Inhibitory Effects of Thai Essential Oils on Potentially Aflatoxigenic Aspergillus parasiticus and Aspergillus flavus

KITTIFA JANTAPAN1, AMNART POAPOLATHEP1, KANJANA IMSILP1, SARANYA POAPOLATHEP1, PHANWIMOL TANHAN1, SUSUMU KUMAGAI2, AND USUMA JERMNAK1

1Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
2Food safety Commission, Minato-ku, Tokyo 107-6122, Japan

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The antiaflatoxigenic and antifungal activities of essential oils (EOs) of finger root (Boesenbergia rotunda (L.) Mansf.), pine (Pinus pinaster), rosewood (Aniba rosaedora), Siam benzoin (Moringa oleifera), and ylang ylang (Cananga odorata) were tested for Aspergillus parasiticus and Aspergillus flavus in potato dextrose broth. Aflatoxin B1 (AFB1) was extracted from culture using a QuEChERS-based extraction procedure and analyzed with high performance liquid chromatography (HPLC) coupled to a fluorescence detector. EO of pine showed the greatest inhibition of growth and AFB1 production of A. parasiticus, followed by EOs of rosewood, finger root, Siam benzoin, and ylang ylang. EO of finger root gave the best inhibitory effects on A. flavus, followed by EOs of rosewood, pine, ylang ylang, and Siam benzoin. EO of Thai moringa did not show any significant inhibition of aflatoxigenic fungi. The antiaflatoxigenic activities of EOs correlated with their antifungal activities in the dose-dependent manner. Comparison of the application of the five selected EOs in peanut pods by direct and vapor exposure indicated that the AFB1 production inhibitory effects of the five EOs by direct exposure were faster and more effective than by vapor exposure. EO of finger root showed the best inhibition of AFB1 production of A. flavus in peanut pods by direct exposure, followed by EOs of pine, rosewood, ylang ylang, and Siam benzoin.

Key words : Essential oils / Aflatoxin B1 production / Fungal growth / Inhibition.

INTRODUCTION

Aflatoxins are the most harmful mycotoxins produced by more than half of the naturally occurring strains of A. parasiticus and A. flavus which are able to contaminate many kinds of food including cereals, fruit, and vegetables (Tian et al., 2013). The Food and Agriculture Organization has estimated that every year a significant percentage of the world’s grain crops are contaminated with hazardous aflatoxins leading to an annual loss around one billion tons (FAO, 2009). Aflatoxin contamination in crops can occur in the field, during storage, as well as in transport. Even though many rapid technological advances during the last few decades have been developed in food production systems in order to obtain healthy, nutritious and technologically safe products, the occurrence of fungi and aflatoxin contamination in food products is not negligible (Kocić-Tanackov and Dimić, 2013). Therefore, many strategies have been developed to prevent fungal growth, subsequent aflatoxin production, and food contamination, including chemical, physical, or biological treatments (Reddy et al., 2010). Nowadays, the use of synthetic compounds for the control of these fungi has raised concerns about the environmental impact and adverse health effects related to their use (Yazdani et al., 2011). The use of natural plant extracts such as EOs provides an opportunity to avoid chemical residue in the environment. The complex substances of EOs have been reported to exhibit broad spectrum antimicrobial activity (Hammer et al., 1999; Haleem Khan and Naseem, 2011). EOs are capable of
reaching pathogenic fungi through the liquid and the gas phase. This bioactivity in the vapor phase of EOs allows their use as possible fumigants for stored commodity during storage (Du et al., 2011). Many EOs have been reported as effective inhibitors of fungal growth and aflatoxin production (Bluma et al., 2008; Bassolé and Juliani, 2012; Jermnak et al., 2012). Thus, a considerable attention has developed in the preservation of grains by the use of EOs as safer and more effective substitutes than synthetic antimicrobial agents and fungicides (Gömöri et al., 2013; Yahyaraeyat et al., 2013). The present study was designed to evaluate the inhibitory effects of six EOs found commonly in Thailand on aflatoxigenic fungi and the application of suitable EOs by direct and vapor exposure on stored peanut pods for controlling aflatoxin production.

MATERIALS AND METHODS

Fungal strains and culture conditions

*Aspergillus parasiticus* NRRL 2999 and *A. flavus* 3041 were obtained from the Postharvest Technology Research Group, Postharvest and Processing Research and Development Office, Department of Agriculture (Bangkok, Thailand) and were used as producers of AFB1. Both strains were maintained on potato dextrose agar (PDA) (Difco, MD, U.S.A) medium. A spore suspension was prepared from a two-week-old culture at a concentration of 1.2x10⁶ CFU/ml and was used as the inoculum.

