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Fumigant and Toxic Activities of Three Essential Oils Against *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae)

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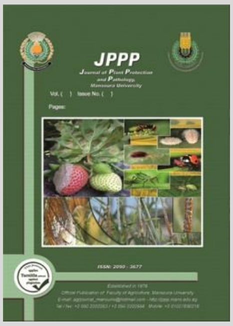

ABSTRACT

Control ways in stored-product soils mainly rely on alternative pesticides. The modification of new environmentally friendly alternatives, such as essential oils (EOs), is a key issue. The aim of the current study was to develop three commercialized essential oils that were examined for fumigant and contact acts against adults of the saw toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). In general, the results of this study showed that with increasing concentration and duration of exposure, toxicity activity increased and weight loss decreased. In case of fumigant tests, orange oil outperformed anise oil in terms of effectiveness against *O. surinamensis* adults. While, anise oil showed a higher toxicity level of contact compared to the other two oils, particularly against adult *O. surinamensis*. The results of this study suggest that these essential oils can be used as viable alternatives to pesticides against *O. surinamensis* in stored grains and stored materials.

Keywords: grain beetle; essential oil; biorational insecticide; fumigant toxicity; contact toxicity.

INTRODUCTION

One of the significant stored grain insects seen globally is the saw toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) (Rossiter *et al.*, 2001; Hashem *et al*., 2012; Hashem *et al*., 2021). This beetle is a secondary pest that depending on storage conditions that are favorable to its developmental stages. It can harm stored cereals in different item forms, including broken kernels, germ, and grain dust (Storey, 1987; Mahroof *et al.*, 2013). It can eat a variety of stored commodities that are essential for human diet, including flours, cereals, wheat bran, dry fruits, and nuts (Govindaraj *et al*., 2014)). Several methods and processes were used to controlling insect that detected in stored items like essential oils. Natural products are alternatively used to control stored product pests (Arthur, 1996). The most practical and cost-effective way to avoid infestation in the majority of storage systems is to utilize chemical and fumigating pesticides (Cao *et al.*, 2019). The ability of this beetle to break down polystyrene and plastic debris has recently attracted significant interest (DeFoliart, 2009, Vigneron *et al.*, 2019).This insect infests a variety of commodities worldwide, primarily cereal, seeds, nuts and so on (Hagstrum *et al.*, 2013). Under ideal circumstances, it quickly completes the immature development (Nika *et al.*, 2021). Both adults and larvae of this beetle can endure frigid temperatures close to 0 °C (Arbogast, 1991). This beetle is also considered a public health pest because of its ability to cause human allergies in all its different stages (Jakubas Zawalska *et al.*, 2016). When this insect attacks a commodity, in addition to causing quantitative damage to the commodity, it also emits a putrid odor that ultimately leads to the destruction of the commodity (Hill, 2003).

Overall, the essential oils (EOs), and their components are routinely tested for their macromolecular insecticidal action against pest species (Benelli *et al.*, 2018). Recently, research has been focused on the environmental safety of using natural plant products in pest control (Isman, 2000). Furthermore, high populations and long times are often required for the evolution of pesticide resistance, and thus essential oils are characterized by favorable insect selectivity, low human toxicity, and insect resistance. (Jindal *et al*., 2013). Therefore, the use of Eos from traditional plants is one of the most efficient alternative biorational control techniques against stored grain pests (Regnault-Roger *et al.*, 2012). According to Çalmaşur *et al.* (2006), these organic compounds found within essential oils are effective against a wide range of stored insect pests, including nematodes, weeds, fungi, mites, and insects. In addition, they exhibit a set of distinct modes of action that lead to several manifestations of toxicity (Mossa, 2016; Hashem *et al*., 2020). These properties lend credence to the hypothesis that essential oils can be used as alternative or complementary tools in Integrated Pest Management (IPM) programs. However, appropriate formulations of EOs are needed to reach their full potential in this field (Benelli *et al.*, 2017).

The saw-toothed grain beetle, *O. surinamensis*, is a small insect with a wide range of hiding places and insecticide resistance (Gautam *et al.*, 2020). Long-term use of fumigants like phosphine and methyl bromide can lead to pesticide residues on stored products, environmental pollution, toxicity to non-target organisms, the emergence of resistant strains, and the toxicity to target organisms (Benhalima *et al.*, 2004; Collins *et al.*, 2005; Jovanovic *et al.*, 2007). Previous studies (Lee *et al.*, 2001; Miresmailli *et al.*, 2006) have examined the insecticidal efficiency of EOs

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against a variety of insect pests; however, there is a lack of information regarding the effects of these oils on the antioxidant defence system, fumigation activity and contact impacts of the tested insect pests. The present study explores the fumigation and contact toxicity of three widely used essential oils (sweet orange, anise, and lemongrass) against adults of *O. surinamensis*. Furthermore, the feasibility of using EOs as a fumigant in a model food system in vivo and their impacts on biological parameters have been examined in order to support their use as a biorational substitute for synthetic pesticides. Since the presence of wheat may affect the results, the toxicity of essential oils has been determined by treating the wheat grains directly.

