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## Chemical composition, antioxidant activity and insecticidal activity of the leaves of the Moroccan variety *Mentha rotundifolia*.

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### ABSTRACT

Essential oils extracted from aromatic or medicinal plants have recently proved different applications in various fields (manufacture of medicines, perfumes and foodstuffs). The objective of this study is to evaluate the antioxidant activity, and the insecticidal activity of *Mentha rotundifolia* essential oil grown in 3 regions of Morocco, additionally to the determination of their chemical composition by gas chromatography (GC) coupled with mass spectrometry (GC-MS), the main component was Cyclohexylideneacetone (36.62%) for the region of salé, Pulégone (31.84%) for the region of Stehat, and Pulégone (37.13%) for the region of Ouezzane. For the antioxidant activity, results shows that those essential oils have a very high IC50 compared to the to the antioxidant ascorbic acid. They have also a greater biocidal effect on *Sitophilus granarius* in 15 minutes.

### 1. Introduction

Morocco is endowed with an abundance of aromatic plants, because of its geographical situation which means that it's bioclimate is conducive to rich and diverse vegetation. Various species and subspecies have been listed, including around a hundred endemics. Many of which have not yet had their chemical characterization and biological potential well studied [1], have been used for a very long time in medicine and are still a vital part of many people's basic care around the globe [2]. Their diverse range of biological characteristics may be ascribed to their complex chemical composition which is greatly influenced by external factors [2]. Biological actions of medicinal and aromatic plants include anti-inflammatory, anti-bacterial, antidiabetic, antioxidant, and anti-obesity and others [2].

the genus *Mentha*, comprises 25 to 30 species, it grows in the moderate regions of Africa, Europe, Australia, Asia, and North America, and belongs to Lamiaceae family [2], that is highly valued because to its widespread usage [3], and known for its biological properties [1].

these include *Mentha rotundifolia*, a hybrid of *Mentha longifolia* and *Mentha suaveolens*. However, according to other authors, *M. rotundifolia* and *M. suaveolens* correspond to the same species [4]. It is incredibly common throughout western Asia, America, and the Mediterranean

region. She has been used as an antiseptic and an antibacterial agent in folk medicine, as well as for their flavor in cooking. This fragrant shrub, also known as "timarssad," is well-known in Morocco and northern Africa [1].

Several studies have examined the chemical content of *M. rotundifolia* essential oils from various parts of the world have been documented, and associated chemotypes have been identified [4], one of them is rich of the oxide of pipériténone, an oxygenated monoterpene with studied biological effects including antibacterial, schistosomicidal, hypotensive, bradycardic, insecticidal, trypanocidal, antinociceptive [4], cardiovascular, and antifungal, toxicity, repellent effects [1]. Additionally, its usage is common. For instance, leaf decoction can be applied topically to treat furunculosis and abscesses, lower fever, and ease tooth discomfort by using it as a mouthwash. The plant is also said to help with ulcerative colitis, cough, and bronchitis. In addition, it is a common spice and used as a tonic, stimulant, stomachic, carminative, analgesic, choleric, antispasmodic, sedative, and hypotensive [3].

*Sitophilus granarius*, also known as the granary weevil, is a widespread insect pest that affects processing factories and storage facilities. *Sitophilus granarius* damages crops such as wheat, rice, beans, corn, sorghum, almonds, and oats [5]. *S. granarius* causes two types of harm to grain: direct loss

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from the pest's eating of the grain and indirect loss from secondary contamination with molts, excrement, or the dead bodies of grain weevils. Additionally, when this bug infests grain, the temperature and moisture of the material is raised. This promotes the growth of mold, including extremely hazardous species, and further reduces the grain's value [6], [7]. Synthetic pesticides, such as phosphine, which is very effective against *S. granarius*, are used to manage this pest. On the other hand, reports of phosphine resistance in certain *S. granarius* populations [5].

The objective of this study is to evaluate the antioxidant activity and insecticidal activity of *Mentha rotundifolia* essential oil of 3 Moroccan regions against *S. granarius*, in addition to the determination of their chemical composition.

## 2. Experimental

### 2.1 Plant material and essential oil extraction

The plant specimens were collected on July 2021 from 3 regions Ouezzane (33.5731104, -7.5898434), Salé (34.0690501, -6.7793343), and Stehat (35.3418917, -4.9515298). One hundred grams of the air-dried plant material of each region was hydrodistilled for 3 hours with a Clevenger-type apparatus. The EO was directly recovered from above the distilled water without adding any solvent and then stored in opaque glass tubes at 4° C until its use.