Reagents and chemicals

Six commercial EOs (100% pure EO) including finger root (*Boesenbergia rotunda* (L.) Mansf.), pine (*Pinus pinaster*), rosewood (*Aniba roseodora*), Siam benzoin (*Styrax tonkinensis*), Thai moringa (*Moringa oleifera*), and ylang ylang (*Cananga odorata*) were purchased from Royal Lotus Co., Ltd. (Bangkok, Thailand) and Kasetsart University Research and Development Institute (Bangkok, Thailand). All EOs were prepared using steam distillation of the leaves, the flowers, the roots, or bark of the plants, except for Thai moringa which cold-pressing from moringa seed was used. AFB1, standard was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A). Standard stock solutions were prepared in methanol. Acetic acid, sodium sulphate anhydrous and sodium acetate anhydrous were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A). HPLC grade methanol and acetonitrile were purchased from RCI Labscan Co., Ltd. (Bangkok, Thailand). Analytical grade mineral oil was purchased from Carlo Erba Reagents (Peypin, France).

**Antiaflatoxigenic and antifungal activities of EOs in liquid medium**

Potato dextrose (Difco, MD, U.S.A) liquid medium was added to 24-well microplate at 1 ml per well. Test EOs were dissolved in methanol at the concentrations of 0, 1, 2, and 4 mg and added to the wells (final concentration of methanol was 0.1%, v/v). A spore suspension (40 µl) was inoculated into the medium and incubated statically at 30°C for 3 d. All concentrations of each EO (0, 1, 2, and 4 mg/mL) were done the assay in the same plate. Each treatment was replicated four times. After cultivation of the strain NRRL 2999 or 3041 for 3 d, the culture broth of each well was filtered through cotton gauze to obtain the mycelia and culture filtrate. The obtained mycelia were washed with 5 ml of distilled water and collected into a 1.5 ml microtube. Mycelia were dried at 80°C for 3 h and subsequently weighted. For the extraction of AFB1 in the culture filtrate, the filtrate (0.5 ml) was collected in a 5 ml plastic tube. Then, the QuEChERS extraction procedure (Frenich et al., 2011) was applied; 1.5 ml of methanol/water solution (80/20, v/v) with 1% acetic acid, 0.3 g of sodium acetate anhydrous and 2 g of sodium sulphate anhydrous were added and the mixture was vortexed for 2 min. Then, the tube was put into a rack in a rotator shaker for 30 min at 50 ×g. After centrifugation at 3,500 × g for 15 min, 1 ml of the supernatant layer was taken and filtered through a 0.22 µm PTFE syringe filter (Shanghai, China), before the sample extract was subjected to HPLC analysis using a 2695 HPLC system (Agilent Technologies, Palo Alto, CA, U.S.A). The separation was performed on a Zorbax eclipse plus C18 column, 5 µm, 150 mm×4.6 mm with a water/methanol/acetonitrile (50/40/10) mobile phase. The flow rate was 1 ml/min and the retention time of AFB1 was 5.9 min. A fluorescence detector (Agilent Technologies, Palo Alto, CA, U.S.A) was used, with the wavelengths of excitation and detection being 362 nm and 455 nm, respectively.

