active against locusts, as estimated by the determination of the LT50 (lethal time 50). LT50 obtained was around 2.25 days for females and 1.9 days in males. This effect can be attributed to one or several compounds of the extract. Given the high humidity of produced faeces, disturbance of water intake by rectum seems to be the most probable cause of mortality.

**Index terms**: Essential oil, acridicide activity, chemotype, *Rosmarinus officinalis*, *Dociostaurus maroccanus.*



## 1. INTRODUCTION

Among the grasshoppers, the Moroccan locust *Dociostaurus maroccanus* remains one of the most harmful pests against agricultural and pastoral production in certain regions of Morocco. It economic impact is higher than other grasshoppers in outbreak areas. Despite all the precautions taken by state services involved in crop protection, this pest problem remains unresolved [1, 2].

To cope with the locust issue, application of conventional chemical insecticides is the most often used technique. Unfortunately, it presents adverse effects for humans and the environment [3]. In addition, the overuse of these insecticides causes an imbalance in the ecosystem and therefore disruption of the food chain such as the eradication of non-target species, auxiliary fauna and the appearance of resistant insects. So an alternative to conventional chemical control is useful for reducing the adverse effects of treatments [4].

The objective of this work is to study the effect of essential oils of *Rosmarinus officinalis*, as natural products, on survival of the locust *D. maroccanus*.

We also compared the composition of these essential oils with other samples from different origins in the Mediterranean Basin.

## 2.MATERIAL AND METHODS

### 2.1 Origin of insects

The insects subject to this study are the third stage instar of the species *Dociostaurus maroccanus*. They were collected in the region of "Timahdite" (Moroccan Middle Atlas) during the year 2013. A mass-rearing was established in wooden cages (Figure-1) at a temperature of 30 ± 1°C and a photoperiod of 12N/12D. Locusts were fed on grass seedlings grown at the Fez university site.

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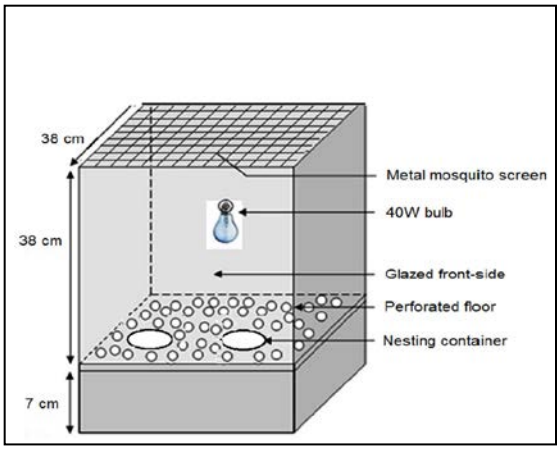


**Figure1.**Mass rearing device for grasshopper

## 2.2. Plant material

The samples of *Rosmarinus officinalis* used for this study were collected during the month of October 2013, in the region of Boulemane at an altitude of 1500 m, in a low matorral vegetation dominated by rosemary, as a result of the degradation of ancient forests of red cedar (*Juniperusn phoenicea*) and holm oak (*Quercus ilex*). The climate in the region is semi-arid with fresh winter [5].

The extraction of essential oils from the leaves of rosemary was performed by hydro distillation in a Clevenger-type apparatus [6].We conducted three distillations by boiling 200 g of dry plant material. The extraction time was about 3 hours in average. Yields are expressed in ml per 100 g of dry matter. The essential oil obtained was dried by anhydrous sodium sulfate and then stored at low temperature (below 4°C) and dark before use. The yield is calculated by the following equation:

$$R = [\frac{V}{ms} \times 100] \pm [\frac{\Delta V}{ms} \times 100]$$

R: yield in essential oils (%); V: volume of essential oils obtained; ΔV: measure error; ms: plant dry mass.

Further analyzes were performed on a gas chromatograph Hewlett Packard (HP 6890 series) with electronic pressure control, equipped with a HP-5 capillary column (30 m × 0.25 mm) with a film thickness 0.25 microns, a FID detector set at 250 °C and fed with a mixture of gas H2/Air and a split-split less injector set at 250 °C. The injection mode is split type (leakage ratio: 1/50, flow rate:66 ml/min). The gas used is nitrogen with a flow rate of 1.7 ml/min. The column temperature is programmed from 50 to 200 °C at a rate of 4°C/min. The unit is controlled by a computer system HP ChemStation, managing the functioning of the device and monitoring chromatographic analyzes.

