

# Fumigant toxicity of five essential oils rich in ketones against *Sitophilus zeamais* (Motschulsky)

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## SUMMARY

Essential oils (EOs) and individual compounds act as fumigants against insects found in stored products. In fumigant assays, *Sitophilus zeamais* Motschulsky adults were treated with essential oils derived from *Aphylllocladus decussatus* Hieron, *Aloysia polystachya* Griseb, *Minthostachys verticillata* Griseb Epling and *Tagetes minuta* L., which are rich in ketones and their major components:  $\alpha$ - thujone, R-carvone, S-carvone, (-) menthone, R (+) pulegone and E-Z- ocimenone. *M. verticillata* oil was the most toxic (LC<sub>50</sub>: 116.6  $\mu$ l /L air) characterized by a high percentage of menthone (40.1%) and pulegone (43.7%). All ketones showed insecticidal activity against *S. zeamais*. However, pulegone (LC<sub>50</sub>: 11.8  $\mu$ l/L air), R- carvone (LC<sub>50</sub>: 17.5  $\mu$ l/L air), S-carvone (LC<sub>50</sub>: 28.1  $\mu$ l/L air) and E-Z-ocimenone (LC<sub>50</sub>: 42.3  $\mu$ l/L air) were the most toxic. These ketones are  $\alpha,\beta$ -unsaturated carbonyl. This feature could play a fundamental role in the increase of insecticidal activity against *S. zeamais*.

**Keywords:** *Sitophilus zeamais*, essential oils, *Aphylllocladus decussatus*, *Aloysia polystachya*, *Minthostachys verticillata*, *Tagetes minuta*,  $\alpha,\beta$ -Unsaturated carbonyl ketones

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## RESUMEN

Los aceites esenciales (AEs) y sus componentes principales actúan como fumigantes contra insectos de granos-almacenados. En ensayos fumigantes, adultos de *Sitophilus zeamais* Motschulsky fueron tratados con AEs, ricos en cetonas, provenientes de *Aphylllocladus decussatus* Hieron, *Aloysia polystachya* Griseb, *Minthostachys verticillata* Griseb Epling y *Tagetes minuta* L., y sus principales compuestos:  $\alpha$ - tujona, R-carvona, S-carvona, (-) mentona, R (+) pulegona y E-Z- ocimenona. El AE de *M. verticillata* fué el mas tóxico (CL<sub>50</sub>: 116,6  $\mu$ l / L aire) caracterizado por un alto contenido de mentona (40,1%) y pulegona (43,7%). Todas las cetonas mostraron actividad insecticida contra *S. zeamais*. Sin embargo, pulegona (CL<sub>50</sub>: 11,8  $\mu$ l/ L aire), R- carvona (CL<sub>50</sub>:

17,5 µl/L aire), S-carvona (CL<sub>50</sub>: 28,1 µl/L aire) y E-Z-ocimenona (CL<sub>50</sub>: 42,3 µl/L aire) fueron las más tóxicas. Estas cetonas presentan α,β-insaturaciones; dicha propiedad puede estar relacionada con el incremento de la actividad insecticida contra *S. zeamais*.

**Palabras clave:** *Sitophilus zeamais*, aceites esenciales, *Aphylocladus decussatus*, *Aloysia polystachya*, *Minthostachys verticillata*, *Tagetes minuta*, cetonas α,β- insaturadas.

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## INTRODUCTION

The central area of Argentina is the most important in the production of corn. The amount of corn exported to the United States, Europe and China during 2011 - 2012 was approximately 75,000 tons (Lezcano, 2012). Worldwide maize grain storage sites are affected by different species of beetle pests. *Sitophilus zeamais* (Motschulsky) is a serious primary pest of stored maize throughout the world causing quantitative and qualitative losses (Boyer *et al.*, 2012). The control of these pests is dependent upon applications of synthetic insecticides: methyl bromide and phosphine (Benhalima, 2004; Athie & Mills, 2005; Pimentel *et al.*, 2012). These control measures have several limitations, such as the development of resistance (Benhalima, 2004; Pimentel *et al.*, 2009; Pimentel *et al.*, 2012) and environmental damage, which cause serious concerns about human health.

