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# Insecticidal effect of *Eucalyptus globulus* and *Rosmarinus officinalis* essential oils on a stored food pest *Ephestia kuehniella* (Lepidoptera, Pyralidea)

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**ABSTRACT**

For the purpose of evaluating the local heritage of eastern Numidia, an ethnobotanical survey was conducted on a population located in the province (department) of El-Tarf (North-East of Algeria) on a frequency of use of two plants known in traditional health; *Eucalyptus globulus* (Myrtaceae) and *Rosmarinus officinalis* (Lamiaceae). After processing data, our results show a female dominance when using these plants on a fairly advanced age group (40 to 60 years old). These plants are used by the people at their fresh state and deem the leaf as the most used part for therapeutic and cosmetic purposes. Furthermore, steam baths and infusion remain the most widespread preparation methods. Most consumers use these plants to treat influenza, respiratory illnesses as well as diseases of the digestive tract. GC-MS of essential oils extracted from the plants studied disclose a dominance of the alcohols portrayed by the monoterpenes (42.73%), sesquiterpenes (32.6%) and oxides (10.48%) in *E. globulus.* Data also indicate a high content of oxides (38.11%) monoterpene alcohols (20.43%) and monoterpenes (19.70%) in *R.officinalis*. Toxicological contact tests were achieved on a store-products pest *Ephestia kuehniella*. The test results were assessed with *E.globulus* ($LC_{50}$=0.013; $LC_{95}$=0.081 µl/cm$^2$) and *R.officinalis* ($LC_{50}$=0.011; $LC_{95}$=0.059 µl/cm$^2$). The lethal time changes according to the dose used and the tested plant. In *E.globulus*, we observe (concentration: 0.005 µl/cm$^2$; $LT_{50}$=56.2 h), (Concentration: 0.01 µl/cm$^2$; $LT_{50}$=49.53h) and (Concentration: 0.04µl/cm$^2$; $LT_{50}$=20.93h). Whereas in *R.officinalis*, we recorded (Concentration: 0.005 µl/cm$^2$; $LT_{50}$=55.7h), (Concentration: 0.01µl/cm$^2$; $LT_{50}$=54.99 h) and (Concentration: 0.04µl/cm$^2$; $LT_{50}$=29.13 h). The bioinsecticide has also been administered by fumigation and underpins toxicity by the reduction of adults longevity in *E.kuehniella* with *R.officinalis* ($LC_{50}$=4.03 µl/l air; $LC_{95}$=14.73 µl/l air) and *E.globulus* ($LC_{50}$=7.76 µl/lair; $LC_{95}$=21.23 µl/l air). In addition, the tested plants show an outstanding repellent effect as long as *E.globulus* and *R.officinalis* essential oils respectively demonstrate a slightly repellent power at 42.22% ($RD_{50}$=0.09 µl/cm$^2$; $RD_{95}$=0.24 µl/cm$^2$) and repellent at 60.00% ($RD_{50}$=0.06 µl/cm$^2$; $RD_{95}$= 0.35 µl/cm$^2$) towards *E.kuehniella* adults. The plant resources represent a genuine reserve of bioactive molecules, which can create solutions to sustainable development issues. These plants might be the source of new molecules of combat against some pests in order to protect human health and safeguard the environment.

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## Introduction

The province (department) of El-Tarf (North East of Algeria) runs along the Mediterranean over 90km. This coastline teems with wild medicinal and aromatic plants (1-15). This harmonious greenery contains ecosystem variables and maintains the ecological stability of the region, which led to several inventories and researches on the vegetation of this region like the works of (16) as well as (17). The empirical knowledge gained by the indigenous people on the use of aromatic and medicinal plants was mostly passed on orally from generation to generation, thereby preserving the heritage knowledge (16, 18, 19). These plants are rich in active ingredients playing a key role in many biological activities and are studied for therapeutic purposes (20, 21). Traditional medicine in

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the region of El-Tarf relies on plants unpromptedly cultivated. In this study, an ethnobotanical survey was carried out on a population from this region to take part in determining and valorizing the local know-how, which focuses on preserving the traditional craft practices while aiming at the potential therapeutic and culinary use of the plants under study. Determining the chemical composition of essential oils from leaves by steam distillation was achieved by GC-MS. Essential oils were also employed to test their compounds that are able to inhibit attacks from insect pests on stored food via the study of the bioinsecticide activity and that on a model of pests *Ephestia kuehniella* (Lepidoptera, Pyralidea). These molecules, naturally synthesized by plants, play roles in down-regulating insects' populations through plants, somewhat of a natural regulator of food insect pests (22). For a brighter future, plant production products also referred to as 'green products' and plant-based biopesticides, are recommended and qualified for their low persistence. Likewise, those made with essential oils can serve as tools in management programs pertaining to pests' resistance to pesticides (23, 24). The aim of this present work is to evaluate the therapeutic and insecticidal usage of two plant from the folk medicinal heritage of eastern Numidia.

## Materials and methods

### Ethnobotanical survey

A direct face-to-face and individual conversation were conducted with people taken randomly from both sexes and from various socio-economic strata. This work covered 13 municipalities of the department of El-Tarf and after four successive years (2017, 2018, 2019 and 2020); so as to identify the several forms and ways to use these two plants *E. globulus and R. officinalis* in traditional herbal medicine. The replies received from conversations were recorded by means of an index card prepared according to the objectives of our research.

### Plant material

*Eucalyptus globulus* was harvested at the region of Ramel Essouk (Altitude 157 m – Latitude 36°47′10″ N – Longitude 8°32′8″ E), whereas *Rosmarinus officinalis* was picked in December at the region of Bougous (Altitude 183 mm – Latitude 36°39′34″ N – Longitude 8°22′10″ E).

### Extracting the essential oil

A quantity of 100 g of leaves dried in the shade of each plant has been hydro-distilled using a Clevenger apparatus for 03 hours. The yield rate was estimated according to (25) using the formula: % oil extraction (p/p) = ((Essential oil weight (g) /plant material weight (g)) x 100. The essential oil obtained was stored at 04°C in opaque glass vials.

### Gas Chromatography of essential oils

GC/mass spectrometry (GC/MS) was carried out following the method described (5).

