The potential use of plant substances extracted from Brazilian flora to control stored grain pest.

Heho T Prates\textsuperscript{1}; Jamilton P. Santos\textsuperscript{2}; José M. Waquil\textsuperscript{3}; Alaide B. Oliveira\textsuperscript{4}

Abstract

The damage caused by stored grain insects in Brazil is estimated to be around 8% of production. Insects responsible for such loss are among others Sitophilus zeamais, Sitophilus oryzae, Rhyzopertha dominica and Tribolium castaneum. Synthetic insecticides are the conventional way to control these insect pests. However, natural products from plants have been studied as an ecologically more effective alternative in protecting stored grains. A screening program of plant substances extracted from Brazilian flora was initiated against those insects. Bioassays to determine the insecticidal activity consisted of tests for fumigation, contact, and contact and/or ingestion. In all assays, twenty individual adults of each insect were used in each of three replicate assays. A control test was prepared the same way but no impregnating substance was used. All tests were carried out at room temperature (26 ± 1°C). Observations on the survival rate of insects were made 24 h after contact and efficacy (%) was calculated using Abbott procedure. Results have shown that cineole, limonene and Eucalyptus globulus and E. camaldulensis essential oils showed toxic effect to insects through cuticle (contact test) and/or by ingestion (contact and or ingestion test) and fumigation activity.

Introduction

The damage caused by stored grain insects in Brazil is estimated to be around 8% of total maize production. Such damage on wheat, rice, dried bean and sorghum is high, but not well known. Insects most responsible for such loss are Sitophilus zeamais, Sitophilus oryzae, Rhyzopertha dominica and Tribolium castaneum. The conventional way to control these insect pests has been with the use of insecticides, either directly applied to grains or by gas fumigation. However, concerns have arisen about the persistence of insecticide residues in grains, which can be harmful to mammals. Another way used for stored grain pest control has been based on methyl bromide and phosphine, which are synthetic insecticides with health and environment hazards. The former has also been identified as one of those responsible for ozone shell destruction, and the later presents some insect resistance problem (Shaaya et al., 1997).

Recently, new ecologically recommendable techniques have been searched in order to discover alternatives to insect control such as biological control. In this sense the interest in natural products from plants relies in the fact that these substances are ecologically more effective in protecting stored grains as compared to synthetic insecticides. Studies of plants biological activities carried out in a screening program can lead to the combat of these pests by direct application of natural compounds or its derivatives (Plummer, 1985).

Some secondary metabolites of plants play an important role in plant-insect interaction, and are commonly responsible for plant resistance to insects (Mann, 1987). Some essential oils have acute toxicity, repellence action, feeding inhibition, or harmful effects on reproductive system of insects. Additionally, secondary metabolites from higher plants have recently been used as pesticides or models for new synthetic pesticides as, for instance, toxaphene (insecticide and herbicide) and omethylin (herbicide) (Duke et al., 1988). These chemicals were developed from plant-derived products such as terpenoids that can be found in the essential oil secreted by the glandular trichomes of Artemisia (Compositae) or closely related genera Pme oil, a by-product of the sulfate wood pulping industry, has the monoterpen e-terpineol among its major constituents. Monoterpenes found in essential oil from different plants have insecticidal activity against termites (Baker and Walsme, 1982). Coats and Colab (1991) described fragrant volatile oils containing monoterpenes or their related compounds: alcohols, ketones, aldehydes, carboxylic acids, and oxanes, such as d-limonene, \( \beta \)-myrcene, \( \alpha \)-terpineol, linalool, and pulegone. These substances have toxicity to house fly (Musca domestica).
L), German cockroach (*Blatella germanica* L.), rice weevil (*Sitophilus oryzae* L.), red flour beetle (*Tribolium castaneum* H.) and Southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) (Rice and Coats, 1994a,b).

