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# Evaluation of essential oils against *Sitophilus zeamais* (Motshulsdy) (Coleoptera: Curculionidae)

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**ABSTRACT**

The objective of the current study was to determine the fumigant toxicity of essential oils for control stored product insects pest. Essential oils from ten plant species currently found in Thailand: pine (*Pinus Palustris*), lemon grass (*Cymbopogon citratus* Stapf), peppermint (*Mentha Piperita*), citronella grass (*Cymbopogon nardus* Linn), sweet acacia (*Acacia farnesiana*), cinnamon (*Cinnamomum verum* J.S. Presl), sweet orange (*Citrus sinensis* Pers), basil (*Ocimum basilicum* L.), clove (*Syzygium aromaticum* L.), and star anise (*Illicium verum* Hook) were extracted by steam distillation and tested for their insecticidal activities against maize weevil (*Sitophilus zeamais* Motshulsdy). Fumigant toxicity test was evaluated on adult of the maize weevil under laboratory conditions. Mortality of the maize weevil was observed and recorded every 12 h until 72 h. Responses varied with the test applied 100 and 10 µl of essential oils from the ten plants species on the tested insects. Three of the essential oils (sweet acacia, basil and star anise) showed high toxicity and were selected for the residuality test to mortality by contact with a surface of treated petri dish and glass jar. The results revealed that diluted 7.5 µl and 205.0 µl of essential oils from three plants (star anise, basil and sweet acacia) achieved a high mortality of the tested insects at 100% within 36 h after exposure to surface of treated petri dish and glass jar respectively.

**Keywords:** Essential oils, fumigant toxicity, maize weevil *Sitophilus zeamais* (Motshulsdy), mortality.

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## INTRODUCTION

According to most of food products lost to various pests during post-harvest storage, consumers demand more natural processed products with long shelf-life but without chemical preservatives. Maize (*Zea mays* L.), rice and wheat are the three most important cereal crops worldwide (Regnault, 1997). Maize damage by *Sitophilus zeamais* causes food loss, increased poverty, and lower nutritional values of grain, increased malnutrition, reduced weight and market values (Keba and Sori, 2013). And also *S. zeamais* reduced germination percentage and maize production as most farmers in developing countries store grain and seed together (Pingali and Pandey, 2001).

Maize weevil, *Sitophilus zeamais* (Motschulsky), is a serious pest of economic importance in stored products in tropical and subtropical countries; infestation often starts in the field, but serious damage is done during maize storage (Suleiman et al., 2015; Fikremariam et al., 2009; Muzemu et al., 2013). It is the damage to grain by feeding activities of the adults and the development of immature stages within the grain. This not only reduces the grain quality but also produces a considerable amount of grain dust mixed with frass (Longstaff, 2010). It causes to weight loss of 20 to 90% for untreated maize in tropical countries (Giga et al., 1991). Maize is stored in commercial structures, with proper monitoring of

temperature and moisture content to control pests in developed countries. But maize is often stored in traditional structures with no environmental control and usually without chemical protectants and usually without chemical protectants in tropical countries (Dhliwayo and Pixley, 2003). Fumigant such as methyl bromide and phosphine are still the most effective for the protection from insect infestation of stored food, feedstuffs, and other agricultural commodities (EPA, 2001). Some stored product insects are found to have developed resistance to methyl bromide and phosphine (Champ and Dyte, 1977).

The use of natural compounds from plants instead synthetic chemical pesticides is an alternative that can reduce the agriculture impact on the environment (Vanichpakorn et al., 2010). The choice of native species as source of oil and/ or extracts employed in pest control could be a strategy to their sustainable use by local communities, and consequently contribute to their conservation. Plant may provide potential alternatives to used for control insect agents because they constitute a rich source of bioactive chemicals (Wink, 1993). Numerous plants have been reported to have a variety of biological activities against insects including insecticidal, repellent, antifeedant, fumigant, growth regulatory, antiovipostion activities (Isman, 2006; Ukeh et al., 2010). Moreover, plant based insecticides often contain a mixture of active substances, which can delay or prevent resistance development (Wang et al., 2007). Plant products can be used for insect pest control in form of essential oils. Aim of this study was carried out to evaluate the fumigant toxicity from ten plants species and three essential oils were assessed against adults of maize weevil, *Sitophilus zeamais* Motshulsdy under laboratory conditions.