### Biological material

*Ephestia kuehniella's* breeding was taken into an oven under optimum conditions of development (27° C temperature, relative humidity near 70% and in the dark). Males and females, in equal numbers, are grown in 01 L plastic containers containing flour. Dating of insects is achieved in days after their ecdysis.

### Effect on adults' longevity through contact

A batch of 10 adult insects aged 0 to 24 h was placed in a Petri dish. A Whatman paper disc was soaked with one of the three essential oils doses (0.25µl; 0.5µl and 2µl) and dispensed in each box. The concentrations obtained are as follows: 0.005 µl/cm$^2$; 0.01 µl/cm$^2$ and 0.04 µl/cm$^2$. Three attempts shall be carried out for the statistical survey. The insect is considered dead when there is no more leg or antenna movement.

### Toxicity on adults' longevity by fumigation

Five (05) adult insects freshly exuviated were placed in a 44ml Plexiglas screw-top container in which there is flour and Whatman paper N°1 soaked with an essential oil that has been studied before the closure of the bottle. For this test, three different concentrations were dispensed (5.68 µl/l air, 11.36 µl/l air and 45.45 µl/l air). Three replications are necessary for the statistical surveys in comparison with the control samples.

### Toxicity by repulsion

This parameter relies on the preferred area method (26). A piece of paper is split into two parts. The first half will be soaked in one of the tested concentrations (0.01 µl/cm$^2$; 0.02 µl/cm$^2$ and 0.08 µl/cm$^2$) and the paper's second half will be soaked in acetone that will serve as a neutral party. After evaporation of the solvent, the piece of paper will be pieced together and placed at the bottom of a box of 09 cm long. 10 adult *E.kuehniella* insects, aged 0 to 24 h, were put in the middle of the box. This procedure is repeated three times for each concentration.

**Ethnobotanical survey**

The logged data on the survey sheets have been entered and processed by IBM SPSS, Statistics 25 software (System Package for Social Sciences, version 25). Reporting our results relied on frequencies and percentages.

**Toxicological tests**

During toxicological tests, the insects' mortality results are further processed according to Abbot, 1925 (27) to rule out any hypothesis of natural mortality and assess the toxic effects of the tested essential oils.

**Insecticidal data analysis**

• Analysis of variance was carried out on the data. Turkey's test was applied at 0.05% to pinpoint the significant differences by way of the IBM SPSS, Statistics 25 software. The data are broken down into tables as averages ± standard errors (m ± s e).

• Identifying $LC_{50}$, $LC_{95}$, $RD_{50}$ and $RD_{95}$ as well as $TL_{50}$, based on Finney et al 1971 (28) was achieved depending on the probit as per the recommendation of Fisher and Yates 1957 (29).

**Table 1.** Survey main results

| Queries | Repartition | Percentage (%) |
|---|---|---|
| Gender | Females | 60.25 |
| | Males | 39.75 |
| Ages | 20 – 40 | 38 |
| | 40 – 60 | 45.5 |
| | > 60 | 16.5 |
| Information inheritance | Ancestral know-how | 48.0 |
| | Studies | 2.54 |
| | Personal experience | 9.64 |
| | Family | 39.82 |

**Table 2.** Uses of different parts of the plant

| Queries | Repartition | *E.globulus* | *R.officinalis* |
|---|---|---|---|
| | | Percentage (%) | |
| Used parts of the plant | Leaf | 73.2 | 53.3 |
| | Flowers | 13.2 | 28.3 |
| | Entire plant | 13.2 | 15.9 |
| | Rod | 00.5 | 01.3 |
| | Grains | 00.0 | 01.2 |
| Fresh/dried plants | Fresh | 75.8 | 61.8 |
| | Dry | 24.2 | 38.2 |
| Manner of use | Steam baths | 69.2 | 07.4 |
| | Cataplasms | 00.4 | 00.0 |
| | Decoctions | 00.0 | 29.7 |
| | Infusions | 27.9 | 46.6 |
| | Powders | 02.5 | 16.3 |
| Manners of consumption | Associated | 10.7 | 31.6 |
| | Alone | 89.3 | 68.4 |
| Treated pathologies | Cardiovascular treatment | 00.0 | 05.8 |
| | Cosmetic use | 00.0 | 8.6 |
| | Food use | 00.0 | 21.0 |
| | Against influenza | 56.5 | 28.2 |
| | Breath and respiratory treatment | 38.2 | 00.0 |
| | Stress treatment | 05.3 | 11.2 |
| | Digestive treatment | 00.0 | 25.2 |
| Banning uses | High doses | 14.6 | 13.9 |
| | Allergies | 02.1 | 06.1 |
| | Asthma | 08.1 | 00.0 |
| | Children | 07.1 | 07.7 |
| | Pregnant | 29.0 | 27.9 |
| | Blood pressure | 09.0 | 08.0 |
| | New born | 30.2 | 28.6 |
| | Any adverse effect | 00.0 | 07.9 |

**Results**

**Results of the ethnobotanical survey**

Based on the statistical treatment of data in the 400 survey sheets, the results are broken down in Tables 1 and 2.

Table 1 shows female dominance (60.25%) compared to men (39.75%). The highest rate (38%) is that of the users aged between 20 and 40. The 40-60 age group displays the highest percentage of users (45.5%). The surveyed population provided us with this information inherited from their loved ones, ancestral know-how (48.0%), families (39.2%) or acquired from personal experiences (9.64%) or out of studies (2.54%).

Table 2 discloses the leaf remains the most used part with a rate of 73.2 % for *E.globulus* and 53.3% for *R.officinalis*. These findings are followed by the use of flowers with 13.2% for *E.globulus* and 28.3% for *R.officinalis*. The whole *E.globulus* plant is used at a 13.2% rate while 15.9% for *R.officinalis*. The citizens of El-Tarf use *E.globulus* and *R.officinalis* fresh rather than dry, with a rate ranging between 75.8% and 61.8%. According to the proportions recorded, 24.2% arises for *E.globulus* while 38.2% for *R.officinalis*. Steam baths are the most used preparation mode for *E.globulus* (69.2%). *As* for *E.globulus*, the most widely used preparation way is an infusion (46.6%) followed by decoction (29.7%).

