Fumigant toxicity of some essential oils against Red Flour Beetles, *Tribolium castaneum* (Herbst) and its safety to mammals

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(With 9 figures)

**Abstract**

Fumigant activities for three essential oils; Garlic oil (*Allium sativum* L); Chili pepper (*Capsicum annuum* L.) and Nigella (*Nigella sativa* L.) were assessed at different concentrations against the adult and 20-days old larval stages of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in the laboratory. The accumulative mortality was observed at different exposure periods (3, 5 and 7 days). The residual effect of garlic oil that was the effective oil, on the treated wheat grains was evaluated with respect to histological changes in the liver, kidney, and stomach of rat fed on this treated wheat. The results showed that the mortality rates of treated stages increased with increasing the time of fumigation treatment. Moreover the highest essential oils toxicity at the Median lethal concentration (LC$_{50}$) values for exposure periods (3, 5 and 7 days) to fumigation were (126, 53, and 47 mg/L air) for adult stage and were (79, 62, and 41 mg/L air) for larval stage, respectively in the case of Garlic oil treatment. While, the lowest essential oils effective was Nigella oil at the Median lethal concentration (LC$_{50}$) values for exposure periods (3, 5 and 7 days) to fumigation were (3594, 629, and 335 mg/L air) for adult stage and were (1040, 416, and 227 mg/L air) for larval stage, respectively.

The toxicity effect of various essential oils against adults and larvae of *T. castaneum* at the LC$_{50}$ at 7 days fumigation could be arranged in descending order as follows: Garlic oil, Chili pepper oil, and Nigella oil. The histological changes showed that the organs slightly affected at the fumigation for 3 days. It may be concluded that the garlic essential oil is the good effective fumigant to control *T. castaneum* in the stored products and it recommended that the fumigation period does not exceed 3 days. The garlic essential oil has the potential for applications in IPM programs for stored-grain pests because of its high volatility and fumigant activity and its safety.

**Keywords:** stored grains, essential oils, fumigation, *Tribolium castaneum*.

Toxicidade fumigante de alguns óleos essenciais contra besouros de farinha vermelha, *Tribolium castaneum* (Herbst) e sua segurança para mamíferos

**Resumo**

Atividades fumigantes de três óleos essenciais - óleo de alho (*Allium sativum* L.), pimenta-malagueta (*Capsicum annuum* L.) e Nigella (*Nigella sativa* L.) - foram avaliadas em diferentes concentrações contra adultos e larvas de *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) com 20 dias de idade em laboratório. A mortalidade acumulada foi observada em diferentes períodos de exposição (3, 5 e 7 dias). O efeito residual do óleo de alho, ou seja, o óleo eficaz, nos grãos de trigo tratados foi avaliado em relação às alterações histológicas no fígado, rim e estômago de ratos alimentados com esse trigo tratado. Os resultados mostraram que as taxas de mortalidade dos estágios tratados cresceram com o aumento do tempo de exposição ao tratamento de fumigação. A maior toxicidade dos óleos essenciais nos valores de Concentração Letal Média (CL$_{50}$) para os períodos de exposição (3, 5 e 7 dias) à fumigação foi (126, 53 e 47 mg/L ar) para a fase adulta e (79, 62 e 41 mg/L ar) para a fase de larva, respectivamente no caso do tratamento com o óleo de alho, enquanto que o óleo essencial menos eficaz foi o óleo de Nigella, com valores de CL$_{50}$ para os períodos de exposição (3, 5 e 7 dias) à fumigação (3594, 629 e 335 mg/L ar) para a fase adulta, e (1040, 416 e 227 mg/L ar) para a fase de larva, respectivamente. O efeito da toxicidade de vários óleos essenciais contra adultos e larvas de *T. castaneum* em LC$_{50}$ aos sete dias de fumigação pôde ser organizado em ordem decrescente, como segue: óleo de alho, óleo de pimenta e óleo de Nigella. As alterações histológicas mostraram que os órgãos foram levemente afetados na fumigação por três dias. Pode-se concluir que o óleo essencial de alho é um bom fumigante efetivo no controle de *T. castaneum* nos produtos armazenados, e recomendou-se que o período de fumigação não ultrapasse três dias. O óleo essencial de alho tem potencial para aplicações em programas de Integrated Pests Management (IPM) para pragas de grãos armazenados, devido à sua alta volatilidade, atividade fumigante e respectiva segurança.