**Antiaflatoxigenic activity of selected EOs in peanut pod**

The experiment was divided into two procedures using either a dipping or a volatile method. Test EOs were diluted in mineral oil at the concentrations of 0, 4, 8, and 16% (v/v) for the dipping (direct exposure) and volatile (vapor exposure) method. In-shell peanuts for human consumption were purchased from a local farmer in Kanchanaburi province, Thailand. The weight of each peanut was around 1.5 g. The raw peanuts were autoclaved at 120°C for 15 min. Each concentration of each EO (0, 4, 8, and 16%). was completely separated into the different Petri dish for doing the assay in the same time. In the dipping method, each of the autoclaved peanuts was dipped in one of the sample solu-
sections (control 1: mineral oil treatment; treatment 2 to 4: 4, 8, or 16% (v/v) of EO). Each peanut was transferred into a Petri dish and inoculated with a spore suspension of A. flavus 3041 (50 µl/pod). Each Petri dish was sealed with a single layer of parafilm. In the volatile method, a 2 x 2 x 2 cm cotton ball was used as a reservoir for each EO. Samples of 1 ml of mineral oil, containing 0, 4, 8, or 16% (v/v) of EO, were added into the cotton ball. A single reservoir was placed in the Petri dish containing four autoclaved peanut pods and inoculated with the spore suspension of A. flavus 3041 (50 µl/pod). Each Petri dish was sealed with a single layer of parafilm immediately following delivery of EO to the reservoir. All Petri dishes were statically incubated at 30°C for 10 d. Each treatment was replicated four times. For the extraction of AFB1 in the peanut kernels, each peanut pod was shelled manually and ground using mortar and pestle to obtain a finely ground peanut sample. Five g of the finely ground peanut sample from each Petri dish was collected into the 50 ml plastic tube. Then, 10 ml of methanol/water solution (80/20, v/v) with 1% acetic acid, 1 g of sodium acetate anhydrous and 4 g of sodium sulphate anhydrous were added and the mixture was vortexed for 2 min. The tube was put into a rack in the rotator shaker at 50 °C for 30 min. After centrifugation at 3,500 x g for 25 min, 1 ml of the supernatant layer was taken and filtered through a 0.22 µm PTFE syringe filter, before the sample extract was subjected to HPLC analysis using the 2695 HPLC system. The separation was performed on the Zorbax eclipse plus C18 column, 5 µm, 150 mm x 4.6 mm with gradient of 0-80% in the acetonitrile mobile phase. The mobile phase was (A) water and (B) acetonitrile. The gradient conditions were 0-2 min 20%B, 2-5 min 20-40%B, 5-8 min 40%B, 8-12 min 40-80%B, and 12-16 min 80% B. The flow rate was 1 ml/min and the retention time of AFB1 was 8.9 min. A fluorescence detector was used, with the wavelengths of excitation and detection being 362 nm and 455 nm, respectively.

**Validation study**

Linearity was tested by spiking the AFB1 standard solution into blank extract liquid medium or extract peanut to yield final concentrations of 1, 4, 10, 40, 100, and 400 ng/ml. For the repeatability study in liquid medium, the blank liquid medium (0.5 ml) was added with the AFB1 standard solution to obtain final concentrations of 12.5, 25, 100, and 400 ng/ml in five replications. For the repeatability study in peanut samples, 5 g of homogenized peanut was added with the AFB1 standard solution to obtain final concentrations of 10, 40, 100, and 200 ng/ml in five replications. The studies were analyzed daily for five days and their results were expressed as relative standard deviation (%RSD). The limit of quantification (LOQ) was calculated by analyzing blank samples spiked at 0.1, 0.4, 1, 4, 8, and 10 ng/ml. LOQ was defined as the lowest concentration of AFB1 that produced a chromatographic peak at a signal-to-noise ratio (S/N) of 10.

**Statistical analyses**

The statistical analyses were conducted using SPSS for Win/v.22 (IBM SPSS Statistics, Somers, NY, U.S.A). The differences between the percentage inhibition of AFB1, production and fungal growth were assessed using one-way ANOVA, followed by the Duncan test with P<0.05 being considered as statistically significant. The AFB1 production and fungal growth were quantified as inhibition percentages in relation to the control treatment without EO using the equation:

\[ \text{Percentage inhibition} = \frac{(C-T)}{C} \times 100 \]

where C is the concentration of AFB1 production or mycelial dry weight (mg) of control plates (without EOs) and T is the concentration of AFB1 production or mycelial dry weight of EO-treated plates.

**RESULTS**

**Method validation**

The results of testing of intra-day and inter-day precision and recovery of the assay in the liquid medium and peanut samples were presented in Tables 1 and 2, respectively. The intra-day precision of the assay in liquid medium and peanut samples ranged from 3.0 to 3.8% and 4.4 to 7.1%, respectively. The inter-day precision of the assay in liquid medium and peanut samples ranged from 5.4 to 7.1% and 6.0 to 8.9%, respectively. The extraction recoveries of AFB1 from liquid medium and peanut samples ranged from 91.6 to 95.8% and 85.0 to 94.9%, respectively. LOQ for AFB1 in liquid medium and peanut samples were 3 and 4 ng/ml, respectively.