### 2.2 Essential oil extraction as a function of time

In order to compare essential oils yield percentage and their chemical composition as a function of time, hydro-distillation was carried out for different times (1h, 2h, 3h, 4h). For each essay, the yield percentage was calculated as the weight (g) of the essential oil per 100 g of dried plant.

### 2.3 Determination of the EO Chemical Composition

Chromatographic analyses were carried out on a Hewlett Packard electronic pressure-regulated gas chromatograph (HP 6890 series), equipped with an HP-5 capillary column (30 m x 0.25 mm) with a film thickness of 0.25µm, an FID detector set at 260°C and fed by a H<sub>2</sub>/air gas mixture and a split - splitless injector set at 275°C, the injection mode is split (leak ratio :1/50, flow rate: 66 ml/min). The gas used is nitrogen at a flow rate of 1.7 ml/min, and the column temperature is programmed from 50 to 250°C at a rate of 4°C/min. The unit is controlled by an HP ChemStation computer system, which manages the unit's operation and monitors the progress of chromatographic analyses. spectrometry (GC-MS), the latter performed on a Hewlett-Packard gas chromatograph. The column used is an HP-5MS capillary column (30 m x 0.25 mm), film thickness 0.25µm, column temperature programmed from 50 to 250°C at 4°C/min, the carrier gas is helium, flow rate fixed at 1.5 ml/min, injection mode is split (leakage ratio: 1/70, flow rate 112 ml/min, the device is connected to a computer system managing a library of NIST 98 mass spectra.

### 2.4 Antioxidant activity: DPPH

The antioxidant activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was determined according to the method described by Brand Williams et al, an ethanolic solution of 0.2 mM DPPH is mixed with different concentrations of the extracts of false pepper, in test tubes, 2.5 ml of each sample were added to 0.5 ml of the ethanolic solution of DPPH, after shaking, the mixtures were placed in the dark at room temperature for 30 minutes, absorbances were measured at 517 nm against a blank containing pure ethanol, the negative control was composed of 2.5 ml ethanol and 0.5 ml ethanolic solution of DPPH

The inhibition of free radical DPPH percentage (I %) is calculated as follows:

$$I (\%) = 100 \times (A [\text{control}] - A [\text{test}]) / A [\text{control}]$$

A[control]: is the absorbance of the control (containing all reagents without the test product).

A[test]: is the absorbance of the test compound (containing all reagents and the test product). All tests were performed in triplicate for each concentration

### 2.5 insecticidal activity

#### 2.5.1 Insect breeding:

Mass rearing involves placing a quantity of healthy wheat in sachets, to which is added wheat contaminated by *Sitophilus granarius*. These sachets are hermetically sealed, and left for a month at room temperature until the adults emerge. the aim being to produce a sufficient mass of individuals.

#### 2.5.2 Determination of the insecticidal activity

This test was carried out to evaluate the percentage of *S. granarius* mortality influenced by essential oils from the three regions. Doses of 25µl, 50µl, 100µl, 150µl and 200µl are diluted in 200µl of acetone for each oil, to ensure even distribution on the filter paper. the essential oil solution was evenly distributed on a filter paper disc, and a control received 200µl of acetone only. Five adult *S. g.* individuals were placed on the filter paper in the middle of the petri dishes, and mortality was monitored by counting the number of dead insects from the first day of treatment until all the individuals had died.

Average Mortality = (deaths of each dose/Total number used per dose) \*100

## 3 Results and discussion

### 3.1 Effect of extraction time

#### 3.1.1 Yield of essential oil

The essential oil yield is expressed as a percentage, and presented in the table below (Table 1).

**Table 1**

Yield of essential oils from different regions as a function of extraction time.

Yields	Time of extraction (hours)	Regions		
		Ouezzane	Salé	Stehat
	1h	1.18	0.46	1.3
	2h	1.36	0.56	1.3
	3h	1.42	1.34	1.9
	4h	1.98	1.7	2.04

The Table (Table 1) show that one hour of extraction yields 1% essential oil for Ouazzane and Stehat, while Salé yields less than 0.5% oil. After 4 hours of extraction, yields increase to 2% for Stehat and Ouazzane and 1.5% for Salé. We can conclude that extraction time influences essential oil yield, and plants from the Stehat and Ouazzane regions are richer in oil than those from Salé.