R-(+)-limonene (p-mentha-1,8-diene) and 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane) are natural products from plants with low toxicity to mammals. Limonene is a major component of *Citrus* spp essential oils, and is also used as an ingredient of soaps, perfumes, and food additives (Karr and Coats, 1988). Cineole (or eucalyptol) is a constituent occurring in variable amounts in the essential oil of *Eucalyptus* spp leaves (Gibson et al., 1991).

The main purpose of the work at Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, was to test natural products extracted from Brazilian flora as an ecologically safe alternative insecticide, which could also be economically produced and used against *Sitophilus zeamais*, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*. The screening program was initiated with monoterpenes (Prates et al., 1998, Santos et al., 1996). This study describes results on monoterpenes characteristics adequate for testing, insecticide activity of cineole and limonene. *Eucalyptus globulus* and *E camaldulensis* essential oils against stored grain pests.

**Material and Methods**

Bioassays to determine the insecticidal activity of natural products consisted of tests for fumigation, contact, and contact and/or ingestion actions. In all assays, twenty individual adults of *Sitophilus zeamais*, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*, obtained from cultures maintained in the laboratory were used in each of three replicate assays. A control test was prepared the same way but no impregnating substance was used. Insect mortality was observed by using a stereo microscope when needed. All tests were carried out at room temperature (26 ± 1°C) in the Entomology Laboratory for Stored Grain Pest Control of Embrapa Maize and Sorghum at Sete Lagoas, MG, Brazil. Substances under test in this work were analytical grade, supplied by Aldrich. Essential oil of *Eucalyptus globulus* (78%, cineole) and *Eucalyptus camaldulensis* (66%, cineole) were obtained from leaves.

**Evaporation test**

An important characteristic in stored grain pest control is the fumigation action of substance. Therefore, monoterpenes were allowed to evaporate as a preparation procedure for the fumigation test. A two-liter tightly closed glass container was used as a fumigation chamber based on a procedure described by Karr and Coats (1988), with some modifications. Sixty-three milligrams of each monoterpenes were separately placed in a watch glass to evaporate, in such a way that the vapour was confined in the chamber. At the end residual substance was weighted and the elapsed time recorded. Results showed in Table 1 indicates that (+)-a-pinene, 1,8-cineole, (−)-β-pinene and R-(+)-limonene were 100% evaporated in the shorter time and therefore eligible for the fumigation test. In this case cineole and limonene, available in the laboratory, were chosen for evaluation.

**Table 1. Evaporation test of monoterpenes. Embrapa Maize and Sorghum, Sete Lagoas, MG, 1998.**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Weight (mg)</th>
<th>Time (h)</th>
<th>Evaporation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-a-Pinene</td>
<td>58</td>
<td>2:15</td>
<td>100.00</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>59</td>
<td>2:30</td>
<td>100.00</td>
</tr>
<tr>
<td>(−)-β-Pinene</td>
<td>63</td>
<td>2:40</td>
<td>100.00</td>
</tr>
<tr>
<td>Limonene</td>
<td>63</td>
<td>4:00</td>
<td>100.00</td>
</tr>
<tr>
<td>(−)-a-Pinene</td>
<td>61</td>
<td>24:00</td>
<td>93.44</td>
</tr>
<tr>
<td>Linalool</td>
<td>61</td>
<td>24:00</td>
<td>72.13</td>
</tr>
<tr>
<td>Citronellal</td>
<td>63</td>
<td>24:00</td>
<td>52.38</td>
</tr>
<tr>
<td>Isopropylcamphene</td>
<td>54</td>
<td>24:00</td>
<td>38.89</td>
</tr>
<tr>
<td>Menthol</td>
<td>65</td>
<td>24:00</td>
<td>30.77</td>
</tr>
<tr>
<td>Citronellol</td>
<td>73</td>
<td>24:00</td>
<td>15.07</td>
</tr>
<tr>
<td>α-Terpeneol</td>
<td>67</td>
<td>24:00</td>
<td>11.94</td>
</tr>
</tbody>
</table>

**Fumigation test**

The fumigation test was carried out in a two-liter tightly closed glass container used as a fumigation chamber. Sixty-three milligrams (three drops) of each cineole and limonene were separately placed in a watch glass to evaporate, in such a way that the vapour was confined in the chamber. The atmosphere inside the fumigation chamber was homogenized by stirring the inner atmosphere with a magnetic stirrer. Insects were exposed to the gas by putting them inside a wire screen cage hanging from a metal support. The fumigant activity was measured 24 h after exposing the insects to the gas and mortality was calculated using Abbott (1925) procedure.

