Essential Oils from Six Herbal Plants for Biocontrol of the Maize Weevil

Orapin Kerchoechnue, Natta Laohakunjit, and Sasathorn Singkornard
School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, Bangkok, Thailand 10150

Frank B. Matta
Department of Plant and Soil Sciences, Mississippi State University, 117 Dorman Hall, P.O. Box 9555, Mississippi State, MS 39762

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Abstract. Essential oils from sweet basil (Ocimum basilicum L.), holy basil (O. americanum L.), hairy basil (O. tenuiflorum L.), lime (Citrus aurantiifolia Swingle), kaffir lime (C. hystrix DC.), and clove (Eugenia caryophyllus L.) were isolated by simultaneous distillation extraction. Toxicity assays against the maize weevil (Sitophilus zeamais Motschulsky) using 30 to 120 μL oil extract from the six plants on 70-mm-diameter filter paper discs resulted in 100% weevil mortality within the 5-h assay period. On rice samples, sweet basil oil extract was the most effective, resulting in 96% to 100% mortality regardless of oil extract volume on the first day during the 5-day assay period. Probit analysis showed that sweet basil oil extract applied on grains had four to five times lower LD₅₀ (lethal dose to kill 50% of the weevils) and two to three times lower LD₉₀ values than the remaining oil extracts. Repellency assay using 1 to 8 μL oil extract showed a different trend, because hairy basil was the most effective among treatments. Based on the effective control of the maize weevil by the oil extracted from herbal plants, such oil extracts could be useful in stored agricultural products and plant protection and reduce the risk associated with synthetic insecticides.

The maize weevil (Sitophilus zeamais (Motschulsky) is a major pest of stored rice and other grains (Asawalam et al., 2008b). In addition, the maize weevil is difficult to control, especially because the treated grain is the marketable product and may be contaminated with undesirable pesticides (Sighamony et al., 1990). There is a need for use of botanical pesticides such as plant essential oils, which are environmental friendly (Asawalam et al., 2008a).

Essential oils are naturally occurring substances with a broad spectrum of bioactivity because of the presence of several a.i. (Isman, 2000). Essential oils have recently attracted particular attention as alternative pest control agents because of their specificity of action against insect pests, biodegradable nature, and potential for commercial application (Park et al., 2003). Increases in oil concentration from leaves of Mentha longifolia L. resulted in an increase in maize weevil mortality and high repellency was reported for the oil at all concentrations tested, thus a potential product for protection of stored products against the maize weevil (Odeyemi et al., 2008). The genus Ocimum of the Lamiaceae family is among major plant sources of essential oils and is composed of annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa, and South America (Darragh, 1988). Ocimum gratissimum L. (Lamiaceae) essential oil was found to be moderately repellent to the maize weevil and had a significant reduction in the number of progeny derived from the maize weevil indicating the potential of the plant in postharvest control (Asawalam et al., 2008b). Sweet basil (O. basilicum L.), for example, is a major essential oil crop that possesses a range of biological activities such as insect repellent, nematocidal, antibacterial, antifungal, and antioxi dant activities (Deshpande and Tipnis, 1997; Lee et al., 2005; Simon et al., 1990). Holy basil is widely known for its health-promoting, medicinal value and as an insect repellent (ORGANICINDIA, 2009). Different types of chemical constituents in essential oils or chemotypes have been obtained from basils depending on the cultivar and growing conditions (Lachowicz et al., 1997; Marotti et al., 1996). Thailand, Madagascar, and Vietnam basils contain high concentrations of methyl chavicol (Simon et al., 1990; Vernin et al., 1984). Other chemotypes characterized in other Ocimum species and varieties include β-caryophyllene in hairy basil or O. tenuiflorum L. (Simon et al., 1990) and cirtal in O. citrorum Visc. (Grayer et al., 1996; Morales et al., 1993; Simon et al., 1990). The toxicity of essential oils often varies as a result of differing modes of action of the chemical constituents and sensitivity of insect pests (Isman, 2000). Pulverized leaves of Chromolaena odorata, a tropical weed, and Citrus limon, a fruit crop, were found to be efficacious in the control of the maize weevil. However, the greenish brown color of C. odorata leaf powder rendered the stored grain unattractive (Mbah and Okoronkwo, 2008). Insecticidal activity of extracts prepared from leaves of plants in Anacardiaceae, Arecaceae, and Amaranthaceae on the maize weevil were evaluated. The insecticidal effect ranged from 20% to 60% by the fifth day depending on the plant extracted (Souza et al., 2008). Various combinations of Niger seed oil and 5% malathion dust provided complete protection to maize seed from the maize weevil up to 90 d after infection (Yuya et al., 2009). Results showed that extracts of alligator pepper and ginger were repellent toward the adult maize weevil, thus demonstrating the potential for their use in stored product protection (Ukeh et al., 2009). Plants is grown in Thailand, which are known to have high levels of essential oils, remain to be exploited for effective use in controlling insect pests of stored agricultural products. Such plants are found in genus Ocimum, Citrus, and Eugenia and, thus, chosen for this study. This study was conducted to 1) characterize essential oil crops from the two common species of Ocimum: sweet basil (O. basilicum L.), holy basil (O. americanum L.), and hairy basil (O. tenuiflorum L.), two species of Rutaceae: Kaffir lime (Citrus hystrix DC), and lime (C. aurantiifolia Swingle), and clove (Eugenia caryophyllus L.); and 2) determine the effectiveness of the extracted essential oils in controlling the maize weevil (Sitophilus zeamais Motschulsky) that infests milled rice during storage.

