

RESEARCH ARTICLE

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EFFICACY OF THREE LOCAL EGYPTIAN ESSENTIAL OILS AGAINST THE RICE WEEVIL, *SITOPHILUS ORYZAE* (COLEOPTERA: CURCULIONIDAE) AND THE COWPEA WEEVIL, *CALLOSBRUCHUS MACULATUS* (COLEOPTERA: BRUCHIDAE)**ABSTRACT:**

Environmental and health concerns of synthetic insecticides highlight the need for new strategies to protect stored grains insect infestation in Egypt. The contact and fumigant toxicity of three local Egyptian essential oils; geranium, *Pelargonium graveolens* Herb, basil, *Ocimum basilicum* Herb, and fennel, *Foeniculum vulgare* seeds to adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Callosobruchus maculatus* (Coleoptera: Bruchidae) were investigated. Three different methods of oil applications were adopted; thin film residue (without grains), mixing with grains, and fumigation. It was observed that, in the absence of rice grains, geranium oil was the most effective against *S. oryzae* and *C. maculatus* with lower LC₅₀ of 710, and 705 ppm, respectively 3 days post exposure, followed by both basil and fennel oils (1305 & 3848 ppm/ 3 days, and 3030 & 1548 ppm/ 3 days), respectively. In the presence of grains, mixing of the same oil concentrations with grains required longer time and higher concentration of oils to achieved 100% mortality of *S. oryzae* and *C. maculatus* adults. However, for *S. oryzae* adults only fennel oil exhibited the lowest LC₅₀ (10956 ppm/ 6 days) followed by basil oil (20 ppm/ 6 days). Geranium oil evoked no detectable mortality of *S. oryzae* adults. Fennel oil induced the highest mortality rate to *C. maculatus* (125 ppm/ 5 days) followed by geranium, and basil oils (485.61 and 2641.43 ppm, respectively). In fumigation bioassay, basil oil achieved the lowest LC₅₀ of 1175 ppm/ 3 days post exposure of *S. oryzae*, followed by geranium, and fennel oils (28675 ppm and 18496 ppm/ 3 days post exposure, respectively). In the case of *C. maculatus*, geranium oil induced the lowest LC₅₀ (21492 ppm/ 3 days) followed by basil (35009 ppm/ 3 days) and fennel oils (50681 ppm/ 3 days). Data were discussed in the light of oil's toxicity and susceptibility of the two weevil species. The results strongly suggest the possibility of using essential oils as toxicant and fumigant against *S. oryzae* and *C. maculatus* adults in storage facilities in Egypt.

KEY WORDS:

Essential oils, rice, rice weevil *Sitophilus oryzae*, *C. maculatus*, cowpea, toxicity, fumigant.

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ARTICLE CODE: 09.01.15**INTRODUCTION:**

Over the years, insect damage to stored grains and cereal products has been of great concern. Among insects that infest stored grains on-farm or in large commercial elevators is the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae).

Legume seeds are considered the main source of protein for human and animal nutrition (Smarrt, 1985). *C. maculatus* causes substantial losses to the pulses in the storage throughout the world (Righi-Assia *et al.*, 2010). It is known to cause up to 100% loss of stored cowpea (Jackai and Daoust, 1986). The pulse weevil causes weight loss, decreased germination potential and reduction in commercial value of the seed (Okunola, 2003). Cereal grains make up the majority of commodities maintained in storage, and represent an important component of the world food supply. *S. oryzae* is one of the most destructive insect pests on cereal grain worldwide. Feeding by *S. oryzae* larvae and adults can reduce grain weight by more than 75% (Dal Bello *et al.*, 2001), as well as, decreases nutritional and aesthetic value of the grain. The weevils reduce germination resulting in lower prices for seed grain (Moino *et al.*, 1998).