In view of the problems with the current fumigants, there is a global interest in generating alternative strategies. There are numerous studies describing essential oils (EOs) and their components as potential insecticides (Pérez *et al.*, 2010; Caballero-Gallardo *et al.*, 2011; Kurdelas *et al.*, 2012). This insecticidal activity has been evaluated against the maize weevil. The results showed that many EOs could control *Sitophilus zeamais* populations in corn grain stores (Liu *et al.*, 2011; Suthisut *et al.*, 2011; Yang *et al.*, 2011). However, the insecticidal activity of synthetic products is far superior to that of EOs but the pressure generated by the consuming public about the use of synthetic pesticides has raised the option of an integrated management of insects affecting food storage sites (Yigezu *et*

*al.*, 2010). In this framework EOs are shown as an important alternative (Correa *et al.*, 2011). Plant essential oils are promising in that they are easily biodegradable, do not present described resistance and they are environmentally friendly (Pérez *et al.*, 2010).

Therefore, in the present work, we report insecticidal activity of EOs rich in ketones of *Aphylocladus decussatus* Hieron, *Aloysia polystachya* Griseb, *Minthostachys verticillata* Griseb Epling, *Tagetes minuta* L. and their major components against *S. zeamais*.

## MATERIALS AND METHODS

### Insects

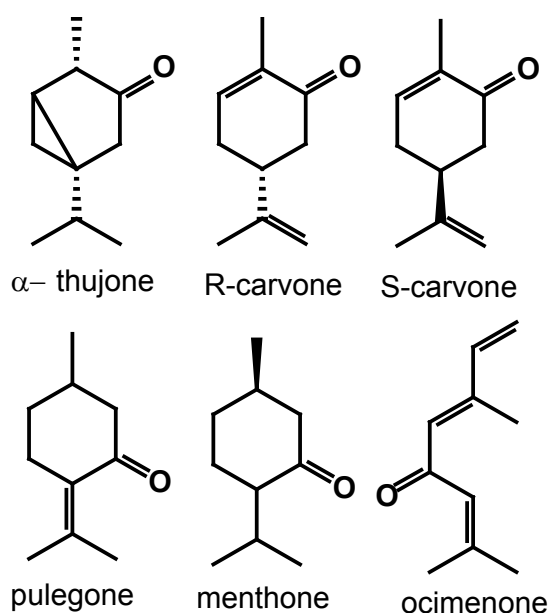
Adults of the strain of *S. zeamais* were obtained from Metán, Salta province, Argentina. The colony was maintained in our laboratory for one year without exposure to insecticides.

*S. zeamais* were reared on sterilized whole maize grain in sealed containers. Insect rearing was carried under controlled temperature and humidity (28 °C and 60-70%) and a light/dark regime of 12:12 h (FAO, 1974). Experiments were conducted under complete darkness, at 28 °C and 60-70% relative humidity. The unsexed adult weevils used were about 2-weeks old.

### Essential oils and pure compounds

*Aphylocladus decussatus* Hieron, *Aloysia polystachya* Griseb (population 1) were collected from La Rioja province, Argentina. Plants of *Aloysia*

*polystachya* Griseb (population 2), *Minthostachys verticillata* Griseb Epling and *Tagetes minuta* L. were collected from Córdoba province, Argentina. We deposited all voucher plant specimens at the Museo Botánico de Córdoba (CORD). The EOs were obtained by hydrodistillation (Demo *et al.*, 2005) and stored at -20°C in airtight microtubes prior to analysis by gas chromatography–mass spectrometry (GC-MS). The  $\alpha$ -thujone, R-carvone, S-carvone, (-) menthone and R (+) pulegone used for bioassays were purchased from Sigma Aldrich (Steinheim, Germany). The E-Z-ocimene was isolated from *T. minuta* EO by supercritical carbon dioxide fractionation. The highest selectivity was obtained at 80 bar and 40 °C, with a static period of 30 min followed by dynamic extraction at a flow rate of 0.1 g/min until complete removal of monoterpenes, leaving a residue of purified ocimene (Figure 1).