Materials and Methods

Extraction and characterization of essential oils. Mature leaves of sweet basil, holy basil, hairy basil, and lime; peel of mature fruit of kaffir lime; and flower buds of clove were used. Sweet basil, holy basil, lime, and kaffir lime were grown organically in orchards located in Rajburi Province, Thailand. Mature leaves of basil (30 d after planting) and lime (45 d after shoot emergence) and mature fruit of kaffir lime (4 months after flowering) were collected at marketable stage of maturity. Flower buds of clove were purchased from a local market in Bangkok. All plant samples were harvested and prepared July 2007. A sample (1 kg) of each plant material was washed, air-dried, and sliced into small pieces before subjecting to hydrodistillation using the modified Clevenger apparatus for simultaneous distillation extraction (SDE). Samples were mixed with 500 mL water and filtered. The volume of the filtrate was adjusted to 7 L in 10-L round-bottom flasks attached to the modified Likens and Nickerson steam distillation continuous extraction head of the SDE apparatus. The solvent, 100 mL...
99% hexane (QReC; Bharat Petroleum Corp, India), was used and contained in a 250-mL flask, placed in a 50 °C water bath, and attached to the other side arm of the head of the SDE apparatus. The extraction process was carried out under normal atmospheric pressure for 24 h. The solvent was separated from the aqueous layer with a rotary evaporator (Rotavapor R-200; Vacuum Controller V-800; Buchi, Switzerland) under reduced pressure of 335 psi at 50 °C. The extracted oils were saved in a 30-mL vial and placed in a refrigerator at 10 °C until used.