## MATERIALS AND METHODS

### Insect preparation

Maize weevil, *Sitophilus zeamais* (Motschulsky), was collected from maize storage silos in Phitsanulok province, Thailand, and reproduced in 1000 ml plastic containing maize (*Zea mays* L.) as a source of food. The insects were maintained in the container at a room temperature of 30 ± 1°C and 75 % RH. They were laboratory-reared with laid eggs on maize, one week after laying eggs, the insect parents were removed. The eggs were kept in the same condition until adult emergence. Ten to fourteen-day olds of adults were used for bioassay tests.

### Plants extract preparation

The essential oils of ten plant species: pine (*Pinus palustris*), lemon grass (*Cymbopogon citratus* Stapf), peppermint (*Mentha piperita*), citronella grass (*Cymbopogon nardus* Linn), sweet acacia (*Acacia farnesiana*), cinnamon (*Cinnamomum verum* J.S. Presl), sweet orange (*Citrus sinensis* Pers), basil (*Ocimum basilicum* L.), clove (*Syzygium aromaticum* L.), and star anise (*Illicium verum* Hook) were extracted from aerial parts of the plants by steam distillation using distilled water (Vogel et al., 1997). Subsequently, the oils were collected in glass recipient and kept in amber colored glass containers at 4°C until the subsequent assays. Pure essential oils were employed in all the tests. The samples were subjected to maize weevil *S. zeamais* (Motshulsdy). Tween 80 was used for emulsion to stabilize the essential oils before testing.

### Insects bioassay test

#### *Fumigant toxicity assay*

This bioassay employed the methodology of Pires et al. (2006) cited by Jessica et al. (2010) which consisted of applying 0 (control), 1000, 100 and 10 µl of the ten essential oils on Whatman N°10 filter paper (Whatman, Maidstone, Kent, UK), which were cut into 3-cm diameter pieces and fixed under the petri dish. Filter papers were impregnated with a series of concentrations of each essential oil. The same procedure was used for the control with filter paper without treatment and placed on the petri dish and then the ten unsexed adult insects was placed on the petri dish after the oils evaporated (10 insects/petri dish). There were four replicates of each treatment. The experimental units were kept in laboratory at a room temperature of 30 ± 1°C. Assessments of mortality were made at 12, 24, 36, 48, 60 and 72 h of exposure.

#### *Mortality by contact with a surface of the treated container (petri dish)*

The methodology of Kouninki et al.(2007) was used. The high toxicities three from ten essential oils were selected to evaluate doses of oils were star anise (*Illicium verum* Hook), basil (*Ocimum basilicum* L.) and sweet acacia (*Acacia farnesiana*). The diluted of the three essential oils, applying 0 (control), 2.5, 5.0 and 7.5 µl (using the filter paper diffusion method with exposed into petri dish). A solution of essential oils in acetone (99% purity), at the required concentration of each treatment was applied on petri dish with 5 g of maize grain. Each petri dish was infested 10 unsexed adult insects, and stored in a room temperature of 30 ± 1°C. Each treatment was carried out in 4 replicates. The mortality was assessed at 12, 24, 48 and 72 h exposure to the toxic.