Many decades before, control of insect pests has been totally based on chemicals especially synthetic insecticides such as persistent organo-chlorine (OC) and organophosphate (OP) insecticides (Anonymous, 2000). However, continuous or heavy uses of synthetic pesticides has created serious problems arising from factors such as direct toxicity to parasites, predators, pollinators, fish and human (Buchanan *et al.*, 2010). They may continue to poison non-target organisms in the environment and increase risk to humans by disruption in the endocrine, reproductive, and immune systems, cancer, neurobehavioral disorders, infertility and mutagenic effects, although very little is currently known about these chronic effects (Ritter, 2007; Jurewicz and Hanke, 2008; Buchanan *et al.*, 2010). Synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use (Keita *et al.*, 2000).

Replacement of conventional synthetic insecticides by bio-rational ones is universal acceptable and practical approach worldwide (Logan *et al.*, 1990). This necessitates continuous research towards substitution of hazardous synthetic insecticides for use of cheaper and eco-friendly natural plant products with active safe components, among which are use of powdered plant parts, oils and extracts that result from secondary metabolism in plants (Lale, 2002).

Essential oils have been widely used as antiparasitical, bactericidal, fungicidal, antiviral and insecticidal (Shaaya *et al.*, 1997). They are volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes cause death of insects by

inhibiting acetyl cholinesterase activity in the nervous system (Houghton *et al.*, 2006). However, in some cases, these chemicals can work synergistically, improving their effectiveness (Nerio *et al.*, 2010).

Moreover, essential oils possess acute contact and fumigant toxicity to insects (Liu and Ho, 1999; Sahaf *et al.*, 2008; Abdelgaleil *et al.*, 2009), repellent activity (Wang *et al.*, 2006; Nerio *et al.*, 2009), antifeedant activity (Huang *et al.*, 1997 & 2000), as well as development and growth inhibitory activity (Tomova *et al.*, 2005; Waliwitiya *et al.*, 2008). The mode of action of oils was partially attributed to interference in normal respiration, resulting in suffocation (Schoonhoven, 1978). Another hypothesis was that oil infiltration under the operculum may block respiration or disrupt the water balance of eggs and developing embryos (Messina and Renwick, 1983). Furthermore, essential oils negatively affect on progeny production, oviposition, longevity of adults, egg hatch rate, and mating behavior (Ho *et al.*, 1997; Huang *et al.*, 1997; Rajapakse and Emden, 1997).

Fumigation is one of the major chemical methods to control stored-product insect infestations worldwide. In this method, fumigant activity of only volatiles of applied constituents was investigated. Fumigation of some essential oils inhibited development of larvae to pupae and the pupae to adults, also resulted in deformities in the different developmental stages of the insect (Chaubey, 2007).

Basil, *Ocimum basilicum* (Lamiaceae) is native to areas in Asia and Africa and grows wild as a perennial shrub. It is one of the most important herbs to many cultures and cuisines. Basil oil has anti-viral, antimicrobial, antioxidant, and anti-cancer properties (Bozin *et al.*, 2007). Geranium *Pelargonium graveolens*, (Geraniaceae) is an aromatic and herbaceous shrub native to South Africa and it is widely cultivated in Egypt. Essential oil of *P. graveolens* is used as a fragrant component in perfumery, food and beverages industry, also as antidepressant and antiseptic remedy (Jain *et al.*, 2009; Džamić *et al.*, 2014). Fennel, *Foeniculum vulgare* Apiaceae (Umbelliferaceae) is a biennial medicinal and aromatic plant. It is generally considered indigenous to the shores of Mediterranean. It is a highly aromatic and flavorful herb with cooking and medicinal uses (Rather *et al.*, 2012).

Basil oil, *Ocimum basilicum*, was evaluated for fumigant toxicity against *Ephestia kuehniella* and *T. castaneum* and for contact toxicity against larvae of both species. Similarly, basil oil showed antifungal and insect-repelling properties (Dube *et al.*, 1989). Preeti *et al.* (2009) confirmed that

extracts from basil are very toxic to mosquitoes. Essential oil of fennel, *Foeniculum vulgare*, was repellent to adults of *S. zeamais*, *C. ferrugineus*, and *Tenebrio molitor* larvae. Repellency was highly dependent upon insect species and oil origin (Cosimi *et al.*, 2009).

Rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the most important pests of stored products in the world. Feeding by *S. oryzae* larvae and adults can reduce grain weight by as much as 75% (Dal Bello *et al.*, 2001), and also decreases nutritional and aesthetic value of the grain. The weevils reduce germination resulting in lower prices for seed grain (Moino *et al.*, 1998). However, few studies have been reported the insecticidal effect of basil, fennel, or geranium oil against adults of *S. oryzae*.

The aim of this study was to assess the bioactivity of these essential oils against two major stored product insects, *S. oryzae* and *C. maculatus* applied to rice and cowpea under laboratory conditions.

MATERIAL AND METHODS:

Insects:

Approximately, 200-400 *S. oryzae* adults were placed in 850 mL glass jars containing 400 g of wheat grains, while *C. maculatus* weevils were reared in glass jars each containing approximately 100-200 adults without sexing containing 300 g cowpea seeds as a food source. The seeds were previously heated at 50°C for 6 hours. This treatment kills any prior infestation by weevils. The openings of jars were covered with muslin cloth, kept in position with rubber bands. *S. oryzae* was maintained in the laboratory at 28 ± 2°C while, *C. maculatus* was maintained at 30 ± 2°C. Both weevils were reared at 70 ± 5% relative humidity (RH) and light: dark photoperiod of 16: 8. In order to obtain adult weevils, the grains were sieved to remove the beetles. Adult insects used for all bioassays were of mixed sex and seven to fourteen days old.

Essential oils:

The essential oils geranium, *P. graveolens* Herb, basil, *O. basilicum* Herb and fennel, *F. vulgare* seeds were purchased from Hashem Bothers Company at Al-Sadat city.

Insecticidal activity of essential oils:

Contact toxicity using thin film residue, grain treatment and fumigant bioassays were designed to test the insecticidal effect of essential oils on adults of *S. oryzae* and *C. maculatus*. Series of dilutions at concentrations of 20000, 10000, 5000, 2500, 1250, and 625 ppm of each essential oil were prepared using acetone as a solvent. All tests were conducted at 30 ± 2°C, 70 ± 5% Relative Humidity and 16: 8 h photophase.

Contact toxicity bioassay using thin film residue:

The insecticidal activity of the different essential oils was assessed by film residue method. Bioassay was done on Petri dishes (9 cm in diameter). Aliquots of 1 ml of the dilutions were spread uniformly along the whole surface of the Petri dishes. The solvent was allowed to evaporate for few minutes leaving thin film of essential oils on the floor of the dishes. Control Petri dishes were treated with acetone alone. Ten *S. oryzae* (7-14 days old) or *C. maculatus* (0-2 days old) adults were released separately into each Petri dish and covered with a lid. Three replicates of each treatment and control were set up. Mortality was recorded after 24, 48, and 72 h.

Contact toxicity bioassay using grain treatment:

The treatment of grains with the essential oils was performed to determine the contact toxicity against *S. oryzae* adults. Different concentrations (20000, 10000, 5000, 2500, and 1250 ppm) of fennel, geranium, and basil oils were diluted with acetone. Twenty grams of grains (rice or cowpea) was placed in a jar (11.5 cm x 6 cm, in diameter). One ml of each concentration was dropped in each jar over the surface of grains using micropipette. The jar was shaken to ensure even spread of the materials over the surface of the grains. The treated grains were left for 20 minutes until the solvent evaporated. Each concentration was replicated three times. Grains treated with solvent only served as control. Ten adults of newly emerged adults of *S. oryzae* or *C. maculatus* were transferred to each jar, covered with muslin cloth and kept under the same laboratory conditions. Mortality counts were recorded 1, 2, and 6 days post exposure for *S. oryzae*, and 1, 2, and 5 days for *C. maculatus*.