The oil extract was weighed and expressed in percentage dry basis (w/w). The refractive index was measured using a handheld refractometer (Atago PAL-1, Tokyo, Japan). For determination of chemical composition, oil samples were dehydrated with anhydrous sodium sulfate and stored at 0 °C in airtight containers. The first harvest oil samples of the six replications per treatment were pooled for analysis by gas chromatography–mass spectrophotometry using Shimadzu GC 17A (Shimadzu, Japan) coupled to a mass spectrophotometer (Focus™; PolarisQ Plus; Thermo Finnigan Corporation, San Jose, CA). Volatile compounds were separated in a DB-5 column (J&W Scientific, Folsom, CA) fused with silica capillary column with 30 m × 0.25-mm internal diameter and 0.25-µm film thickness. The gas chromatography injector was in a split mode (split ratio of 1:20 v/v). The injector and detector temperatures were 230 °C. The column temperature was set at 40 °C for 5 min, programmed to increase to 250 °C at 3 °C/min, and then held at 250 °C for 5 min. All mass spectra were acquired in electron impact and chemical ionization modes with ionization voltage of 70 eV and mass range of 40 to 350 m/z. Identification of chemical compounds was carried out by comparison with the mass spectra of standard compounds reported in the US National Institute of Standards and Technology. Linear retention indices were calculated after analysis under the same conditions of an alkane series (C₈-C₃₂).

**Insect culture.** Cultures of maize weevil were maintained in a controlled temperature–humidity chamber (28 ± 2 °C and 70% ± 5% relative humidity) with light and dark duration of 12 h each. Adults were obtained from stock cultures at the Postharvest and Processed Agricultural Products Laboratory, Department of Agriculture, Bangkok, Thailand. Whole rice grains were used as food media for the insects.

**Toxicity/mortality assay.** The toxicity of the oil extract from the six plants to the maize weevil was assayed following the method of Tapondjou et al. (2005) with modifications and was performed under conditions with and without whole rice grains. In the toxicity assay without grains, the oil extract was applied on filter paper discs (Whatman No.1, 70-mm diameter, surface area of 38.5 cm²) and placed in glass petri dishes (7-cm diameter). Different volumes of the oil extract were used: 30, 60, 90, and 120 µL diluted in 99.8% acetone equivalent to 0.78, 1.56, 2.34, and 3.12 µL cm⁻² filter paper, respectively. Acetone solution alone served as the control. The acetone was allowed to evaporate for 10 min before the introduction of 20 unsexed adults (5 to 9 d old) into each dish. All treatments were replicated four times. Mortality (number of dead weevils from a total of 20 live weevils per dish) was recorded hourly for 5 h and expressed as percentages.

In the toxicity assay with rice grains, 40 g were placed in a glass bottle (150 mL) and mixed with 30, 60, 90, or 120 µL of the extracted essential oils in 1 mL of 99.8% acetone equivalent to 0.75, 1.50, 2.25, and 3.00 mg kg⁻¹, respectively. Acetone alone served as a control. The solution was mixed and stirred continuously with the grains to evenly spread it over the surface. The acetone was allowed to evaporate for 10 min before the introduction of 20 adult weevils (5 to 9 d old) into each bottle. All treatments were replicated four times. Mortality (number of dead weevils from a total of 20 live weevils per bottle) was recorded daily for 5 d and expressed in as percentages.

Probit analysis was performed to calculate the lethal dose for 50% (LD₅₀) and 99% (LD₉₉) insect mortality or LD₅₀ and LD₉₉, respectively.

Table 1. Yield and refractive index of essential oils extracted from six plant sources.

| Plant source | Yield (% dry basis: w/w) | Refractive index (°Brix) |
|--------------|--------------------------|-------------------------|
| Sweet basil leaves | 0.09 | 1.5 |
| Holy basil leaves (Ocimum basilicum) | 0.04 | 1.5 |
| Hairy basil leaves (O. americanum) | 0.05 | 1.5 |
| Kaffir lime fruit peel (Citrus hystrix) | 1.71 | 1.5 |
| Lime leaves (C. aurantiifolia) | 0.07 | 1.5 |
| Clove flower bud (Eugenia caryophyllus) | 1.28 | 1.5 |

Table 2. Retention index and percent chemical composition of essential oils extracted from six plant sources and retention index based on standard synthetic compounds.

