Evaluation of Six Plant Essential Oils against Three Stored Product Insects and Their Effects on the Haemogram under Laboratory Conditions

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ABSTRACT

The effect of six plant essential oils against three stored grain insects, *Sitophilus oryzae, Rhizopertha dominica* and *Tribolium castaneum* were studied under laboratory conditions. The tested oils are namely, fennel oil (*Foeniculum vulgare*), caraway oil (*Carum carvi*), cinnamon oil (*Cinnamomum verum*), citronella oil (*Cymbopogon winterianus*), nutmeg oil (*Myristica fragrans*) and black cumin oil (*Nigella sativa*). The result showed that increasing of the oil concentration and exposure times significantly increased the mortality percentage.

Generally, the toxicity of essential oils at all tested concentrations increased with the increasing of exposure time. So, the results indicated that toxicity of all tested essential oil against the three stored grain insects at the high concentration was recorded high mortality faster than low concentration.

The tested essential oils significantly decreased the different haemocyte counts in tested insects, cinnamon oil markedly increased the haemocyte surface areas in the tested insects especially the surface area of oenocytoides in *T. castaneum.*

The recorded results indicate that the essential oils of fennel, caraway, cinnamon and citronella could be applicable to the management of *S. oryzae, R. dominica* and *T. castaneum* adults.

Key words: *Sitophilus oryzae, Rhizopertha dominica*, *Tribolium castaneum*, plant essential oils, haemocytes.

INTRODUCTION

Stored product insects have been considered serious pests over the world. They cause great losses in weight and quality of the stored products (Pugazhvendan *et al*., 2009). Losses of grain in storage due to insects are main components of the insect damage in agricultural production like several crops, wheat, rice, pasta, beans, nuts and many other crops. The stored products are liable to be attacked by many coleopteran insect pests; e.g. red flour beetle (*Tribolium castaneum*), rice weevil beetle (*Sitophilus oryzae*) and lesser grain borer (*Rhizopertha dominica*).

Control of stored product insects depend strongly on the use of synthetic insecticides and fumigants, which has led to problems such as environmental disturbances, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms, in addition to direct toxicity to users (Isman 2006). Biological control, such as green pesticides, may be an effective way for stored-product pest management. Green or botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules (Isman, 2008).

Plant essential oils in general have been recognized as an important natural resource of insecticides (Gbolade *et al*., 2000), which are often responsible for a plant distinctive scent or taste. Essential oils extracted from more than 75 plant species have been evaluated for fumigant toxicity against stored product insects. Fumigant toxicity tests conducted with essential oils of plants (mainly belonging to Apiaceae, Lamiaceae, Lauraceae and Myrtaceae), which have strongly effect on stored product insects such as *T.castaneum*, *R. dominica*, *S. oryzae* and *S. zeamais* (Rajendran and Srianjini, 2008). There are 17.500 aromatic species that occur in higher plants (Bruneton, 1999). The plant essential oils major constituents, monoterpenes, are also of interest because of their toxicity to insects and other potent biological activities (Kubo *et al*., 1994; Basilico and Basilico, 1999). Plant essential oils and their components have been shown to possess potential for development as new fumigants and they may have advantages over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (Isman, 2008).

The present study aims to evaluate the efficacy of six plant essential oils against three stored insects *S. oryzae, R. dominica* and *T. castaneum*, and their effects on haemolymph cells.

MATERIALS AND METHODS

1- Insect culture

Adults of rice weevil (*Sitophilus oryzae*), lesser grain borer (*Rhizopertha dominica*) and red flour beetle (*Tribolium castaneum*), were collected from infested grains and flour from local market, and

separately rearing under laboratory conditions of 30 ±2 °C and 70-75% R.H.

1.1- Culture of *Sitophilus oryzae* and *Rhizopertha dominica*.

The adults of *S. oryzae* and *R. dominica* collected from infested grains were brought to the laboratory and kept in plastic jar (32 X 50 cm). Wheat was sterilized by heating at 70 °C for 1 hr., then cooled and allowed to reabsorb before use. After that, it was transferred to separate jar until depth of 10 cm. Adults (200 - 400 insects) were collected from the infested grains and added into each jar, which contain sterilized culture of wheat. All jars were painted by aband of Vaseline around the inside edge of the jar to prevent the escaping of insects, and sealed with muslin. The jars were placed at room temperature about (30 ±2 °C) and relative humidity of 70-75%. After two weeks; insects were sieved out and transferred to another jar. Then, adults were used for experimental work, according to El-Disouky (2002).

1.2- Culture of *Tribolium castaneum*.

The adult of *T. Castaneum* collected from infested wheat flour was brought to the laboratory and placed in plastic jar (32 X 50 cm). *T. Castaneum* kept in a media consists of four parts of wheat flour and one part of brewer's yeast. The whole wheat flour has been sieved to remove all of the large particles in the flour. This culture will be maintained at the same previous conditions.

2- Source of essential oils

Six of the tested plant essential oils, e.g. fennel oil (*Foeniculum vulgare*), caraway (*Carum carvi*), cinnamon (*Cinnamomum verum*), citronella (*Cymbopogon winterianus*), nutmeg (*Myristica fragrans*) and black cumin (*Nigella sativa*) were provided by Faculty of pharmacy, Alexandria University (Table 1).

