Evaluation of *Cymbopogon martini*ii Oil Extract for Control of Postharvest Insect Deterioration in Cereals and Legumes

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ABSTRACT

The essential oil of *Cymbopogon martini*ii was tested for its potency as a botanical pesticide to protect stored wheat (*Triticum aestivum*) and gram (garbanzo bean, *Cicer arietinum*) from insect infestation. The *C. martini*ii oil was potent as a fumigant in stored grain. The oil was an effective repellent against the beetles *Callosobruchus chinensis* and *Tribolium castaneum*. Geraniol, the major component of the oil, was not as effective as the oil itself. *C. martini*ii oil significantly affected oviposition, adult development, and mortality of *C. chinensis* in cow peas (*Vigna unguiculata*). The *C. martini*ii oil when used as a fumigant did not affect viability, germination, and seedling growth of grain. Because of its insecticidal and semiochemical nature, the oil could be used as an alternative to synthetic pesticides in an integrated pest management program to protect stored food commodities.

Cereals and pulses (legumes) have great biological and nutritional value. They play a major role in correcting malnutrition in developing countries. Bruchid beetles (e.g., *Callosobruchus chinensis* (L.), Bruchidae) which are internal feeders, are major pests of stored legumes (1, 30). Losses from bruchid damage occur in 20 to 60% of stored pulse samples (5). Weevils and grain beetles (e.g., *Rhyzopertha dominica* (F.), Bostrichidae; *Sitophilus oryzae* (L.), Curculionidae; and *Tribolium castaneum* (Herbst), Tenebrionidae), which are internal and external feeders, are the most common causes of insect deterioration of wheat samples and are responsible for the losses of 10 to 40% (4, 31). The widespread use of different insecticides for stored product pest control has led to the development of insecticide resistance (2, 11, 28, 29, 34, 43), harmful effects on nontarget organisms, persistence in the environment, and bioaccumulation in the food web (9). Hence, there is growing interest in the use of safer alternatives for pest control. Because of greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become popular. During recent years, products of some pesticidal plants, e.g., *Azadirachta indica* and *Chrysanthemum cinerariaefolium*, have received global attention for protection of several food commodities because of their insecticidal properties (39). Such plant products have been formulated as botanical pesticides, which are used as alternatives to synthetic pesticides in crop protection. Plant products, because of their natural origin, are biodegradable and normally do not leave toxic residues on the food commodities after application (7). The essential oil obtained from leaves of the palmarosa (*Cymbopogon martini*ii (Roxb) Wats, Poaceae) was tested in the present study for its in vivo efficacy as a fumigant against insect pests in the management of biodeterioration of stored wheat and grain. Although there are some preliminary reports on the biological activity of the essential oil of *C. martini*ii (3, 10, 42), investigations on various aspects of its practical efficacy as a botanical pesticide are lacking.

This study was a comparative investigation of the efficacy of crude *Cymbopogon* oil and geraniol, its major component, in protection of food commodities from insect infestation. The effect of the oil on oviposition and adult development in *C. chinensis* and the efficacy of the oil as a repellent also was determined.

MATERIALS AND METHODS

Rearing of test insects. *Callosobruchus chinensis* (L.), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), and *Tribolium castaneum* (Herbst) were collected from stored gram (garbanzo bean, *Cicer arietinum*) and wheat (*Triticum aestivum*) samples, and their identity was confirmed by the Department of Entomology, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, India. *C. chinensis* were reared on cow pea seeds (*Vigna unguiculata* (L.) Walp.) (5, 19). *S. oryzae* and *R. dominica* were maintained on wheat grains and *T. castaneum* was maintained on wheat flour at 27 ± 2°C and 80 ± 5% relative humidity (26). Fifty adult insects were released separately into 250-g samples of wheat or cow peas in plastic containers covered by muslin cloth. After 24 h, adult insects were removed from the samples, which were then incubated in a temperature-humidity control cabinet (27 ± 2°C and 80 ± 5% relative humidity) to obtain insects of the same age. Adult insects were 3 days old when used in bioassays.