| Compound | Retention index | Sweet basil | Holy basil | Hairy basil | Kaffir lime | Lime | Clove |
|----------|----------------|-------------|------------|-------------|-------------|------|-------|
| α-pinene | 936            | —           | —          | —           | —           | —    | —     |
| β-pinene | 978            | —           | —          | —           | —           | —    | —     |
| 1,8-cineole | 1024        | 1.3         | —          | —           | —           | —    | —     |
| Limonene | 1025           | —           | —          | —           | —           | —    | —     |
| Ocimene  | 1029           | 2.5         | —          | —           | —           | —    | —     |
| cis-ocimene | 1041       | —           | —          | —           | —           | —    | —     |
| β-terpinene | 1049        | —           | —          | —           | —           | —    | —     |
| d-cembrene | 1069         | —           | —          | —           | —           | —    | —     |
| cis-linalooloxide | 1072 | —           | —          | —           | —           | —    | —     |
| 6-camphenene | 1082         | —           | —          | —           | —           | —    | —     |
| α-terpinolene | 1082      | —           | —          | —           | —           | —    | —     |
| α-campholenol | 1105        | —           | —          | —           | —           | —    | —     |
| β-citronellol | 1129       | —           | —          | —           | —           | —    | —     |
| Isoeugenol | 1130          | —           | —          | —           | —           | —    | —     |
| cis-verbeneol | 1132         | —           | —          | —           | —           | —    | —     |
| methyl salicylate | 1171       | —           | —          | —           | —           | —    | —     |
| Verbeneol | 1183          | —           | —          | —           | —           | —    | —     |
| α-terpeneol | 1188          | —           | —          | —           | —           | —    | —     |
| cis-β-citral (nerol) | 1210     | —           | —          | —           | —           | —    | —     |
| Chavicol | 1219          | —           | —          | —           | —           | —    | —     |
| cis-α-citral (geranial) | 1244       | 6.8         | —          | —           | —           | —    | —     |
| methyl chavicol | 1331       | 86.3        | —          | —           | —           | —    | —     |
| Eugenol | 1355          | 23.9        | —          | —           | —           | —    | —     |
| α-cubebene | 1355         | 12.5        | —          | —           | —           | —    | —     |
| methyleugenol | 1369        | 53.9        | —          | —           | —           | —    | —     |
| β-elemene | 1389          | 1.9         | —          | —           | —           | —    | —     |
| β-caryophyllene | 1421    | 17.7        | 10.8       | 10.4        | 25.8        | —    | —     |
| trans-α-bergamotene | 1434     | 5.9         | 3.7        | 0.8         | —           | —    | —     |
| α-caryophyllene | 1455      | —           | 1.2        | 3.6         | 0.2         | 1.3  | 3.4   |
| α-amorphone | 1477          | —           | —          | —           | —           | —    | —     |
| β-chamigene | 1483          | 2.2         | —          | —           | —           | —    | —     |
| Valencene | 1483          | —           | —          | —           | —           | —    | —     |
| eremophilene | 1486         | —           | —          | —           | —           | —    | —     |
| α-farnesene | 1498         | —           | —          | —           | —           | —    | —     |
| δ-cadinene | 1507          | 1.2         | 1.2        | —           | —           | —    | —     |
| α-bisabolene | 1530        | —           | 10.2       | —           | 2.5         | —    | —     |
| eugenol acetate | 1532       | —           | —          | —           | —           | —    | —     |
| Caryophyllene oxide | 1578      | 1.3         | —          | —           | —           | —    | —     |

Column on DB-5 column. **—** indicates no compound detected.
respectively (Finney, 1971). Probit-transformed percentage insect mortality was regressed on log doses and the regression equation was noted.