**Contact test**

The contact effect was evaluated by treating a 7 cm diameter (38.5 cm²) Whatman n° 1 filter paper with the substances dissolved in acetone. The treated filter paper was placed on top of a three pin point support for two minutes to allow the acetone to evaporate before putting it inside a Petri dish. Glass rings (5.0 cm internal diameter and 2.5 cm
high) treated on the inside with a fine powder (carnauba) to prevent the insects from climbing the glass ring wall were used to force them to stay on the filter paper. The glass rings were also covered with a fine cloth to prevent the insects from flying away. The filter papers were treated by evenly spreading doses of the substances diluted in acetone at the ratios (substance: acetone) of 10:0, 8:2, 6:4, 4:6, 2:8, 1:9, 0:10. One dose of limonene or one of cineole means 21.00 mg and 19.67 mg, respectively. After a 48-h period the flask was open and mortality was calculated using Abbott (1925) procedure. Same procedure was carried out with Eucalyptus essential oil.

Contact and/or ingestion

The contact and/or ingestion activity was evaluated by infesting samples of treated wheat grains of 20 g, stored in a 30 mL glass vial, using the same doses as above. The applied concentrations of limonene were the same as in the contact test. The substances were allowed to penetrate the insects, through the contact with the cuticle (palps) also at least the legs, probably other body parts as the insects moved through the grain and by ingesting treated particles of grain. After a 48-h period the flask was open and mortality was calculated using Abbott (1925) procedure. Same procedure was carried out with Eucalyptus essential oil.

Results and Discussion

Fumigant activity

The fumigant activity of cineole and limonene, evaluated by the efficiency (mortality, %) to kill insects are presented in Table 2. Limonene had about the same effect (≥95 %) to all insects except for S. oryzae (5 %), but was more effective against T. castaneum than cineole (58.3 %). The present results agree with those reported by Karr and Coats (1988), in the sense that limonene has high toxic fumigant activity against cockroaches and rice weevils.

Table 2. Fumigant efficiency of 1, 8-cineole and R-(+)-limonene to kill stored grain pest Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 1998.

<table>
<thead>
<tr>
<th>Insect</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cineole</td>
</tr>
<tr>
<td>S. zeamais</td>
<td>100.0</td>
</tr>
<tr>
<td>S. oryzae</td>
<td>53.3</td>
</tr>
<tr>
<td>R. dominica</td>
<td>100.0</td>
</tr>
<tr>
<td>T. castaneum</td>
<td>58.3</td>
</tr>
</tbody>
</table>

Essential oil of E. globulus (Table 3) was very effective against all insects. On the other hand, E. camaldulensis had about the same efficiency except for T. castaneum (28.3 %), but was less effective against S. zeamais (70.6 %).

Table 3. Fumigant efficiency of Eucalyptus globulus and Eucalyptus camaldulensis essential oil to kill stored grain pest Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 1998.

<table>
<thead>
<tr>
<th>Insect</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. globulus</td>
</tr>
<tr>
<td>S. zeamais</td>
<td>87.0</td>
</tr>
<tr>
<td>S. oryzae</td>
<td>88.0</td>
</tr>
<tr>
<td>R. dominica</td>
<td>98.3</td>
</tr>
<tr>
<td>T. castaneum</td>
<td>98.3</td>
</tr>
</tbody>
</table>

Contact activity with treated filter paper and contact/ingestion activity with treated wheat grains

The contact and/or ingestion activity efficiency of cineole and limonene against R. dominica, T. castaneum, S. zeamais and S. oryzae are illustrated in Tables 4 and 5, respectively. Cineole was generally more effective to all pests than limonene.