#### *Mortality by contact with a surface of the treated container (glass jar)*

The methodology of Kouninki et al. (2007) was used, with slight modifications that consisted of using 2600 $cm^3$ glass jar (using the filter paper diffusion method with exposed into the jar) and applying 68, 137 and 205 µl instead of 2.5, 5.0 and 7.5 µl, respectively. A solution of the three essential oils in acetone, at the required concentration applied on 6 cm of filter paper and place on the jar. The jar was agitated for 1 min for the oil to cover the interior surface. The oil was allowed to evaporate at ambient temperature for 1 h, then 10 unsexed adult insects were placed in each jar with 5 g of maize. Four replicates were made per treatment. The treatments were kept at room temperature at 30 ± 1°C. Insect mortality was assessed at 24, 48 and 72 h of exposure to the essential oils.

As the mortality rate in the control was lower than 5%, this was corrected with the Abbott formula (Abbott, 1925). An insect was considered dead when there was no movement after prodding it with a dissection needle.

$$\% \text{ Mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

**Statistical analysis**

The significance of treatments was calculated by one way Analysis of Variance (ANOVA) and effective treatment was separated by the Duncan's New Multiple Ranges Test (DMRT). Differences between means were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

### Fumigant toxicity of 10 essential oils against maize weevil *Sitophilus zeamais* (Motshulsdy)

Fumigant toxicity of ten essential oils with 100 and 10 µl as shown in Tables 1 and 2. The mortality of *S. zeamais* increased with the increase of volume of the oils and exposure time. Almost of the essential oils show fumigant toxicity with high mortalities ranging from 82.5 to 100% at 72 h after treatment with 100 µl (Table 1). When the decrease essential oils to 10 µl were applied on tested insects, the high fumigant toxicity occurred on the three essential oils against *S. zeamais* with significant differences ($P < 0.01$). Only three of essential oils showed significantly higher fumigant toxicity against *S. zeamais* than the other treatment with mortality of 100% at 72 h, whereas the other essential oils could not achieve up to 100 % insect mortality. Sweet acacia (*Acacia farnesiana*), basil (*Ocimum basilicum* L.) and star anise (*Illicium verum* Hook) achieved 100 % mortality of *S. zeamais* within 36 h after treatment. No mortality was observed in the untreated controls (Table 2). Therefore the three essential oils were selected to apply for testing mortality by contact with a surface of treated container with petri dish and glass jar as shown on Tables 3 and 4, respectively.

In general, mortality increased with increased exposure time to the essential oil, which concurs with Bittner et al. (2008). The 10 µl of essential oils from sweet acacia, basil and star anise exceeded 100% mortality at 36 h (Table 2). Particularly, the sweet acacia caused 100% mortality at 24 h after treatment, meantime mortality of basil and star anise was 87.5%. Similarly, it was observed in the fumigant action bioassay on *S. zeamais* (Motschulsky) by Jessica et al. (2010) that 35 µl of *Peumus boldus* Molina oil in a volume of 0.15 L has a rapid toxic effect, producing 100% mortality in 6 h. At 24 h, the treatments higher than 20 µl of the essential oil in 0.15 L caused 100% mortality. Also the mortality of *S. zeamais* after 24 h exposure to different dosage of *C. dinisii* oil demonstrated by Vedovatto et al. (2015). Vogel et al. (2005) reported that the essential oil of *Rosmarinus officinalis* L. and *Eucalyptus blakelyi* Maiden had fumigant action against the mite *Tetranychus urticae* Koch (Miresmailli et al., 2006); *Sitophilus oryzae* L. and *Tribolium castaneum* Herbst (Lee et al., 2003).