Extraction of essential oil. Fresh leaves of *C. martini*ii (250 g) were cut into small pieces with scissors and thoroughly washed with sterilized water. The volatile fraction (essential oil) was extracted by hydrodistillation in a Clevenger apparatus. The isolated fraction had two distinct layers, an upper oily layer and a lower aqueous layer. The layers were separated, water traces were removed with capillary tubes and anhydrous sodium sulfate (37), and the essential oil was stored in clean glass vials.

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Chemical characterization. The C. martinii essential oil was subjected to chemical analysis on a gas chromatograph–mass spectrophotometer (GC-MS; Shimadzu QP 2000, Kyoto, Japan) at 70 eV and 250°C with a ULBON HR-1 GC column (unless otherwise specified equivalent to an OV-1 fused silica capillary, 0.25 mm by 50 m, with a film thickness of 0.25 μm helium as the carrier gas at 2 ml/min). The compounds were identified by comparing their retention times and mass spectra with authentic standards on the GC-MS system used for analysis. Geraniol was the major component of C. martinii oil.

Fumigation of wheat and gram samples by C. martinii essential oil and geraniol. C. martinii essential oil and purified geraniol (Sigma, Steinheim, Germany) were used to fumigate wheat and gram samples separately by the method adapted by Shaaya et al. (31) and Varma and Dubey (40). Wheat samples (500 g, var. Malviya 234, 11.13% moisture) were kept in closed plastic containers (35 cm diameter by 16 cm). Care was taken to use uninfested freshly harvested wheat samples. Twenty-five individuals of each insect species, i.e. R. dominica, S. oryzae, and T. castaneum of the same age and both sexes were introduced separately in the wheat samples. Cotton swabs soaked in C. martinii oil and geraniol were introduced separately into the plastic containers to produce a concentration of 500 mg/liter. The containers were airtight. Freshly harvested uninfested gram samples (var. PUSA 362, 10.8% moisture) were inoculated similarly with C. martinii oil and geraniol at 500 mg/liter. Insect-inoculated wheat and gram samples without oil or geraniol treatment were used as controls. After 24 months of storage in the laboratory in a temperature-humidity control cabinet (27 ± 2°C and 80 ± 5% relative humidity), the efficacy of C. martinii oil and geraniol for protection of the fumigated wheat and gram from insect damage was determined on the basis of two parameters: grain damage (percentage) and weight loss (percentage) of treated and control samples.

The grain damage was determined by observing feeding injuries and emergence holes on the surface of grain. Infestation samples. The grain damage was determined by observing feeding (percentage) and weight loss (percentage) of treated and control samples was calculated on a fresh weight basis using the formula suggested by Parkin (25):

\[
\text{weight loss (\%)} = \frac{W - W_I}{W_I} \times 100
\]

where \(W_I\) is the weight of the grains before the experiment and \(W\) is the weight of the grains at the end of the experiment.

The feeding deterrent action of the oil treatments as fumigants was observed by calculating the feeding deterrent index (FDI) following the method of Ismam et al. (17):

\[
\text{FDI} = \frac{W - W_I}{W_I} \times 100
\]

where \(W_I\) is the weight of the grains before the experiment and \(W\) is the weight of the grains at the end of the experiment.
TABLE 2. Fumigant efficacy of geraniol as fumigant on stored wheat and gram infested with four insect pests.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight loss (%)</th>
<th>FDI (%)</th>
<th>Grain damage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geraniol</td>
<td>1.76 ± 0.40</td>
<td>11.56</td>
<td>32.05</td>
</tr>
<tr>
<td>Control</td>
<td>1.79 ± 0.06</td>
<td>5.96</td>
<td>1.79</td>
</tr>
</tbody>
</table>

where C is weight loss in the control sample group and T is weight loss in the treated sample group.