Our results are highest to those reported for the same species in Tunisia, where the highest yield was 1.26% and lower one was 1.04 according to the site of sampling [8]. Moreover, results of Algerian *Mentha rotundifolia* (L.) Huds essential oil yields were also lower than Moroccan ones ranging from 0,7 % to 0,9 % [9], similar results were obtained by Fatiha et al [10].

Variations in climatic factors may have contributed to produce diversity in the total essential oil concentration within the same species of mint. According to reports, *Mentha* species produced more essential oils during the summer when they were in full bloom than during the winter when they were nearing the end of their growth cycle [10].

### 3.1.2 Chemical composition

Gas chromatography (GC) of *M. rotundifolia* EO from 3 extractions (1h, 2H, 3h) isolated 3 main compounds Pulegone, Terpinen-4-ol, Eucalyptol.

The results of the table 2 show that the 3 main compounds are present in different percentages during the 4 hours of extraction. During the first three hours, Pulegone accounts for a very high percentage, between 30 and 50%, while Terpinen-4-ol and Eucalyptol do not exceed 20% of the chemical composition, extraction in 4 hours is characterized by a large presence of Terpinen, which exceeds 40%, followed by Eucalyptol and then Eucalyptol, which is beginning to disappear, so we can see that 3 hours of extraction remains the best time to obtain good yields of these three compounds, so this test gives us information on the optimum time for extracting each of these compounds.

**Table 2**  
Chemical composition of essential oils as a function of extraction time

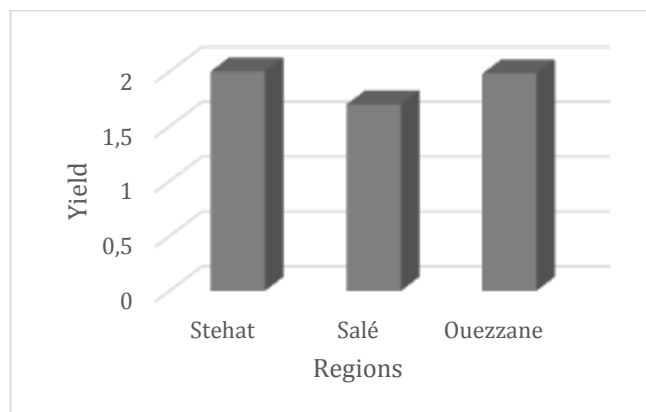
Extraction time	1H	
Compounds	Retention time	Stehat 1H
<b>Pulegone</b>	39.919	40.59
<b>Terpinen-4-ol</b>	50.625	14.90
<b>Eucalyptol</b>	32.686	7.12
Extraction time	2H	
Compounds	Retention time	Stehat 2H
<b>Pulegone</b>	39.75	34.95
<b>Terpinen-4-ol</b>	50.694	19.56
<b>Eucalyptol</b>	32.634	6.43
Extraction time	3H	
Compounds	Retention time	Stehat 3H
<b>Pulegone</b>	39.953	47.95
<b>Terpinen-4-ol</b>	50.41	8.88
<b>Eucalyptol</b>	32.563	5.01
Extraction time	4H	
Compounds	Retention time	Stehat 4H
<b>Pulegone</b>	38.797	3.29
<b>Terpinen-4-ol</b>	50.574	51.88
<b>Eucalyptol</b>	32.327	32.33

## 3.2 Region effect

### 3.2.1 Yield of essential oil

The essential oils obtained from round-leaf mint are light yellow in color, with a very spicy aromatic scent in all three regions.

Figure 2 shows the yield of essential oils extracted from Round-leaved Mint harvested from the 3 regions of Morocco. The histograms show that the plant from the Stehat region remains the richest in essential oil (2%), followed by Ouazzane (1.98%) and Salé (1,7%)



**Figure 1:** Yield of essential oils from plants of the 3 regions

### 3.2.2 Chemical composition:

Gas chromatography (GC) of *M. rotundifolia* EOs from the 3 regions isolated 38 compounds representing 90.97% for the Salé region, 92.96% for the Stehat region and 94.64% for the Ouezzane region.

The essential oil of the salé region is dominated by Cyclohexylideneacetone (36.62%), Beta-Copaene (6.45%), Caryophyllene (3.71), P-menth-8-en-1-ol (3.35%) and finally Cinerolon (3,33%).

For the Stehat region, the essential oil is dominated by Pulégone (31.84%), 3,5 Dimethylhydroquinone (8,9), Terpinen-4-OL (7.79%), P-menth-8-en-1-ol (6.01%) and Eucalyptol (5,71%).

