Fumigant and repellent activities of different essential oils alone and combined against the maize weevil (Sitophilus zeamais motschulsky)

Abstract

The insecticidal and repellent activity of five essential oils (EOs) was studied separately and in binary combinations against Sitophilus zeamais. Minthostachys verticillata EO showed the highest fumigant activity with a LC₅₀ value of 28.2µl/l air. A moderate toxicity was observed with Eucalyptus globulus EO (LC₅₀=335.7µl/l air), whereas the EOs from Aloysia citriodora, Coriandrum sativum and Mentha sp. did not show insecticidal effect at 600µg/l air. All combinations that include M. verticillata EO showed strong fumigant activity with LC₅₀ values lesser than 78µl/l air. The co-toxicity coefficient (CTC) of M. verticillata and E. globulus EO combination indicating an additive effect (CTC=119.1). Repellent activity was evaluated using two-choice olfactometer assay. All EOs and their combinations had repellent effect on adults of S. zeamais (P<0.05).

Keywords: biopesticides, fumigant toxicity, repellent effect, stored maize pest

Abbreviations: EO, essential oil; ANOVA, analysis of variance; CTC, co-toxicity coefficients; DDVP, 2,2-dichlorovinyl dimethyl phosphate; RI, retention index; GC-MS, gas chromatography-mass spectrometry; EI-MS, electron impact mass spectra; TI, toxicity index

Introduction

The maize weevil Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) is a worldwide primary pest of stored maize. Both larvae and adults feed on corn grains reducing their weight, nutritional value, commercial value and germination rate. The damage produced on grains also favors the occurrence of secondary pests and fungi. Fumigation is the most widespread method to control stored-product pests, however the overuse of conventional fumigants including DDVP, 2,2-dichlorovinyl dimethyl phosphate have led to the development of resistance by insects, environmental pollution and negative effects on non-target organisms and human health. Consequently, the interest in generating different strategies of control has increased.

Essential oils (EOs) are complex mixtures of volatile secondary metabolites produced by aromatic plants. They constitute an important source of bioactive chemicals and provide interesting alternatives to conventional insecticides due to their limited persistence on the environment, low mammalian toxicity and low probability of generate resistance. Numerous studies have demonstrated that EOs have a great potential as insecticides and repellents. Furthermore, many EOs or their constituents were studied for their fumigant and repellent effect on maize pests, including S. zeamais.

The activity of an EO generally depends on its major constituents, but sometimes the sum of the activities of individual constituents does not explain the overall activity of the oil, evidencing synergistic or antagonistic effects. These effects also occur among constituents of different EOs. Therefore, combinations of EOs could significantly enhance their biological activity. For example, Benelli et al. observed that the binary mixture of EOs from Satureja montana L. and Aloysia citriodora Palau has higher larvicidal toxicity than the individual oils against Culex quinquefasciatus Say.

The aim of the current study was to evaluate the fumigant and repellent activities of EOs from some locally available plants: Aloysia citriodora Palau (Verbenaceae), Coriandrum sativum L. (Apiaceae), Eucalyptus globulus Labill (Myrtaceae), Mentha sp. (Lamiaceae) and Minthostachys verticillata (Grisb.) Epling (Lamiaceae) and separately and in binary combinations, against S. zeamais.

Materials and methods

Essential oils

Leaves of A. citriodora, E. globulus, M. sp. and M. verticillata and C. sativum seeds were collected in commercial crops in Córdoba, Argentina. The samples were air dried and subjected to hydrodistillation for 2 hours in a Clevenger’s apparatus in order to extract their vaporized EOs, which were stored in dark glass tubes under refrigeration (4°C) until evaluation.

Identification of the EOs constituents was determined using electron impact mass spectra (EI-MS) obtained from gas chromatography-mass spectrometry (GC-MS), and by co-injection of standards (Sigma Aldrich Co. Buenos Aires, Argentina), with the mass spectra libraries Adams, NIST and a homemade library being utilized. Compound concentrations were expressed as a percentage of the peaks area, and the retention index RI of each compound was obtained for a homologous series of n-alkanes C₄–C₃₀ (Sigma Aldrich Co. Buenos Aires, Argentina). Identifications were made by

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matching both their mass spectra and RI values with those reported in the literature and those of pure compounds, whenever possible. GC-MS was performed on a GC-MS Perkin Elmer 680, equipped with a mass selective detector in the electron impact mode (70eV). The chromatography conditions being as follows: DB-5 capillary column (30mx0.25mm, film thickness 0.25mm), the oven temperature was programmed linearly at 60°C for 5minutes, ramped up to 170°C at 4°C/minute, and then to 240°C at 20°C/minute; injector temperature 250°C; detector temperature 250°C; carrier gas, H2, at 45cm/second, split into 50ml/minute and samples of 1µL (1/100 in n-heptane, v/v) injected manually in the split-less mode.