**Repellency assay.** The repellency test of McDonald et al. (1970) was adapted for this assay. Each solution was prepared by diluting 1, 2, 4, and 8 μL of the oil extract in 99.8% acetone equivalent to 0.05, 0.10, 0.21, and 0.42 μL·cm⁻² filter paper, respectively, when treated to half of a filter paper disc (Whatman No.1, 70-mm diameter, surface area of 38.5 cm²). The filter paper discs were cut into two halves, the first half treated with the oil extract solution and the other half with acetone as control. The treated and control disc halves were then attached with a clear adhesive tape and placed in a petri dish (7-cm diameter). The acetone was allowed to evaporate for 10 min before the introduction of 20 unsexed adult maize weevils (Tn) at the middle of the filter paper disc and then the dish was sealed. All treatments were replicated four times. The number of weevils present at the control half (Nc) and treated half (Nt) of the filter paper disc was recorded after 2 h. Percentage repellency (PR) was calculated by the formula: PR = [(Nc – Nt)/Tn] 100. Mean PR values were classified using the method of Juliana and Su (1983).

**Results and Discussion**

**Characteristics of extracted essential oils.** Yield of essential oils was lower in leaf sources (sweet basil, holy basil, hairy basil, and lime) and ranged from 0.04% to 0.09 compared with the flower buds of clove with 1.28% and kaffir lime fruit peel with 1.71% (Table 1). However, the refractive index of the oil extracts from the various plant sources did not vary and was 1.5° Brix. There was no direct relationship between essential oil yield and refractive index (Table 1).

Oil extracts from leaves of sweet and holy basil and clove flower buds had less diverse

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Fig. 1. Percent mortality of maize weevil during the 5-h toxicity assay on filter paper (without rice grains) using 0 to 120 μL of essential oils extracted from sweet basil (A), holy basil (B), hairy basil (C), kaffir lime (D), lime (E), and clove (F). (Volumes of 0, 30, 60, 90, and 120 μL oil extract are equivalent to 0, 0.78, 1.56, 2.34, and 3.12 μL oil extract per cm² filter paper, respectively.)
chemical composition (six to seven compounds) than hairy basil and lime leaves (15 to 16 compounds) (Table 2). Oil extract from kaffir lime fruit peel had an intermediate number of chemical constituents (10 compounds). Chemical compounds present in highest quantity in the oil extract were methyl chavicol in sweet basil (86.3%), methyl eugenol in holy basil (53.9%), α-cubebene in hairy basil (12.5%), eugenol in clove (62.1%), and limonene in kaffir lime (38.6%) and lime (48.6%). β-caryophyllene was present in moderate amounts (greater than 10%) in holy basil, hairy basil, lime, and clove. A moderate amount of cis-α-citral (11.3%) was obtained in lime and α-terpineol (11.7%) in kaffir lime. Kaffir lime had 30.5% β-pinene and 38.6% limonene. Hairy basil oil extract had no dominant compound; however, α-cubebene, β-caryophyllene, cis-β-citral, and α-bisabolene were present at 12.5%, 10.8%, 12.3%, and 10.2%, respectively (Table 2).

In sweet basil, methyl chavicol was the most abundant compound as found by Vernin et al. (1984). However, in other studies, linalool and 1,8-cineole were found as the major components in sweet basil (Lachowicz et al., 1997; Marotti et al., 1996; Simon et al., 1990). In this study, linalool was not found in our sampling of sweet basil. Again, this discrepancy might be the result of a difference in extraction technique, variety of sweet basil, and difference in cultural practices. In hairy basil, Djibo et al. (2004) showed that 1,8-cineole comprised ≈45% to 60% of the oil extract, but this compound was not among the 16 compounds obtained from hairy basil in the present study. This discrepancy may be the result of a different technique for extraction. Four compounds (α-cubebene, cis-β-citral, β-caryophyllene, and α-bisabolene) accounted for ≈45% of the oil extract of hairy basil.

Fig. 2. Percent mortality of maize weevil during the 5-d toxicity assay on rice grains using 0 to 3.00 mL kg⁻¹ grains of extracted essential oils from sweet basil (A), holy basil (B), hairy basil (C), kaffir lime (D), lime (E), and clove (F). (Concentrations of 0, 30, 60, 90, and 120 μL oil extract are equivalent to 0.0, 0.75, 1.50, 2.25, and 3.00 mg kg⁻¹, respectively).
basil in this study. On the other hand, results with holy basil were consistent with the findings of Simon et al. (1990) and Kothari et al. (2004), whereas results with clove concurred with the findings of Sefidkon and Jamzad (2005) and Omidbeigi et al. (2007).