MATERIALS AND METHODS

Rearing of the saw-toothed grain beetles

This beetle was collected from different parts Mansoura government, and mainly grain silos. Insect samples were brought to f Economic Entomology Department, Faculty of Agriculture, Mansoura University. Insects were kept at 32 ± 2°C, 60 ± 5% Rh, and a 16:8 (L:D) photoperiod. Insects were reared in plastic containers (500 ml) for three generations. Males and females of adults aged 7-15 days were then used in the following experiments.

Essential oils derived plants:

Hashem Brothers Company for Essential Oils and Aromatic Products (Kafr-Elsohby, Kalyoubeya, Egypt) supported this study with the essential oils of sweet orange (*Citrus sinensis* L.), anise (*Pimpinella anisum* L.), and lemongrass (*Cymbopogon citratus*) as a present.

Fumigant assay

Fumigant tests were conducted against *O. surinamensis* beetles in a plastic jar with a volume of 60 ml containing 5 g of rice grain (three adult / 1g of rice). Using a micropipette, the essential oil was deposed on a 7.5cm diameter filter paper disc (Whatman No. 1 paper) (Abbott, 1925). The four doses used were 10000, 15000, 20000, and 25000 ppm. The plastic cover was then closed to keep out insects after the paper had been placed within it and given 10 minutes to allow the essential oils to concentrate. Instead of EO, controls just received pure water. Using adhesive tapes, the filter paper was fastened to the desiccator's interior surface. The number of dead insects was counted after checking the insects after 1, 2, and 3 hours.

Contact and Ingestion assay

Authors have decided to directly treat the grains with a standardized amount of oils in an effort to approximate realistic application conditions as much as feasible. Then, a predetermined number of insects belonging to the same age group were placed immediately on the grains. As a result, exposure to the treated grain and attempts at nutrition are to blame for the mortality that has been reported. Ten grammes of modified rice were used in 15 mL plastic jars for the toxicity experiments. At concentrations of 10,000, 15,000, 20,000, and 25000 ppm, one mL of essential oil was diluted in acetone before being applied to the rice.

To homogenize the treatment after applying the EOs, samples were stirred in a vortex for one minute. Five plastic jars containing 10 g of rice were used as the control treatment, and they only received 1 mL of acetone. To get rid of the acetone, treated rice was dried for 15 minutes within the hood. Then, twenty insects were placed to each plastic jar of rice before the jars were sealed with tulle to allow air to circulate. Jars were positioned in a controlled environment. Each concentration received five repeats. The mortality was recorded after 24 h and 7 days of exposure.

After 45 days of storage in controlling chamber (30±2° C and 60±5% RH), the percentage weight loss of rice grain compared to the control was used to determine the efficiency of EOs. The weight loss (%) of the samples was calculated based on their fresh weight:

$$\textbf{Weight loss } \% = \boldsymbol{W1} - \left(\frac{\boldsymbol{W}}{\boldsymbol{W1}}\right) \textbf{X 100}$$

Where, W1 is the weight of the samples before the experiment and W is the weight of samples at the end of the experiment.

Statistical Analysis:

Data of activity of three EOs, concentrations and exposure hours were analyzed using two-way ANOVA with concentrations and hours as independent variables. Once the interaction term was significant, each variable was analyzed separately by one-way ANOVA, and means were compared using Student- Newman-Keuls Test (Costat Software, 2004).

RESULTS AND DISCUSSION

Result:

Fumigation toxicity

The diverse tested oil kinds and concentrations generally had an impact on the survival of adult saw-toothed grain beetle insects. The findings demonstrated that mortality rates increased as concentration and exposure times increased. In addition, orange oil proved more toxic to the saw-toothed grain beetle at higher doses than other essential oils. Table 1 provides a summary of the fumigant test results of the oils against *O. surinamensis*. Using the fumigation toxicity bioassay, orange oils displayed a strong activity against *O. surinamensis* at all concentrations, indicating that orange oils could be more toxic than lemon grass and anise oils complete mortality rate was reached after 3 days post exposure at concentrations of 15,000, 20,000, and 25,000 ppm for orange oil, while the mortality rate did not exceed 39% and 66% at the highest concentration (25000 ppm) after the same period for Lemon grass and anise oils, respectively.