Both of these plants are mostly used singly, with a rate of 89.3% for *E.globulus* and 68.4 % for *R.officinalis*. Utilization of these plants together is less common, with 10.7% for *E.globulus* and 31.6% for *R.officinalis*. We can see that *E.globulus* is used to treat influenza (56.5%), respiratory illnesses (38.2%) and stress (5.3%). The most treated diseases by *R.officinalis* are influenza (28.2%) and digestive tract diseases (25.2%). This plant is also known for its use in the kitchen with 21.0%. This plant is little used in cosmetics (8.6%) or treating stress (11.2%) along with cardiovascular diseases (5.8%). The surveyed population agrees with banning using both plants at a high dose for newborns, pregnant women, children, people with high blood pressure and allergy sufferers. Moreover, 8.1% forbid giving *E.globulus* to people with asthma, whereas 7.9% did not notice any adverse effects after using *R.officinalis*.

**Yield of obtained essential oils**

A significant variety in terms of quantity of oil derived from the plants studied, of which the *E.globulus* produced a yield of 1.65% while *R.officinalis* produced a yield of 0.36%.

**Chromatographic profile of essential oils**

According to Figures 1 and 2, *E.globulus* essential oil is made up of monoterpenes (42.73%), sesquiterpenes (32.6%) and oxides (10.48%. Parallel to this, *R.officinalis* essential oil is made up of oxide (38.11%), monoterpenes alcohols (20.43%), monoterpenes (19.70%), ketones (13.53%) and characterized by the presence of low phenols levels (1.11%).

**Insecticide activity of essential oils**

***Contact Toxicity test***

After contact of *E.kuehniella* adults with different essential oils concentrations (Fig. 3) and recording the mortality rate, the statistical processing shows the impact of concentrations on the increase of the mortality rate of 48h insects($F_{1, 12} = 33.25$, $p \leq 0.001$) and at 72h of exposure ($F_{1, 12} = 7.58$, $p \leq 0{,}01$). The variety in essential oil shows no major difference at 48h of test ($F_{1,12} = 0.083$, $p \geq 0.05$). On the other hand, results of 72h test reveal a significant influence of essential oil variety on the rise of mortality rates of *E.kuehniella* adults ($F_{1, 12} = 5.33$, $p \leq 0.05$).

**Table 3.** Chemical composition of *Eucalyptus globulus* essential oil

| RT | Name | Rekioua (present work) | (30) | (31) | (32) |
|---|---|---|---|---|---|
| 5.520 | 3-Carene | **4.18%** | | | |
| 6.808 | β-Pinene | 0.28% | | 0.217 | 0.400 |
| 7.784 | α-Phellandrene | 0.17% | | | |
| 8.553 | o-Cymene | 0.69% | | | 6.60 |
| 8.859 | Eucalyptol | **10.46%** | | 51.083 | 80.20 |
| 9.916 | γ-Terpinene | 0.22% | | | |
| 11.165 | Terpinolene | 0.43% | 7.12 | | |
| 11.678 | Linalool | 0.13% | 5.54 | | |
| 12.186 | Fenchol | 0.12% | | 0.179 | |
| 13.295 | Pinocarveol | 1.21% | | 9.987 | |
| 14.369 | Pinocarvone | 0.51% | | | |
| 14.506 | endo-Borneol | 0.21% | | 0.346 | |
| 15.059 | Terpinen-4-ol | 0.39% | 2.07 | 0.178 | 0.10 |
| 15.455 | ND | 0.14% | | | |
| 15.565 | Isocarveol | 0.90% | | | |
| 15.721 | α-Terpineol | **2.22%** | | 0.486 | 0.40 |
| 15.941 | (-)-Myrtenol | 0.20% | 1.50 | 0.202 | |
| 16.986 | cis-Carveol | 0.48% | | 0.187 | |
| 17.396 | p-Mentha-1(7).8-dien-2-ol | 0.97% | 2.36 | | |
| 17.529 | Verbenone | 0.43% | | | |
| 18.101 | D-Carvone | 0.16% | 1.59 | | |
| 18.442 | Carveol | 0.08% | | | |
| 18.69 | Nerol | 0.32% | | | |
| 22.63 | Hydroxycineole acetate | 0.34% | | | |
| 23.028 | α-Terpinyl acetate | **5.82%** | | | |
| 23.941 | Isoledene | 0.48% | | | |
| 24.062 | α-Copaene | 0.34% | | | |
| 24.565 | Nerol acetate | 0.45% | | | |
| 25.541 | α-Gurjunene | 1.75% | | | |
| 26.475 | β-Gurjunene | 1.30% | | | |
| 26.904 | Aromandendrene | **14.48%** | | | |
| 27.008 | Selina-5.11-diene | 0.88% | | | |
| 27.759 | Alloaromadendrene | **5.35%** | | | |
| 28.221 | γ-Gurjunene | 0.45% | | | |
| 28.412 | γ-Muurolene | 0.39% | | | |
| 28.793 | β-Selinene | 0.87% | | | |
| 28.885 | Longifolene | 0.70% | | | |
| 29.215 | Ledene | **3.28%** | | | |
| 29.44 | α-Muurolene | 0.10% | | | |
| 29.989 | γ-Cadinene | 0.45% | | | |
| 30.174 | Aromadendrene. dehydro- | 0.55% | | | |
| 31.837 | Epiglobulol | **3.92%** | | | |
| 32.12 | Globulol | 1.76% | | 2.817 | |
| 32.559 | Spathulenol | 0.43% | | | |
| 32.917 | Ledol | **12.24%** | | | |
| 33.16 | Viridiflorol | **3.19%** | | | |
| 33.235 | Guaiol | 1.35% | | | |
| 33.558 | cis-Eudesm-6-en-11-ol | 1.93% | | | |
| 34.373 | β-Selinenol | **2.53%** | | | |
| 34.61 | β-Guaiene | 1.18% | | | |
| 34.708 | γ-Eudesmol | 1.52% | | | |
| 35.101 | .tau.-Cadinol | 0.36% | | | |
| 35.436 | β-Eudesmol | **4.34%** | | | |
| 35.543 | α-Eudesmol | **2.37%** | | | |