**Fig. 1.** Chemical structures of natural ketones compounds studied in the present work.

### Analysis of essential Oils

The EOs were analyzed by Perkin Elmer Clarus 500 chromatograph equipped with a detector FID and a capillary column DB-5 (60 m x 0.25 mm i.d. and 0.25  $\mu$ m coating thickness). The temperature of the column was programmed from 60 °C to 240 °C at 4 °C/min. The detector and injector temperatures were 250 °C. Helium was used as carrier gas with a flow rate of 0.9 ml/min. The quantitative composition

was obtained by peak area normalization, and the response factor for each component was considered to equal 1. For the determination of the composition, EOs samples were diluted with n-hexane. The injection volume was 1  $\mu$ l. The identification of the EOs' components was carried out by GC-MS. A Perkin-Elmer Clarus 600 GC-MS coupled with an ion trap mass detector which was employed for the identification. A capillary column DB-5 (60 m x 0.25 mm i.d. and 0.25  $\mu$ m coating thickness) was used for the separation of the components. Helium was used as carrier gas with a flow rate of 0.9 ml/min. The temperature program for the oven and injector was the same as that for the GC-FID. Ionization was realized by electron impact at 70 eV. Mass spectral data were acquired in the scan mode in the m/z range 35-250. Retention indices (RI) of the sample components were determined on the basis of homologous n-alkane hydrocarbons under the same conditions. The compounds were identified by comparing their retention indices and mass spectra with published data (Adams, 2007) and libraries NIST and Adams. The main components were further identified by coinjection of authentic standards (SIGMA, USA).

### Fumigant Toxicity Assay

The insecticidal activity against *S. zeamais* was evaluated using fumigant toxicity assay described by Huang *et al.* (2000), with some modifications. Briefly, glass vials (30 ml) were used as fumigation chambers. Different amounts of EOs and pure compounds were applied to Whatman filter paper disks (2 cm diameter) placed on the underside of the screwcap of a glass vials at the doses corresponding to 15- 600  $\mu$ l/L air. A series of concentrations of each EOs and pure compound were prepared in n-hexane. Solvent was allowed to evaporate for 2 min prior to introduction of insects. Ten adults *S. zeamais* were placed into each vial (5 replicas / dose). Control insects were kept under same conditions without EOs and pure compounds. Insect mortality was checked after 24 h. The mortality percentages and LC<sub>50</sub> values were calculated according to Finney (1971).

### Statistical analysis

The concentration-mortality data were subjected to Probit analysis to obtain the LC<sub>50</sub> values. The lethal concentrations LC<sub>50</sub> and LC<sub>95</sub> were calculated using SPSS Statistics program version 17.0 (SPSS Inc). The values of LC<sub>50</sub> were considered to be significantly different, if 95% confidence limits did not

overlap. Treatment means were compared and separated by Duncan's test at  $p = 0.05$  using the InfoStat software Professional 2010 (Di Rienzo *et al.*, 2010).

## RESULTS AND DISCUSSION

### Essential oils

The main compounds in the EOs extracted from *A. decussates*, *A. polystachya* (population 1 and 2), *M. verticillata* and *Tagetes minuta* are presented in Table 1. We identified 32, 9, 15, 22

and 7 compounds from *A. decussates*, *A. polystachya* (population 1), *A. polystachya* (population 2), *M. verticillata* and *T. minuta* EOs, respectively. The principal compounds of EOs obtained from *A. decussates* were sabinene (12.4%), 1,8-cineole (14.6%),  $\alpha$ -thujone (39.4%) and  $\beta$ -thujone (17.9%). The major component of *A. polystachya* (population 1) was  $\alpha$ -thujone (98.7%), while *A. polystachya* (population 2) had as main components  $\alpha$ -thujone (27.6%) and carvone (62.9%). *M. verticillata* EO was characterized by a high percentage of menthone (40.1%) and pulegone (43.7%). Ocimenone (43.5%) and *cis*- $\beta$ -ocimene (42.4%) were the main