3- Bioassay tests

3.1- Evoluation of plant essential oils against three stored insects.

The efficacy of six plant (Table 1) essential oils was evaluated against the adults of *S. oryzae*, *R. dominica* and *T. Castaneum* at different concentrations. All concentrations of tested essential oils were diluted in acetone. The contact toxicity of essential oils was tested by using residual film technique and appllied directly on petri dish (9 cm) without any grains according to Qi and Burkholder (1981). One ml. of each concentration was pipetted in Petri-dish. After evaporation of acetone, 20 adults were placed in each Petri-dish. Four replicates were carried out of each concentration and control. Mortality percentage was recoded after 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days. The percentage of mortality was corrected for control mortality using Abbott's formula (Abbott, 1925). Toxicity factor was calculated as following:-

Toxicity factor = LC_{50} of the compound / LC_{50} of more toxic compound

3.2- Effects of the essential oils on the haemocytes of the tested insects.

The haemocytes of adults were examined by light microscope. Differential haemocytes count (DHC) was determined in citronella oil at concentrations of 0.0037, 0.0322 and 0.052 mg/cm^2 for *S. oryzae, R. dominica* and *T. castaneum*, respectively. Also, cinnamon oil at concentrations of 0.0063, 0.0072 and 0.0064 mg/cm^2 for *S. oryzae, R. dominica* and *T. castaneum*, respectively. While, caraway oil examined at concentrations of 0.008, 0.028 and 0.041 mg/cm^2 on *S. oryzae*, *R. dominica* and *T. castaneum,* respectively. Fennel oil at concentrations of 0.0098, 0.012 and 0.019 mg/cm^2 on *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. Furthermore, nutmeg oil examined at concentrations of 0.027 and 0.041 mg/cm^2, finally black cumin at concentrations of 0.028 and 0.032 mg/cm^2 on *S. oryzae* and *R. dominica*, respectively.

Blood samples obtained from 5 adults of each treated insects with tested oils and control was prepared for (DHC) (Gad, 2006). Each blood sample was replicated five times. The appropriate number of each tested oil and control were separately smeared to thin film between two glass slides.

Table 1: Plant essential oils; common name, scientific name; family, and series of concentrations used in the study.

Common name	Scientific name & Family	Concentrations used (mg / cm^2)
Fennel	*Foeniculum vulgare* (Apiaceae)	0.004, 0.008, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06
Caraway	*Carum carvi* (Umbelliferae)	0.004, 0.008, 0.01, 0.03, 0.05, 0.06, 0.08, 0.09 and 0.11
Cinnamon	*Cinnamomum verum* (Lauraceae)	0.004, 0.008, 0.01, 0.02, 0.03 and 0.04
Citronella	*Cymbopogon winterianus* (Poaceae)	0.004, 0.006, 0.008, 0.01, 0.02, 0.03, 0.05, 0.08, 0.09, 0.11 and 0.13
Nutmeg	*Myristica fragrans* (Myristicaceae)	0.008, 0.01, 0.03, 0.05, 0.06, 0.08, 0.09, 0.11 and 0.13
Black cumin	*Nigella sativa* (Ranunculaceae)	0.004, 0.006, 0.008, 0.01, 0.03, 0.05, 0.06 and 0.08

The smeared blood were air dried, stained with Wright's blood stain (Martha and Hachiro, 1971) for 1 minute and distained for 2 min. with 70% ethyl alcohol.

The blood cell types were examined and identified under oil immersion (100x) using a light microscope. DHC were carried out in random scan of blood films (approximately 100 haemocytes for each film). The identification of haemocytes types were performed according to Arnold (1974). Haemocyte surface areas were measured by micrometric slide.

4- Statistical analysis

All the tested criteria were statistically analyzed compared using (F) test and Least Significant Differences (L. S. D) at 0.01 probability level, (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

1- Effect of six essential oils against three stored grain insects.

1.1- *Sitophilus oryzae*

Mortality percentages of six essential oils on the *S. oryzae* adults were evaluated at different concentrations after different exposure times by using a residual film method. The median lethal concentration (LC_{50} values) and the slope values were calculated.

Results showed that the fennel oil at concentration of 0.01 mg/cm^2 was achieved 100% mortality after 10 days of exposure. The same effect with the caraway oil and nutmeg oil at low concentrations of 0.008 and 0.01 mg/cm^2. While, the concentration of 0.008 mg/cm^2 of citronella oil and black cumin oil gave the same percentage after 8 days of exposure.

On the other hand, the toxic effects with the high concentrations of fennel oil 0.05 and 0.06 mg/cm^2 were achieved 100% mortality after 5 days of exposure. Also, the same trend with the caraway oil at concentrations of 0.05, 0.06, 0.08, 0.09 and 0.1 mg/cm^2. Citronella oil at concentration of 0.08 mg/cm^2 gave the same percentage after the 2nd day. Approximately, cinnamon oil was achieved the best toxicity against the *S. oryzae*. It was caused 100% mortality at concentration of 0.01 mg/cm^2 after 8 days of exposure compering with the other tested essential oils.