Table 1. Effect of three essential oils, as fumigants, at different concentrations on mortality rates of the saw-toothed grain beetle, *Oryzaephilus surinamensis*.

Concentrations (ppm)	Essential oils Lemon grass	Orange	Anise
10000	11.86 ± 3.95 Ba	14.66 ± 5.33 Ba	32.00 ± 3.88 B a
15000	15.93 ± 5.28 Bc	100 ± 0 Aa	37.34 ± 2.67 Bb
20000	28 ± 2.49 ABc	100 ± 0 Aa	53.33 ± 3.65 Ab
25000	38.64 ± 9.75 A a	100 ± 0 A c	65.34 ± 8.79Ab

Means followed by the same Capital letters in a column of the same oil, and those followed by the same small letters among oils in a raw are not significantly different at 5% level of probability (Student-Newan-Keuls Test).

For both between and between exposure intervals, all main effects and related interactions were significant (Table 2). By using 2-way ANOVA, the interaction between hours and concentrations showed no significant differences in the lemon oil (F=2.93, df= 6, P=0.042), and the analysis of variance (ANOVA) indicated that there was a significant difference of concentrations (F= 6.71, df =3, P =0.001).

Meanwhile, there were no considerable differences among the three hours (F= 0.64, df =2, P = 0.53). There were significant differences for the concentrations and the interaction between hours and concentrations in orange oil (F= 15.13, df= 3, P=< 0.001 and F= 2.38, df= 6, P= 0.04). However, there were no considerable differences among hours (F= 2.38, df= 2, P= 0.10). There were significant differences for the concentrations and hours in Anise oil (F= 8.55, df= 3, P=0.0002 and F= 31.73, df= 6, P= 0.0001). Meanwhile, there were no considerable differences among the interaction between hours and concentrations (F= 0.92, df= 2, P= 0.49).

Table 2. Effect of three essential oils with different concentrations on the saw-toothed grain beetle, *Oryzaephilus surinamensis* by fumigation treatments

Source of Variation	Degrees of freedom	F	P
	Lemon		
Concentrations	3	6.71	0.0007
Hours	2	0.64	0.53
Concentration * Hour	6	2.93	0.04
	Orange		
Concentrations	3	15.13	0.0001
Hours	2	2.38	0.10
Concentration * Hour	6	2.38	0.04
	Anise		
Concentrations	3	8.55	0.0001
Hours	2	31.73	0.0001
Concentration * Hour	6	0.92	0.49

Contact and Ingestion toxicity

The insecticidal activity of three essential oils against the adults of the *O. surinamensis* are reported in Table 3. The studied oils were shown to have a positive correlation with mortality and concentration. Additionally, mortality rates differed greatly amongst oils. Less than 90% of the people who were exposed to anise oil after 24 hours died after being exposed to tested oils at the highest concentration of 25000 ppm. This result was considered while deciding that in order to provide sufficient efficiency in use, this threshold (10 % mortality) had to be crossed. The results showed that at low concentrations, the EOs listed in Table 3 had little effect on *O. surinamensis* adults.

Table 3. Effect of three essential oils at different concentrations on mortality rates of the saw-toothed grain beetle, *Oryzaephilus surinamensis* by mixing with the rice grains

Concentrations (ppm)	Essential oils name		
	Lemon grass	Orange	Anise
10000	1.33 ±1.33 Ab	0 ± 0 A b	33.33 ±12.9 A a
15000	2.67 ± 1.63 A b	5.33 ± 5.33 Ab	34.66 ± 12.18 A a
20000	2.67 ± 1.63 Ab	6.67 ±2.98 Ab	46.67 ± 16.05 Aa
25000	4 ±1.63 Ab	16.0 ±14.39 Ab	68.00 ±18.31 Aa

Means followed by the same capital letters in a column of the same oil, and those bearing the same lowecase letters in a raw are not significantly different at 5% level of probability (Student- Newan-Keuls Test).

All major effects and associated interactions were not significant for exposure periods within or between (Table 4). There were no significant differences for the concentrations, days and the interaction between days and concentrations in lemon oil (F= 0.38, df= 3, P=0.77, F= 1, df= 2, P=0.38 and F= 1.38 df= 6, P= 0.2415). By using 2-way ANOVA, the concentrations, days and the interaction between hours and concentrations, showed no significant differences in the orange oil (F=0.76, df= 3, P=0.52, F=0.85, df= 2, P=0.43 and F=1.27, df= 2, P=0.29, respectively). There were no significant effect of the concentrations, hours and their interactions in Anise oil (F= 0.71, df= 3, P=0.55, F= 0.72, df= 2, P= 0.04897 and F= 1.05, df= 6, P= 0.40).