**Palavras-chave:** grãos armazenados, óleos essenciais, fumigação, *Tribolium castaneum*.
1. Introduction

Insect pests of stored products are responsible for considerable economic losses to stored grains. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is an important pest of stored grains in Egypt. It presents secondary eating habits and is cosmopolitan, can attack different products, as like flour, bran, feed, grain, biscuits, etc. (Trematerra and Sciarratta, 2004; Daglish, 2006). Control of this insect relies heavily on the use of synthetic insecticides and fumigants, which has led to problems to the environment. This included, increases costs of application, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms in addition to direct toxicity to users (Muniz et al., 2008; Boyer et al., 2012; Darwish et al., 2015; El-Gizawy, 2012). Nowadays, the plants are tested in the form of powder, vegetable oil; essential oil (EO), aqueous and organic extracts in the laboratories against the insect pests (Boeke et al., 2004). There are several scientific reports that describe various biological effects which include toxic, repellent and anti-feeding effects (Jacobson, 1990; Koul et al., 2008; Mossa, 2016).

Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect-control agents. Although many studies have addressed the potential toxicity of essential oils as protectants for stored products, the residue effect studies of some of them are still required. Therefore, the present study was conducted to evaluate the effect of the toxicity of some essential oils against the two stages of *T. castaneum* and identify the impact of effective once which exhibits residue on treated wheat on histological changes in some rat organs.

2. Material and Methods

2.1. Insect culture

*Tribolium castaneum* was reared in glass containers (250 mL) containing wheat flour covered with a fine mesh cloth for ventilation. The cultures were maintained in the dark in an incubator at 28 ± 2 °C and 60 ± 5% RH (Ayvaz et al., 2002). Adults were obtained from laboratory stock cultures maintained at the Plant Protection Dept. Faculty of Agric., Moshtohor, Benha University, Egypt.

2.2. Essential oils

Three essential oils belonging to different families; Amaryllidaceae, Solanaceae; and Ranunculaceae were used in this study. All the essential oils were bought from Al-Gomhuria Company of drugs, chemicals, and medical supplies in Egypt. The used oils were Garlic oil (*Allium sativum* L.); Chili pepper (*Capsicum annuum* L.); and Nigella (*Nigella sativa* L.). The fumigant toxicity of these oils was tested to the 7-days old adults and 20-days old larvae of *T. castaneum* at 28 ± 2 °C and 60 ± 5% RH.

2.3. Fumigant toxicity of essential oils against the insect pest stages

Ten grams from each pure oil were diluted with 50 mL acetone to obtain 20% (w/v) stock solution which was diluted to obtain 0.625, 1.25, 2.5, 5, and 10% (w/v) concentrations. In this experiment, 200 ml glass jars with tinted covers were used as fumigation chambers for the plant oil. The tested dosages of oil inside the jars were 62.5, 125, 250, 500, and 1000 mg/L air. Tested essential oils on mentioned concentrations were applied on filter paper individually and were inserted at the bottom of the jars. For every jar, one filter paper was inserted at the bottom and six jars were used in each treatment. Thirty adults or larvae were put inside each jar in cotton bags (2×1 cm) with a few amounts of crushed wheat. The jars were well closed and incubated at 27 ± 2 °C and 65 ± 5% R.H. Three replicates (30 larva or adults per replicate) were used for each treatment and control, thus we thought that this numbers are enough to make block based experimental design. The same steps were followed in the control treatment using only acetone without oil. The mortality of larvae and/or adults was calculated for the exposure times 3, 5, and 7 days after fumigation treatment. The insect mortality data was corrected by Abbott’s (1925) formula.

2.4. Histological study

Twenty-seven male Albino Wister rats, weighing 150 ± 25 g were obtained from Rodents laboratory at Faculty of Agriculture, Moshtohor, Benha University. The used oils were Garlic oil (*Allium sativum* L.); Chili pepper oil (*Capsicum annuum* L.); and Nigella (*Nigella sativa* L.). The effect of the three plant oils Garlic oil (*Allium sativum*), Chili pepper oil (*Capsicum annuum*), and Nigella oil (*Nigella sativa*), on *T. castaneum* was summarized in Table 1. The probit statistics estimates of sublethal concentrations and their 95% confidence limits and the
Table 1. The median lethal concentrations and their confidence limits of tested oils against *Tribolium castaneum* (Herbst) stages.