The identification of the compounds was based on their IK (Kováts indices) and on gas chromatography coupled to mass spectrometry (GC-MS). The latter is carried on a gas chromatograph Hewlett-Packard (HP 6890 series) coupled with a mass spectrometer (HP 5973 series). Fragmentation is performed by electron impact in a field of 70 eV. The capillary column HP-5MS (30 m x 0.25 mm) was used, and the film thickness is 0.25 microns.

The column temperature is programmed from 50 to 250 °C at a rate of 4°C/min. The carrier gas was helium with a flow rate set at 1.5 ml/min. The injection mode is split (split ratio: 1/70 flow 112 ml/min. The camera is connected to a computer system running a mass spectrum library NIST 98.

## 2.3 Acridicide effect of essential oils

The locusts were distributed at 10 individuals per 3 liters glass box, with both sides airy. Males and females were separated to study the impact of treatments on sex factor. For each sex, we made three batches with three replicates each. The three batches are made up of individuals fed grass coated with water (control group 1), ethanol 75% (control group 2), and essential oil and 75% ethanol (1V/9V) (treated group 3). We have considered 90 males and 90 females, making a total of 180 individuals. Tested individuals were previously made fast for 24 hours before feeding the above diets. Behavioral observations were made throughout the experiment. The death toll was thus observed once per day until the total mortality of individuals. The young shoots of grass were replaced once a day to avoid evaporation of the essential oil, water and ethanol used.

### 2.4 Statistical analyses

To assess the closeness of the EO (essential oil) composition between the different localities, a cluster analysis was performed using Euclidean distance as a measure of dissimilarity. To test whether the different groups of localities have significant different compositions, an ANOSIM (analysis of similarity) was conducted, again with Euclidean distance. Briefly, a p-value <0.05 (deduced from 10000 permutations) associated to 2 given localities was considered to indicate a significant difference in EO composition. To classify the contribution of the different compounds in the differences between the different groups, SIMPER analysis (similarity percentage) was used. All the analyses were conducted using PAST 2.17c [7].

## 3. RESULTS AND DISCUSSION

### 3.1 Yield and chemical composition of essential oils (EO)

The average yield of essential oils extracted from the plant is studied in the range of 0.8%. This performance is comparable to that obtained for the same species studied in the region of Tlemcen (Algeria) whose yield was 0.8 % for the wildlife species and 0.6 % for the cultivated species [8]. In contrast, this performance was revealed lower than that given by the same species from different regions in Tunisia [9] and Sardinia [10] whose performance can reach 1.2% to 1.75%. This difference can be attributed to several factors such as climate and geographical conditions and the period of harvesting and drying conditions. It has been shown that drying affects the performance of essential oils: a plant dried in non-optimal condition may lose all of its essential oils [11]. In addition, the content of essential oils of rosemary leaves dried in shade for one week is four times higher than that of a fresh plant [12]. Overall, EO of Moroccan *Rosmarinus officinalis* have a relatively constant chemical composition comparable to works done on the same plant. However, the proportions of some compound can result in significant variations. Chroma- tographic analyzes of EO extracted (Table 1) showed the predominance of two mono- terpenes, 1,8-cineole (42.24%) and α-pinene (16.31%), among 29 constituents representing 97.57% of this extract. Some monoterpenes are present with relatively high percentages such as camphor (10.81%), sabinene (8.64%) and myrtenol (5.01%). Other components are minor with a percentage close to 1%, as α-cubebene, and γ-gurjunene and viridiflorol.

**Table 1.**Chemical composition of EO of *Rosmarinus officinalis* (KI = Kováts index)

| KI | Compounds | Percentages (%) |
|---|---|---|
| 931 | α-Thujene | 0.11 |
| 939 | α-Pinene | **16.31** |
| 953 | Sabinene | **8.64** |
| 976 | Camphene | **3.48** |
| 1011 | δ-3-Carene | 0.05 |
| 1033 | 1,8-cineole | **42.24** |
| 1068 | Trans-sabinene hydrate | 0.57 |
| 1097 | Cis-sabinene hydrate | 0.04 |
| 1125 | α-Campholene | 0.02 |
| 1143 | Camphor | **10.81** |
| 1165 | Borneol | 2.84 |
| 1204 | Verbenone | 0.02 |
| 1227 | Trans-2-Caren-4-ol | 0.03 |
| 1185 | α-Terpineol | 0.61 |
| 1194 | Myrtenol | **5.01** |
| 1235 | Myrtenyl acetate | 0.02 |
| 1285 | Bornyl acetate | 0.13 |
| 1351 | α-Cubebene | 0.01 |
| 1376 | α-Copaene | 0.02 |
| 1384 | β-Bourbonene | 0.02 |
| 1418 | β-Caryophyllene | 1.45 |
| 1473 | γ-Gurjunene | 0.01 |
| 1499 | α-Muurolene | 0.15 |
| 1513 | γ-Cadinene | 0.64 |
| 1581 | Caryophylleneoxyde | 4.22 |
| 1584 | Copaen-4-α-ol | 0.04 |
| 1590 | Viridiflorol | 0.01 |
| 1611 | Tetradecanal | 0.03 |
| 1653 | τ-Cadinol | 0.04 |
| Total (%) | | **97.57** |