**Antiaflatoxigenic and antifungal activities of EOs in liquid medium**

The effects of six EOs on AFB1 production and growth of A. parasiticus and A. flavus in liquid medium were presented in Tables 3 and 4, respectively. The inhibitory effect of each EO was compared to its control (0 mg/mL) in the same plate. Among the tested EOs, EO of pine showed the greatest inhibitory activity on AFB1 production of A. parasiticus (98.6%) at the concentration of 1 mg/ml and showed the almost complete inhibition of AFB1 production at the concentration of 2 mg/ml. EOs of finger root and rosewood were also found the almost complete inhibition of AFB1 production of A. parasiticus at the concentration of 2 mg/ml. EOs of Siam benzoin and ylang ylang showed 82.6% and
### TABLE 1. Intra-day and inter-day accuracy and precision of AFB1 in liquid medium \((n=5)\)

| Quality control sample concentration (ng/ml) | Recovery (%) | Precision (%RSD) |
|---------------------------------------------|--------------|------------------|
| Intra-day (5 days)                           |              |                  |
| 12.5                                        | 95.8         | 3.2              |
| 25                                          | 93.2         | 3.8              |
| 100                                         | 93.1         | 3.0              |
| 400                                         | 91.6         | 3.0              |
| Inter-day (5 days)                           |              |                  |
| 12.5                                        | 92.8         | 5.4              |
| 25                                          | 93.8         | 7.1              |
| 100                                         | 94.3         | 7.1              |
| 400                                         | 92.1         | 5.6              |

### TABLE 2. Intra-day and inter-day accuracy and precision of AFB1 in peanut samples \((n=5)\)

| Quality control sample concentration (ng/ml) | Recovery (%) | Precision (%RSD) |
|---------------------------------------------|--------------|------------------|
| Intra-day (5 days)                           |              |                  |
| 10                                          | 89.0         | 7.1              |
| 40                                          | 85.0         | 4.4              |
| 100                                         | 92.2         | 6.7              |
| 200                                         | 93.5         | 6.0              |
| Inter-day (5 days)                           |              |                  |
| 10                                          | 89.0         | 8.2              |
| 40                                          | 85.1         | 6.0              |
| 100                                         | 94.9         | 8.9              |
| 200                                         | 90.7         | 8.6              |

### TABLE 3. Effects of six EOs on AFB1 production of A. parasiticus and A. flavus extracted from liquid medium

| EO source     | Concentration (mg/ml) | AFB1 production (µg/ml) | Percentage inhibition of AFB1 production of A. parasiticus* | AFB1 production (µg/ml) | Percentage inhibition of AFB1 production of A. flavus* |
|---------------|-----------------------|-------------------------|------------------------------------------------------------|-------------------------|---------------------------------------------------------|
| Finger root   | 0                     | 2.23±0.21               | 0.0aA                                                      | 0.45±0.03               | 0.0aA                                                   |
|               | 1                     | 0.73±0.14               | 67.3±3.8bB                                                | 0.006±0.002             | 98.7±1.2bC                                              |
|               | 2                     | nd                      | >99.9cC                                                   | nd                      | >99.9bD                                                 |
|               | 4                     | nd                      | >99.9cD                                                   | nd                      | >99.9bC                                                 |
| Pine          | 0                     | 1.93±0.42               | 0.0aA                                                      | 0.37±0.07               | 0.0aA                                                   |
|               | 1                     | 0.03±0.01               | 98.6±0.5bC                                                | 0.082±0.022             | 77.8±2.8bB                                              |
|               | 2                     | 0.01±0.01               | 99.5±0.2cC                                                | 0.035±0.012             | 90.6±2.2cC                                              |
|               | 4                     | 0.01±0.02               | 99.5±0.1cD                                                | nd                      | >99.9dD                                                 |
| Rosewood      | 0                     | 2.10±0.20               | 0.0aA                                                      | 0.33±0.03               | 0.0aA                                                   |
|               | 1                     | 0.52±0.17               | 75.3±1.3bB                                                | 0.09±0.03               | 72.8±4.2bB                                              |
|               | 2                     | nd                      | >99.9cC                                                   | nd                      | >99.9bC                                                 |
|               | 4                     | nd                      | >99.9cD                                                   | nd                      | >99.9bC                                                 |
| Siam benzoin  | 0                     | 2.57±0.41               | 0.0aA                                                      | 0.39±0.01               | 0.0aA                                                   |
|               | 1                     | 0.79±0.14               | 69.2±3.8bB                                                | 0.12±0.07               | 69.3±2.9bB                                              |
|               | 2                     | 0.73±0.22               | 71.7±4.9bB                                                | 0.07±0.02               | 82.1±4.6bB                                              |
|               | 4                     | 0.45±0.23               | 82.6±3.0cC                                                | 0.04±0.01               | 90.0±1.5dB                                              |
| Thai moringa  | 0                     | 1.90±0.16               | 0.0aA                                                      | 0.47±0.05               | 0.0aA                                                   |
|               | 1                     | 1.44±0.32               | 24.2±6.1cA                                                | 0.80±0.06               | 0.0aA                                                   |
|               | 2                     | 1.60±0.25               | 15.8±5.2bA                                                | 1.10±0.3                | 0.0aA                                                   |
|               | 4                     | 2.06±0.23               | 0.0aA                                                      | 0.55±0.2                | 0.0aA                                                   |
| Ylang Ylang   | 0                     | 2.24±0.36               | 0.0aA                                                      | 0.41±0.08               | 0.0aA                                                   |
|               | 1                     | 1.43±0.17               | 36.3±6.5bA                                                | 0.11±0.02               | 73.5±3.9bB                                              |
|               | 2                     | 0.69±0.06               | 69.2±1.8cB                                                | 0.02±0.01               | 95.2±0.9cC                                              |
|               | 4                     | 0.67±0.13               | 70.1±6.0cB                                                | 0.006±0.001             | 98.6±0.4cC                                              |