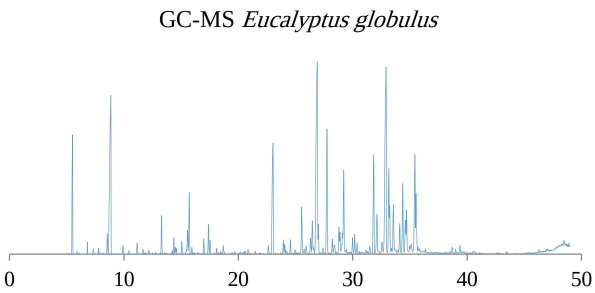


**Figure 1.** GC-MS profile of *Eucalyptus globulus* essential oil.

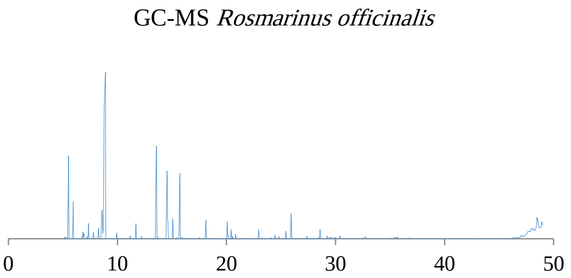


**Figure 3.** GC-MS profile of *Rosmarinus officinalis* essential oil.

**Table 4.** Chemical composition of *Rosmarinus officinalis* essential oil

| RT | Name | Rekioua (present work) | (30) | (33) | (34) |
|---|---|---|---|---|---|
| 5.503 | 3-Carene | **8.22%** | 0.07 | | **0.18** |
| 5.93 | Camphene | **2.97%** | 0.02 | 9.18 | **5.34** |
| 6.727 | β-Phellandrene | 0.13% | | | |
| 6.82 | β-Pinene | 0.55% | | 5.53 | **5.74** |
| 6.906 | 1-Octen-3-ol | 0.39% | | | |
| 7.172 | ND | 0.15% | | | |
| 7.334 | β-Myrcene | 1.19% | | 1.27 | **1.48** |
| 7.79 | α-Phellandrene | 0.55% | | | **0.19** |
| 8.252 | (+)-4-Carene | 0.95% | | | |
| 8.57 | β-Cymene | **4.09%** | | 2.76 | **1.27** |
| 8.888 | Eucalyptol | **37.51%** | | 21.53 | **52.06** |
| 9.916 | γ-Terpinene | 0.46% | | | **0.65** |
| 11.152 | Terpinolene | 0.30% | | | **0.26** |
| 11.678 | Linalool | 1.22% | | 0.87 | **0.64** |
| 12.192 | Fenchol | 0.18% | | | |
| 13.567 | Camphor | **11.70%** | 31.16 | 21.84 | **7.69** |
| 14.543 | endo-Borneol | **9.64%** | | 2.15 | **2.28** |
| 15.063 | Terpinen-4-ol | 1.71% | | | **0.59** |
| 15.71 | L-.alpha.-Terpineol | **6.97%** | | 1.92 | **1.92** |
| 18.098 | D-Carvone | 1.62% | 0.06 | | |
| 20.064 | Bornyl acetate | 1.54% | 3.44 | 2.45 | **0.25** |
| 20.152 | Thymol | 0.23% | | | |
| 20.417 | ND | 0.77% | | | |
| 20.544 | Carvacrol | 0.20% | | | |
| 20.822 | ND | 0.37% | | | |
| 22.942 | α-Terpinyl acetate | 0.83% | | 0.40 | |
| 24.432 | (-)-β-Bourbonene | 0.33% | | | |
| 24.802 | β-Elemene | 0.17% | | | |
| 25.431 | Methyleugenol | 0.67% | | | |
| 25.928 | Caryophyllene | **2.43%** | | 1.32 | |
| 27.384 | Humulene | 0.22% | | 0.07 | **0.22** |
| 28.574 | β-Copaene | 0.86% | | | **0.17** |
| 29.232 | Aromandendrene | 0.30% | 0.09 | | |
| 29.573 | ND | 0.15% | | | |
| 29.983 | ND | 0.13% | | | |
| 30.387 | δ-Cadinene | 0.33% | 0.24 | | **0.19** |

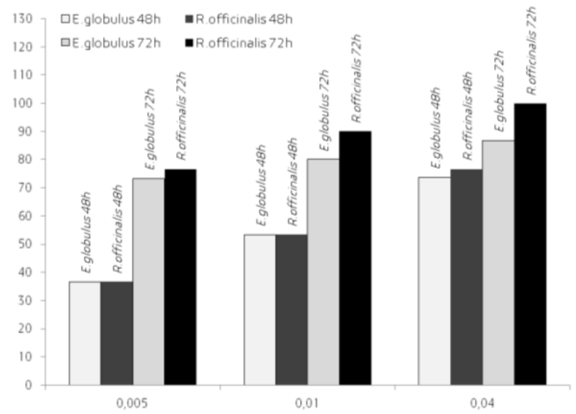


**Figure 3.** Mortality progression (%) of *E.kuehniella* adults at 48h and 72h. For each concentration, the same lowercase letters are not significantly different (p> 0.05) between the means of the two essential oils, according to Tukey's test.

**Table 5.** Determination of LC 50 and LC 95 of the Toxic Effect by contact of *E.globulus* and *R.officinalis* essential oils on *E.kuehniella* adults.

| Oils essential | $LC_{50}$ (µl / $cm^2$) | $LC_{95}$ (µl / $cm^2$) | $\chi 2$ | Slope ± SE | Sig | Degrees of freedom |
|---|---|---|---|---|---|---|
| *E.globulus* | 0.013 (0.06 - 0.019) | 0.081 (0.062 - 0.124) | 2.91 | 24.22 ± 4.93 | 0.08 | 1 |
| *R.officinalis* | 0.011 (0.006 - 0.016) | 0.059 (0.048 - 0.078) | 1.99 | 34.78 ± 5.31 | 0.15 | 1 |

**Table 6.** Lethal time (LT50) of the Toxic Effect by contact of essential oils of *E. globulus* and *R.officinalis* on adults of *Ephestia kuehniella*.