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Studies on *Rosmarinus officinalis* harvested in Morocco [13, 14], Algeria [8, 15], Tunisia [9], Spain and France [13], Italy [16], Turkey [17] and Iran [18] showed variations in the composition of EO. To identify groups of chemotypes among the different samplings, we considered the 27 major compounds, accounting for a mean value of at least 0.15% of total composition. A first cluster analysis using Euclidean distance as a measure of dissimilarity gave four groups (figure 2).

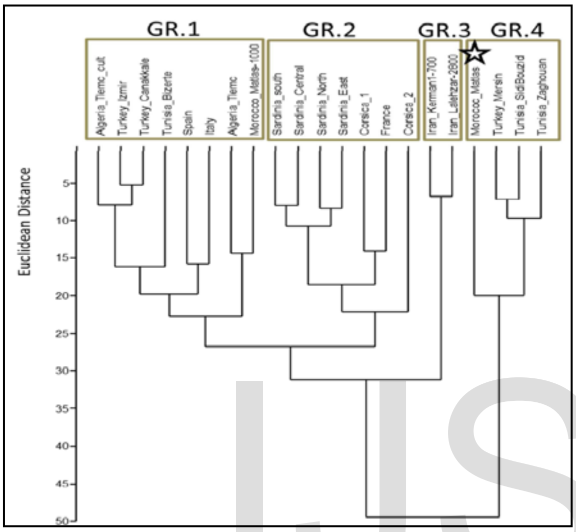


**Figure2.**Cluster analysis of the four groups of chemotypes, using Euclidean distance as a measure of dissimilarity. [The 27 majority compounds were taken into account for calculations. The star corresponds to our sampling].

The first one concerns individuals collected in localities widespread in the Mediterranean Basin from Morocco to Turkey; the second one is composed of populations from Corsica, Sardinia and South of France; the third one is limited to samples from Iran; the fourth one relates to different countries situated in the South and East parts of Mediterranean Sea. To assess the statistical significance of the different groups, we then applied an ANOSIM (Analysis of similarity). It revealed that all the combinations between groups had significant different chemical compositions (Table 2), except for the case of groups 3 and 4, where the difference were only marginally significant (p=.067). It means that each group of localities has its own EO chemotype, at least for the major compounds. SIMPER analysis indicates that the 27 majority compounds account for 99.8 % of dissimilarity between groups (Table 3).

**Table 2**. ANOSIM between the four groups of chemotypes (p-values given from 10000 permutations)

| | Gp 1 | Gp 2 | Gp 3 | Gp 4 |
|---|---|---|---|---|
| Gp 1 | | 0.0001 | 0.0207 | 0.0022 |
| Gp 2 | 0.0001 | | 0.0275 | 0.0041 |
| Gp 3 | 0.0207 | 0.0275 | | 0.0674 |
| Gp 4 | 0.0022 | 0.0041 | 0.0674 | |

[P-values <0.05 correspond to significant differences between each couple of chemotype groups.]

The particular features of each chemotype are easy to resume: Group 3 and 4 (including the Moroccan sample of the present work) are characterized by high percentages of α-pinene and 1,8 cineole respectively, while groups 1 and 2 share intermediary values for both compounds (figure 3).