Insects

Sitophilus zeamais adults were obtained from Metán, Salta, Argentina. Insects were maintained in sealed containers (10 l) with whole maize grains under controlled conditions (26°C and 60% relative humidity), in darkness. The colony was kept in our laboratory for two years without exposure to insecticides before testing. The unsexed adult weevils used in all the experiments were approximately 2 weeks old.

Fumigant toxicity assay

Susceptibility of S. zeamais adults to volatile compounds from A. citriodora, C. sativum, E. globulus, Mentha sp. and M. verticillata EOs and all their possibly binary combinations were evaluated using fumigant toxicity assay described by Peschiutta et al. with some modifications. Different doses (10-600µl/l air) of the EOs or their combinations were applied to Whatman filter paper disks of 2cm diameter placed on the underside of the screw cap of a fumigation chamber (30ml-glass vial). The EOs was mixed in 1:1 ratio (v/v) in all binary combinations. A piece of voile was also placed under the screw cap to avoid direct contact of the weevils with the EOs. In each vial 5g of whole maize grains were deposited in order to mimic the natural conditions in a silo. Ten adults of S. zeamais were placed in each fumigation chamber. Control treatments were performed without EO (negative control) and with 2,2-dichlorovinyl dimethyl phosphate (DDVP) at 0.06µl/l air (positive control). The assays were carried out in complete darkness at 28°C and 60±5% relative humidity. Five replicates per dose were performed and insect mortality was recorded after 90 minutes. The experiment was repeated five times per dose. For each test the response index (RI) was calculated with the following equation: RI=([T-C]/C)×100, where T is the number responding to treatment, C is the number responding to control, and T-C is the total number of insects released. Positive RI indicates attraction to the treatment and negative RI indicates repellency.

Statistical analysis

The concentration-mortality data recorded after 24 hours of exposure to the EOs was subjected to a statistical analysis using the log-logistic model available in the “drc” package and compiled by the statistical software R. Lethal concentrations causing 50 and 95% of mortality (LC50 and LC95) were determined, as well as their confidence limits at 95%.

The significance of the mean RI in each treatment of the two-choice olfactometer bioassay was evaluated by the Student’s t test for paired comparisons. Mean values of RI were first analyzed by analysis of variance (ANOVA) followed by a Dunnett’s test (P<0.05).

Results and discussion

The composition of EOs from A. citriodora, C. sativum, E. globulus, Mentha sp. and M. verticillata are shown in Table 1. According to the analysis the main components were geranial (43.43 %) and nerol (36.36 %) in A. citriodora EO; linalool (93.81 %) in C. sativum EO; 1,8-cineole (32.18 %) and p-cymene (17.04 %) in E. globulus EO; carvone (76.14 %) in Mentha sp. EO; and pulegone (57.09 %) and menthone (36.36 %) in M. verticillata EO.

The fumigant activity of the EOs and their binary combinations was evaluated against S. zeamais. Behavioral response of S. zeamais adults to these compounds was measured using two-way olfactometer. Two 250ml-Erlenmeyer were connected by a glass tube 30x1cm diameter in which was opened a small window 1x1cm equidistant from the two Erlenmeyer flasks. Corn kernels (6g) and a filter paper of 2cm diameter with the test compound (treatment) or with the solvent alone (control) were placed in each Erlenmeyer flask. The EOs and combinations were tested at 4µl/l air. Twenty insects deprived of food for at least 12hours were placed in the center of the tube through the window made for that purpose, which subsequently was closed. The experiments were performed under dark conditions at 28°C and 60±5% relative humidity. The number of insects in each container was recorded after 90 minutes. The experiment was repeated five times per dose. For each test the response index (RI) was calculated with the following equation: RI=([T-C]/C)×100, where T is the number responding to treatment, C is the number responding to control, and T-C is the total number of insects released. Positive RI indicates attraction to the treatment and negative RI indicates repellency.