**Table 1.** Fumigant toxicities of 10 essential oils (100 µl) on mortality (%) *Sitophilus zeamais* Motshulsdy.

| Treatment | % Mortality | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12 h | 24 h | 36 h | 48 h | 60 h | 72 h | df |
| Control (water) | $0^{d}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | 0 |
| Pine oil | $15.0^{d}$ | $22.5^{c}$ | $70.0^{ab}$ | $87.5^{ab}$ | $97.5^{a}$ | $97.5^{a}$ | * |
| Lemon grass | $0^{d}$ | $0^{c}$ | $7.5^{c}$ | $7.5^{c}$ | $10.0^{c}$ | $12.5^{c}$ | ns |
| Peppermint | $92.5^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Citronella grass | $5.0^{d}$ | $17.5^{c}$ | $22.5^{bc}$ | $35.0^{bc}$ | $47.5^{bc}$ | $55.0^{bc}$ | ns |
| Sweet acacia | $55.0^{bc}$ | $75.0^{ab}$ | $95.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Cinnamon | $22.5^{cd}$ | $45.0^{bc}$ | $62.0^{bc}$ | $69.5^{bc}$ | $79.5^{ab}$ | $84.5^{ab}$ | * |
| Sweet orange | $65.0^{ab}$ | $70.0^{ab}$ | $77.5^{ab}$ | $80.0^{ab}$ | $80.0^{ab}$ | $82.5^{ab}$ | * |
| Basil oil | $80.0^{ab}$ | $97.5^{a}$ | $97.5^{a}$ | $97.5^{a}$ | $97.5^{a}$ | $97.5^{a}$ | * |
| Clove Oil | $32.5^{cd}$ | $57.5^{bc}$ | $82.5^{ab}$ | $82.5^{ab}$ | $82.5^{ab}$ | $82.5^{ab}$ | * |
| Star anise | $70.0^{ab}$ | $85.0^{ab}$ | $97.5^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |

ns = non significant; * = significant difference, means (followed by the same letter) are not significantly different at 5% level by DMRT.

### Mortality by contact with a surface of treated container (petri dish) with the 3 selected essential oils

The three selected of essential oils (sweet acacia , basil and star anise) were applied on petri dish, the mortality by contact with a surface of the treated petri dish showed mortality increases with increases volume of the essential oil. At 12 h, *S. zeamais* response to the sweet acacia oil with mortality was 75, 92.5 and 95% at treatment of 2.5, 5.0 and 7.5 µl respectively. However, star anise oil given the low mortality to *S. zeamais* only 22.5 and 30.0% at treatment of 2.5 and 5.0 µl respectively, but sharply increase reached a mortality to 100% at treatment of 7.5 µl at the first 12 h after testing. There was no mortality in the untreated control (Table 3). In 2003, some researcher

**Table 2.** Fumigant toxicities of essential oils (10 µl) on *Sitophilus zeamais* Motshulsdy.

| Treatment | % Mortality | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12 h | 24 h | 36 h | 48 h | 60 h | 72 h | df |
| Control (water) | $0^{d}$ | $0^{c}$ | $0^{c}$ | $0^{d}$ | $0^{d}$ | $0^{d}$ | ns |
| Pine oil | $2.5^{d}$ | $15.0^{bc}$ | $17.5^{bc}$ | $17.5^{c}$ | $17.5^{b}$ | $17.5^{cd}$ | ns |
| Lemon grass | $2.5^{d}$ | $2.5^{c}$ | $5.0^{bc}$ | $7.5^{cd}$ | $7.5^{c}$ | $10.0^{d}$ | ns |
| Peppermint | $30.0^{c}$ | $32.5^{b}$ | $37.5.0^{b}$ | $40.0^{b}$ | $50.0^{b}$ | $70.0^{ab}$ | * |
| Citronella grass | $2.5^{d}$ | $5.0^{c}$ | $5.0^{bc}$ | $7.5^{cd}$ | $10.0^{c}$ | $15.0^{cd}$ | ns |
| Sweet Acacia | $95.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Cinnamon | $17.5^{cd}$ | $22.5^{bc}$ | $32.5^{b}$ | $40.0^{b}$ | $52.5^{b}$ | $52.5^{bc}$ | * |
| Sweet orange | $0^{d}$ | $0^{c}$ | $0^{c}$ | $0^{d}$ | $7.5^{c}$ | $17.5^{cd}$ | ns |
| Basil oil | $62.5^{bc}$ | $87.5^{ab}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Clove Oil | $10.0^{cd}$ | $15.0^{bc}$ | $32.5^{b}$ | $35.0^{b}$ | $47.5^{b}$ | $50.0^{bc}$ | ns |
| Star anise | $50.0^{bc}$ | $87.5^{ab}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |

ns = non significant; * = significant difference, means (followed by the same letter) are not significantly different at 5% level by DMRT.