| Oils essential | Concentration (µl / $cm^2$) | $LT_{50}$ (hours) | $\chi 2$ | Slope ± SE | Sig | Degrees of freedom |
|---|---|---|---|---|---|---|
| *E.globulus* | 0.005 | 56.21 (53.35-58.96) | 0.18 | 0.03 ± 0.005 | 0.99 | 4 |
| | 0.01 | 49.53 (46.08-52.14) | 5.05 | 0.04 ± 0.005 | 0.28 | 4 |
| | 0.04 | 20.93 (16.46-33.46) | 0.12 | 0.02 ± 0.006 | 0.99 | 4 |
| *R.officinalis* | 0.005 | 55.7 (52.22- 57.69) | 1.00 | 0.04 ± 0.005 | 0.90 | 4 |
| | 0.01 | 45.99 (41.94-48.87) | 2.58 | 0.04 ± 0.006 | 0.63 | 4 |
| | 0.04 | 29.13 (17.89-35.17) | 2.90 | 0.05 ± 0.008 | 0.57 | 4 |

Based on Tables 5 and 6, we notice that $LC_{50}$ lethal concentrations causing mortality of 50% *Ephestia kuehniella* adults are close: 0.013 and 0.011 µl/$cm^2$. Likewise, for $LT_{50}$ lethal times values. This confirms that both plants have the same influence on the mortality of the insects tested.

### *Toxicity test by fumigation*

The unidirectional ANOVA shows the major influence of essential oils ($F_{1,36}$ = 33.88 ; $p \leq 0.001$), inhalation time ($F_{2.36}$ = 63.77 ; $p \leq 0.001$) and tested doses ($F_{2,36}$ = 7.47, $p \leq 0.01$) on the increase of mortality of *E.kuehniella* adults.

Exposure of *E.kuehniella* adults to various essential oils doses caused their mortality which was phased in Figure 4. The mortality rate of *E.kuehniella* adults increases over time depending on the dose and the quality of the essential oil. At the lowest dose (5.68 µl/l air), *R.officinalis* essential oil causes all adults at 66h, whereas *E.globulus* essential oil mortality of all insects is achieved only at 78h. The results of the second dose (11.36µl/l air) show that inhaling *R.officinalis* essential oil for 60h leads to the death of all *E.kuehniella* adults. This same phenomenon occurs with *E.globulus* essential oil at 72h. After 54h of exposure to the third dose (45.45µl/l air) of *R.officinalis* essential oil, we noticed the death of all *E.kuehniella* adults, when in *E.globulus* we recorded this rate at 60h (Fig.4).

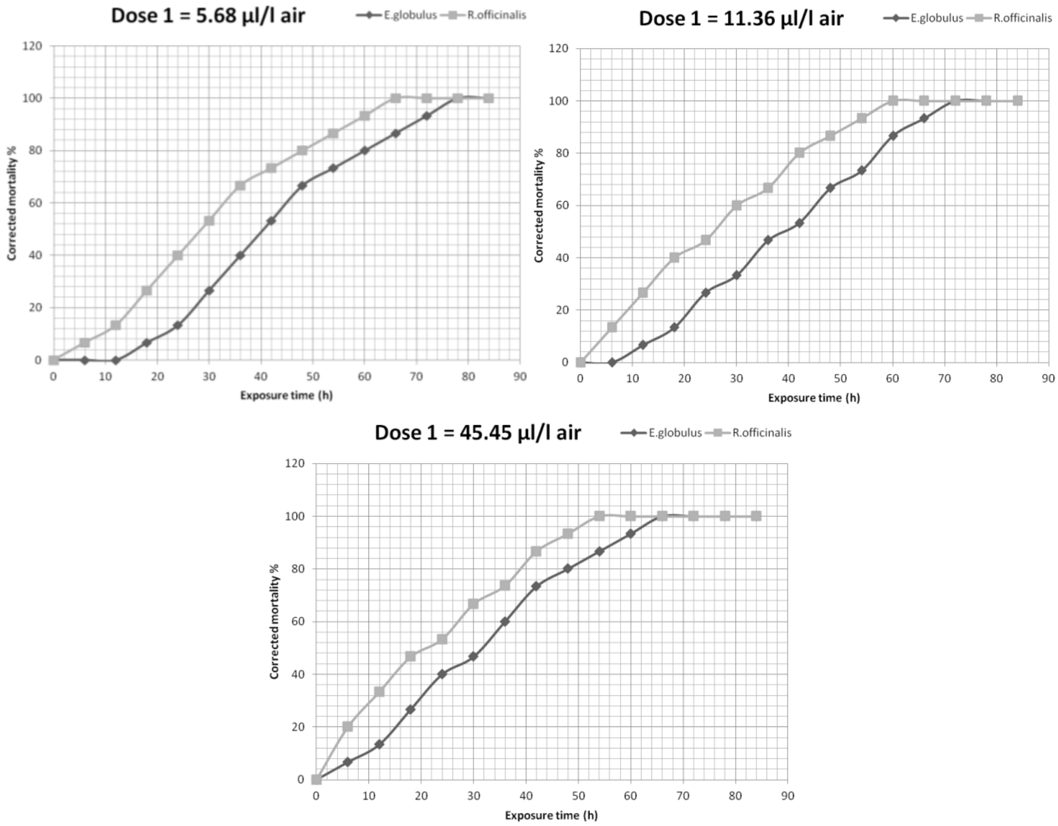


**Figure 4.** Mortality (%) of *Ephestia kuehniella* adults after inhalation of different doses of *E. globulus* and *R.officinalis* essential oils.

Based on Tables 7 and 8, *R.officinalis* essential oil seems to be more effective than *E.globulus* essential oil using the following lethal doses of 4.032 µl/l air and 7.756 µl/l air, respectively. The same goes for the lethal time, *R.officinalis* essential oil provokes mortality of *E.kuehniella* adults in a shorter time interval than that caused by *E.globulus* essential oil.

***Toxicity Test by repellency***

The statistical survey brings out that the repellent properties obtained underscore the dependency of essential oils variety ($F_{1.24}$ = 5.44. $p \leq 0.05$). The tested concentrations ($F_{2.24}$ = 61.00. $p \leq 0.001$) and the duration of exposure various essential oils concentrations $F_{2.12}$ = 51.00. $p \leq 0.001$) to keep *E.kuehniella* adults away. The coefficient of determination ($R^2$ = 0.927) indicates a high and positive correlation between the studied factors, proving their insect-repellent effect on *E.kuehniella* adults at 24 h of the treatment.

