FUMIGANT TOXIC ACTIVITY OF ESSENTIAL OILS ON Sitophilus granarius (Linné)

A. Lamiri1, S. Lhaloui2,*, B. Benjilali3, M. Berrada4

1 Université Hassan I, Faculté des Sciences et Techniques, PoBox 577, Settat, Morocco
2 Institut National de La Recherche Agronomique, Centre Aridoculture, PoBox 589, Settat, Morocco
3 Institut Agronomique et Veterinaire Hassan II, Rabat, PoBox 6202-Rabat-Instituts, 10101-Rabat, Morocco
4 Université Hassan II, Faculté des Sciences Ben M’sik, PoBox 7955, Casablanca, Morocco

* Corresponding author. E-mail: Lhaloui@hotmail.com

Received 08 December 2000

Abstract

Granary weevil (Sitophilus granarius L.) is the most important and oldest known pest of stored products. To protect the grain wheat against this pest, nineteen essential oils extracted from aromatic and medicinal plants were tested for their insecticidal effect by determination of percentage of mortality after 24 and 48 hours of exposure. Several extracts were toxic to this pest, however the strongest activity was attributed to the essential oil of Mentha pulegium (100% of mortality) followed by Mentha viridis, Origanum compactum and Eucalyptus globulus.

Keywords: Sitophilus granarius; Wheat; Aromatic plants; Essential oils; Insecticidal effects.

1. Introduction

Plant oils are considered to be potent natural products as insecticides [1], and insect reproduction retardant [2,3]. The use of botanical pesticides is an ancient practice and tradition all over the world. With the advent of synthetic insecticides, these traditions have been largely neglected by farmers. However the serious health hazards for mamalia associated with synthetic insecticides and the negative ecological consequences have led to search for new classes of pesticides that are efficient and environmentally safe. In fact, pesticides developed on the basis of active molecules obtained from natural products are considered to be biodegradable, with a lesser persistence in the environment. The best known compound of this new class of insecticides is azadiractin, a limonoid from leaves and fruits of the neem tree, Azadirachta indica A. Juss [4].

Aromatic plants, widely spread in Morocco, which possess a very rich and diversified flora, are famous for their aromatic and medicinal characteristics. They are used in various industries such as cosmetics, perfumes, detergents, as well as in pharmacology and food aromatization. To these rapidly evolving traditional sectors, a new industrial development could be added in the phytosanitary domain. In fact, in addition to the antiseptic and fungicidal properties already identified in many plants [5-9], it has been shown that many aromatic plant species, as well as their essential oils, present a high level of efficiency in protecting crops and stored food against insect pests [4, 10-14].

The insect studied in this paper, the granary weevil (Sitophilus granarius L.), (Curculionidae) is the most damaging of the stored cereals pests (e.g. wheat) [15]. Fumigation is still one of the most effective methods for the prevention of storage losses. The main fumigants used at present are synthetic insecticides, such as methyl bromide and phosphine. The former is suspected to leave residues that are harmful to warm-blooded animals [16], while the latter, which is widely used, has shown alarming indications of insect resistance development [17-18]. Besides these insecticides are always associated with residuals that are dangerous for the consumer and the environment, and present the risk of developing resistance, in addition to the high cost benefit ratio. All these factors pushed research towards developing alternative insecticides.

The objective of this study is to examine the pesticide effect of some natural products "essential oils" on the granary weevil in attempts to reduce losses caused by the insect and to replace synthetic insecticides by bio-insecticides non toxic to the environment.

2. Material and methods

2.1. Chemical analysis

Seventeen aromatic and medicinal plant species, representing 7 families and 14 genera (Table 1) were collected from the Rif and Atlas mountains of Morocco. Nineteen essential oils were obtained from these plants by hydrodistillation in the laboratory using a Clevenger apparatus as modified by Miquel [19]. Three samples of the essential oil from A. herba-alba were used to check for chemotypic variability of this species.

The chemical composition of the essential oils was determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques.

The GC analysis was carried out using a DANI gas chromatography apparatus equipped with FID and a
DB-5 capillary column (25m x 0.25mm). Analytical conditions were: injector and detector temperatures 240°C and 260°C, respectively, oven temperature programmed from 50°C to 230°C at 4°C/min. Isothermal temperature at 230°C for 10 min; carrier gas 1mL N₂/min. Relative concentrations were calculated using peak areas as given by Shimadzu CR 6A integrator, without correction for response factors. The GC-MS analysis was done using an HP 5980 Series II gas chromatograph equipped with DB-5 capillary column (25 m x 0.3 mm; film thickness 0.25 µm) and an HP 5772A mass selective detector. Analytical conditions were: injector and detector temperatures 240°C and respectively, oven temperature programmed from 50°C to 230°C at 4°C/min, then isothermal temperature at 230°C for 10 min; carrier gas 1mL He/min; source 70eV. The oil constituents were identified by mass spectra data using NBS library and other literature data. Because A. herba-alba presents several chemotypes, three different samples were tested to examine the effect of chemical races of this species.