Generally, the toxicity of essential oils at all tested concentrations increased with the increasing of exposure time. Hence, the results indicate that toxicity of essential oil against *S. oryzae* at the high concentration was recorded 100% mortality faster than low concentration.

The mentioned results showed that the toxicity of all tested oils against *S. oryzae* was depended on oil concentration and time of exposure. According to the LC_{50} values after 6 days of exposure (Table 2), cinnamon oil was more toxic than caraway oil by about 8.75 times. The desending order of toxicity as following: cinnamon, black cumin, citronella, nutmeg, fennel and caraway oil, with the LC_{50} values 0.0012, 0.0047, 0.0050, 0.0053, 0.0059 and 0.0105 mg/cm^2, respectively.

1.2 - *Rhizopertha dominica*

The tested essential oils caused more mortality in high concentrations and the toxicity was increased with the increasing of exposure time. Fennel oil at concentrations of 0.06 and 0.05 mg/cm^2 achieved 97.5% and 95% mortality after 1 day of exposure, respectively. While, Fennel oil at concentration of 0.04 mg/cm^2 caused 93.8% mortality after 6 days of exposure. In respect of the caraway oil, the results revealed that the toxicity at the high concentration of 0.11 mg/cm^2 was recorded a high mortality 90% and 98.8% after 2 and 10 days of exposure, respectively. Only, citronella oil at the same concentration achieved 100 % mortality after 7 days of exposure. While, cinnamon oil was gave the same mortality percentage at concentrations of 0.03 and 0.04 mg/cm^2 after 9 days exposure.

On the other hand, the toxicity of caraway oil and black cumin oil at the low concentration of 0.03 mg/cm^2 achieved 90% mortality after 10 days of exposure, respectively. But, nutmeg oil was gave the same percentage at concentrations of 0.09 and 0.13 mg/cm^2 after 10 and 6 days of exposure, respectively. While, black cumin oil at concentration of 0.08 mg/cm^2 achieved 98.8% mortality after 10 days exposure and 95% after 6 days of exposure.

Table 2: Relative toxicity of six essential oils against *S. oryzae* after 6 days of exposure.

Essential oils	LC_{50} (mg/cm^2)	Confidence limits		Slope ± SE	Toxicity factor
		Lower	Upper		
Cinnamon	0.0012	0.0001	0.0026	0.96 ± 0.24	1.00
Black cumin	0.0047	0.0037	0.0053	2.90 ± 0.5	3.92
Citronella	0.0050	0.0042	0.0056	3.10 ± 0.5	4.17
Nutmeg	0.0053	0.0018	0.0086	1.05 ± 0.2	4.42
Fennel	0.0059	0.0047	0.0070	2.08 ± 0.3	4.92
Caraway	0.0105	0.0087	0.0135	1.80 ± 0.3	8.75

Relative toxicity of six essential oils against *R.dominica* shown according to the LC_{50} values after 6 days of exposure, cinnamon oil was the most toxic oil. The desending order of toxicity as following: cinnamon, fennel, caraway, citronella, black cumin and nutmeg oil, with the LC_{50} values 0.0074, 0.014, 0.029, 0.029, 0.035 and 0.063 mg/cm^2, respectively (Table 3). At the same trend in *S. oryzae,* the toxicity of all tested oils against *R.dominica* was depended on concentration and time of exposure.

1.3- *Tribolium castaneum*

The essential oils were evaluated against the adult of *T. castaneum* by using the residual film method. *T. castaneum* has shown tolerance to the nutmeg oil and the black cumin oil until 1000 mg/cm^2. The mortality percentage was recorded after 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. Also, the median lethal concentration (LC_{50}) and the slope values were calculated.

The effect of fennel oil against *T. castaneum* showed high mortality with high concentration and after exposure times. Fennel oil at concentrations of 0.06 and 0.05 mg/cm^2 achieved 80% and 78.8% mortality, respectively, after 10 days of exposure. The same trend, were observed with caraway oil, which gave the same mortality percentage (80%) at the high concentration of 0.11 mg/cm^2 after 8 days of exposure. While, citronella oil at the same concentration (0.11 mg/cm^2) achieved 95% mortality after 9 days of exposure. On the other hand, cinnamon oil was recorded 91.3% mortality at concentrations of 0.04 and 0.03 mg/cm^2 after 1 day and 10 days of exposure, respectively.

Results in (Table 4) showed relative toxicity of four tested essential oils against *T. castaneum*. Based on the LC_{50} values after 6 days of exposure, cinnamon oil was more toxic than fennel oil by about 2.74 times, 3.58 than citronella and 6.06 times than caraway oil, with the LC_{50} values 0.0125, 0.0343, 0.0447 and 0.0758 mg/cm^2, respectively.