Repellent potency of oil against four insect pests of stored products. The essential oil was tested for its insect repellent potency against C. chinensis, S. oryzae, T. castaneum, and R. dominica following the methods of Dubey et al. (12) and Varma and Dubey (38). A Y-shaped olfactometer was used (common arm 24 cm, each branch 14.5 cm). The repellent experiment was carried out at a light intensity of 37.3 Lux and 25°C. Cotton swabs soaked in 0.01, 0.05, and 0.1 ml of the essential oil were kept separately in the experimental arm. The control arm contained a cotton swab soaked in an equal amount of distilled water. The apparatus was then attached to a suction pump to create a vacuum. For each insect species, 30 adult insects of the same age and both sexes were introduced separately into the base of the stem of the Y-arm of the olfactometer. After 30 min, the numbers of insects in the experimental and control arms were counted. The experiment was replicated six times, and percent repellence was calculated following the formula of Gundupurao and Majumdar (16):

\[
% \text{ repellency} = \frac{C - T}{T} \times 100
\]

where C is the number of insects in the control arm, E is the number of insects in the experimental arm, and T is the total number of insects released.

Effect of essential oil on mortality, oviposition, and adult development of bruchids on cow peas. The C. martini essential oil was tested for its in vivo effects on C. chinensis mortality, oviposition, and adult development (8). A stock solution of oil was prepared separately by dissolving 100 μl in acetone. Fifty cow pea seeds were placed in each petri plate (9.0 cm diameter) and were treated separately with different doses of C. martini oil, i.e., 100, 10, 1.0, and 0.1 μl. The seeds were treated by continuous shaking for 5 min for proper coating of the seeds with oil. For control samples, the seeds were dressed in acetone instead of the oil. The treated samples were kept in a temperature-humidity control cabinet (27 ± 2°C and 80 ± 5% relative humidity). After 24 h, five bruchids of the same age and both sexes were introduced into each plate. Requisite control samples were kept for each treatment group. After 24 h, the mortality of insects was observed in each plate, and all insects (live plus dead) were removed. The adults showing any movement of legs and antennae were considered alive. The eggs laid on treated and control seeds were counted 3 days after the introduction of insects. The number of adult insects emerging in cow peas in each treated and control group was counted after various storage periods, i.e., 30, 33, 35, and 38 days.

Effect of C. martini oil on germination and viability of fumigated gram. The effect of the C. martini oil on germination and seedling growth of treated gram samples was determined following the method of Mishra and Dubey (23). Ten gram seeds in each of the fumigated and control groups were placed on Whatman no. 1 moistened filter paper in sterilized petri plates. The germination percentage and growth of seedlings in fumigated and control groups were determined from day 2 to day 5. Three replicates were conducted for each sample group. The number of germinated seeds in each treated and control group was calculated for all the replicates, and the percent germination was determined. The viability of the gram seed was determined based on the tet-
TABLE 3. Insect repellent activity of C. martinii oil extract against four stored grain insect pests

<table>
<thead>
<tr>
<th>Dose of essential oil (ml)</th>
<th>C. chinensis</th>
<th>R. dominica</th>
<th>S. oryzae</th>
<th>T. castaneum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>43.00 ± 5.18 A</td>
<td>16.67 ± 7.18 A</td>
<td>16.10 ± 5.12 A</td>
<td>21.67 ± 2.54 A</td>
</tr>
<tr>
<td>0.05</td>
<td>46.00 ± 7.53 A</td>
<td>22.78 ± 17.48 A</td>
<td>17.20 ± 6.80 A</td>
<td>26.23 ± 2.10 A</td>
</tr>
<tr>
<td>0.1</td>
<td>50.00 ± 5.55 A</td>
<td>26.10 ± 1.05 A</td>
<td>25.10 ± 11.15 A</td>
<td>56.67 ± 5.44 B</td>
</tr>
</tbody>
</table>

*Values are means (n = 6) ± SE. Within each column, means with different letters are significantly different (P < 0.05, LSD).