*The inhibitory effect of EOs on AFB1 production in liquid medium is expressed as the percentage inhibition of AFB1 production compared to the control. Values with different letters in the same column are significantly different at \(P<0.05\) (small letters indicate differences within EOs; capital letters indicate differences between EOs at the same concentration.). The data represent mean values ± SD from four replications.
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EOs of Siam benzoin and ylang ylang showed 90.0% and 98.6% inhibition of the AFB₁ production of A. flavus at the highest concentration tested, respectively. Among the six EOs, EO of finger root showed the best inhibitory effect on the growth of A. flavus. The growth inhibitory effect of EOs of pine, rosewood, and ylang ylang was approximately 40% at the highest concentrations tested. EO of Siam benzoin showed little inhibitory effect of A. flavus growth. No significant difference was observed in the inhibition of AFB₁ production and growth on both strains caused by EO of Thai moringa used in a dose-dependent manner.

Anti-aflatoxigenic activity of selected EOs in peanut pod

Based on the above results, we found that the anti-

### TABLE 4. Effects of six EOs on growth of A. parasiticus and A. flavus harvested from liquid medium

| EO source       | Concentration (mg/ml) | Mycelial dry weight (mg) | Percentage inhibition of growth of A. parasiticus* (%) | Mycelial dry weight (mg) | Percentage inhibition of growth of A. flavus* (%) |
|-----------------|-----------------------|--------------------------|------------------------------------------------------|--------------------------|---------------------------------------------------|
| Finger root     | 0                     | 3.18±0.2                 | 0.0aA                                                | 3.50±0.3                 | 0.0aA                                             |
|                 | 1                     | 2.75±0.5                 | 13.6±1.6bA                                           | 0.30±0.1                 | 91.3±0.8bc                                        |
|                 | 2                     | nd                       | >99.9cC                                              | nd                       | >99.9bD                                           |
|                 | 4                     | nd                       | >99.9cD                                              | nd                       | >99.9bD                                           |
| Pine            | 0                     | 3.25±0.2                 | 0.0aA                                                | 3.15±0.3                 | 0.0aA                                             |
|                 | 1                     | 2.55±0.1                 | 21.5±5.9bB                                          | 2.95±0.1                 | 6.4±3.2bA                                         |
|                 | 2                     | nd                       | >99.9cC                                              | 2.90±0.1                 | 8.1±3.0bA                                         |
|                 | 4                     | nd                       | >99.9cD                                              | 1.85±0.2                 | 41.3±5.5cC                                        |
| Rosewood        | 0                     | 3.30±0.2                 | 0.0aA                                                | 3.00±0.9                 | 0.0aA                                             |
|                 | 1                     | 2.78±0.7                 | 15.9±8.6bA                                          | 2.10±0.2                 | 30.0±2.1bB                                        |
|                 | 2                     | nd                       | >99.9cC                                              | 2.10±0.6                 | 30.0±3.2bB                                        |
|                 | 4                     | nd                       | >99.9cD                                              | 1.80±0.2                 | 40.0±6.1bC                                        |
| Siam benzoin    | 0                     | 3.50±0.6                 | 0.0aA                                                | 3.40±0.3                 | 0.0aA                                             |
|                 | 1                     | 2.73±0.1                 | 21.9±4.8bB                                          | 3.80±0.4                 | 0.0aA                                             |
|                 | 2                     | 0.90±0.2                 | 74.3±5.9cB                                          | 3.10±0.4                 | 9.0±3.5bA                                         |
|                 | 4                     | 0.67±0.1                 | 80.7±1.4cC                                          | 3.00±0.3                 | 11.7±8.3bB                                        |
| Thai moringa    | 0                     | 2.70±0.5                 | 0.0aA                                                | 2.20±0.4                 | 0.0aA                                             |
|                 | 1                     | 2.40±0.3                 | 11.1±1.9bA                                          | 2.10±0.5                 | 5.0±0.8bA                                         |
|                 | 2                     | 2.35±0.6                 | 13.0±1.5bA                                          | 2.10±0.3                 | 5.0±1.3bA                                         |
|                 | 4                     | 3.10±0.0                 | 0.0aA                                                | 2.90±0.4                 | 0.0aA                                             |
| Ylang Ylang     | 0                     | 2.20±0.3                 | 0.0aA                                                | 3.00±0.1                 | 0.0aA                                             |
|                 | 1                     | 1.95±0.1                 | 11.4±5.7bA                                          | 2.20±0.3                 | 26.7±1.8bB                                        |
|                 | 2                     | 1.93±0.1                 | 12.4±4.5bA                                          | 2.20±0.3                 | 26.7±1.8bB                                        |
|                 | 4                     | 1.35±0.1                 | 38.6±5.8cB                                          | 1.80±0.1                 | 40.0±7.1bC                                        |