Toxicity (mortality) of essential oils. Percent mortality of the maize weevil after 1 h inside the petri dish increased with greater volumes of all plant oil extracts impregnated onto the filter paper, except for clove oil extract, which was effective at all volumes (30 to 120 μL), killing 96% to 100% of the weevils within 1 h (Fig. 1). Mortality increased over time until all the weevils were killed, which was reached at different time periods depending on the plant source and volume of the oil extract. Clove oil extract appeared to be the most toxic, because at the lowest volume of 30 μL, it took only 2 h to obtain 100% mortality, whereas the extracts from the other five plant sources took 3 to 5 h. Kaffir lime and lime oil extract volumes of 90 to 120 μL and sweet basil at 120 μL killed all the insects in 1 h. In the control (acetonitrile alone), all weevils were still alive at the end of the 5-h assay period.

The toxicity assay of oil extracts applied on rice grains indicated a volume effect was manifested, i.e., weevil mortality increased with greater volumes of the oil extract (Fig. 2). Sweet basil oil extract seemed to be the most effective at all volumes used after 1-d posttreatment. At the lowest volume (30 μL oil extract), sweet basil caused >80% mortality, whereas at the higher concentration (120 μL oil extract), it caused 98% to 100% mortality in 1 d. The oil extracts from the other five plant sources did not cause weevil mortality after 1 d when applied on rice grains at 30 μL oil extract.

In comparing the different assays at volumes of 30 to 90 μL of oil extract, weevil mortality correspondingly increased but was lower than the effects of these oil extracts when applied on filter paper. In addition, volumes lower than 90 μL of holy basil, hairy basil, kaffir lime, lime, and clove oil extracts did not cause 100% weevil mortality after the 5-d assay period, unlike the toxicity assays without grains (i.e., on filter paper) in which all volumes of the essential oils resulted in 100% mortality within 5 h. In the control, no weevil mortality was noted after 5 d.

As indicated by the regression equations, the response (percentage mortality) was positive and linear where mortality increased with increased volume of the essential oil regardless of the assay (Table 3). Without rice grains, the LD₉₀ values for sweet basil, holy basil, and clove did not differ but were lower compared with the LD₉₀ values of hairy basil, lime, and Kaffir lime. Kaffir lime had the highest LD₉₀ value, indicating that it was the least effective. The LD₉₀ values for sweet basil, holy basil, hairy basil, and clove did not differ but were lower compared with LD₉₀ values of kaffir lime and lime. LD₉₀ values did not differ between kaffir lime and lime and were least effective. When applied on rice grains, sweet basil oil extract had the lowest LD₉₀ (0.43 mL kg⁻¹ grains) and lowest LD₉₀ (1.52 mL kg⁻¹ grains). Oil extracts from the other five plant sources were four to five times higher for the LD₉₀ and two to three times higher for the LD₉₀ values compared with sweet basil oil extract. The least effective extracts were hairy basil and lime (Table 3).