The repellent test results are summarized in Table 9 and Figure 5. The repellent effect rate increases according to the concentration and quality and of the tested essential oil. *Eucalyptus globulus* essential oil has a relatively repellent power PR=43.22). However, *Rosmarinus officinalis* essential oil has a repellent power with PR=60.00%.

**Table 7.** Determination of the LC50 and LC95 of the toxic effect by fumigation of *E.globulus* and *R.officinalis* essential oils on *E.kuehniella* adults.

| Oils essential | $LC_{50}$ (µl / l air) | $LC_{95}$ (µl / l air) | χ2 | Slope ± SE | Sig | Degrees of freedom |
|---|---|---|---|---|---|---|
| *E.globulus* | 7.756 (5.746 - 9.271) | 21.228 (16.746 - 34.754) | 0.000 | 0.122 ± 0.032 | 0.988 | 1 |
| *R.officinalis* | 4.032 (0.377 - 5.656) | 14.733 (12.340 - 20.731) | 0.000 | 0.154 ± 0.036 | 1.000 | 1 |

**Table 8.** Determination of the lethal time (LT50) of the toxic effect by fumigation of *E.globulus* and *R.officinalis* essential oils on *E.kuehniella* adults.

| Oils essential | Concentration (µl / l air) | $LT_{50}$ (hours) | χ2 | Slope ± SE | Sig | Degrees of freedom |
|---|---|---|---|---|---|---|
| *E.globulus* | **5.68** | 42.486 (36.979 - 53.244) | 58.228 | 0.056 ± 0.005 | 0.000 | 7 |
| | **11.36** | 38.088 (32.353 - 45.408) | 36.358 | 0.044 ± 0.004 | 0.000 | 7 |
| | **45.45** | 29.857 (27.435 - 31.853) | 0.198 | 0.045 ± 0.005 | 1.000 | 7 |
| *R.officinalis* | **5.68** | 28.573 (17.069 - 35.575) | 66.689 | 0.046 ± 0.005 | 0.000 | 7 |
| | **11.36** | 26.526 (13.598 - 31.986) | 64.382 | 0.062 ± 0.005 | 0. 000 | 7 |
| | **45.45** | 19.149 (5.085 - 24.545) | 34.503 | 0.067 ± 0.007 | 0.000 | 7 |

**Table 9.** Repellency (mean ± se) of essential oils of *E.globulus* and *R.officinalis* on adults of *E.kuehniella* during 24 hours of exposure.

| | Concentration (µl / cm²) | | | Average repulsion |
|---|---|---|---|---|
| | **0.01** | **0.02** | **0.08** | |
| *E.globulus* | 26.67 ± 6.67 a | 46.67 ± 6.67 b | 53.33 ± 6.67 c | **42.22** |
| Repellency classes | **III : Moderately repellent** | | | |
| *R.officinalis* | 33.33 ± 6.67 a | 66.67 ± 6.67 b | 80 ± 0.00 c | **60.00** |
| Repellency classes | **IV : Repellent** | | | |

For each concentration, the same lower case letters are not significantly different (p> 0.05) between the means of the two essential oils, according to Tukey's test.

**Table 10.** Repellency doses RD50 and RD95 of the essential oil of *E.globulus* and *R.officinalis* against adults of *E. kuehniella* after 24 h of exposure.

| Oils essential | $RD_{50}$ (a,b) (µl / cm²) | $RD_{95}$ (a,b) (µl / cm²) | χ2 | Slope ± SE | Sig | Degrees of freedom |
|---|---|---|---|---|---|---|
| *E.globulus* | 0.09 (0.07 -0.13) | 0.24 (0.18 - 0.42) | 0.35 | 10.44 ± 2.44 | 0.56 | 1 |
| *R.officinalis* | 0.06 (0.04 - 0.18) | 0.35 (0.21 - 1.70) | 2.64 | 5.70 ± 2.36 | 0.10 | 1 |

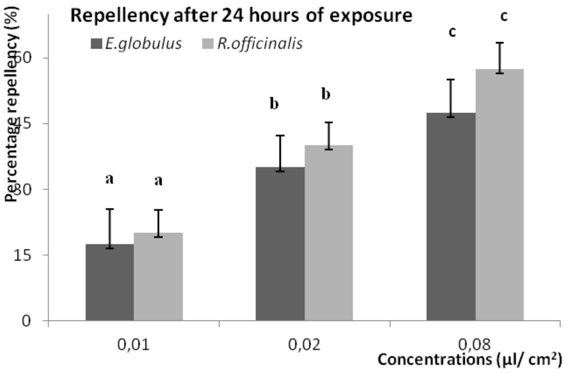


**Figure 5.** Repellency of *E.globulus* and *R.officinalis* essential oil on *E. kuehniella* adults after 24 hours of exposure. For each concentration, the same lowercase letters are not significantly different (p> 0.05) between the means of the two HEs, according to Tukey's test.

**Table 11.** Lethal time repellent TL50 of the essential oil of *E.globulus* and *R.officinalis* against adults of *E. kuehniella* after 24 h of exposure.

| Oils essential | Concentration (µl / cm²) | $TL_{50}$ (hours) | χ2 | Slope ± SE | Sig | Degrees of freedom |
|---|---|---|---|---|---|---|
| *E.globulus* | 0.01 | 42.07 (31.22 - 74.88) | 1.702 | 0.03 ± 0.01 | 0.19 | 1 |
| | 0.02 | 27.41 (19.00 - 63.35) | 0.62 | 0.02 ± 0.008 | 0.43 | 1 |
| | 0.08 | 18.52 (10.57 - 62.84) | 3.01 | 0.02 ± 0.008 | 0.08 | 1 |
| *R.officinalis* | 0.01 | 34.12 (23.95- 76.35) | 0.76 | 0.02 ± 0.008 | 0.38 | 1 |
| | 0.02 | 9.62 (3.83 - 14.87) | 0.37 | 0.03 ± 0.008 | 0.54 | 1 |
| | 0.08 | 7.39 (1.08 - 12.94) | 2.54 | 0.03 ± 0.008 | 0.11 | 1 |