*The inhibitory effect of EOs on growth of fungi in liquid medium is expressed as the percentage inhibition of fungal growth compared to the control. Values with different letters in the same column are significantly different at P<0.05 (small letters indicate differences within EOs; capital letters indicate differences between EOs at the same concentration.) The data represent mean values ± SD from four replications.
FIG. 1. Effects of five selected EOs at different concentrations with dipping method (direct exposure) on A. flavus in peanut sample after 10 days of incubation at 30°C.

(a) Control group: peanut sample was dipped into mineral oil.
(b, c, and d) Treatment groups: peanut sample was dipped into 4, 8 or 16% (v/v) of EOs of finger root (1), pine (2), rosewood (3), Siam benzoin (4), and ylang ylang (5), respectively.

FIG. 2. Effects of five selected EOs at different concentrations with volatile method (vapor exposure) on A. flavus in peanut sample after 10 days of incubation at 30°C. The cotton ball was used as the reservoir for each EO.

(a) Control group: 1 ml of mineral oil was added into the cotton ball.
(b, c, and d) Treatment groups: 1 ml of 4, 8, or 16% (v/v) of EOs of finger root (1), pine (2), rosewood (3), Siam benzoin (4), and ylang ylang (5) was added into the cotton ball, respectively.
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Production inhibition at the higher concentration tested. However, at the same concentration of EOs using the volatile method there was no significant inhibition of AFB1 production. At the concentration of 16% of all EOs, using the dipping method could almost completely inhibit AFB1 production of *A. flavus* in peanut samples whereas using the volatile method varied between 12.9 and 25.0%.

**DISCUSSION**

Since antiquity, EOs from aromatic and medicinal plants have been known to possess biological activity and constitute one of the most investigated groups of secondary metabolites (Soković et al. 2013). Many studies have revealed that *Aspergillus* growth and mycotoxin production were inhibited by many plant EOs (Alpsoy, 2010; Silva et al., 2010). Some components of EOs such as flavonoids, terpenoids, and phenolics aflatoxigenic effect of five EOs on *A. flavus* was better than on *A. parasiticus*. Moreover, it is known that *A. flavus* is predominantly a fungus which produces aflatoxin on peanut production Thailand (Ehrlich et al., 2007; Rostami et al., 2009). Therefore EOs of finger root, pine, rosewood, Siam benzoin, and ylang ylang were selected for further experimentation. The effects of five selected EOs at different concentrations with dipping and volatile methods on *A. flavus* in peanut sample after 10 days were presented in Figures 1 and 2, respectively. The amounts of AFB1 production were detected from peanut samples and were presented in Tables 5. The inhibitory effect of treatment groups was compared to the same control group (0 mg/mL) in the same time. EO of finger root was very effective in the inhibition of AFB1 production using the dipping method. It showed more than 90% AFB1 production inhibition at the lowest concentration tested. EOs of pine, rosewood, and ylang ylang showed more than 70% AFB1 production inhibition at the higher concentration tested. However, at the same concentration of EOs using the volatile method there was no significant inhibition of AFB1 production. At the concentration of 16% of all EOs, using the dipping method could almost completely inhibit AFB1 production of *A. flavus* in peanut samples whereas using the volatile method varied between 12.9 and 25.0%.