Table 4. Effect of three essential oils with different concentrations on the saw-toothed grain beetle, *Oryzaephilus surinamensis* by mixing with the rice grains

Source of Variation	Degrees of freedom	F	P
	Lemon		
Concentrations	3	0.38	0.7672
Days	2	1	0.3754
Con * days	6	1.38	0.2415
	Orange		
Concentrations	3	0.76	0.5200
Days	2	0.85	0.4335
Con * days	6	1.27	0.2891
	Anise		
Concentrations	3	0.71	0.5458
Days	2	0.72	0.04897
Con * days	6	1.05	0.4025

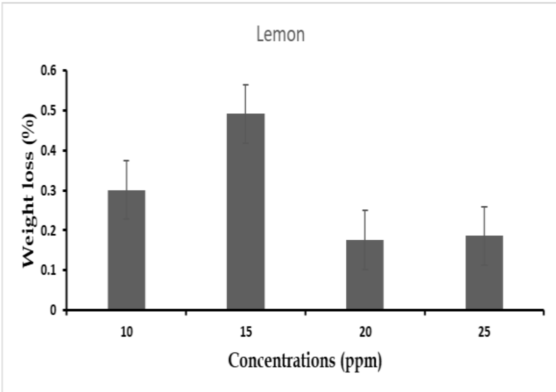

Fig. 1. The percentage of the weight losses after feeding of O. *surinamensis* on rice mixed with the essential Lemongrass oil.

Figure 1 shows that the concentration of 15000 ppm of lemon was the most effect on the weight losses by *O. surinamensis.*

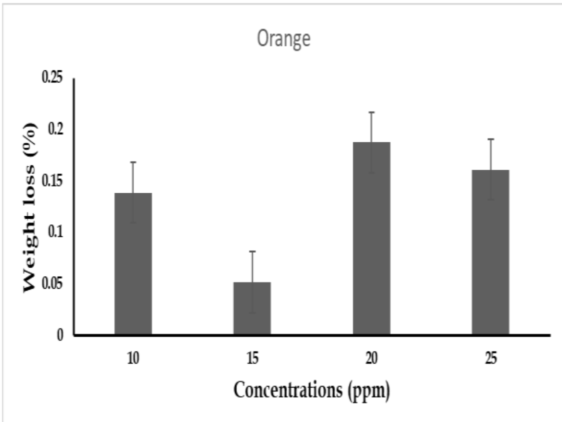

Fig. 2. The percentage of the weight losses after feeding of O. *surinamensis* on rice mixed with the essential *Citrus sinensis* oil.

Figure 2 shows that the concentration of 20000 ppm of orange was the most effect on the weight losses by *O. surinamensis*.

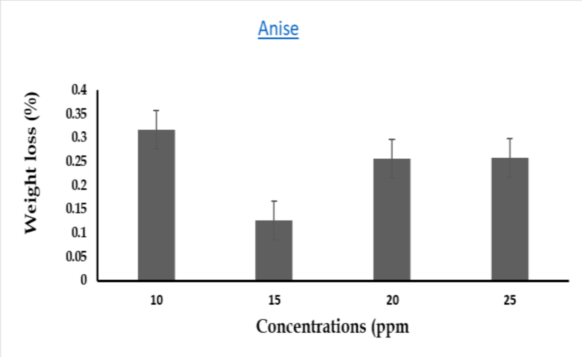

Fig. 3. The percentage of the weight loses after feeding of O. *surinamensis* on rice mixed with the essential *Pimpinella anisum* oil.

Figure 3 shows that the concentration of 10000 ppm of anise oil was the most effect on the weight losses by *O. surinamensis* and the percentage of weight loses was equaled in the concentrations of 20000 and 25000 ppm.