<table>
<thead>
<tr>
<th>Botanical name of the plant</th>
<th>Family</th>
<th>Main components of the essential oils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Eucalyptus globulus</td>
<td>Myrtaceae</td>
<td>1,8-cineole (74.5), α-pinene (9.5), α-terpineole (3.1)</td>
</tr>
<tr>
<td>2- Myrtus communis</td>
<td>Myrtaceae</td>
<td>1,8-cineole (49.3), α-pinene (20), myrtenal acetate (15.5)</td>
</tr>
<tr>
<td>3- Pistacia lentiscus</td>
<td>Anacardiaceae</td>
<td>myrcene (38), limonene (15.5), p-cymene(10.1), α-phellandrene (7.6)</td>
</tr>
<tr>
<td>4- Indra graveolens</td>
<td>Compositae</td>
<td>bornyl acetate (63.9), bornel (25.6), camphene (5), camphor (3.6)</td>
</tr>
<tr>
<td>5- Cedrus atlantica</td>
<td>Pinaceae</td>
<td>limonene (29.2), myrcene (16.9), ocimen + α-pinene (18.6), β-pinene (6.4)</td>
</tr>
<tr>
<td>6- Origanum compactum</td>
<td>Labiatae</td>
<td>carvacrol (35.1), p-cymene (22.5), thymol (20), γ-terpinene (7.1)</td>
</tr>
<tr>
<td>7- Ammi-visnaga</td>
<td>Umbelliferaceae</td>
<td>linalol (70.1), 3-pentylmethylbutanoate (4.3)</td>
</tr>
<tr>
<td>8- Citrus sinensis</td>
<td>Rutaceae</td>
<td>limonene (87.2)</td>
</tr>
<tr>
<td>9- Tanacetum annum</td>
<td>Compositae</td>
<td>sabinene (21.5), camphor (9.3), β-pinene (9), myrcene (8.5)</td>
</tr>
<tr>
<td>10- Mentha pulegium</td>
<td>Labiatae</td>
<td>pulegone (66.5), carvone (5.8), caryophyllene (3.9)</td>
</tr>
<tr>
<td>11- Rosmarinus officinalis</td>
<td>Labiatae</td>
<td>1,8-cineole (58.8), camphor (9.6), α-pinene (7.7), camphene (3.9), β-pinene (9)</td>
</tr>
<tr>
<td>12- Thymus satureoides</td>
<td>Labiatae</td>
<td>borneol (23.4), camphene (14.4), α-terpineol (12.5), α-pinene (7.3)</td>
</tr>
<tr>
<td>13- Mentha viridis</td>
<td>Labiatae</td>
<td>carvone (60.3), limonene (25.4), dihydrocarvone (2.7)</td>
</tr>
<tr>
<td>14- Origanum majorana</td>
<td>Labiatae</td>
<td>linalol (27.2), terpine-4-ol (17.1), γ-terpinene (9.7), α-terpinene (6.3)</td>
</tr>
<tr>
<td>15- Lavandula officinalis</td>
<td>Labiatae</td>
<td>1,8-cineole (48.1), β-pinene (15.4), linalol (8.3), α-pinene (5.5)</td>
</tr>
<tr>
<td>16- Artemisia arborescens</td>
<td>Compositae</td>
<td>β-thujone (40.1), camphor (20.1), myrcene (7.3), limonene (5.4)</td>
</tr>
<tr>
<td>17- A. herba-alba (α-thujone)</td>
<td>Compositae</td>
<td>α-thujone (65.3), β-thujone (10.5), camphor (12.6)</td>
</tr>
<tr>
<td>18- A. herba-alba (camphor)</td>
<td>Compositae</td>
<td>camphor (60.8), β-thujone (14.5)</td>
</tr>
<tr>
<td>19- A. herba-alba(α-thujone+camphor)</td>
<td>Compositae</td>
<td>camphor (32.6), α-thujone (20.3), 1,8-cineole (5.7)</td>
</tr>
</tbody>
</table>

Table 1: Main components of essential oils tested on Sitophilus granarius L.