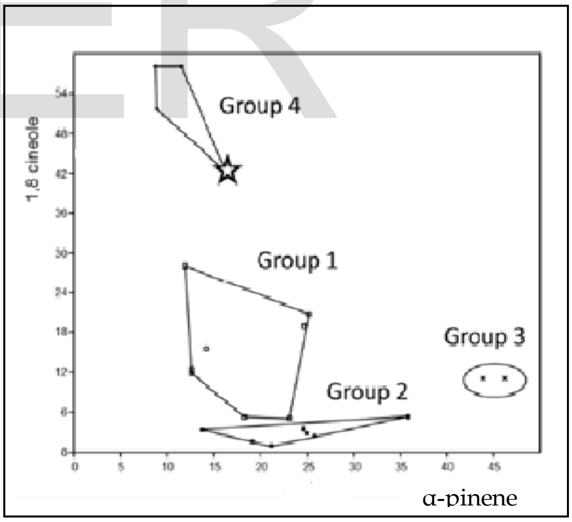


**Figure3**. Mean percentages of α-pinene and 1,8 cineole in the four chemotypes of Rosemary. The star corresponds to sampling.

Group 1 has the greatest yields in Camphor, and group 2 the highest ones for Bornyl acetate, Verbenol and Borneol.

From the present synthesis, it is difficult to assess the significance of these different chemotypes in terms of phylogenetic aspects within rosemary species, and of adaptation to various environmental constraints.

Table3: Mean composition of the four chemotype groups of Rosemary from SIMPER analysis

| Compound | Contrib. % | Cumu-lative % | M. abund. Gr1 | M abund. Gr2 | M abund. Gr3 | M. abund. Gr4 |
|---|---|---|---|---|---|---|
| 1,8-Cineole | 59.2 | 59.2 | 14.7 | 2.84 | 11.1 | 52.5 |
| α-Pinene | 17.2 | 76.4 | 17.8 | 23.6 | 45.1 | 11.4 |
| Bornyl acetate | 5.27 | 81.7 | 1.79 | 12.3 | 2.65 | 0.23 |
| Camphor | 4.7 | 86.4 | 13.1 | 3.7 | 3.85 | 11.8 |
| Verbenone | 3.98 | 90.3 | 5.54 | 9.57 | 2.45 | 0.81 |
| Borneol | 3.82 | 94.1 | 7.12 | 11.5 | 3.4 | 2.44 |
| Camphene | 0.96 | 95.1 | 4.71 | 6.82 | 9.1 | 3.54 |
| β-Pinene | 0.84 | 96 | 3.12 | 1.04 | 1.9 | 1.1 |
| Sabinene | 0.72 | 96.7 | 0.17 | 1.87 | 2.3 | 2.16 |
| Limonene | 0.42 | 97.1 | 3.12 | 3.9 | 0.6 | 0.6 |
| β-Cymene | 0.34 | 97.4 | 0.25 | 2.58 | 0 | 0 |
| Linalool | 0.31 | 97.7 | 2.65 | 2.24 | 1.9 | 0.53 |
| Myrcene | 0.28 | 98 | 1.97 | 2.03 | 3.9 | 2.63 |
| Iso-Pinocamphone | 0.22 | 98.2 | 1.79 | 0.56 | 0 | 0 |
| β-Phellandrene | 0.22 | 98.5 | 0.08 | 1.96 | 0.65 | 0 |
| Geraniol | 0.22 | 98.7 | 0 | 0.93 | 0 | 0 |
| α-Phellandrene | 0.22 | 98.9 | 0 | 1.8 | 0.1 | 0 |
| Myrtenal | 0.18 | 99.1 | 0.31 | 0 | 0 | 1.33 |
| P-Cymene | 0.14 | 99.2 | 0.56 | 0.01 | 0.65 | 1.88 |
| Caryophyllene oxide | 0.12 | 99.3 | 0.36 | 0 | 0.1 | 1.08 |
| α -Terpineol | 0.11 | 99.4 | 1.62 | 1.22 | 0.4 | 0.8 |
| Trans-verbenol | 0.09 | 99.5 | 0.11 | 1.02 | 1.15 | 0.05 |
| β-caryophyllene | 0.07 | 99.6 | 1.33 | 0.73 | 0.85 | 0.64 |
| O-cymene | 0.05 | 99.7 | 0.56 | 0 | 0 | 0 |
| d-3-Carene | 0.04 | 99.7 | 0.74 | 0.47 | 0 | 0.04 |
| Terpinen-4-ol (4-Terpineol) | 0.04 | 99.7 | 1.01 | 0.77 | 0.1 | 0.48 |
| Cis-Calamenene | 0.02 | 99.8 | 0.38 | 0 | 0 | 0 |

### 3.2. Acridicide activity of essential oils

Behavioural observations made on the treated individuals show a perturbation at their locomotor activities. Treated insects are weak and accomplish very limited movement compared to control individuals. Similarly, we noticed a decrease in the amount of grass consumed over time. Furthermore, these individuals treated exhibit a digestive disturbance evidenced by high humidity of their feces. This may be explained by the disrupting and antifeedant effects of essential oils and especially by a dysregulation of the water control following inhibition of water absorption by the rectum. This phenomenon has been widely observed in the migratory locust *Locusta migratoria* exposed to synthetic insecticides [19, 20], in the desert locust *Schistocerca gregaria* fed on *Pergamum harmala* [21], and *Euchorthippus albolineatus* treated by Artemisia herba-alba EO [22].