In the Ouezzane region, the essential oil is dominated by Pulégone (37.13%), Terpinen-4-OL (8.57%), Eucalyptol (5.71%),  $\beta$ -Copaene (5.71%),  $\alpha$ -Thujol (3.47).

Examination of Table 2 shows that a number of identical compounds are present in the *Mentha rotundifolia* EO samples, but they differ in abundance, the main compounds in EO from both the Ouezzane and Stehat regions Stehat regions are Pulegone, which is in the majority in EO from both Ouezzane and Stehat in the Salé region EO, Terpene-4-ol and Eucalyptol are in the majority in the Stehat region EO. the EO of the Stehat and Ouezzane regions, P-menth-8-en-1-ol is in the majority in the EO of the stehat region and a minority in the EO of the other two regions, Cyclohexylideneacetone is in the majority in EO from the Salé region, but absent in EO from the other two regions.

**Table 3**  
Chemical composition of essential oils from the 3 regions.

Compounds	Retention time	Sale	Stehat	Ouezzane
$\beta$ -Terpinen	11.31	1.59	1.01	0.96
3-Carene	12	2.08	1.96	1.83
$\beta$ -Myrcene	12.12	1.74	1.45	1.46
$\beta$ - Myrcene	12.2	1.23	1.04	1.03
(+)-4- Carene	12.74	-	0.91	1.15
D-Limonene	12.98	3.21	1.69	3.47
Eucalyptol	13.07	2.76	5.71	6.7
$\gamma$ -Terpinene	13.44	0.28	1.49	1.81
P-Menth-8-en-1-ol	13.65	3.35	6.01	3.32
2-Carene	13.95	0.3	0.6	0.71
1-Octen-3-YL-Acetate	14.13	1.72	1.23	1.25
$\alpha$ -Thujol	14.73	0.34	3.88	5.31
Terpinen-4-ol	15.42	0.62	7.79	8.57
Anethole	15.62	1.02	-	-
Estragole	15.69	2.91	0.5	-
$\alpha$ -Terpineol	15.99	0.95	-	1.54
Pulegone	16.39	2.85	31.84	37.13
p-Menth-1-en-3-one	16.6	1.54	-	-
cis-p-Menth-8-ene	16.68	1.3	0.29	0.23
Carvone	16.84	0.52	0.33	0.24
Verbenone	17.87	2.58	2.37	2.17
3,5-Dimethylhydroquinone	18.18	-	8.9	2.24
Cyclohexylideneacetone	18.46	36.62	-	-
Methyleugenol	18.55	1.46	0.39	0.7
Cinerson	18.79	3.33	-	-
Caryophyllene	19.06	3.71	1.25	1.6
$\beta$ -Farnesene	19.14	0.43	1.29	0.21
$\alpha$ -Cubebene	19.31	0.98	1.1	1.14
Cis-Muuroala-4(14).5-diene	19.56	1.5	1.28	1.33
$\beta$ -Copaene	19.82	6.45	4.8	4.44
Alloaromadendrene	19.92	0.33	0.37	0.35
$\alpha$ -Copanene	20.18	0.35	0.22	0.3
Trans-Calamenene	20.24	0.68	0.32	0.38
$\alpha$ -Muurolene	20.39	0.3	0.23	0.31
Selina-3.7(11)-diene	21.18	0.24	0.74	0.37
4-Epi-Cubedol	21.37	0.79	0.78	0.86
Tau.-Cadinol	21.62	0.38	0.36	0.47
$\alpha$ -Cadinol	21.8	0.53	0.83	1.06

Our results are similar to those obtained by [11] who studied the chemical composition and antimicrobial and insecticidal activities of the essential oil of *Mentha rotundifolia* from the Béni Mellal region (Morocco), where the majority compound was Pulegone (85%) [11].