2.2. Biological

In this study, nineteen essential oils, extracted from aromatic and medicinal plants, were evaluated for their insecticidal effect against the granary weevil, at the entomological laboratory of the National Institute of Agronomic Research of Settat, Morocco. Biological model: The insects used in the study were taken out of a granary weevil population maintained at the laboratory. Ten newly emerged insects were confined in a chamber and submitted to the different tests. Dead adults were counted after 24 and 48 hr of exposure to the essential oils.

2.3. Bioassay

The insecticidal test was based on ten individual adults, confined within an experimental chamber (Petri dish with interior volume of 0.098 liter). The essential oils were deposited on a Whatman filter paper, in a small fumigation room, closed by a gauze cloth on the top, thus avoiding direct contact between the oils and the insects. Different amounts of essential oil from 0.5µl to 20µl were applied. Each experiment was repeated three times. The percent of mortality was recorded after 24 and 48 hr of exposure. All experiments were conducted in a growth chamber set at 20 ± 2°C, 12h:12h (dark light) photoperiod, and 70% RH.

2.4. Statistical analysis

The data were analyzed using proc GLM (SAS Statistical Analysis System, SAS Institute, [20]). To decrease variability, root square (angular) transformations were performed on the data (Table A.10 from Steel and Torrie [21]). Analysis of variance was performed on the transformations. Means were separated using Fisher’s Least Significant Difference test (LSD) at 0.05 significance level. Pretransformed means are reported.
3. Results and discussion

Table 1 shows the main components of the different oils used in this study. The insecticidal effects of all the tested essential oils were confirmed after a series of preliminary tests using different oil concentrations.

At 24h of exposure, toxicity varied slowly as oil concentration increased, and the percent of mortality was low to null for 0.5, 1, 5 and 10µl. However, only Mentha pulegium showed an above average toxicity at 5 and 10µl (Table 2). At 20µl, 3 more oils (Eucalyptus globulus, Origanum compactum, and Ammi-visnaga) caused more than 50% mortality.

No mortality was recorded for oils of 3 plant species, Pistacia lentiscus, A. visnaga and Artemesia herba-alba (α-thujone+camphor) as we increased the concentration from 0.5 to 5µl.

<table>
<thead>
<tr>
<th>Botanical name of the plant</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus globulus</td>
<td>7dBC</td>
<td>13cdBCD</td>
<td>27bcBCD</td>
<td>37ahBC</td>
<td>63aB</td>
</tr>
<tr>
<td>Myrtus communis</td>
<td>7bC</td>
<td>10bBCD</td>
<td>13abDE</td>
<td>27aBDE</td>
<td>33aDEF</td>
</tr>
<tr>
<td>Pistacia graveolens</td>
<td>0bD</td>
<td>0bE</td>
<td>0bG</td>
<td>3bG</td>
<td>23aFGH</td>
</tr>
<tr>
<td>Cedrus atlantica</td>
<td>0bD</td>
<td>0bE</td>
<td>3bFG</td>
<td>7abG</td>
<td>17aH</td>
</tr>
<tr>
<td>Origanum compactum</td>
<td>3dCD</td>
<td>20aABC</td>
<td>33bcB</td>
<td>40abB</td>
<td>53aBC</td>
</tr>
<tr>
<td>Ammi-visnaga</td>
<td>0cD</td>
<td>0cE</td>
<td>0cG</td>
<td>17bEF</td>
<td>57aBC</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>0dD</td>
<td>3bDE</td>
<td>7abEF</td>
<td>10abFG</td>
<td>17aH</td>
</tr>
<tr>
<td>Tanacetum annuum</td>
<td>0bD</td>
<td>7bDE</td>
<td>17aCD</td>
<td>20aCDE</td>
<td>30aEFG</td>
</tr>
<tr>
<td>Mentha pulegium</td>
<td>17aA</td>
<td>37aA</td>
<td>60aA</td>
<td>87bA</td>
<td>100aA</td>
</tr>
<tr>
<td>Rosmarinus officinalis</td>
<td>7bBC</td>
<td>7bCDE</td>
<td>27aBCE</td>
<td>33aBCD</td>
<td>43aCDE</td>
</tr>
<tr>
<td>Thymus saturoides</td>
<td>17aA</td>
<td>20bcABC</td>
<td>20bcBCE</td>
<td>37abBC</td>
<td>47aCD</td>
</tr>
<tr>
<td>Mentha viridis</td>
<td>13dAB</td>
<td>23aAB</td>
<td>30bcBC</td>
<td>37abBC</td>
<td>43aCDE</td>
</tr>
<tr>
<td>Origanum majorana</td>
<td>3bCD</td>
<td>3bDE</td>
<td>20aBCD</td>
<td>27aBCDE</td>
<td>33aDEFG</td>
</tr>
<tr>
<td>Lavandula officinalis</td>
<td>0cD</td>
<td>3cDE</td>
<td>17bCD</td>
<td>30abcBDE</td>
<td>37aDEF</td>
</tr>
<tr>
<td>Artemisia arborescens</td>
<td>0cD</td>
<td>0cE</td>
<td>7bEF</td>
<td>17aEF</td>
<td>23aFGH</td>
</tr>
<tr>
<td>A. herba-alba (α-thujone)</td>
<td>0bD</td>
<td>0bE</td>
<td>3bFG</td>
<td>20aDE</td>
<td>27aFGH</td>
</tr>
<tr>
<td>A. herba-alba (camphor)</td>
<td>0cD</td>
<td>0cE</td>
<td>7bEF</td>
<td>27aBCE</td>
<td>33aDEFG</td>
</tr>
<tr>
<td>A. herba-alba (α-thujone+camphor)</td>
<td>0bD</td>
<td>0bE</td>
<td>0bG</td>
<td>20aDEF</td>
<td>20aGH</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* values followed by the same letter within rows (small letters) and within columns (capital letters) are not significantly different at p = 0.05