Discussion

Biological activity of the commercialized essential oils on agricultural arthropod pests and food storage pests have been well examined. The fumigant and contact activity of commercially manufactured essential oils of anise, cumin, and lemongrass against *O. surinamensis* adults were examined. After 72 hours of treatment, the three evaluated essential oils killed 90% of the stored insect pests, with anise and orange oils having stronger fumigant activities than cumin oil (Rani, 2012). Several previous investigations (Kéita *et al.,* 2001) have been reported that essential oils derived by steam distillation from the Guinean plants *Ocimum gratissimum* and *Ocimum basilicum* caused 80 and 70% mortality in *Callosobruchus maculatus* adults after they were exposed to a 12-hour fumigation. Basil oil exhibits concentration- and time-dependent oviposition prevention and ovicidal potentials against female Aedes aegypti mosquitoes (Khani and Rahdari 2012), and also *Tribolium confusum* species adults die at higher rates when exposed to coriander oil (Warikoo *et al.,* 2011). The use of essential oils as a fumigant for dried grains and legumes may become more significant when methyl-bromide is no longer in use. Plant extracts must be able to penetrate grain bulks in order to reproduce fumigant gases, however there is little evidence to back up this assertion. When thinking about using essential oils as a fumigant, it's also necessary to comprehend sorption and residue on the target grain (Lee *et al.,* 2002).

In this current study, after being exposed for 24 hours, two commercially available essential oils—orange and anise oils—exhibited potent fumigant and contact actions against *O. surinamensis* adults. Anise oil had more active in the contact assay, whereas orange oil demonstrated had higher activity in the fumigant trial. The principal constituents of both steam-distilled oils are limonene and methyl chavicol (estragole) (Kubeczka and Forácek, 2002, Lis-Balchin, 2006). Strong contact and fumigant activity was observed in the fruits of *Zanthoxylum schinifolium* Sieb. et Zucc against *S. zeamais*; estragol constituted a significant element (69.5%) of the essential oil (Wang *et al.,* 2011). Minor ingredients with beneficial effects were linalool, -myrcene, and -pinene. Terpenes and terpenoids, such as linalool, geraniol, menthol, carvacrol, borneol citral, citronellal, pulegone, and eugenol, are present in substantial proportions in many plant essential oils (Esen *et al.,* 2007, Kordali *et al.,* 2008, Kim *et al.,* 2010). The effectiveness of insect fumigants against *S. oryzae* and *T. castaneum* is enhanced by these volatile chemicals (Singh *et al.*, 1989; Prates *et al.,* 1998; Tripathi *et al.,* 2001; Lee *et al.,* 2002). Except for -humulene, the primary hazardous pathway for the other chemicals in our investigation was fumigation by vapour action. This is true even though certain elements of the two essential oils under study demonstrated fumigant as well as contact effects on *O. surinamensis* adults. It is therefore likely that the respiratory systems of insects are where these toxic mono- and sesquiterpene chemicals carry out their fumigant activity; more research will be needed to determine the precise mechanism of action. Octopaminergic, -aminobutyric acid, and acetylcholine esterases have been proposed as novel target sites for various essential oil components, albeit this has not been confirmed (Kostyukovsky *et al.,* 2002; Priestley *et al.,* 2003; Yeom *et al.,* 2012).

These findings and previous research outputs demonstrate that essential oils could be applied as fumigants or pesticides in the future to manage pests of stored commodities that reside in protected spaces such as buildings, greenhouses, and storage bins. Therefore, more investigation is needed into the safety of the essential oils and their constituents for the environment and human health before they may be employed in a practical way as novel fumigants. Furthermore, formulation research is necessary to minimize production costs, create standards for quality control, and boost the potency and stability of products based on essential oils.

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الانشطة المبخرة والسامة لثلاثة زيوت اساسية ضد خنفساء السورينام ***Oryzaephilus surinamensis*** **(L.) (coleoptera: Silvanidae)**

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الملخص

تعتمد طرق المكافحة في صوامع الحبوب المخزونة بشكل أساسي على مبيدات الآفات البديلة. التطور الحديث فى استخدام بدائل مبيدات الافات الصديقة للبيئة مثل الزيوت الاساسية تعتبر غاية اساسية الهدف منها هو تطوير استخدام ثلاثة انواع من الزيوت الاساسية الاقتصادية والتى لها نشاط سام بالتبخير والملامسة ضد الحشرات الكاملة لخنفساء السورينام . ونتائج هذة الدراسة بصفة عامة أوضحت ان زيادة التركيز وفترة التعرض لمثل هذة الزيوت الطيارة نتج عنه زيادة فى تأثير السمية ونقص فى وزن الحشرة.بالنسبة للتأثير السام لأبخره الزيوت فقد اوضحت النتائج أن زيت البرتقال أكثر فاعلية من زيت الينسون ضد الحشرات الكاملة لخنفساء السورينام .وأوضحت النتائج ان التأثير السام بالملامسة لزيت الينسون كان اكثر سمية من الزيوت الاخرى. وقد أظهرت نتائج هذة الدراسة ان الزيوت المختبرة تعتبر بدائل واعدة ضد الافات الحشرية للحبوب المخزونة اعتمادا على اطوار نموها