**Table 1** Chemical constituents of essential oils from *A. decussates*, *A. polystachya* (1), *A. polystachya* (2), *M. verticillata* and *T. minuta* plants collected from Córdoba and La Rioja provinces, Argentina.

| RI <sup>1</sup> | Compounds                       | <i>A. decussatus</i> | <i>A. polystachya</i> (P1) | <i>A. polystachya</i> (P2) | <i>M. verticillata</i> | <i>T. minuta</i> | Methods of identification <sup>2</sup> |
|-----------------|---------------------------------|----------------------|----------------------------|----------------------------|------------------------|------------------|--|
| 947             | $\alpha$ -Pinene                | 1.3                  | tr <sup>2</sup>            |                            | 0.3                    |                  | GCMS                                   |
| 990             | Sabinene                        | <b>12.4</b>          | 0.3                        | 0.1                        | 0.1                    |                  | GCMS                                   |
| 994             | $\beta$ -Pinene                 | 0.4                  | tr                         |                            | 0.3                    |                  | GCMS                                   |
| 1001            | $\beta$ -Myrcene                | 0.5                  |                            |                            | 0.2                    |                  | GCMS                                   |
| 1044            | Limonene                        | tr                   |                            | 0.8                        | 1.8                    | 1.9              | GCMS                                   |
| 1045            | 1,8-Cineole                     | <b>14.6</b>          |                            |                            | 0.2                    |                  | GCMS                                   |
| 1051            | <i>cis</i> - $\beta$ -Ocimene   |                      |                            |                            | 0.7                    | <b>42.4</b>      | GCMS                                   |
| 1059            | <i>trans</i> - $\beta$ -Ocimene | 0.7                  |                            |                            |                        |                  | GCMS                                   |
| 1064            | Dihydrotagetone                 |                      |                            |                            |                        | 3.4              | GCMS                                   |
| 1072            | $\gamma$ -Terpinene             | 0.3                  |                            |                            |                        |                  | GCMS                                   |
| 1097            | Linalool                        |                      |                            | 0.9                        |                        |                  | GCMS. Co                               |
| 1134            | $\alpha$ - Thujone              | <b>39.4</b>          | <b>98.7</b>                | <b>27.6</b>                | 1.6                    |                  | GCMS. Co                               |
| 1144            | $\beta$ - Thujone               | <b>17.9</b>          | 0.7                        | 0.8                        |                        |                  | GCMS                                   |
| 1146            | <i>allo</i> - Ocimene           |                      |                            |                            |                        | 3.7              | GCMS                                   |
| 1158            | <i>trans</i> -Sabinol           | 0.5                  |                            |                            |                        |                  | GCMS                                   |
| 1159            | <i>trans</i> - Tagetone         |                      |                            |                            |                        | 1.1              | GCMS                                   |
| 1162            | Menthone                        |                      |                            | 0.4                        | <b>40.1</b>            |                  | GCMS. Co                               |
| 1175            | Isomenthone                     |                      |                            |                            | 2.0                    |                  | GCMS                                   |
| 1176            | Pinocarvone                     | 0.1                  |                            |                            |                        |                  | GCMS                                   |
| 1183            | 4-Terpineol                     | 0.7                  |                            |                            |                        |                  | GCMS                                   |
| 1193            | $\alpha$ -Terpineol             |                      |                            | 0.7                        |                        |                  | GCMS. Co                               |
| 1197            | Dihydrocarvone                  |                      |                            |                            | 1.2                    |                  | GCMS                                   |
| 1235            | (Z)-Ocimenone                   |                      |                            |                            |                        | 3.9              | GCMS                                   |
| 1244            | Pulegone                        |                      |                            | 0.8                        | <b>43.7</b>            |                  | GCMS                                   |
| 1245            | (E)-Ocimenone                   |                      |                            |                            |                        | <b>43.5</b>      | GCMS                                   |
| 1258            | Carvone                         | 0.2                  | tr                         | <b>62.9</b>                |                        |                  | GCMS. Co                               |
| 1299            | <i>trans</i> -Sabinil acetate   | 3.8                  |                            |                            |                        |                  | GCMS                                   |
| 1345            | Piperitenone                    |                      |                            | 0.2                        | 0.6                    |                  | GCMS                                   |
| 1431            | $\beta$ -Caryophyllene          |                      |                            |                            | 0.7                    |                  | GCMS                                   |
| 1492            | $\beta$ -Gurjunene              | 0.4                  |                            |                            | 0.4                    |                  | GCMS                                   |
| 1510            | Germacrene B                    |                      |                            |                            | 3.6                    |                  | GCMS                                   |
|                 | Unknown compounds               | 6.8                  | 0.3                        | 4.8                        | 2.5                    | 0.1              |  |