Previous studies in other countries have revealed the existence of different major components, such as Rotundifolone (65%) [12] and piperitenone oxide (23.5 and 38.6%), cis-piperitone oxide (28.1 and 30.5%) in Algeria [13]. Moreover another study done with the same species from China showed that the essential oil were dominated by cis-jasmone (36.77 %, 32.43 %), germacrene D (11.89 %, 10.88 %),  $\beta$ -ocimene (10.51 %, 11.27 %), viridiflorol (8.20 %, 8.47 %), octenyl acetate (6.41 %, 6.74 %) and  $\beta$ -

farnesene (4.22 %, 5.66 %) [14], in another one the major component was menthol (40.50%), with the presence of other ones such as : menthone (5.0%), menthyl acetate (4.50%), menthofuran (4.20%), neomenthol (3.80%), linalyl acetate (3.50%), piperitone oxyde (3.20%), piperitone (3.10%), isomenthone(2.50%), 1,8-cineole (2.40%), linalool (2.0%), limonene (1.80%), geraniol (1.70%), myrcene (1.60%), geranyl acetate (1.50%) and trans-Sabinene hydrate (1.40%) [15]. however pulegone oxide dominated a Moroccan one with a percentage of 83.5% [16].

### 3.3 Antioxidant activity: DPPH

From the results shown in the table above, we can see that the 3 extracted oils do not have significant antioxidant activity, as they have a very high IC<sub>50</sub> compared to the to the antioxidant ascorbic acid.

**Table 4**  
IC<sub>50</sub> of essential oils and ascorbic acid

Extracts	IC <sub>50</sub> ( $\mu$ g/mL)
Sale EO	113.693 $\pm$ 1.116
Ouazzane EO	561.948 $\pm$ 1.308
Stehat EO	72.873 $\pm$ 1.204
Ascorbic acid	1.907 $\pm$ 0.038

In the light of the above results, the percentages of DPPH radicals increased with increasing concentration of HE methanolic solutions, For the Stehat and Salé regions, the inhibition percentages are 85.46% and 81.39% respectively at a concentration of 200  $\mu$ g/ml, demonstrating that their antioxidant activity than Ouazzane.

The findings of this study are closely consistent with those reported by another work where the lowest IC<sub>50</sub> value of the *M. rotundifolia* essential oil was determined as 2222.2  $\pm$  25.2  $\mu$ g/ml, which means that the oil has been found less effective [10], Less values was determined by Riahi et al, where the lowest IC<sub>50</sub> value that was observed was 26.11  $\pm$  1.04 g/ml [8]. Tunisian species exhibited less activity with a lower IC<sub>50</sub> value of 3.66 mg/mL [17]. These values are highest then other reports, where the lowest value was 0.36  $\pm$  0.03 mg/mL [18].

### 3.4 Insecticidal activity

#### 3.4.1 Insecticidal activity of the essential oil of Stehat region:

For the plant in Stehat, the low concentration of 25 $\mu$ L oils had an average influence of 40% mortality rate within 10 minutes of closing the boxes, then 5 minutes later to reach 100% mortality rate. In the case of high concentrations mortality rates (20% and 40%) for 150 $\mu$ L and 200 $\mu$ L within 5 minutes, and a and a maximum mortality rate in 10 minutes.

The higher the concentration, the higher the mortality rate in 10 minutes. minutes, while 15 minutes is long enough to reach the maximum mortality rate for all concentrations.

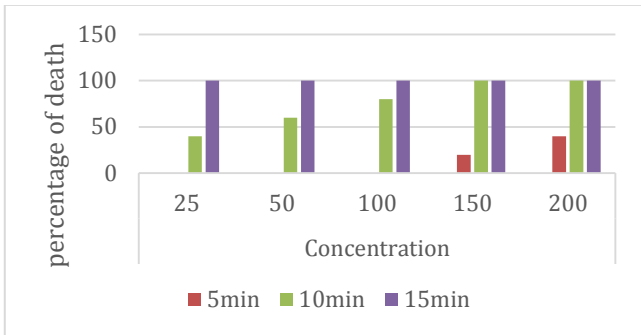


Figure 2: Histogram of the insecticidal activity of Stehat essential oil.

3.4.2 Insecticidal activity of the essential oil of Salé region:

Essential oil from Salé, its effect starts after 15 minutes at 25µL with a mortality rate of mortality rate of 40%, rising to 80% in 20 minutes and 100% in 25 minutes. The mortality rate doubles from 50 µL to 100 µL in 10 minutes. For higher concentrations, 150 µL and 200 µL show an insecticidal effect in 5 minutes. Mortality rates of 20% and 40% respectively.