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The significance of the mean RI in each treatment of the two-choice olfactometer bioassay was evaluated by the Student’s t test for paired comparisons. Mean values of RI were first analyzed by analysis of variance (ANOVA) followed by a Dunnett’s test (P<0.05).

Results and discussion

The composition of EOs from A. citriodora, C. sativum, E. globulus, Mentha sp. and M. verticillata are shown in Table 1. According to the analysis the main components were geranial (43.43 %) and nerol (28.89 %) in A. citriodora EO; linalool (93.81 %) in C. sativum EO; 1,8-cineole (32.18 %) and p-cymene (17.04 %) in E. globulus EO; carvone (76.14 %) in Mentha sp. EO; and pulegone (57.09 %) and menthone (36.36 %) in M. verticillata EO.

The fumigant activity of the EOs and their binary combinations was evaluated against adults of S. zeamais. Minthostachys verticalata EO showed the highest fumigant toxicity with a LC50 value of 28.2µl/l air (Table 2). A moderate toxicity was observed with E. globulus EO (LC50=335.7µl/l air), whereas the EOs from A. citriodora, C. sativum and Mentha sp. did not show fumigant activity at 600µl/l air. Similarly, Herrera et al. found that the EO from M. verticillata was the most bioactive among the tested EOs against S. zeamais, however they registered a higher LC50 value that could be attributed to the natural variation in the composition of the EOs. In another previous study M. verticillata EO also was the most toxic against Musca domestica L. equaling the LC50 of the reference insecticide DDVP. The strong fumigant toxicity of M. verticillata EO can be due to its elevated content of pulegone and menthone.

Combinations of M. verticillata EO with the EOs from Mentha sp., E. globulus, C. sativum and A. citriodora showed high fumigant activity with LC50 values of 41.8, 43.7, 57.1 and 77.6µl/l air respectively (Table 2). On the other hand, all binary combinations of EOs from A. citriodora, C. sativum and Mentha sp. were not toxic
against *S. zeamais* at 600µl/l air. Due to it was not possible to obtain the LC50 values of all the EOs and combinations, we could only calculate the CTC of *M. verticillata* and *E. globulus* EO combination (CTC=119.1), which indicates an additive effect.19

All the tested EOs and combinations had repellent effect on adults of *S. zeamais* (P<0.05) (Table 3). Although there were no statistically significant differences among treatments, the combination of *Mentha* sp. and *A. citriodora* EOs showed the highest response index value (-85.75±5.43). This value was even higher than those observed for *Mentha* sp. and *A. citriodora* EOs separately (-56.40±10.13 and -44.67±17.72 respectively), which could be due to the synergistic action of their main compounds. Similarly, Liu et al.25 found that repellent activity of the mixture of EOs from *Artemisia princeps* Pamp and *Cinnamomum camphora* (L.) Presl. against adults of *Sitophilus oryzae* L. and *Bruchus rugimanus* Bohem was significantly higher than that elicited by individual oils. The mechanisms involved in how the interactions among the components of each EO result in the improvement of the repellent activity need further investigation.26

Summing up, *M. verticillata* EO alone or in combination with EOs from *A. citriodora*, *C. sativum*, *E. globulus* or *Mentha* sp. has strong fumigant activity, while all the tested EOs and combinations have repellent effect on adults of *S. zeamais*, offering interesting alternatives to traditional pesticides to control *S. zeamais*.