<sup>1</sup>Retention index on a DB-5 column relative to homologous series of n-alkanes. GCMS: peak identifications are based on MS comparison with file spectra (The similarity is over 97%). Co: peak identification is based on standard comparison with relative retention time.<sup>2</sup> tr = concentration less than 0.05%

components of *T. minuta* EO. All EOs were characterized by a high concentration of ketone type compounds, in the cases studied these compounds represented over 40% of the total EO. The exception were sabinene and 1,8-cineol in *A. decussates* with a concentration above 10% and the hydrocarbon *cis*- $\beta$ -sabinene with a concentration of 42.4% in *T. minuta*. The following compounds showed a concentration less than 0.05% and they are components of only one or two species, from *A. decussates*:  $\alpha$ -thujene, camphene,  $\alpha$ -terpinene, *cis*-pinocarveol acetate, carvacrol, *trans*-carvil acetate, myrtenol, (neo-3)-thujanol acetate,  $\alpha$ -terpinil acetate, *neois*-3-thujanol, sabinone, eugenol, *cis*-carvil acetate,  $\alpha$ -humulene, calamenene, oxide caryophyllene, from *A. polystachya* (1),  $\alpha$ -myrcene, myrtenol,  $\alpha$ -curcumene, from *A. polystachya* (2), *cis*-carveol, thymol,  $\alpha$ -humulene, spathulenol, from *M. verticillata*,  $\delta$ -2-carene, *p*-cymene, *cis* sabinene hydrate acetate, eugenol,  $\beta$ -bourbonene, 1*S*,*cis*-calamenene.

### Toxicity of essential oils

Comparison of LC<sub>50</sub> values for the five EOs against *S. zeamais* showed that *M. verticillata* oil was the most toxic (LC<sub>50</sub> = 116.6  $\mu$ L air), toxicity being twice lower at 24 h after exposure than in the rest of the EOs studied. LC<sub>50</sub> of EO of *T. minuta* could not be calculated because it did not show dose dependent linear behavior (Table 2).

**Table 2.** Fumigant toxicity of essential oils from *A. decussates*, *A. polystachya* (1), *A. polystachya* (2), *M. verticillata* and *T. minuta* plants against adults of *S. zeamais* at 24 h after exposure.

| Source                             | % Mortality <sup>1</sup><br>(concentration<br>at 150 ( $\mu$ L air)) | LC <sub>50</sub><br>( $\mu$ L air) | 95%<br>confidence<br>interval<br>( $\mu$ L air) |
|------------------------------------|--|------------------------------------|---|
| <i>A. decussates</i>               | 25.00(5.77) a  | 212.12                             | 198.44 226.97                                   |
| <i>A. polystachya</i> <sup>1</sup> | 32.00(10.95)a  | 230.74                             | 201.11 266.24                                   |
| <i>A. polystachya</i> <sup>2</sup> | 36.00(11.40)a  | 218.65                             | 192.16 245.28                                   |
| <i>M. verticillata</i>             | 82.50(15.00)b  | 116.61                             | 58.12 161.65                                    |
| <i>T. minuta</i>                   | 56.7(5.8) c  | ND <sup>2</sup>                    |   |