Previous studies obtained differences in effectiveness of oil extracts from different plant sources. Tapondjou et al. (2005) showed that Eucalyptus oil was more toxic to maize weevil than Cupressus oil. Several factors may account for the differences in toxicity of plant oil extracts such as chemical composition of the essential oils and insect susceptibility (Casida, 1990). In the present study, variations in toxicity to maize weevil of the six plant oil extracts may indicate differing modes of action. Under similar conditions when the oil extract was applied on rice grains, methyl chavicol, which is comprised of more than 80% sweet basil oil extract, seemed to be the most toxic. Eugenol (=62% in clove), methyl eugenol (=54% in holy basil), and linalone (=26% to 48% in kaffir lime and lime) were present in lower quantities than methyl chavicol in sweet basil, which may account for the lower effectiveness of the oil extracts in killing the weevil. It was noted that the oil extracts of clove, holy basil, kaffir lime, and lime required higher levels of 2.25 to 3.00 mL kg⁻¹ grains to cause weevil mortality comparable to that of sweet basil oil extract at 0.75 to 1.50 mL kg⁻¹ grains. Furthermore, it has been reported that other chemical constituents may contribute to the overall toxic effect of oil extracts (Prates et al., 1998). Obeng-Ofori et al. (1997) demonstrated that other constituents of essential oils such as 1,8-cineole, terpinol, and α-pinene can exert a toxic effect on the maize weevil. Ojimelukwe and Adler (1999) also found that α-pinene and terpinol produced a potent toxic effect on rice flour beetle (Tricholobus confusum). When present in an oil extract, these compounds may act in concert and cause higher toxicity to insect pests. Pinenes (α and β types) and α-terpineol were detected from kaffir lime oil extract at relatively higher concentrations (4.5% to 30.5%) than that of 1,8-cineole in sweet basil oil extract (1.3%) (Table 2). Hairy basil and lime oil extracts had 15 to 16 chemical constituents but were less effective than the other plant sources in the toxicity assay on rice grains, implying that the more diverse the chemical composition of an oil extract, the less toxic it would be against the maize weevil. These results suggest that the other compounds may exert a depressing effect on the a.i. (s) in the oil extract. The major component of X. aeriopica was found to be largely responsible for the toxic action of essential oil against the maize weevil and the toxic action may have been imparted by the result of the combined effects of its constituents (Asawalam et al., 2008a). The mechanism underlying this possible interaction could be understood by elucidating the toxic effect of each chemical compound in the oil extract by applying them singularly or in combination on the weevil.

Nevertheless, the results show the potential of essential oils from the six plant sources in controlling the maize weevil, with sweet basil oil extract having the greatest potential. Extensive rice storage trials are needed to establish the practicality and effectiveness of this technique in using oil extracts against the weevil. It is noteworthy that volatile oils are not particularly dangerous to consumers because they are removed during washing and easily evaporate during cooking (Bauer et al., 1990; Tapondjou et al., 2005).

Repellency of essential oils. Repellent action of the oil extracts increased as essential oil volume, except for hairy basil oil extract, which was totally repellent at all volumes (Table 4). Oil extracts from sweet basil and lime at 4 to 8 μL and clove and holy basil at 8 μL were also rated to have the highest repellency (Class V) PR. Holy basil oil extract at the lowest volume of 1 μL did not exhibit any repellent action. At volumes from 2 to 8 μL, holy basil was the least effective among the six oil extracts.

The response of maize weevil to hairy basil oil extract may illustrate that oil extract, which is the most effective as a repellent, may not necessarily be the most effective as a toxicant. Hairy basil oil extract was the most effective repellent, but as a toxicant, it was the least effective among the six oil extracts. 

Table 3. LD₉₀ and LD₉₀ calculated by Probit analysis of essential oils extracted from six plant sources against the maize weevil in toxicity assays on filter paper and on rice grains.

| Plant source of essential oils | Regression equation | LD₉₀ (μL cm⁻²) | LD₉₀ (μL cm⁻²) |
|-------------------------------|---------------------|----------------|----------------|
| Sweet basil, O. cinnamomeum   | y = 0.0949x + 1.11  | 0.20 a         | 0.3 a          |
| Holy basil, O. americanum     | y = 0.0982x - 1.13  | 0.21 a         | 0.3 a          |
| Hairy basil, O. tenuiflorum   | y = 0.0577x - 0.87  | 0.26 b         | 0.35 a         |
| Kaffir lime, Citrus hystrix   | y = 0.193x - 1.38   | 0.40 c         | 1.08 b         |
| Lime, C. austrofyllata       | y = 0.234x + 1.71   | 0.29 b         | 1.00 b         |
| Clove, Eugenia caryophyllus  | y = 0.078x - 1.03   | 0.22 a         | 0.34 a         |

Means in columns separated by Duncan’s multiple range test (P ≤ 0.05).