In concoulison, citronella oil was more toxic against *S. oryzae* than *R. dominica* and *T. castaneum* by about 5.8 and 8.8 times, respectively (Table 5). While, the treatment with cinnamon oil against *S. oryzae* was more toxic than *R. dominica* and *T. castaneum* by about 6.2 and 10.4 times, respectively (Table 6). On the other hand, the caraway oil was more toxic against *S. oryzae* than *R. dominica* and *T. castaneum* by about 2.8 and 7.2 times, repectively (Table 7). While, the effect of fennel oil was more toxic against *S. oryzae* than *R. dominica* and *T. castaneum* by about 2.4 and 5.8 times, respectively (Table 8). Only, the nutmeg oil and black cumin oil affected on *S. oryzae* and *R. dominica*. The treatment with nutmeg oil and black cumin oil against *S. oryzae* were more toxic than *R. dominica* by about 11.9 and 7.5 times, respectively (Tables 9 and 10).

Table 3: Relative toxicity of six essential oils against *R. dominica* after 6 days of exposure.

Essential oils	LC_{50} (mg/cm^2)	Confidence limits		Slope ± SE	Toxicity factor
		Lower	Upper		
Cinnamon	0.0074	0.0057	0.009	1.60 ± 0.23	1.00
Fennel	0.014	0.010	0.017	3.00 ± 0.70	1.89
Caraway	0.029	0.020	0.035	2.20 ± 0.37	3.92
Citronella	0.029	0.021	0.041	0.72 ± 0.17	3.92
Black cumin	0.035	0.028	0.044	1.30 ± 0.20	4.73
Nutmeg	0.063	0.057	0.069	3.50 ± 0.47	8.51

Table 4: Relative toxicity of four essential oils against *T. castaneum* after 6 days of exposure.

Essential oils	LC_{50} (mg/cm^2)	Confidence limits		Slope ± SE	Toxicity factor
		Lower	Upper		
Cinnamon	0.0125	0.011	0.014	2.5 ± 0.21	1.00
Fennel	0.0343	0.031	0.037	2.9 ± 0.30	2.74
Citronella	0.0447	0.038	0.051	1.9 ± 0.22	3.58
Caraway	0.0758	0.070	0.081	3.7 ± 0.34	6.06

Table 5: Relative toxicity of citronella oil against three stored insects after 6 days of exposure.

Insects	LC_{50}(mg/cm^2)	Confidence limits		Toxicity factor
		Lower	Upper	
S. oryzae	0.005	0.004	0.005	1.0
R. dominica	0.029	0.021	0.041	5.8
T. castaneum	0.044	0.007	0.022	8.8

Table 6: Relative toxicity of cinnamon oil against three stored insects after 6 days of exposure.

Insects	LC_{50}(mg/cm^2)	Confidence limits		Toxicity factor
		Lower	Upper	
S. oryzae	0.0012	0.0001	0.0026	1.0
R. dominica	0.0074	0.0057	0.009	6.2
T. castaneum	0.0125	0.011	0.014	10.4

Table 7: Relative toxicity of caraway oil against three stored insects after 6 days of exposure.

Insects	LC_{50}(mg/cm^2)	Confidence limits		Toxicity factor
		Lower	Upper	
S. oryzae	0.0105	0.0087	0.013	1.0
R. dominica	0.029	0.020	0.035	2.8
T. castaneum	0.0758	0.070	0.081	7.2

Table 8: Relative toxicity of fennel oil against three stored insects after 6 days of exposure.

Insects	LC_{50}(mg/cm^2)	Confidence limits		Toxicity factor
		Lower	Upper	
S. oryzae	0.0059	0.0047	0.0070	**1.0**
R. dominica	0.014	0.010	0.017	**2.4**
T. castaneum	0.0343	0.031	0.037	**5.8**

Table 9: Relative toxicity of nutmeg oil against *S. oryzae* and *R. dominica* after 6 days of exposure.

Insects	LC_{50}(mg/cm^2)	Confidence limits		Toxicity factor
		Lower	Upper	
S.oryzae	0.0053	0.0018	0.0086	1.0
R. dominica	0.063	0.057	0.069	11.9

Table 10: Relative toxicity of black cumin oil against *S. oryzae* and *R. dominica* after 6 days of exposure.

Insects	LC_{50}(mg/cm^2)	Confidence limits		Toxicity factor
		Lower	Upper	
S. oryzae	0.0047	0.0037	0.0053	1.0
R. dominica	0.035	0.028	0.044	7.5

In the present study, the contact toxicity of six essential oils (fennel, carway, cinnamon, citronella, black cumin and nutmeg) using a residual film method were evaluated against the main three stored-products pests, *S. oryzae*, *R. dominica* and *T. castaneum* adults. Similar investigations are reporte by several authors, Kim *et al*., (2003) achieved a good insecticidal activity against *Lasioderma serricorne* adults with extracts of some parts of plants such as cinnamon (*Cinnamomum cassia*) bark and fennel (*Foeniculum vulgare*) fruit. Kim *et al*., (2001) demonestreated that the *F. vulgare* fruit-derived materials could be useful for managing populations of three coleopteran stored-product insect. Also, Chaubey (2008) compared between seven extracted of essential oils, he found that black cumin oil (*Nigella sativa*) was the most potent oil against the coleopteran insect *Callosobruchus chinensis* by fumigation. The essential oils caused chronic toxicity as the insect fumigants, which caused less damage to the stored grains. In addition, the toxicity of essential oils to stored-product insects was influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used (Don-Pedro, 1996 and Lee *et al*., 2001). A large number of powders and essential oils from natural products have been used as bio-pesticides against different insect pests since they present no risk to humans and the environment, unlike more conventional pesticides (Jayasekara *et al*., 2005). The growth-inhibiting, reproduction retarding, and repellent effects of bio-pesticides have also been explained against storage pests (Tunc *et al*., 2000 and Tripathi *et al*., 2002).