**Table 1** Relative percentage concentrations of the components of the essential oils

| RI (Literature) | RI (Calculated) | Compound names | Minthostachys verticillata | Coriandrum sativum | Aloysia citriodora | Eucalyptus globulus | Mentha sp. | Methods of identification |
|----------------|-----------------|----------------|---------------------------|---------------------|-------------------|-------------------|------------|--------------------------|
| 924            | 928             | α-thujene      |                           | 2.33                | GC-MS, RI         |
| 932            | 935             | α-pinene       | 0.27                      | 0.8                 | 0.17              | 0.83              | GC-MS, RI, Co |
| 969            | 972             | sabinene       | 0.15                      | 0.32                | GC-MS, RI, Co     |
| 974            | 973             | 1-octen-3-ol   |                           | 2.71                | GC-MS, RI         |
| 974            | 978             | β-pinene       | 0.37                      | 1.25                | 0.65              | GC-MS, RI, Co     |
| 988            | 984             | β-myrcene      | tr                        | 1.06                | 0.3               | GC-MS, RI         |
| 1026           | 1002            | α-phellandrene |                           | 9.68                | GC-MS, RI         |
| 1020           | 1005            | p-cymene       | tr                        | 0.52                | 0.37              | 17.04            | tr          | GC-MS, RI, Co |
| 1024           | 1027            | limonene       | 0.86                      | 4.56                | tr                | 3.46              | GC-MS, RI |
| 1026           | 1026            | 1,8-cineole    | 0.31                      | 32.18               | 5.19              | GC-MS, RI, Co     |
| 1054           | 1056            | g-terpinene    | 1.44                      | 1.07                | GC-MS, RI         |
| 1086           | 1084            | terpinolene    |                           | 0.34                | GC-MS, RI         |
| 1095           | 1093            | linalool       | 93.81                     | 1.51                | GC-MS, RI         |
| 1100           | 1103            | undecane       |                           | 0.23                | GC-MS, RI         |
| 1137           | 1150            | cis-verbenol   |                           | 1.97                | GC-MS, RI         |
| 1141           | 1151            | camphor        |                           | 2.41                | GC-MS, RI         |
| 1148           | 1164            | menthone       | 36.36                     | 0.22                | GC-MS, RI         |
| 1158           | 1165            | isomenthone    | 1.7                       | 0.34                | GC-MS, RI         |
| 1159           | 1167            | menthofuran    |                           | 0.75                | GC-MS, RI         |
| 1165           | 1177            | borneol        |                           | 0.49                | GC-MS, RI         |
| 1167           | 1181            | isopulegone    | 0.79                      | GC-MS, RI          |
| 1174           | 1184            | 4-terpineol    | 0.12                      | 5.89                | GC-MS, RI         |
| 1183           | 1193            | cryptone       |                           | 9.13                | GC-MS, RI         |
| 1186           | 1201            | α-terpineol    |                           | 3.65                | GC-MS, RI         |
| 1191           | 1201            | cis-dihydrocarvone |                     | 2.2                | GC-MS, RI         |
| 1193           | 1208            | dihydro carveol neo-iso |         | 0.46                | GC-MS, RI         |
| 1226           | 1235            | carveol cis    |                           | 0.33                | GC-MS, RI         |
| 1233           | 1240            | pulegone       | 57.09                     | tr                  | 0.41              | GC-MS, RI         |
| 1235           | 1243            | neral          | 28.89                     | GC-MS, RI          |
| 1238           | 1249            | cuminaldehyde  |                           | 2.16                | GC-MS, RI         |
| 1239           | 1261            | carvone        |                           | 76.14               | GC-MS, RI         |
| 1249           | 1266            | piperitone      |                           | 0.56                | GC-MS, RI         |

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Table Continued...

| RI (Literature) | RI (Calculated) | Compound names | Minthostachys verticillata | Coriandrum sativum | Aloysia citriodora | Eucalyptus globulus | Mentha sp. | Methods of identification |
|-----------------|-----------------|----------------|----------------------------|--------------------|--------------------|----------------------|-------------|--------------------------|
| 1264            | 1274            | geranial       | 43.43                      | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1274            | 1285            | phellandral    | 1.97                       | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1283            | 1349            | α-terpinen-7 al | 0.52                      | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1374            | 1378            | α-copaene      | 0.29                       | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1387            | 1388            | β-bourbonene   | 0.36                       | 0.88               | -                  | -                    | -           | GC-MS, RI                |
| 1410            | 1420            | α-cedrene      | 0.86                       | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1418            | 1426            | β-cariophylene | 1.84                       | 2.78               | -                  | -                    | -           | GC-MS, RI                |
| 1434            | 1427            | g-elemene      | 1.42                       | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1451            | 1460            | allo-aromadendrene | 0.35                 | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1452            | 1464            | α-humulene     | 0.25                       | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1454            | 1469            | cis β-farmasene| tr                         | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1475            | 1477            | g-gurjunene    | tr                         | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1478            | 1480            | g-murolene     | 4.11                       | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1479            | 1481            | α-curcumene    | 2.3                        | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1484            | 1487            | germacrene d   | 0.46                       | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1522            | 1522            | δ-cadinene     | 0.4                        | -                  | -                  | -                    | -           | GC-MS, RI                |
| 1577            | 1593            | spathulenol    | 0.66                       | 4.35               | -                  | -                    | -           | GC-MS, RI                |
| 1582            | 1595            | cariophylene oxide | 0.14                    | 5.8                | -                  | -                    | -           | GC-MS, RI                |
| 1677            | 1660            | nerolidol acetate | 1.51                    | -                  | -                  | -                    | -           | GC-MS, RI                |

tr: traces (<0.1%).