| Exposure period (days) | Stage | Garlic oil (*Allium sativum*) | Chili pepper oil (*Capsicum annuum*) | Nigella oil (*Nigella sativa*) |
|-----------------------|-------|-------------------------------|-------------------------------------|--------------------------------|
|                       |       | **LC₅₀ mg/L air** | **Slope ± SD** | **R** | **LC₅₀ mg/L air** | **Slope ± SD** | **R** | **LC₅₀ mg/L air** | **Slope ± SD** | **R** |
| 3 days                | adult | 126 (69-232) | 0.82 ± 0.009 | 0.992 | 194 (132-287) | 1.12 ± 0.12 | 0.949 | 3594 (618-20878) | 0.75 ± 0.02 | 0.980 |
|                       | larvae| 79 (47.34-132) | 1.25 ± 0.06 | 0.978 | 97 (67-140) | 1.58 ± 0.40 | 0.921 | 1040 (467-2316) | 0.93 ± 0.06 | 0.962 |
| 5 days                | adult | 53 (26-104) | 1.19 ± 0.008 | 0.996 | 96 (58-160) | 1.14 ± 0.17 | 0.933 | 629 (372-1064) | 1.08 ± 0.01 | 0.993 |
|                       | larvae| 62 (43.72-88) | 2.28 ± 0.30 | 0.969 | 71 (46-108) | 1.68 ± 0.28 | 0.949 | 416 (267-649) | 1.07 ± 0.02 | 0.985 |
| 7 days                | adult | 47 (28-78) | 2.06 ± 0.34 | 0.956 | 67 (42-108) | 1.54 ± 0.11 | 0.974 | 335 (226-498) | 1.11 ± 0.03 | 0.986 |
|                       | larvae| 41 (24-70) | 2.17 ± 0.03 | 0.996 | 58 (40-83) | 1.33 ± 0.31 | 0.996 | 227 (147-350) | 0.98 ± 0.06 | 0.963 |

R = Correlation Coefficient of regression line; SD = Standard deviation of the mortality regression.
The toxicity effect of various essential oils against adults and larvae of *T. castaneum* at the LC$_{50}$ and LC$_{95}$ at 7 days exposure period could be arranged in descending order as follows: Garlic oil, Chili pepper oil and Nigella oil which was the least effective (Table 2). The results indicated also that Garlic oils were much more effective as fumigants against *T. castaneum* adult more than against the larvae. For both adults and larval stages the toxicities of Chili pepper oil was similar to that of Garlic oil, while, Nigella oil was shown a much lower level of activity. The relative toxicity was up to 18.3, and 3.0% at the LC$_{50}$ and LC$_{95}$ values respectively, for adult stage While it was 14.1, and 2.3% at the LC$_{50}$ and LC$_{95}$ values respectively for larvae stage when compared with Garlic oil (100%).

Our results on fumigant toxicity of *Allium sativum* and *Capsicum annum* on *Tribolium castaneum* (Herbst) larval and adult stages indicated that these essential oils had good fumigation toxicity. These findings relate with Mobki et al. (2014) who reported that mortality of *T. castaneum* increased with increasing garlic extract with concentration and time. The concentration of 225.8 µl/l air of the garlic extract generated 83.3% larval mortality after 48 hours. The current findings are in partial resemblance with the results obtained by Huang et al. (2000) who stated that the contact and fumigant toxicities of compounds extracted from garlic, *A. sativum* were greater against the adults. These two compounds (methyl allyl disulfide and diallyl trisulfide) of garlic were also more toxic to *T. castaneum* adults than to *S. zeamais*. Mona et al. (2009) reported similar results that garlic (*A. sativum*), mint (*Mentha piperita*), basil (*Ocimum basilicum*), thyme (*Thymus vulgaris*), sesame (*Sesamum indicum*) and chamomile (*Chamaemelum nobile*) plant extracts showed both toxicity and repellency actions against *T. castaneum*.

### 3.3. Histological studies of the effect of LC$_{50}$ Garlic oil on some rat organs

#### 3.3.1. Liver

The histological examination of liver tissues in rats fed on fumigant or non-fumigant wheat grains were presented in Figures 1-3. The control animals showed a normal histological structure and, minimal fatty change but no liver cell degeneration (Figure 1). Compared to the liver of rats of the control group, the animals’ group was tested after feeding on the wheat fumigated for 3 days, the liver tissues showed slight histological changes. In these liver tissues, some hepatocytes appeared as fat cells of empty cytoplasm with peripheral nuclei while the other hepatocytes showed normal histological features (Figure 2).

### Table 2. Toxicity index of various essential oils against the *Tribolium castaneum* (Herbst) stages for fumigation period at 7 days.

| Insect pest stage | Essential oils       | LC$_{50}$ mg/L air | LC$_{95}$ mg/L air | Relative toxicity at LC$_{50}$ | Folds       |
|-------------------|----------------------|--------------------|--------------------|-------------------------------|-------------|
|                   |                      |                    |                    | LC$_{50}$ | LC$_{95}$ | LC$_{50}$ | LC$_{95}$ | LC$_{50}$ | LC$_{95}$ |
| Adult             | Garlic oil           | 41.7               | 297                | 100.0   | 100      | 5.5      | 33.4      |
|                   | Chili pepper oil     | 58.4               | 783                | 71.4    | 37.9     | 3.9      | 12.7      |
|                   | Nigella oil          | 227.8              | 9918               | 18.3    | 3.0      | 1.0      | 1.0       |
| 20-days old larvae| Garlic oil           | 47.5               | 238                | 100.0   | 100      | 7.1      | 43.7      |
|                   | Chili pepper oil     | 67.9               | 276                | 70.0    | 86.2     | 4.9      | 37.4      |
|                   | Nigella oil          | 336.0              | 10394              | 14.1    | 2.3      | 1.0      | 1.0       |

The relative toxicity was calculated as [(the least LC$_{50}$ value) / (LC$_{50}$ value of other oils)] × 100.
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At five days of treatment, the affected hepatocytes increased showing multiple large lipid vacuoles representing severe fatty degeneration while normal hepatocytes decreased (Figure 3). Because of the multiple vacuoles and empty cytoplasm, the glycogen content in livers of this group showed a lower amount in comparison to control liver.