Results of the present study showed that the toxicity of all tested oils depended on the concentration and time of exposure. Based on the LC_{50} values after 6-days of exposure, all tested oils were effective on *S. oryzae* and *R. dominica*. But, toxicity of four tested oils except nutmeg oil and black cumin oil on *T. castaneum* were demonstrated in the results. Previous studies reported that adults of *T. castaneum* were more susceptible to essential

oils or their components than those of other insect species. By Shaaya *et al*., (1997) evaluated the fumigant toxicity of a large number of essential oils extracted from various species and herb plants against several major stored-product insects. *T. castaneum* was found to be the most resistant, compared with *S. oryzae*, *R. dominica* and *Oryzaephilus surinamensis*, to most tested essential oils. Also, the essential oils were found to be toxic when applied topically or by fumigation. So, the oils may be explored as a potential natural insecticide towards *T. castaneum* because of their high repellency and insecticidal activities (Zapata and Smagghe, 2010). Other results are reporte by Al-Hadidi *et al*., (2014) who compared between four plant extracts against *T.castaneum*. They found that the cinnamon oil and nutmeg oil gave excellent results against the *T. castaneum* using the plant powder. In contrast, Padin *et al*., (2000) evaluated the repellence, attractance, and mortality of essential oils from several herb species cultivated in Argentina on the stored grain pests *S. oryzae* and *T. castaneum*. No repellent activity was observed with any of the essential oils tested.

Finelly, The essential oil of plants may contain hundreds of different constituents but certain components will be present in larger quantities. Among the essential oil components, the monoterpenoids have drawn the greatest attention for fumigant activity against stored-product insects (Lopez and Pascual-Villalobos., 2008).

On the other hand, the data obtained are in agreement with the (El-Nahal, 1989), who reported that the period of exposure rather than the dosage appeared to be the most important factor affecting the efficiency of the vapours of the essential oil of Indian, *Acorus calamus*, on the adults of several stored-product insect pests such as S. *oryzae*, *T. confusum* and *R. dominica*. Our results are comparable with El-Disouky (2002) who found that citronella and nutmeg oils achieved 100% mortality against *S. oryzae* adults at concentration of 0.5% after 72 hrs of exposure. Also, anise and eucalyptus oils achieved high mortality (over 95%) with high doses within shorter exposure periods between (24hr and144 hr.) against adults of *T. confusum* and *S. oryzae* (Sarac and Tunc, 1995). Under laboratory conditions, El- Disouky *et al*., (2009) evaluated some essential oils isolated from Egyptian plants (*Mentha microphylla*, *Artemisia judaica*, *Eucalyptus camaldulensis* and *Majorana hortensis*) against *S. oryzae* and *T. castaneum* in stored wheat grains. Most of essential oils treatments showed significantly higher mortality of adults of both insects after one and two weeks compared with untreated wheat grains. Also, tulsi and wintergreen oils showed maximum percentage of mortality 92% and 86% at 48 and 72 hours after treatment, respectively. Tripathi *et al*., (2000) showed that a significant negative correlation between dose–response relationship of *Artemisia annua* oil and survival of *T. castaneum* (i.e., increase in dose caused decrease in survival and adult emergence).

2- Effect of some essential oils on the insect haemogram.

2.1- The different haemocyte types' counts.

The effects of some essential oils on the different haemocyte counts in the adults of *S. oryzae*, *R. dominica* and *T. castaneum* were evaluated.

Examination of stained blood smears from treated adult's of *S. oryzae, R. dominica* and *T. castaneum* at different concentrations of tested essential oils after 48 hours (Gad and Alzahofi, 2009). The present results revealed that five primary types of haemocytes in *S. oryzae* and *R. dominica* haemolymph of the adult. There were Prohaemocytes (Pr), Granulocytes (Gr), Plasmatocytes (Pl), Oenocytoides (Oe) and Spherule cells (Sp). While, the haemolymph of *T. castaneum* adults contain four types of haemocytes, Prohaemocytes (Pr), Granulocytes (Gr), Plasmatocytes (Pl) and Oenocytoides (Oe). These results supported with those obtained by Giulianini *et al.*, (2003).