**Table 3**. Mortality (%) of *Sitophilus zeamais* Motshulsdy exposed to petri dish surface treated with the 3 selected essential oils.

| Treatment | % Mortality | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12 h | 24 h | 36 h | 48 h | 60 h | 72 h | df |
| Control (water) | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | |
| Sweet acacia oil 2.5 µl | $75.0^{ab}$ | $82.5^{ab}$ | $82.5^{ab}$ | $85.0^{ab}$ | $87.5^{ab}$ | $87.5^{ab}$ | * |
| Basil oil 2.5 µl | $12.5^{b}$ | $30.0^{b}$ | $60.0^{ab}$ | $62.5^{ab}$ | $75.0^{ab}$ | $75.0^{ab}$ | * |
| Star anise oil 2.5 µl | $22.5^{b}$ | $32.5^{b}$ | $52.5^{ab}$ | $55.0^{ab}$ | $60.0^{ab}$ | $62.5^{ab}$ | * |
| Sweet acacia oil 5.0 µl | $92.5^{a}$ | $97.5^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Basil oil 5.0 µl | $20.0^{b}$ | $44.5^{ab}$ | $69.5^{ab}$ | $74.5^{ab}$ | $74.5^{ab}$ | $79.5^{ab}$ | * |
| Star anise oil 5.0 µl | $30.0^{b}$ | $67.5^{ab}$ | $97.5^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Sweet acacia oil 7.5 µl | $95.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Basil oil 7.5 µl | $35.0^{b}$ | $55.0^{ab}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Star anise oil 7.5 µl | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |

* = significant difference, means (followed by the same letter) are not significantly different at 5% level by DMRT.

**Table 4.** Mortality (%) of *Sitophilus zeamais* Motshulsdy exposed to 2600 $cm^3$ glass jar surface treated with the 3 selected essential oils.

| Treatment | % Mortality | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12 h | 24 h | 36 h | 48 h | 60 h | 72 h | df |
| Control (water) | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | $0^{c}$ | |
| Sweet acacia oil 68.0 µl | $27.5^{b}$ | $72.5^{ab}$ | $82.5^{ab}$ | $82.5^{ab}$ | $82.5^{ab}$ | $82.5^{ab}$ | * |
| Basil oil 68.0 µl | $67.5^{ab}$ | $85.0^{ab}$ | $97.5^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Star anise oil 68.0 µl | $72.5^{ab}$ | $75.0^{ab}$ | $90.0^{a}$ | $97.5^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Sweet acacia oil 137.0 µl | $47.5^{ab}$ | $62.5^{b}$ | $92.5^{a}$ | $97.5^{a}$ | $97.5^{a}$ | $97.5^{a}$ | * |
| Basil oil 137.0 µl | $57.5^{ab}$ | $82.5^{ab}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Star anise oil 137.0 µl | $57.5^{ab}$ | $77.5^{ab}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Sweet acacia oil 205.0 µl | $90.0^{a}$ | $95.0^{a}$ | $95.0^{a}$ | $97.5^{a}$ | $97.5^{a}$ | $97.5^{a}$ | * |
| Basil oil 205.0 µl | $87.5^{a}$ | $90.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |
| Star anise oil 205.0 µl | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ | * |

ns = non significant; * = significant difference, means (followed by the same letter) are not significantly different at 5% level by DMRT.