On the other hand, activity efficiency of E. globulus and E. camaldulensis essential oil are shown in Tables 6 and 7. It was not observed significant differences in the efficiency of both essential oils against those insects as compared to pure substances (cineole and limonene), but the former were more effective in controlling R. dominica and T. castaneum, probably due to other substances present in the oil.

The mortality on filter paper was slightly lower than that of the treated grain method, in general. A possible reason for this result would be that insects may intake the toxic substance through the cuticle or by eating (oral intake) when exposed to the treated grains.

Conclusion

Cineole, limonene and Eucalyptus essential oil showed contact and/or ingestion and fumigant activity. The glass vials used to test for contact and/or ingestion activity with wheat grain, although open to the air, were slender, creating the possibility for the fumigant compound, slowly released, to be retained sufficient time to affect insects and cause some mortality even before the contact and/or ingestion activity takes place. Thus, the observed mortality of the insects placed in contact with wheat treated grain cannot be credited only to the contact and/or ingestion effect.
Table 4. Contact with filter paper and contact and/or ingestion efficiency of 1,8-cineole e R-(+)-limonene to kill *Sitophilus zeamais* and *S. oryzae*. Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 1998.

<table>
<thead>
<tr>
<th>Dose</th>
<th><em>S. zeamais</em></th>
<th><em>S. oryzae</em></th>
<th><em>S. zeamais</em></th>
<th><em>S. oryzae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact/ingestion</td>
<td>Contact</td>
<td>Contact/ingestion</td>
<td>Contact</td>
</tr>
<tr>
<td>10 + 00</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>08 + 02</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>06 + 04</td>
<td>95.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>04 + 06</td>
<td>95.0</td>
<td>100.0</td>
<td>65.0</td>
<td>100.0</td>
</tr>
<tr>
<td>02 + 08</td>
<td>48.3</td>
<td>78 3</td>
<td>8.5</td>
<td>96.5</td>
</tr>
<tr>
<td>01 + 09</td>
<td>3.3</td>
<td>0</td>
<td>0</td>
<td>8.5</td>
</tr>
<tr>
<td>00 + 10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

1 One dose of cineole equals to 19.67 mg, and one dose of limonene equals to 21.00 mg

Table 5. Contact with filter paper and contact and/or ingestion efficiency of 1,8-cineole e R-(+)-limonene to kill *Rhyzopertha dominica* and *Tribolium castaneum*. Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 1998.

<table>
<thead>
<tr>
<th>Dose</th>
<th><em>R. dominica</em></th>
<th><em>T. castaneum</em></th>
<th><em>R. dominica</em></th>
<th><em>T. castaneum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact/ingestion</td>
<td>Contact</td>
<td>Contact/ingestion</td>
<td>Contact</td>
</tr>
<tr>
<td>10 + 00</td>
<td>97.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>08 + 02</td>
<td>93.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>06 + 04</td>
<td>87.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>04 + 06</td>
<td>77.0</td>
<td>100.0</td>
<td>98.3</td>
<td>100.0</td>
</tr>
<tr>
<td>02 + 08</td>
<td>10.0</td>
<td>97.0</td>
<td>10.2</td>
<td>100.0</td>
</tr>
<tr>
<td>01 + 09</td>
<td>3.0</td>
<td>76.0</td>
<td>1.7</td>
<td>45.0</td>
</tr>
<tr>
<td>00 + 10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 One dose of cineole equals to 19.67 mg, and one dose of limonene equals to 21.00 mg

Table 6. Contact with filter paper and contact and/or ingestion efficiency of *Eucalyptus globulus* and *Eucalyptus camaldulensis* essential oil to kill *Sitophilus zeamais* and *S. oryzae*. Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 1998.