The different haemocyte counts were clearly affected by the tested essential oils in *S. oryzae*. All tested essential oils reduced the number of (Pr, Gr and Pl). The maximum decrease was observed in cinnamon oil at concentration 0.0063 and 0.0072 mg/cm^2 in *S. oryzae* and *R. dominica*, respectively after 48 hrs of treatmet. The percentage of (Pr) decreased significantly to be 4.3 and 7%, when compared with the control 14.2 and 15% in *S. oryzae* and *R. dominica*, respectively. Also, the percentage of (Pl) decreased from 35% in the control of *S. oryzae* to 20.2% after treatment and decreased from 34% in the control of *R. dominica* to 25.6% after treatment. Furthermore, the percentage of (Gr) significantly decreased from 45.3% in control of *S. oryzae* and 42% in *R. dominica*, to be 25% in the treatment in *S. oryzae* and 28% in *R. dominica*. The percentage of (Oe) and (Sp) significantly increased to be 19.2 and 13.2%, when compared with control 7.6 and 5%, respectively in *S. oryzae*. Moreover, in *R. dominica* the percentage of (Oe) and (Sp) markedly increased to be 17 and 12%, while control was 6 and 5%, respectively. The same trend was observed in black cumin oil treatment followed by citronella oil, nutmeg oil and caraway oil, respectively (Figures 1 and 2).

As shown in Figure (3) treatment the adults of *T. castaneum* with the cinnamon oil at a concentration of 0.0072 and citronella oil at a concentration of 0.0322 mg/cm^2 caused significant decrease in the number of (Pr, Gr and Pl) and increased the number of (Oe) except the black cumin and nutmeg oil, which have no effects

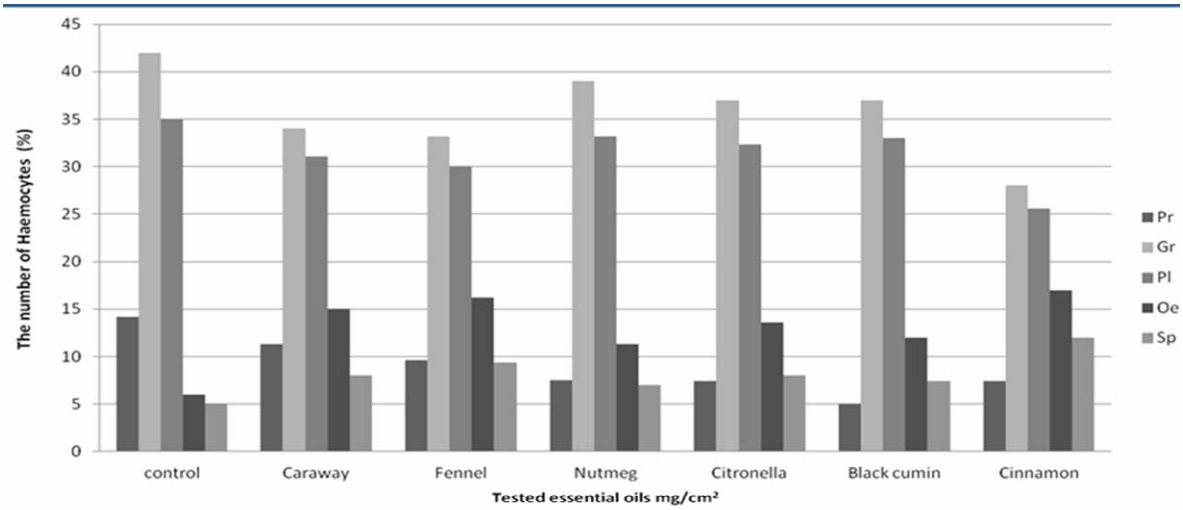

Figure 1: Effect of tested essential oils on different haemocyte types'count in *S. oryzae*.

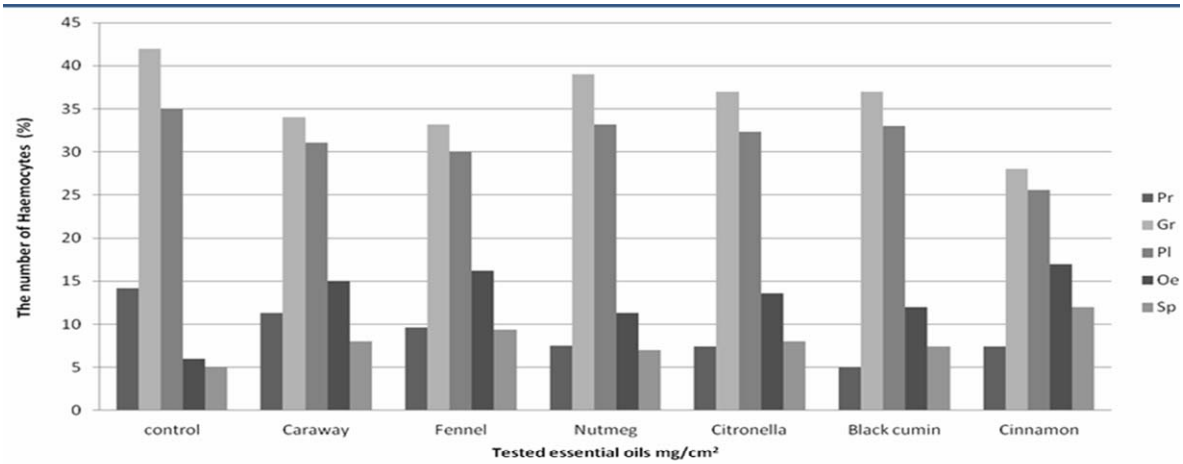

Figure 2: Effect of tested essential oils on the different haemocyte types'count in *R. dominica*.