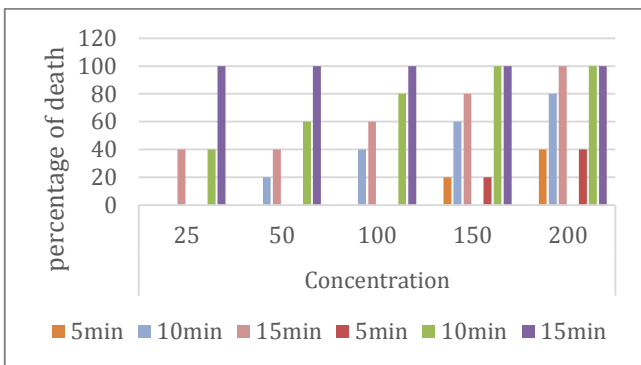


Figure 3: Histogram of the insecticidal activity of Salé essential oil

3.4.3 Insecticidal activity of the essential oil of Ouazzane region:

For oil from Ouazzane region, the 25 µL dose gives average mortality rates of 40% and 60% in 15, 20 and 25 minutes respectively, then the effect of the 150 µL and 200 µL doses begins in 5 minutes, with mortality rates of 20 and 40% respectively, so after 25 minutes, the maximum mortality rate is marked for all doses.

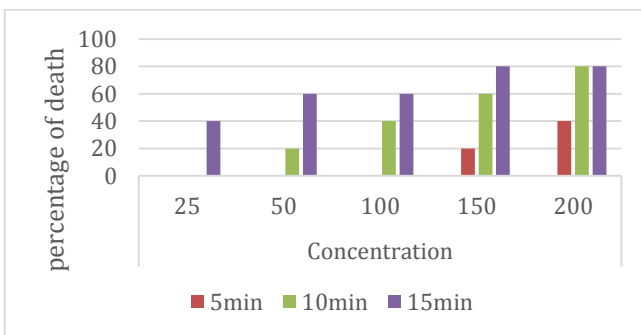


Figure 4: Histogram of the insecticidal activity of Ouezzane essential oil.

We can therefore conclude that the insecticidal effect of essential oils depends on the concentration of the oils used and the duration of contact with the insect, as well as the effect of the regions indicated in our study, such as Stehat is characterized by a very strong insecticidal effect since it gives the maximum mortality rate in 15 minutes, followed by Salé lasting 25 minutes to achieve 100% mortality and finally Ouazzane requires the longest duration to reach the maximum rate.

According to the results, S.g mortality rates increased as a function of the doses of oils used and the duration of exposure per contact. The lowest mortality values were recorded in the control batches, at 25% after 7 days of testing, the EOs used at doses of 200, 150 and 100 µl showed higher mortality rates mortality rates from day one onwards.

In general, Mentha rotundifolia essential oil had strong toxicity against S. granaries, this extract significantly affected the mortality of pests, and the findings indicate strong insecticidal activity of essential oil as a fumigant for insects. In our observations, the oil was characterized by a rapid knockdown effect, convulsion, paralysis, and death. The rapid kill is one of the main points because no progeny is produced if insects die quickly. Those results are comparable with those reported by other authors who found a highest concentration of EO (0.8 µL/L), leading to a 100 % mortality of all beetle pests after exposure time ≥ 4 h, additionally the 0.2 µL concentration and 5 h exposure time was enough to attain 100 % mortality of all the insects, they also conclude that an exposure time ≥ 5 h for S. granarius is extremely enough to obtain 100 % kill of the insects in all concentrations tested. It has been shown as well that this essential oil is effective against other pests such as Tribolium confusum[4], [19], Rhopalosiphum padi and Sitobion avenae [20], but it works better on S. granaries [4]. Overall, antioxidant activity and insecticidal activity of M. rotundifolia essential oil could be attributed to its chemical composition, which is determined by the genotype and influenced by environmental and agronomic conditions [8] generally and particularly to its major compound and its high level [20], According to recent research, essential oils have a variety of effects on insect physiology. Furthermore, the neurotoxicity of oxygenated monoterpenes was investigated. These compounds are generally volatile and lipophilic, allowing them to enter insects quickly and interfering with their physiological processes by preventing acetylcholinesterase activity and acting on the octopaminergic effect sites of insects[20].

4 Conclusion

As a conclusion, a good yield percentage depends on the duration of essential oil extraction, and plants from Stehat and Ouazzane have a higher yield than those from Salé, which means that the geographical position also plays a role on this parameter.

Basing on the analysis of the antioxidant activity of the oils studied, we can conclude that they have a less activity when compared with ascorbic acid, while Stehat and Salé oils have a higher antioxidant activity than Ouazzane.

Analysis of the insecticidal effect of EOs revealed significant differences for the following factors duration of exposure and regions of origin, making it possible to classify the oils used according to their toxicity. used according to their toxicity.

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