Table 2: Fumigant toxicity (in % mortality) of essential oils on Sitophilus granarius, using 5 amounts and 24 h exposure time.

This indicates that toxicity is not only due to the concentration of the oil, but also to the insecticidal effect of a specific plant oil. According to these results, 3 groups of activity could be distinguished. M. pulegium stands alone with a significantly high toxicity level (group1). In the second group, we can pull E. globulus, O. compactum and A. visnaga, which had a moderate toxicity at 20µl concentration. All the remaining oils presented a low toxicity and could be categorized together (group 3).

At the 48h exposure test, toxicity also increased with the increase in oil concentration, and was low to null for 0.5, 1 and 5µl (Table 3). M. pulegium and O. compactum caused, however, more than 50% mortality starting at 5µl.

Three oil categories could be distinguished as far as their toxicity to the granary weevil is concerned: M. pulegium, E. globulus, M. viridis and O. compactum were the most toxic. The oils of P. lentiscus, Inula graveolens, Citrus cinensis, and A. herba-alba (α-thujone+camphor) were the least toxic. The remaining oils were all moderately toxic.

The test of the chemical races of A. herba-alba indicated that the camphor chemotype was the most toxic, the α-thujone had a medium toxicity, and the (α-thujone +camphor) was the least toxic (Tables 2 and 3). It seems that there is an antagonism between both constituents (α-thujone +camphor) when they are present together. This shows that toxicity of an essential oil could vary depending on some parameters such as the chemotypes or chemical races of the same species. Therefore, it is important to check all the available cheomotypes of a given plant species.

Over all, the results of this study indicate that the percent of adult mortality increased with the increase of amount of oil used in the test. Also, that there is a
very striking difference between the level of mortality caused by the essential oil of *M. pulegium*, being the most toxic, and those caused by all the other essential oils.

Previously this oil was used medicinally as an abortifacient and to induce menstruation at a dose of 1-3 minimis, (1minim = ca.0.065g). Cellular necrosis and acute lung and liver damage were also observed at doses of 400mg/Kg and higher given intraperitoneally to mice [22]. The authors concluded that the isopropylidene grouping of pulegone and its derivatives is responsible for these effects. In the present study, the oil is used as a fumigant.

The use of fumigants to control stored products insects is extremely important because they act fast and penetrate the infested commodities entirely, thus controlling hidden infestations inside grain kernels. Because of the serious health hazards for mamalia associated with synthetic insecticides and the negative ecological consequences, pressure was put on scientists to search for more environmentally and toxicologically safe, and more selective and efficient pesticides. Natural pesticidal products, also called botanical pesticides, are available as alternatives to synthetic chemical formulations.

### Table 3: Fumigant toxicity (in % mortality) of essential oils on *Sitophilus granarius*, using 5 amounts and 48h exposure time.