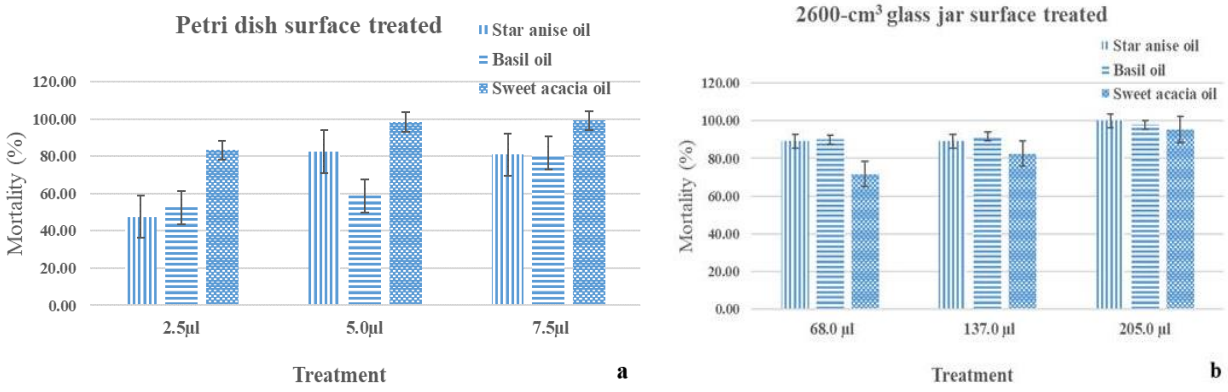


**Figure 1.** The effect of the 3 selected essential oils on % mortality of *Sitophilus zeamais* Motshulsdy exposed to petri dish surface (a) and exposed to 2600 $cm^3$ glass jar surface (b).