## Discussion

### Survey discussion

The surveyed population throughout 13 municipalities of the province (department) of El-Tarf tells us that ladies (60.25%) are most interested in using the two studied plants compared to men (39.75%). The inventory undertaken by (16) in the same area under study also reveals that ladies (71.8%) are more interested in traditional medicine than men (28.2%). Furthermore, an ethnobotanical survey conducted by (35) at El-Tarf in four (04) medicinal plant families pointed out that ladies (62%) hold the information and phytotherapeutic know-how. Other ethnobotanical works also corroborate this (18; 37). The same investigation shows that it is people aged 40 to 60 who use the two plants studied (45.5%) relative to the age group 20 to 40 (38%). Our results are confirmed by the work of (9) and (36). Although (16) in their study employed divergent age groups yet results are closer to ours, where people aged 50 to 75 are represented by a rate of 48.7% and those of age group 30 to 50 representing 32.5% of the surveyed ones. The most used part of the plant as a herbal remedy is the leaf for *E.globulus* (73.2 %) and *R.officinalis* (53.3 %). According to (37), the predominance of the use of an organ in relation to another in the therapeutic field comes from the concentration of its active ingredients. This is corroborated by the inventory of (16) as well as the results of (18). Leaves are the most commonly used because they serve at the same time as the location of photochemical reactions as well as a reservoir of organic matter derived there from (38). They supply most alkaloids, heterosides and essential oils (39). These findings are consistent with the work of (40) as in which they point out that those local populations in the regions of Aguelmouss and Kénitra in Morocco mainly use leaves of medicinal plants in the traditional phytotherapy of that region. Using fresh plants substantiates the findings of (16). This can be explained by the annual availability of plants. Broadly speaking, fresh or dry plant usage hinges majorly on their availability in the environment of the surveyed people (34-35).

The most familiar mode of preparation to speed up the therapeutic action of *E.globulus,* is a steam bath (69.2%) followed by infusion (27.9%). An ethnobotanical survey carried out in Fès by (42) on four medical and aromatic plant's families' show that infusion is the most widely used preparation method by the surveyed persons. Regarding *R.officinalis*, infusion (46.6%) is the most widely used preparation mode, followed by decoction (29.7%). (16) along with (43) reported similar results.

Phytotherapy is definitely the best approach in health medicine focused as much on prevention as to treatment (44). The most treated diseases by *Eucalyptus* are influenza (56.5%), followed by respiratory illnesses (38.2%). Most rosemary usages treat influenza (28.2%) and disorders of the digestive duct (25.2%). However, the research of (16) report that rosemary is very valuable to respectively treat liver problems, digestive and abdominal disorders together with blood circulation (43).

The data of the interviewees are based on the knowledge of ancestors (48.0%) and their families or their entourage (39.82%), which is consistent with other researches (36).

The plant's most used part as a remedy is the leaf; (73.2%) for the *E.globulus* and (53.3 %) for *R.officinalis*. According to (37), the predominance of using an organ versus another in the therapeutic field is due to its concentration in active ingredients. That is confirmed in the inventory of (16) together with the results of (18). Leaves are the most often used as they are at the same time the location of phytochemical reactions and a reservoir or organic matter derived there from (38). They supply the largest part of alkaloids, heterosides and essential oils (39). These findings are in line with the works of (40) along with (41) where they tell that the local populations in of Aguelmouss et Kenitra in Morocco use chiefly leaves of medicinal plants in traditional phytotherapy in that region.

Using fresh plants corroborates with the results of (16). The annual availability of plants can explain this. In general, the usage of fresh or dry plants primarily depends on their availability in the environment of the interviewees (35).

The most familiar mode of preparation to speed up the therapeutic action of *E.globulus*, is the steam bath (69.2%), followed by infusion (27.9%). An ethnobotanical survey conducted in Fez by (42) on four MAP families shows that infusion is the most practiced by the interviewees. As for *R. officinalis,* infusion (46.6%) is the most practiced preparation method, followed by decoction (29.7%). (16) together with (43) pointed out these same results. Phytotherapy is definitely the best approach in health medicine, focused as much on prevention as treatment (44). The most treated diseases by *Eucalyptus* are influenza (56.5%), followed by respiratory diseases (38.2%). Most rosemary usages treat influenza (28.2%) and disorders of the digestive duct (25.2%). However, the research (16) reports that rosemary is very valuable to respectively treat liver problems and digestive and abdominal disorders together with blood circulation (43). The data of the interviewees are based on the knowledge of ancestors (48.0%) and their families or their entourage (39.82%), which is consistent with other researches (36).

**The yield and chromatographic profile**

The *E. globulus* looked into provided a yield of 1.65 %, higher than that obtained (0.17%) by (45) and lower (2.53 %) than the results of (46) of the same species in the region of Moyancón in Ecuador and the region de Tizi Ouzou in Algeria. In parallel, *R. officinalis* has given a yield of 0.36 % rather low in comparison with when compared to that obtained by (35) as well as (47) valued respectively at 1.5 % and 1.03 %for the same species in Tunisia and Germany. This yield variance between the same species might be due to multiple biotic factors (the vegetation cycle, plant age, the growth stage and the part that is subjected to distillation). The analyzed *E. globulus* essential oil by CPG/SM reports a dominance of Aromandendrene (14.48 %), Ledol (12.24 %), Eucalyptol (10.46 %), α-Terpinylacetate (5.82 %) and Aromadendrene (5.35 %). Nonetheless, (30), who analyzed the essential oil of a similar species in Morocco observed an ascendancy of Estragole (28.14%), terpinolene (7.12%), the 1.4-hexadiene-methyl-3-(1-methylethylidene) (7.01%), linalool (5.54%) and furfural (4.66%). *Eucalyptus globulus* essential oil is best known for its expectorant and mucolytic properties associated with its high content of 1.8-cineole. Though it also has antiviral, antifungal, insecticidal and painkiller properties. Besides, the researched *R. officinalis* essential oil contains Eucalyptol (37.51%) and Camphor (11.70%). That is confirmed by (34). The *R.officinalis* of Tunisia was rich in eucalyptol (52.06 %) and camphor (7.67%). In Morocco, results of (30), illustrate the dominance of Camphor (31.16 %) and 2,4-Hexadiene, 3,4-dimethyl (9.08 %). Variety in the chemical composition of essential oils of the same plant species could be explained by the presence of volatile substances. The latter could undergo some modifications during distillation. The presence of Terpinen-4-ol, γ-Terpinene and terpinolene is not but a rearrangement of sabinene (48). During hydro-distillation, *E.globulus* and *R.officinalis* essential oils have been exposed to some modifications, which lead to the existence of multiple sabinene derivatives (48). In addition, the biotic factors have their own impacts on the chemical composition and final amount of essential oils from wild and cultivated plants such as temperature, altitude and sunshine.