The essential oil of C. martinii had higher activity as a botanical fumigant for protection of stored grain and wheat than did geraniol, its major component. Thus, the mixture of the components of the C. martinii oil is responsible for its insecticidal activity. These findings are in accordance with those of Tapondjou et al. (36), who found that crude oil extracts from Cupressus sempervirens and Eucalyptus saligna had greater toxic effects against Sitophilus zeamais, and Tribolium confusum than did cymol (the major component), indicating that the crude oils con-
The effect of C. martinii oil on seed germination and seedling growth of fumigated gram sample is shown in Table 5. The oil had a statistically significant effect on seed germination and seedling growth as compared to the control. The oil-treated seeds showed higher germination and seedling growth percentages than the control. The oil was more efficacious for reducing Callosobruchus chinensis infestation in gram than for reducing T. castaneum, S. oryzae, and R. dominica infestation in wheat. Such differential susceptibility of insect pests of stored products to different botanical pesticides has been reported previously (6, 32) and indicates that certain plant-based treatments may be effective against some but not all insect pests. The developmental stage of the particular insect species may be important in determining resistance or susceptibility to chemicals used as pesticides. Newly emerged beetles have been reported to be more susceptible than older beetles (41). In the present study, C. chinensis individuals may have been at a stage at which they were highly susceptible to the C. martinii oil. Thus, a chemical under investigation should be tested on different developmental stages of an insect species.

At different doses, the C. martinii oil had different affects on the behavior of the insect pests. The repellent activity of the oil indicates its possible use as a protective band around bulk grain bins or as part of packaging material to inhibit invasion by pests. The repellent methyl salicylate recently has received regulatory approval in the United States for use in packaging (24).

Because the C. martinii oil was more insecticidal against C. chinensis than the other insects, its effect on oviposition and adult mortality was evaluated. Callosobruchus females generally prefer smooth rather than rough seed varieties for oviposition (15). Therefore, the cow pea was selected for oviposition and adult emergence trails in place of gram. Oviposition inhibitors have the advantage of attacking a pest at the start of its life cycle. When the insect is deterred from laying its eggs on the foodstuff, the pest population cannot increase. In the present study, the emergence of adults in cow pea samples started on day 30 of storage, and maximum emergence was noticed on day 38. Therefore, the effect of C. martinii on adult emergence was recorded from day 30 to day 38 of storage. Not adult emergence was noted for the cow pea samples treated with high doses of oil, even up to day 38 of storage, indicating the highly adverse effect of oil on adult emergence. The reduction in adult emergence could be due to egg or larval mortality or reduced hatching of the eggs. It has been reported that the larvae of Callosobruchus species must penetrate the seed to survive. The larvae are unable to do this unless the egg is firmly attached to the seed surface (14).

In the present study, eggs on the oil-treated seeds were

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% germination</th>
<th>Radicle (mm)</th>
<th>Plumule (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. martinii</td>
<td>3.00 ± 1.54</td>
<td>70.00 ± 10.00</td>
<td>86.67 ± 3.30</td>
</tr>
<tr>
<td>Control</td>
<td>4.16 ± 1.40</td>
<td>96.67 ± 3.30</td>
<td>96.67 ± 3.30</td>
</tr>
</tbody>
</table>

Values are means (n = 3 ± SE). Within each column, means are not significantly different (P > 0.05, Student’s t-test).
loosely attached to the cow pea surface. Thus, the oil may have successfully inhibited larval penetration into the seed.

Various synthetic pesticides have been reported to affect the germination of and seedling growth from treated seeds. However, in the present investigation the C. martinii oil did not adversely affect the viability and germination of the fumigated gram. This property of the oil supports its safety for use in plant protection. A similar observation has been reported for other essential oils that have been used to protecting seeds without having an adverse effect on germination (20, 21, 23).

Semiochemicals are generally considered to be safer and more environmentally acceptable than conventional pesticides (27). Products of some other plants, e.g., Aphanixix polysachyra (35), Caesalia axillaris, Mentha arvensis (38, 40), and Securidaca longependunculata (18), have also shown efficacy as semiochemicals against pests of stored grains.

C. martinii oil can be recommended as a botanical fumigant for the management of insect infestations in stored food commodities. At different doses, it may be used as a botanical insecticide or as a semiochemical against insect pests of stored products.

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