3.3.2. Kidney

The histological section on the kidneys of rats which fed on non-fumigated wheat grains treatment, which showed renal tubules both Proximal tubule (PCT), Distal tubule (DCT), also Glomerula, Parietal layer, Visceral layer and Collecting tubule (Figure 4) the histological section on the kidneys in this group showed no histological abnormalities. In rat which was fed on fumigated wheat treatment for 3 days, some degenerative changes were noticed mainly fatty degeneration in renal tubules around glomerulus Figure 5. Concerning the rat feeding on fumigated wheat treatment for 5 days, the histological section on the kidneys showed moderate degenerative changes in renal tubules with degenerated material and desquamated cells in the lumen of tubules Figure 6.

3.3.3. Stomach

Photomicrograph of rat Stomach in control group showed no histological abnormalities, the mucous secreting cells were at the neck of glands only (Figure 7). While the
Figure 4. Photomicrograph of normal histological structure of kidney in control group which showed the glomeruli (A), Parietal layer (B), Visceral layer (C), Proximal tubule (D), Distal tubule (E) and Collecting tubule (F) Masson’s trichrome stain.

Figure 5. Photomicrograph of rat kidney which feeds on fumigated wheat treatment for 3 days showed degenerative changes mainly fatty degeneration (FD) in renal tubules around glomerulus (H&E stain).

Figure 6. Photomicrograph of rat kidney which feeds on fumigated wheat treatment for 5 days showed degenerative changes in renal tubules with degenerated material and desquamated cells (DC) in the lumen of tubule (H&E stain).

Figure 7. Photomicrograph of control stomach showed Serosa (S), Tunica muscularis (TM), Lamina propria (LP), Epithelium (E), and mucous secreting cells (MS) at the neck of glands only. PAS technique.
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Stomach in the animals fed on fumigated wheat treatment for 3 days there is an increase of inflammatory mononuclear cellular infiltration in submucosa just beneath the gastric glands (Figure 8). Moreover, the stomach in the group which was fed on fumigated wheat treatment for 5 days showed an increase of mucous secreting cells which appeared in 2 levels; one was at the neck of glands and the other was at the mid of glands. These mucous secreting cells showed strongly PAS positive reaction (Figure 9). Although the fumigation with Garlic oil appeared to be safe in the 3-days exposure period, it exhibited some toxic effects in the 5-days exposure period of the study period. Where, the microscopic examinations of the different tissues (liver, kidney, and stomach) showed slightly changes especially, at the short exposure time. In the liver, mild to severe necrosis of the hepato cells of animals fed on fumigated wheat with the garlic oil was observed in this study. Necrosis of the liver has been reported by Sudakin and Power (2007) and Turkez and Togar (2013) who concluded that inhalation of phostoxin may cause severe pulmonary irritation leading to acute pulmonary edema, renal and hepatic damage. Disruption of the liver cell as reflected by the altered morphological structure is therefore suggested as the cause of raised serum level of liver enzymes as previously reported (Jimoh and Odutuga, 2001). It was reported that the phostoxin are causing activated signals lead to increase necrosis factors resulted inflammation, malignancy and cell death (Arora et al., 1995; Sinha et al., 2005; Saleki et al., 2007). In addition, Ozmen et al. (2009) and Mogilner et al. (2009) conducted the histological changes after exposure to CO2 as a result of hypoxia and/or ischemia that lead to acute cellular swelling, cytoplasmic vacuoles, hydropic degeneration, focal hemorrhage and epithelial cells within the tubular lumens. It could be concluded that the Garlic essential oil was the most effective oil to control T. castaneum based on the LC50 value.

And based on the histological findings Garlic essential oil was safe to protect the stored products as a fumigant, provided as a treatment of not more than 3-days.

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Figure 8. Photomicrograph of treated stomach showed a number of inflammatory mononuclear cellular infiltration (A) in submucosa just beneath the gastric glands (H&E stain).

Figure 9. Photomicrograph of treated stomach showed Submucosa increase of mucous secreting cells that appeared in 2 levels; one was at the neck of glands (A) and the other was at the mid of glands (B). PAS-technique.
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