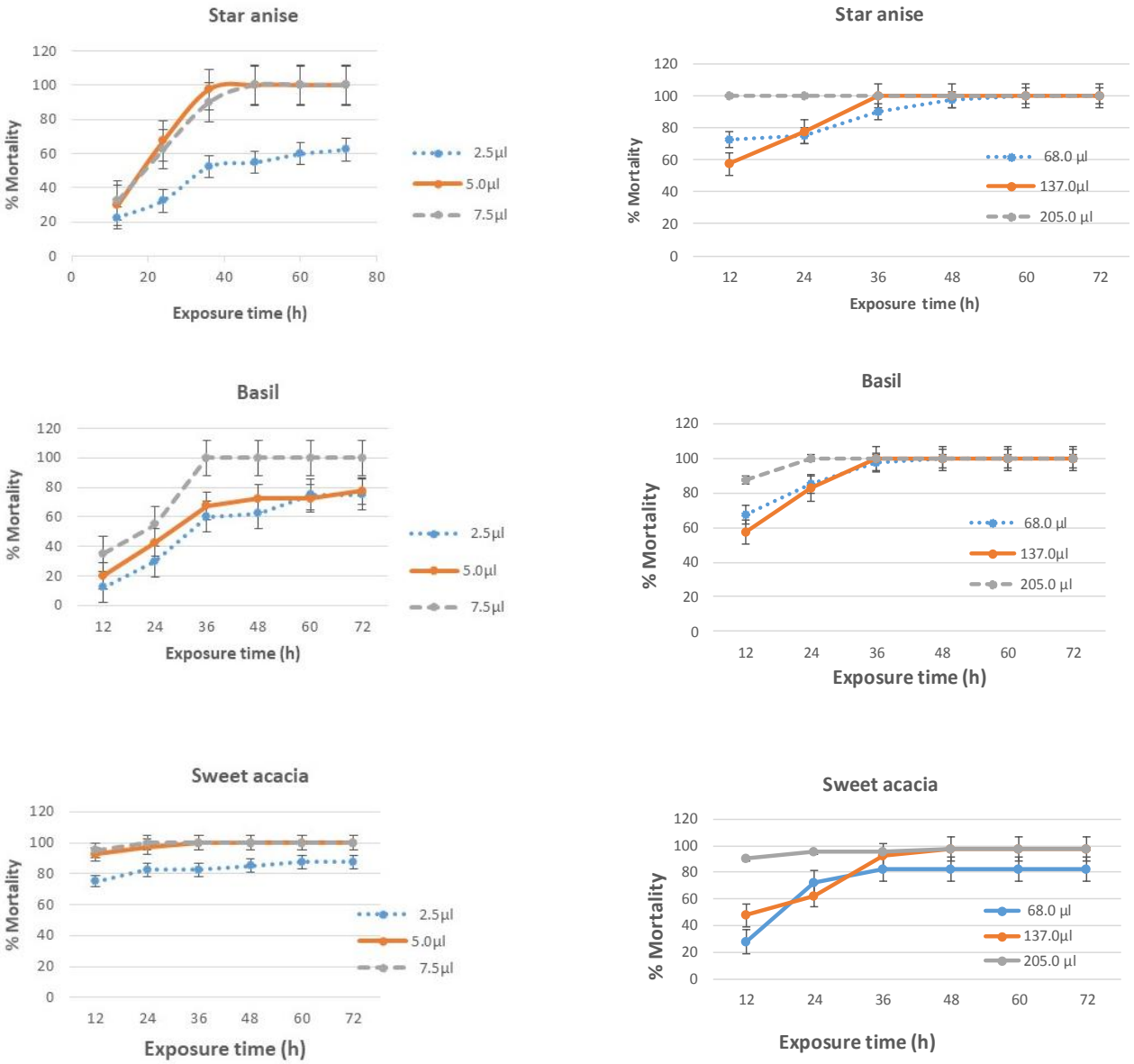


**Figure 2.** Percentage mortality of *Sitophilus zeamais* Motshulsdy exposed to various periods of time to the 3 selected essential oils on filter paper discs.

point out a test with a filter paper diffusion method that cinnamon oil, mustard oil, and horseradish oil produced 100% mortality within 1 day after treatment of 0.7 mg/cm$^2$ for insecticidal activity against adult *Sitophilus oryzae*. Additional, extracts of *Acorus calamus*, cinnamon oil *C. cassia* and *C. sieboldii* caused 100% mortality within 1 day after treatment of 3.5 mg/cm$^2$ against adult *Callosobruchus chinensis* L. (Kim et al., 2003).

### Mortality by contact with a surface of treated container (glass jar) with the 3 selected essential oils

Additional, the treated container was slightly modified that consisted of using 2600 cm$^3$ glass jar instead of the petri dish and applying of three selected essential oils was 68.0, 137.0 and 205.0 µl. Essential oil of star anise at treatment of 205.0 µl resulted the highest of mortality of 100% at 12 h. No mortality was observed in the untreated controls (Table 4). As the time increases between observations the mortality of adults *S. zeamais*, mortality of all treatments reached at 100% at 36 h. Therefore, the mortality rate that was obtained by contact with a surface of treated container of petri dish or glass jar with the 3 selected essential oils given highest at 36 h. Aslan et al. (2004) reported that the level of mortality has been reached at 48 h with the essential oils of *Achillea biebersteinii* Afan and *A. wilhelmsii*, and at 96 h with oil of Pistacia spp. (Aslan et al., 2004).

These experimental were conducted to determine whether the insecticidal activity of the 3 selected essential oils were attributable to fumigant activity. In all cases, considerable differences in insect mortality were noted with different doses and exposure times as shown on Figures 1 and 2. It can be concluded that for control of *S. zeamais*, higher doses for a relatively short period are much more effective than lower doses for longer periods. Fumigant toxicity of the three essential oils to bruchid increase of exposure time from 6 to 48 h resulted in an increase of larval mortality, whilst further increases of exposure time gave no additional detrimental effect (Papachristos and Stamopoulos, 2002).

## CONCLUSION

In conclusion, essential oil of *Acacia farnesiana*, *Ocimum basilicum* L. and *Illicium verum* Hook had fumigant toxicity on *Sitophilus zeamais* Motshulsdy. All essential oils are toxic for adults of *S. zeamais*, whether by exposure to the surface of the treated container petri dish or exposure to the surface of the treated container glass jar.

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