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Table 4. Percent repellency of essential oils extracted from six plant sources against the maize weevil.

| Plant source of essential oils | Volume (µL) | Repellency (%) | Repellency class |
|-------------------------------|-------------|----------------|-----------------|
| Sweet basil, *Ocimum basilicum* | 0           | 0              | 0               |
|                              | 1           | 62.5           | IV              |
|                              | 2           | 72.5           | IV              |
|                              | 4           | 92.5           | V               |
|                              | 8           | 100.0          | V               |
| Holy basil, *O. americanum*  | 0           | 0              | 0               |
|                              | 1           | 52.5           | III             |
|                              | 2           | 75.0           | IV              |
|                              | 4           | 85.0           | V               |
| Hairy basil, *O. tenuiflorum* | 0           | 0              | 0               |
|                              | 1           | 100.0          | V               |
|                              | 2           | 100.0          | V               |
|                              | 4           | 100.0          | V               |
|                              | 8           | 100.0          | V               |
| Kaffir lime, *Citrus hystrix* | 0           | 0              | 0               |
|                              | 1           | 55.0           | III             |
|                              | 2           | 50.0           | III             |
|                              | 4           | 67.5           | IV              |
|                              | 8           | 65.0           | IV              |
| Lime, *C. aurantiifolia*     | 0           | 0              | 0               |
|                              | 1           | 65.0           | IV              |
|                              | 2           | 77.5           | IV              |
|                              | 4           | 90.0           | V               |
|                              | 8           | 91.25          | V               |
| Clove, *Eugenia caryophyllus* | 0           | 0              | 0               |
|                              | 1           | 25.0           | II              |
|                              | 2           | 75.0           | IV              |
|                              | 4           | 80.0           | V               |
|                              | 8           | 85.0           | V               |

Volumes are equivalent to 0.05, 0.10, 0.21, and 0.42 µL oil extract per cm² filter paper, respectively.

Repellency class: 0 = less than 0.1 PR, 1 = 0.1 to 20 PR, 2 = 20.1 to 40 PR, III = 41.0 to 60 PR, IV = 61.0 to 80 PR, and V = 80.1% to 100 repellency (Juliana and Su, 1983).

Sweet basil oil extract was the most effective toxicant, but as a repellent, it was less effective than hairy basil. These responses may again be attributed to differences in chemical composition of oil extracts and the modes of action of these chemical constituents, particularly as an insect repellent (Casida, 1990). However, these findings show that at appropriate rates of application, the essential oils from the six plant sources could exhibit both repellent and toxic actions against the maize weevil. Maize weevil control with essential oils may also be possible on a larger scale by applying to field-grown crops. The effectiveness period for weevil control by such chemicals must be determined.

**Conclusion**

A good level of control of the test insects (maize weevil, *Sitophilus zeamais*) was achieved with essential oils of sweet basil (*O. basilicum*), holy basil (*O. americanum*), hairy basil (*O. tenuiflorum*), clove (*S. aromaticum*), lime (*C. aurantiifolia*), and kaffir lime (*C. hystrix*). This demonstrates a scientific rationale for the incorporation of these herbal plant extracts into grain protection practices or in organic agricultural products. Sweet basil oil induced a relatively high mortality rate (greater than 90%) at a low volume of 30 µL against the maize weevil on 40 g of rice grain the first day of exposure and also completely suppressed insect progeny production thereafter. Hairy basil oil resulted in complete insect repellency of maize weevil. Overall, essential oils extracted from herbal plants could play an important role in plant protection and reduce the risk associated with the use of synthetic insecticides.

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