<table>
<thead>
<tr>
<th>Botanical name of the plant</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus globulus</td>
<td>27cA</td>
<td>27cB</td>
<td>33bcD</td>
<td>43bcDE</td>
<td>73aBC</td>
</tr>
<tr>
<td>Myrtus communis</td>
<td>17cABC</td>
<td>23cBC</td>
<td>37cC</td>
<td>40abCDEF</td>
<td>53aD</td>
</tr>
<tr>
<td>Pistacia lentiscus</td>
<td>0f</td>
<td>0ef</td>
<td>0fH</td>
<td>7bh</td>
<td>27af</td>
</tr>
<tr>
<td>Inula graveolens</td>
<td>0bf</td>
<td>3bf</td>
<td>17aDEF</td>
<td>25afG</td>
<td>30af</td>
</tr>
<tr>
<td>Cedrus atlantica</td>
<td>17cD</td>
<td>27bcB</td>
<td>37abC</td>
<td>40abCDEF</td>
<td>53aD</td>
</tr>
<tr>
<td>Origanum compactum</td>
<td>13bBCD</td>
<td>27cB</td>
<td>63bB</td>
<td>63bB</td>
<td>77aBC</td>
</tr>
<tr>
<td>Ammi-visnaga</td>
<td>0df</td>
<td>0df</td>
<td>7cFGH</td>
<td>33bDEFG</td>
<td>63aCD</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>0cf</td>
<td>7bf</td>
<td>13abEF</td>
<td>20aG</td>
<td>23af</td>
</tr>
<tr>
<td>Tanacetum annum</td>
<td>3ceF</td>
<td>10beCDE</td>
<td>30abCDE</td>
<td>40aCDEFG</td>
<td>43aF</td>
</tr>
<tr>
<td>Mentha pulegium</td>
<td>30dA</td>
<td>53aA</td>
<td>93bA</td>
<td>100a4</td>
<td>100a4</td>
</tr>
<tr>
<td>Rosmarinus officinalis</td>
<td>13eBCD</td>
<td>23cBC</td>
<td>40cC</td>
<td>46bBCD</td>
<td>63aCD</td>
</tr>
<tr>
<td>Thymus sativoides</td>
<td>23cAB</td>
<td>30cB</td>
<td>37bcC</td>
<td>50abBCD</td>
<td>53aD</td>
</tr>
<tr>
<td>Mentha viridis</td>
<td>20dABC</td>
<td>30cdB</td>
<td>43bcBC</td>
<td>57bBC</td>
<td>80aB</td>
</tr>
<tr>
<td>Origanum majorana</td>
<td>3bDE</td>
<td>20bDE</td>
<td>30aCDE</td>
<td>47aBCD</td>
<td>50aDE</td>
</tr>
<tr>
<td>Lavandula officinalis</td>
<td>0df</td>
<td>20cBCD</td>
<td>33bCD</td>
<td>43abCDE</td>
<td>50aDE</td>
</tr>
<tr>
<td>Artemisia arborescens</td>
<td>0cf</td>
<td>3ef</td>
<td>10bcFG</td>
<td>27abEFG</td>
<td>37aEF</td>
</tr>
<tr>
<td><em>A. herba-alba</em> (α-thujone)</td>
<td>0bf</td>
<td>0bf</td>
<td>3bGH</td>
<td>33aDEFG</td>
<td>37aEF</td>
</tr>
<tr>
<td><em>A. herba-alba</em> (camphor)</td>
<td>0cf</td>
<td>0ef</td>
<td>10bFG</td>
<td>27aEFG</td>
<td>50aDE</td>
</tr>
<tr>
<td><em>A. herba-alba</em> (α-thujone+camphor)</td>
<td>0bf</td>
<td>0bf</td>
<td>7bFGH</td>
<td>27aEFG</td>
<td>33aEF</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* values followed by the same letter within rows (small letters) and within columns (capital letters) are not significantly different at p = 0.05.

### 4. Conclusion

The results observed in this study confirm the high insecticidal effect of some plant essential oils, which may have important applications to prevent losses of stored crops and other products. The development of natural or biological insecticides will help decrease the negative effects associated with chemical insecticides (such as residues, resistance and environmental hazards). Bio-insecticides that will be effective, selective, bio-degradable, associated with little or no resistance of the target pest, and non toxic to the environment, will better contribute to the quality and quantity of agricultural production of the world.

More studies need to be carried in order to investigate the mechanism of toxicity of the essential oil on insect development, and to establish the relation between the chemical composition of the oils and the insecticidal activity against *sitophilus granarius* Linné.
Acknowledgments

The author is grateful to Dr. Hadarbach for advice on statistical analysis.

References