Table 2 Fumigant toxicity of the essential oils and their combinations against Stilophillus zeamais

| Essential oils | LC_{50} (µl/l air) | 95% CL (µl/l air) | LC_{95} (µl/l air) | 95% CL (µl/l air) | (X^2)^* |
|----------------|--------------------|-------------------|--------------------|-------------------|---------|
| Minthostachys verticillata | 28.2               | 18.4-43.1         | 106.4              | 40.4-280.5        | 13.45   |
| Eucalyptus globulus         | 335.7              | 250.3-450.3       | 896.6              | 417.2-1927.0      | 8.77    |
| Mentha sp.                  | >600               | -                 | -                  | -                 | -       |
| Aloysia citriodora          | >600               | -                 | -                  | -                 | -       |
| Coriandrum sativum          | >600               | -                 | -                  | -                 | -       |
| M. verticillata+Mentha sp.  | 41.8               | 31.1-56.3         | 72.7               | 38.7-136.8        | 2.5     |
| M. verticillata+E. globulus | 43.7               | 32.4-58.9         | 88                 | 50.6-153.2        | 39.05   |
| M. verticillata+C. sativum  | 57.1               | 43.7-74.6         | 133.6              | 57.9-308.3        | 6.71    |
| M. verticillata+A. citriodora | 77.6               | 52.4-114.9        | 262                | 71.2-964.2        | 7.96    |
| E. globulus+Mentha sp.      | >600               | -                 | -                  | -                 | -       |
| E. globulus+A. citriodora   | >600               | -                 | -                  | -                 | -       |
| E. globulus+C. sativum      | >300               | -                 | -                  | -                 | -       |
| Mentha+A. citriodora        | >600               | -                 | -                  | -                 | -       |
| Mentha+C. sativum           | >600               | -                 | -                  | -                 | -       |
| A. citriodora+C. sativum    | >600               | -                 | -                  | -                 | -       |

*Chi-square values, significant at P<0.05 level
CL: confidence limits

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Table 3 Response of Sitophilus zeamais to five essential oils and their binary combinations at 4µl/l air in a two-choice olfactometer bioassay

| Essential oils                  | Response index (RI) |
|--------------------------------|---------------------|
| Coriandrum sativum             | -72.04±13.03 ** a    |
| Eucalyptus globulus           | -71.90±5.75 *** a    |
| Mentha sp.                    | -56.40±10.13 ** a    |
| Aloysia citriodora             | -46.67±17.72 ** a    |
| Minthostachys verticillata    | -38.40±1.64 ** a     |
| A. citriodora + C. sativum    | -78.33±1.67 *** a    |
| E. globulus + A. citriodora   | -67.34±6.30 *** a    |
| E. globulus + C. sativum      | -48.03±5.41 ** a     |
| E. globulus + Mentha sp.      | -74.08±3.22 *** a    |
| M. verticillata + A. citriodora| -61.18±2.33 *** a    |
| M. verticillata + C. sativum  | -55.85±8.95 ** a     |
| M. verticillata + E. globulus | -43.99±10.18 ** a    |
| M. verticillata + Mentha sp.  | -55.80±4.14 ** a     |
| Mentha sp. + A. citriodora    | -85.75±5.43 *** a    |
| Mentha sp. + C. sativum       | -60.48±12.64 *** a   |
| Control                       | 1.49±1.4 b           |

*(P<0.05) and ** (P<0.01); *** (P<0.001); N=5 (significant response to experimental stimulus; paired-sample t-test). Mean responses to different treatments followed by different letters are significantly different (ANOVA, P<0.05, means comparison by Dunnet’s test).

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**Conflict of interest**

The author declares no conflict of interest.

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