**Toxicity of essential oils**

Some MAP essential oils can play the role of insecticide, larvicides or ovicide on some pest insects, though their different effects according to the type of insect or used plant (49-56).

***Contact toxicity***

Recorded lethal concentrations for *E.globulus* and *R.officinalis* were respectively $LC_{50}$ = 0,013 µl/cm$^2$; $LC_{95}$ = 0,081 µl/cm$^2$; $LC_{50}$ = 0,011 µl/cm$^2$ and $LC_{95}$ = 0,059 µl/cm$^2$. Similarly, the lethal times values as the obtained results on *R.officinalis* essential oils indicate mortality of all *E.kuehniella* adults in relation to *E.globulus* essential oil, apart from the third concentration **(**0,04 µl /cm$^2$) where *E.globulus* was rated stronger than *R.officinalis* ($LC_{50}$ : 20,93 µl /cm$^2$ and $LC_{50}$: 29,13 µl /cm$^2$**)**. Some findings on the same type of stored food pest *E.kuehniella* show a varied performance of this insect to plant species. (58) found an $LC_{50}$ estimated at 0,61 µl/cm$^2$ using *Zataria* essential oil. Conversely, (58) tested *Mentha rotundifolia* essential oil on *E.kuehniella* adults and figured out an efficiency that exceeds that of the other analyzed essential oils, with an $LC_{\mathbf{50}}$ estimated at 0.004 µl/cm$^2$.

***Toxicity by fumigation***

Toxicity was measured based on the $LC_{50}$ obtained in our findings. Previous studies state that the adult longevity rate of *E.kuehniella* is disrupted after inhaling several varieties of essential oils of MAP. Several $LC_{50}$ values were recorded and explained through the chemical characteristics of the tested essential oils. That is proved by (59) with *Pistacia lentiscus* essential oil ($LC_{50}$= 1,84 µl/l air) as well as *Ziziphora clinopodioides* essential oil where $LC_{50}$ was around 1,39 µl/l air (60). Besides, (58) recorded some $LC_{50}$ at 0,54 µl/l air and 2,91 µl/l air for *M.rotundifolia* et *M.communis* respectively. The work of (30) shows that *E.globulus* and *R.officinalis* essential oils are highly toxic compared with *Tribolium confusum* essential oils.

***Toxicity by repulsion***

Many studies on the repellent effect of different essential oils and natural extracts with respect to the lepidopteran *E.kuehniella*, confirm their efficiency. The behavior of *E.kuehniella* diverges according to the extracted chemical component substances. In line with the protocol of (26), *Origanum vulgaris* essential oil was considered as a reasonably repellent essential oil against adults of the same insect (15). Nevertheless, white *Artemisia herba alba* EO tested by (37) on larvae and *E.kuheniella* adults was classified in category 4 as repellent oil. As for *E.kuehniella* adults, the tests of (22) enable to classify the extract derived from the Laperrine's olive tree *Olea europea* in class 4 in respect of which the analyzed substance distinguishes itself by the repellent property towards adults and reasonably repellent against larvae of the same insect.

## Conclusions

After investigating the data from the native people of 13 municipalities in El-Tarf, our findings show a female dominance interested in traditional phytotherapy and holds more information and knowledge passed on from ancestors. Moreover, traditional phytotherapy is the cheapest means money-wise. According to the citizens of this region, foliage is the richest part in therapy elements. The survey population shows a great affinity to both fresh and dry leaves for the preparation of remedies. The residents of the region of El-Tarf know the explored plants and their therapeutic modalities and trust the prerequisite knowledge using the two plants studied. People are thoroughly familiar with the healing properties since they use them for the same purposes. Steam baths were the most familiar preparations of *E.globulus* by the local inhabitants, whereas infusion was the most prevalent modus operandi for *R.officinalis.* The diseases treated by *E.globulus* are influenza and respiratory illnesses. On the other hand, *R.officinalis* is used to treat digestive duct disorders. The plants surveyed show a significant variation in essential oil quality. Based on CPG/SM results through the analysis of *E. globulus* essential oil, we figured out that alcohols are dominant throughout monoterpenes (42.73 %), sesquiterpenes (32.6 %) and oxide (10.48 %). Concurrently, *R.officinalis* essential oil is made up of oxides (38.11 %), monoterpene alcohols (20.43 %) and Monoterpenes (19.70%), followed by the existence of Ketones (13.53 %) and marked by the presence of phenols (1.11 %). The Insecticidal activity confirms to us that by further exposing *E.kuehniella* adults to different concentrations either by contact, fumigation or repulsion, the statistic processing reveals the impact of essential oils of *E.globulus* and *R.officinalis.* The tested concentrations or doses, along with the exposure period, affect the increasing rate of insects' mortality. Contact toxicity brings out that at 48 hours from the test, no difference was reported in the mortality rise of *E.kuehniella* adults. Nevertheless, at 72 hours from the test, we have observed a major power in the rise in the mortality rate of the other *E.kuehniella* adults. Hence, the essential oils studied have the same efficiency in protecting the stored food from pests like *E.kuehniella.* In fine, our results indicate that the repellent properties increase in proportion to the concentration, exposure time and chemical characteristics of the tested natural substances. *E.globulus* essential oil has a moderately repellent property of 42.22% ($DR_{50}$=0.09 µl /cm$^2$; $DR_{95}$= 0.24 µl/ cm$^2$). On the other hand, *R.officinalis* essential oil has a repellent property of 60.00% ($DR_{50}$=0.06 µl /cm$^2$. $DR_{95}$= 0.35 µl/ cm$^2$). These findings open a new research track to explore in an effort to upgrade the mechanisms of molecular actions of these extracts. The plants studied may be a source of new molecules to safeguard human health, protect the environment and post-harvest food products.

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## Interest conflict

The authors declare that they have no conflict of interest.

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