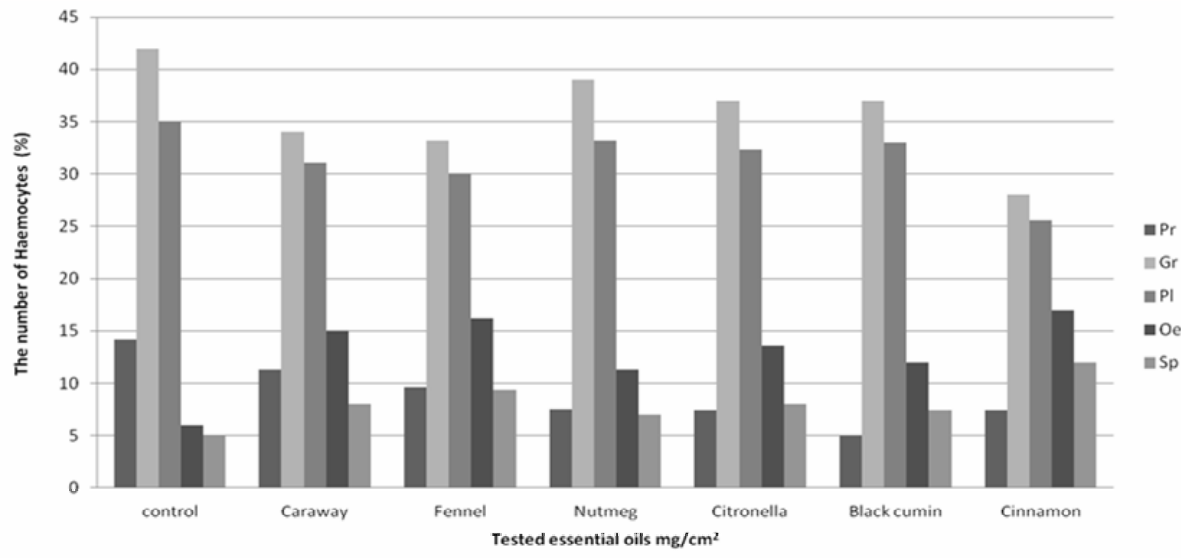

Figure 3: Effect of tested essential oils on the different haemocyte types'count in *T. castaneum*.

on the adults of *T. castaneum*. Cinnamon oil significant decreased the percentage of (Pr) to be 8%, while control was 12% and also, the percentage of (Gr) significantly decreased from 43% in the control to be 30% in the treatment. The same trend was observed in the percentage of (Pl) decreased from 34% in the control to 27%, while the percentage of (Oe) significantly increased from 6% in the control to 16.3% in the treatment.

2.2- The haemocyte surface area

Table (11) shows the variations in the surface area of each haemocyte type in cinnamon oil treatment. The obtained results proved that all haemocytes surface areas were markedly increased when compared with the control in the haemolymph of *S. oryzae* and *T. castaneum***.** On the other hand, treatment the adults of *R. dominica* with cinnamon oil caused slightly increase in the haemocytes surface area.

The surface area of (Pr) was (3.7µm^2) in *S. oryzae*, (4.1µm^2) in *R. dominica* and (5.2µm^2) in *T. castaneum*, while in the control was (3.1µm^2), (3.6µm^2) and (4.5µm^2), respectively. The same trend observed in the surface area of (Gr) was (5.5µm^2) in *S. oryzae*, (8.2µm^2) in *R. dominica* and (13.3µm^2) in *T. castaneum*, while in the control was (5.1µm^2), (7.3µm^2) and (12.6 µm^2), respectively. Also, in (Pl) was (6.0µm^2) in *S. oryzae*, (8.0µm^2) in *R. dominica* and (12.5 µm^2) in *T. castaneum*, while in the control was (5.4µm^2), (7.3 µm^2) and (11.2µm^2), respectively.

The same trend observed with (Oe) in *S. oryzae* (5.6µm^2) and *R. dominica* (12.5µm^2), while in the control was (5.6µm^2) and (9.3µm^2), respectively. Besides, there was a significant increased in the (Oe) surface area in the haemolymph of *T. castaneum* treatment with cinnamon oil (20.3µm^2) when compare with the control (13.2µm^2). The surface area of (Sp) was (4.7µm^2) in *S. oryzae* and (9.3µm^2) in *R. dominica***,** while in the control was (4.2µm^2) and (8.1µm^2), respectively.