<sup>1</sup> Values (means  $\pm$  SE) with different letters in the same column are significantly different from each other according to Duncan's multiple range test at  $P \leq 0.05$ . Each datum represents the mean of five replicates, each set up with 10 adults. <sup>2</sup> ND: Not determined

### Toxicity of individual compounds

All ketones showed insecticidal activity against *S. zeamais*. The toxicity of pure compounds can be divided into two groups from the most to the least toxic: Group 1: pulegone (LC<sub>50</sub>: 11.8  $\mu$ L air), R-carvone (LC<sub>50</sub>: 17.5  $\mu$ L air), S-carvone (LC<sub>50</sub>: 28.1  $\mu$ L air) and ocimene (LC<sub>50</sub>: 42.3  $\mu$ L air); Group

2:  $\alpha$ -thujone (LC<sub>50</sub>: 65.5  $\mu$ L air) and menthone (LC<sub>50</sub>: 85.4  $\mu$ L air) (Table 3).

The EOs and the individual compounds act as fumigants against insects found in stored products. In the study, EO of *M. verticillata* was the most bioactive, which can be attributed to its content in pulegone (43.6 %) (Table 1). However, *S. zeamais* showed greater sensitivity to pure compounds than EOs.

Pulegone was more toxic than carvone and E-Z-ocimene (Table 3). In addition, carvone isomers presented differences in toxicity. Carvone R was more active than S isomer. Controversial results of insecticidal activity of isomers of carvone were shown by Lee *et al.* (2003) and Tripathi *et al.* (2003).

**Table 3.** Fumigant toxicity of pure compounds against *S. zeamais* adults at 24 h after exposure.

| Compound          | % Mortality <sup>1</sup><br>(concentration<br>at 50 ( $\mu$ L air)) | LC <sub>50</sub><br>( $\mu$ L air) | 95% confidence<br>interval<br>( $\mu$ L air) |
|-------------------|---|------------------------------------|--|
| R-Carvone         | 98.00(4.47) a   | 17.56                              | 15.17 19.88                                  |
| S-Carvone         | 82.00(21.68) a  | 28.10                              | 23.59 33.26                                  |
| Pulegone          | 100(0) a  | 11.81                              | 11.22 12.64                                  |
| $\alpha$ -Thujone | 30.00(25.00) b  | 65.53                              | 57.31 73.40                                  |
| Menthone          | 24.00(11.22) b  | 85.46                              | 72.15 97.95                                  |
| Ocimene           | 96.00 (8.9) a   | 42.30                              | 37.87 47.55                                  |

<sup>1</sup> Values (means  $\pm$  SE) with different letters in the same column are significantly different from each other according to Duncan's multiple range test at  $P \leq 0.05$ . Each datum represents the mean of five replicates, each set up with 10 adults.

Numerous studies have shown ketones with highest toxicity against *Sitophilus* in fumigant and contact assays (Lee *et al.*, 2003; Tripathi *et al.*, 2003; Liu *et al.*, 2011; Germinara *et al.*, 2012). These results suggest that the presence of carbonyl groups augments toxicity. On the other hand, ketones belonging to Group 1 were  $\alpha,\beta$ -unsaturated. This feature could be playing a fundamental role in the increase of insecticidal activity. Thus, ketones of Group 1 were more toxic than those of Group 2 (Table 3). Xavier & Rauter (2008) revealed that compounds containing the  $\alpha,\beta$ -unsaturated act as Michael acceptors for the addition of protein nucleophilic groups. Also,  $\alpha,\beta$ -unsaturated carbonyl compounds were described as potent inhibitors of the enzymes Glutathione-S-transferases that contribute to the phase II metabolism of xenobiotics (Yu & Abo-Elghar, 2000).

In conclusion, the present results indicate that  $\alpha,\beta$ -unsaturated carbonyl ketones act as potential fumigants against *S. zeamais*. Based on these findings, they could serve as viable alternative to synthetic insecticides.

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