**TABLE 5.** Effects of five selected EOs at different concentrations with two methods on AFB1 production of *A. flavus* in peanut sample

| EO source   | Concentration (% v/v) | AFB1 production (µg/g) | Percentage inhibition of AFB1 production | AFB1 production (µg/g) | Percentage inhibition of AFB1 production |
|------------|------------------------|------------------------|------------------------------------------|------------------------|------------------------------------------|
|            |                        |                        | Dipping method*                          |                        | Volatile method*                         |
|            |                        |                        | (%)                                      |                        | (%)                                      |
| Finger root| 0                      | 10.80±1.8              | 0.0aA                                    | 16.90±3.2              | 0.0aA                                    |
|            | 4                      | 0.55±0.7               | 94.9±7.2bC                              | 23.09±1.4              | 0.0aA                                    |
|            | 8                      | 0.21±0.2               | 98.1±2.1bC                              | 16.96±1.3              | 0.0aA                                    |
|            | 16                     | 0.19±0.2               | 98.3±1.7bB                              | 13.85±2.4              | 18.0±3.3bA                              |
| Pine       | 0                      | 10.80±1.8              | 0.0aA                                    | 16.90±3.2              | 0.0aA                                    |
|            | 4                      | 1.39±0.3               | 86.9±3.2bB                              | 23.72±2.9              | 0.0aA                                    |
|            | 8                      | 0.23±0.2               | 97.8±1.8cC                              | 21.21±1.8              | 0.0aA                                    |
|            | 16                     | 0.21±0.1               | 98.1±1.2cB                              | 14.72±0.3              | 12.9±1.9bA                              |
| Rosewood   | 0                      | 10.80±1.8              | 0.0aA                                    | 16.90±3.2              | 0.0aA                                    |
|            | 4                      | 1.30±0.8               | 88.0±7.2bB                              | 25.45±1.4              | 0.0aA                                    |
|            | 8                      | 0.48±0.3               | 95.5±2.6bBC                             | 23.19±2.8              | 0.0aA                                    |
|            | 16                     | 0.16±0.3               | 98.5±0.2cB                              | 14.00±1.8              | 17.2±7.9bA                              |
| Siam benzoin| 0                     | 10.80±1.8              | 0.0aA                                    | 16.90±3.2              | 0.0aA                                    |
|            | 4                      | 6.40±1.1               | 41.0±4.5bA                              | 19.51±1.1              | 0.0aA                                    |
|            | 8                      | 5.70±1.8               | 47.4±4.4bA                              | 18.52±2.9              | 0.0aA                                    |
|            | 16                     | 0.46±0.2               | 95.8±2.3cA                              | 13.50±3.2              | 20.2±7.5bA                              |
| Ylang ylang| 0                      | 10.80±1.8              | 0.0aA                                    | 16.90±3.2              | 0.0aA                                    |
|            | 4                      | 2.95±0.8               | 72.7±8.9bB                              | 20.18±1.2              | 0.0aA                                    |
|            | 8                      | 1.75±0.7               | 83.7±7.2bB                              | 20.64±1.3              | 0.0aA                                    |
|            | 16                     | 0.39±0.2               | 96.4±1.7cA                              | 12.67±1.5              | 25.1±5.4bA                              |