The present results in this concern were in agreement with the results of Saxena and Tikku (1990). They observed that plumbagin, a phytochemical, eliminated the prohaemocytes while granulocytes and plasmatocytes decline continuously after 24 - 48 hrs of treatment of *Dysdercus koenigii* F. (Heteroptera). Also, Arnold (1974) noted a high mitotic and a rapid turnover of Oenocytoids, possibly as a mechanism of releasing products of their metabolism into the haemolymph. On the other hand, Sharma *et al*., (2008) studied the effect of sweet flag rhizome oil (*Acorus calamus*) on haemogram of *Spodoptera litura*. They observed that all the concentrations of oil caused the injury to both (Pl) and (Gr) and also affected the haemogram. While, Wael *et al.*, (2016) reaveled that apricot kernel extract treatment, caused a reduction in the percentage of the (Pr), (Gr) and (Pl) in 2nd larval instar of *S. littoralis*. It is clear that, irrespect of haemocytes, all different haemocyte types' surface areas especially (Oe), in cinnamon oil always exceed those of the control. The present findings agreed with those obtained by Gad and El–DaKheel (2009) who proved that cinnamon and chamomile oils treatment has increased the haemocyte surface area than control in *Culex quinquefasciatus* larvae. Also, Abou-Taleb *et al.*, (2015) reported a decrease in the different haemocyte counts and corpora allata (CA) activity in 4th larval instar of *S. littoralis* after treatment with insecticides lufenuron and chlorfluazuron. Moreover, Saxena and Tikku (1990) demonstrated that plumbagin treatment caused damage in haemocyte and suppression of filopodial elongations of plasmatocytes and granulocytes (the types that are active in defense mechanism). While, Arnold and Hinks (1983) noticed a high mitotic and a rapid turnover of Oenocytoids, possibly as a mechanism of releasing products of their metabolism into the haemolymph. Also, Sharma *et al*., (2008) demonestrated that the major effect of oil treatment was observed on plasmatocytes (Pl) and granular haemocytes (Gr).

Table 11: Effect of cinnamon oil on surface area of different haemocyte types' in tested insects.

Insects	Essential oils	Haemocytes surface area (µm^2)				
		Pr *	Gr *	Pl *	Oe *	Sp *
S. oryzae	Control	3.1±0.4^a	5.1±0.9^a	5.4±1.1^a	5.6±0.6^a	4.2±0.5^a
	Cinnamon (0.0063 mg/cm^2)	3.7±0.5^a	5.5±0.8^a	6.0±0.8^a	6.2±0.8^a	4.7±0.8^a
R. dominica	Control	3.6±0.5^a	7.3±1.2^a	7.3±1.2^a	9.3±1.2^a	8.1±0.7^a
	Cinnamon (0.0072 mg/cm^2)	4.1±0.3^a	8.2±1.2^a	8.0±0.6^a	12.5±0.6^b	9.3±0.6^a
T. castaneum	Control	4.5±0.4^a	12.6±0.7^a	11.2±1.6^a	13.2±1.6^a	------
	Cinnamon (0.0064 mg/cm^2)	5.2±0.8^a	13.3±1.3^a	12.5±1.2^a	20.3±1.2^b	------

***** Pr, Prohaemocytes; Gr, Granulocytes; Pl, Plasmatocytes; Oe, Oenocytoides and Sp, Spherule cells.

*Each value represents the mean ± SE.

*Mean in same column followed by the same letters are not significant.

Probability level at 0.05.

In the conclusion, the investigated plant essential oils can play an important role in control of stored-grain insects and reduce the need for the use of insecticides. It could be recommended for use as a part of integrated pest management program of *S. oryzae*, *R. dominica* and *T. castaneum* in stored-grains.

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الملخص العربي

تقييم ستة زيوت نباتية ضد ثلاث أنواع من حشرات المواد المخزونة وتأثيراتها على صورة الدم تحت الظروف المعملية

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تم دراسة تأثير ستة زيوت نباتية (زيت الشمر- زيت الكراوية - زيت القرفة - زيت السترونيلا - زيت جـوز الهند - زيت الحبة السوداء) ضد ثلاث أنواع من حشرات المواد المخزونة وهي سوسة الأرز، خنفـساء الحبـوب وخنفساء الدقيق الصدئية. وقد أظهرت النتائج أن زيادة تركيز وأزمنة تعرض الحشرات موضع الدراسـة للزيـوت أدت إلى زيادة كبيرة في نسبة الموت للحشرات المعاملة.

كما أوضحت النتائج زيادة سمية الزيوت النباتية موضع الدراسة في جميع التركيزات المختبرة مع زيـادة مـدة التعرض. وقد أشارت النتائج إلى أن سمية جميع الزيوت النباتية المختبرة ضد الثلاث حشرات موضع الدراسة تزيد بزيادة التركيز المستخدم للزيت.

وأنخفضت أعداد أنواع خلايا الدم المختلفة للحشرات موضع الدراسة وقد أدت المعاملة بزيت القرفة إلـى زيـادة ملحوظة في مـساحة سـطح خلايـا الـدم فـي هيموليمـف الحـشرات المعاملـة خاصـة الخلايـا النبيذيـة. وتشير النتائج إلى أن الزيوت النباتية مثل الشمر والكراوية والقرفة والسترونيلا يمكن أن تطبق في برنـامج لإدارة حشرات المواد المخزونة مثل(سوسة الأرز وخنفساء الحبوب وخنفساء الدقيق الصدئية) من خلال مزيد من الدراسات حول كيفية التطبيق فى المخازن والصوامع.