<table>
<thead>
<tr>
<th>Dose</th>
<th><em>E. globulus</em></th>
<th><em>E. camaldulensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. zeamais</em></td>
<td><em>S. oryzae</em></td>
</tr>
<tr>
<td></td>
<td>Contact/ingestion</td>
<td>Contact</td>
</tr>
<tr>
<td>10 + 00</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>08 + 02</td>
<td>98.3</td>
<td>100.0</td>
</tr>
<tr>
<td>06 + 04</td>
<td>96.0</td>
<td>100.0</td>
</tr>
<tr>
<td>04 + 06</td>
<td>88.8</td>
<td>88.5</td>
</tr>
<tr>
<td>02 + 08</td>
<td>56</td>
<td>83.3</td>
</tr>
<tr>
<td>01 + 09</td>
<td>2.3</td>
<td>20.0</td>
</tr>
<tr>
<td>00 + 10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 One dose of *E. globulus* and *E. camaldulensis* equals to 21.00 mg

823
Table 7. Contact with filter paper and contact and/or ingestion efficiency of *Eucalyptus globulus* and *Eucalyptus camaldulensis* essential oil to kill *Rhysopertha dominica* and *Tribolium castaneum*. Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 1998

<table>
<thead>
<tr>
<th>Dose(^1)</th>
<th><em>R. dominica</em></th>
<th><em>T. castaneum</em></th>
<th><em>R. dominica</em></th>
<th><em>T. castaneum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact</td>
<td>Contact/ingestion</td>
<td>Contact</td>
<td>Contact/ingestion</td>
</tr>
<tr>
<td>10 + 00</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>08 + 02</td>
<td>100.0</td>
<td>100.0</td>
<td>80.0</td>
<td>100.0</td>
</tr>
<tr>
<td>06 + 04</td>
<td>100.0</td>
<td>100.0</td>
<td>20.0</td>
<td>100.0</td>
</tr>
<tr>
<td>04 + 06</td>
<td>100.0</td>
<td>100.0</td>
<td>30.0</td>
<td>100.0</td>
</tr>
<tr>
<td>02 + 08</td>
<td>90.0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>01 + 09</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>50.0</td>
</tr>
<tr>
<td>00 + 10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\) One dose of *E. globulus* and *E. camaldulensis* equals to 21.00 mg

Based on the present results it can be concluded for the monoterpenes that: a) the cineole and limonene have significant insecticide effect, being lethal to all insects; b) these substances are toxic by penetrating the insect body via the respiratory system (fumigant effect), the cuticle (contact effect) or the digestive system (ingestion effect); c) cineole was more effective than limonene, except in fumigation test against *T. castaneum*; d) limonene was more effective for *T. castaneum* than for *R. dominica*; e) the test with treated wheat grain was somewhat more sensitive than the one with the filter paper.

Essential oil of *Eucalyptus* were very efficient against all insects being *E. globulus* slightly more effective than *E. camaldulensis* except in fumigation test against *T. castaneum*. It should be stressed that cineole is not the only substance in the essential oil of *Eucalyptus* what might have other effects due to other substances present in the oil.

**Acknowledgments**

This work was grant by EMBRAPA/CNPMS and in part with financial support from FAPEMIG, CNPq and FINEP. We thank Dr. Mara Cristina Mazza from EMBRAPA/CNPQ for collecting *Eucalyptus globulus* and *Eucalyptus camaldulensis* leaves and to Dr. Midori Koketsu from EMBRAPA/CTAA in providing both *Eucalyptus* essential oil samples.

**References**


Rice, P. J. and Coats, J. R., 1994a Insecticidal
properties of monoterpenoid derivatives to the house fly (Diptera: Muscidae) and red flour beetle (Coleoptera: Tenebrionidae). Pesticide Science 41, 195–202.
Rice, P. J. and Coats, J. R., 1994b. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology 87, 1172–1179.