The essential oils of the Moroccan locust have a detrimental effect on their survival, as for males and females. This effect is expressed in the first day of treatment with a mortality rate of 35% in females and 30% in males. It gradually increases to reach its maximum on the fifth day in males and the sixth day in females. In contrast, the mortality rate in the control did not exceed 20% until the end of the experiment (Fig. 4).



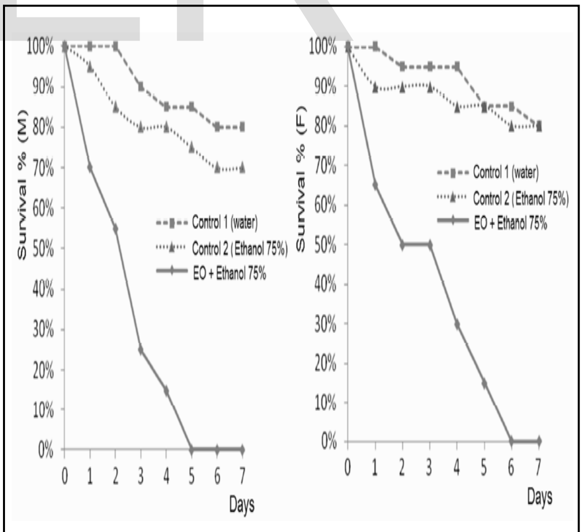


**Figure4:** Temporal variations of survival in males (M) and females (F)

The importance of toxicity of these EO toward locusts is estimated by determining the LT50 (lethal time 50), based on the regression of mortality rates over time (Fig. 5). LT50 obtained is around 2.30 days in females and 1.85 days in males.

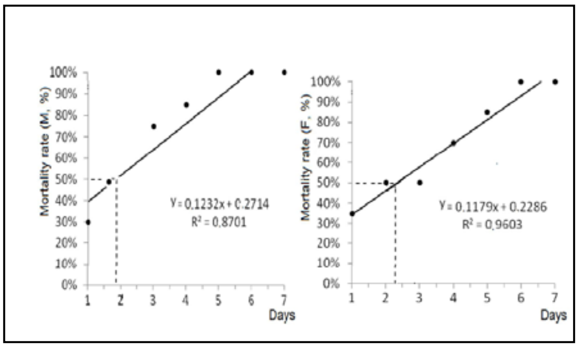


**Figure 5**: Temporal variations of regression of mortality rate in male (M) and female (F).

Behavioral and physiological problems posed by locusts treated with the essential oil of *Rosmarinus officinalis* can be explained by the effect of certain toxic compounds of this plant species. Thus, it was demonstrated that the monoterpene α-pinene has insecticidal activity [23], piperitone showed insecticidal activity against the Chrysomelidae *Callosobruchus maculatus* [24]. In addition, it has been reported the toxicity of myrcene on the Curculionidae *Sitophilus oryzae* [25]. Other effects *of Azadira chaindica* leave consumption are the interruption of insect molting by inhibiting the secretion of the hormone in the insect ecdysone. Similarly, these essential oils can also be used as a repellent against insects such as desert locusts and reduce its ability to fly [26].

## 4. CONCLUSION

The chromatographic analysis of the essential oil of *Rosmarinus officinalis* allowed the identification of 29 different compounds dominated by 1,8-cineole. Comparison analyses on samples collected in different areas mainly around Mediterranean Sea revealed 4 different chemotypes well defined and delimited. It is striking that the previous analysis performed in the same Moroccan region in Moyen-Atlas, but at around 1000 m A.S.L., corresponds to another chemotype group, less rich in 1,8 cineole. The remarkable toxicity of the essential oil of our Rosemary sampling toward Moroccan locust, in the conditions of laboratory at least, seems of great importance in the potential substitution of chemical pesticides widely used to fight insect pests. This knowledge therefore provides a basis for suitable formulation process or synthesis of molecules that could be used in field experiments.

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