*The inhibitory effect of EOs on AFB1 production in peanut samples is expressed as the percentage inhibition of AFB1 production compared to the control. Values with different letters in the same column are significantly different at P<0.05 (small letters indicate differences within EOs; capital letters indicate differences between EOs at the same concentration.). The data represent mean values ± SD from four replications.
were found to have inhibitory activities against *A. parasiticus* and *A. flavus* (Holmes, 2008). The extent of inhibition of fungal growth and aflatoxin production was dependent on the concentration of the EOs used and the high percentage of active components in the EOs (Soliman and Badeaa, 2002). In current study, the six EOs were evaluated based on their inhibitory effects on *A. flavus* and *A. parasiticus* growth and AFB1 production in potato dextrose liquid medium. EO of pine had the best inhibitory effect on AFB production, of *A. parasiticus* (IC50 = 0.53 mg/ml) followed by EO of rosewood (IC50 = 0.89 mg/ml), finger root (IC50 = 1.01 mg/ml), Siam benzoin (IC50 = 1.41 mg/ml), and ylang ylang (IC50 = 2.09 mg/ml), whereas EO of finger root gave the best AFB1 production inhibitory effect on *A. flavus* (IC50 = 0.52 mg/ml) followed by rosewood (IC50 = 0.93 mg/ml), pine (IC50 = 0.97 mg/ml), ylang ylang (IC50 = 0.99 mg/ml), and Siam benzoin (IC50 = 1.22 mg/ml). Only EO of Thai moringa showed no significant difference in inhibitory activity on both strains. Almost all EOs showed strong growth inhibition of the fungi at the highest concentration tested. Their anti-aflatoxigenic activities may correlate with their antifungal activities in the dose-dependent manner. However, EO of Siam benzoin inhibited AFB1 production of *A. flavus* with weak growth inhibitory activity. This result may indicate that essential oil of Siam benzoin has an antiaflatoxigenic property at concentration lower than its fungitoxic concentration (Prakash et al., 2012).

The inhibition of AFB1 production of EO of finger root is directly related to its three major components of nerol, camphor, and 1, 8 cineole, which exhibited very good antifungal properties (Pattaratanawadee et al., 2006; Mokbel and Alharbi, 2015). The fungal growth and AFB1 production inhibitory effects of EO of pine could be correlated with the presence of α-pinene, a major flavonoid component. It seems possible that the strong inhibitory activity of α-pinene may relate to its antioxidant activity (Okamura et al., 1994; Moghtader et al., 2011). The efficacy of EO of rosewood may be attributed to having linalool, a major monoterpene component (Simić et al., 2004). The combination of the terpenoid compounds which consist of linalool, limonene, and α-pinene may relate to the antiaflatoxigenic effect of EO of ylang ylang (Brokl et al., 2013; Pinto et al., 2013). The antiaflatoxigenic activity of EO of Siam benzoin could be correlated with containing a high percentage of benzoic acid and its ester (Dixit and Singh, 2011). Furthermore, the antimicrobial properties of these essential oils have been tested against various pathogenic fungi. The EOs of finger root and pine have been reported against *A. niger*, *Fusarium oxysporum*, and *Alternaria alternata* in the medium (Pattaratanawadee et al., 2006; Amri et al., 2013). The EOs of Siam benzoin and ylang ylang showed the inhibitory activity against *Trichophyton* spp. in the medium (Shin and Lim, 2004; Inouye et al., 2006). Whereas the EO of rosewood showed the antifungal activity against *A. terreus* and *Trichoderma viride* (Simić et al., 2004).

Many papers have reported using herb and spices EO in direct contact and via a vapor phase application for controlling pathogenic and spoilage fungi in foods (Lopez et al., 2005; Tyagi et al., 2012). The dipping and volatile methods of five selected EOs were tested to corroborate the results of antifungal, antiaflatoxigenic activities of the EOs in liquid medium, and to apply the feasibility of the method for controlling aflatoxin contamination in peanut. In the dipping method, EO of finger root had the best effect on the inhibition of AFB1 production of *A. flavus* (IC50 = 2.5%, v/v), followed by EOs of pine (IC50 = 2.8%, v/v), rosewood (IC50 = 3.0%, v/v), ylang ylang (IC50 = 4.5%, v/v), and Siam benzoin (IC50 = 7.1%, v/v). All tested EOs strongly inhibited the mycelial growth of *A. flavus* at the highest concentration tested. The AFB1 production inhibitory effect of EOs using the volatile method was much lower than for the dipping method. A significant inhibition of each EO was observed at the concentration of 16%, v/v. The results of the dipping and volatile methods suggested that the major components of these EOs may inhibit spore germination or mycelia growth upon contact. In the volatile method, the active vapor components of EO may play a role in the inhibition of fungal growth or aflatoxin production. Therefore, using a higher concentration of EOs in the volatile method may be effective in controlling aflatoxin production. The application of these EOs in the vapor exposure should further be investigated in the large scale experiment with the appropriate reservoir and package. This present work showed the possibility of protecting stored in-shell peanut against *A. flavus* by applying EOs of finger root, pine, rosewood, Siam benzoin, and ylang ylang. All five EOs could be used against aflatoxigenic fungi in stored grains. However, the appropriate application of these EOs as fungal-control agents should further be investigated on safety issues for human and animal health. For example, the combination of application the higher concentration of EOs and using the edible films or coatings preserve peanut during storage.

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