Fumigation effect:

In order to evaluate the fumigant toxicity of essential oils, the bioassay described by Moravvej and Abbar (2008) was used. Series of dilutions of essential oils at concentrations of 160000, 80000, 40000, and 20000 ppm were prepared using acetone as a solvent. Plastic gars (170 cm³) with screw cap were used for the bioassays. Six cm diameter circular pieces of filter papers (Whatman No. 1) impregnated with the different concentrations of the essential oils were attached to the undersurface of the screw cap. Ten *S. oryzae* (7-14 days old) or *C. maculatus* (0-2 days old) adults were transferred into jars, each contain 20 g of grains (rice or cowpea) then closed with their screw caps with the attached treated filter paper. The same procedure was used for the control using filter paper treated with acetone. Three replicates of each treatment and control

were set up. Assessments of mortality were recorded 24, 48, and 72 h post exposure.

Data analysis:

Mortality rate was estimated and corrected according to Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected Mortality} = \frac{(\text{Mortality\% of treated insects} - \text{Mortality\% of control} \times 100) / (100 - \text{Mortality\% of control})}$$

The toxicity data was analyzed using probit analysis to estimate the LC₅₀ (LDP line).

RESULTS:

Insecticidal activity of essential oils:

Table 1. Insecticidal effect of basil, *Ocimum basilicum*, fennel, *Foeniculum vulgare* and geranium, *Pelargonium graveolens* oils on *Sitophilus oryzae* adults using thin film residue after 24, 48, and 72 h post-exposure

Essential oils	Sub lethal concentration LC ₅₀ (ppm)	Confidence limits		Slope value (SV)	Toxicity index (TI)
		Lower	Upper		
24 h post exposure					
Basil oil	5963.82	4393.35	6505.52	5.18	39.68
Fennel oil	5662.95	4884.41	6353.99	3.78	41.79
Geranium oil	2366.49	1860.76	2800.76	2.82	100.00
48 h post exposure					
Basil oil	4058.35	3701.37	4439.52	3.84	34.68
Fennel oil	4722.31	4211.36	5764.94	3.69	29.80
Geranium oil	1407.57	972.45	2042.74	2.64	100.00
72 h post exposure					
Basil oil	1305.37	759.67	1871.72	2.131	54.95
Fennel oil	3030.57	1924.59	4373.11	2.930	23.45
Geranium oil	710.83	283.05	953.13	2.060	100.00

Each essential oil at concentrations of (20000, 10000, 5000, 2500, 1250, and 625 ppm) was tested.

Film residue contact toxicity to *C. maculatus* adults:

Data in table 2 revealed that geranium oil achieved the highest insecticidal activity to *C. maculatus* with LC₅₀ values of 2346.27, 1076.16, and 705.08 ppm after 1, 2, and 3 days post treatment, respectively (Table 2).

Film residue contact toxicity to *S. oryzae* adults:

Data in table 1 show that in absence of rice grains, direct contact toxicity of geranium oil against *S. oryzae* adults was higher than both basil or fennel oils with lower LC₅₀ values (2366, 1407, and 710 ppm, respectively) after 1, 2, and 3 days post exposure (Table 1). After the same periods of exposure, LC₅₀ values of basil oil were (5963, 4058, and 1305 ppm) followed by fennel oil which were (5662.95, 4722.31, and 3030.57 ppm) after 1, 2, and 3 days post exposure, respectively (Table 1). Values of LC₅₀ of the different oils decreased with increasing the exposure time.

On the other hand, fennel oil was the least effective with LC₅₀ values of 10082.00, 6062.75, and 3848.03 ppm after 1, 2, and 3 days, respectively. The LC₅₀ values after 24 and 48 hours post exposure of *S. oryzae*, were higher than those of *C. maculatus* (Tables 1 & 2).

Table 2 Insecticidal effect of basil, *Ocimum basilicum*, fennel, *Foeniculum vulgare* and geranium, *Pelargonium graveolens* oils on *Callosobruchus maculatus* adults using thin film residue 24, 48, and 72 h post-treatment

Essential oils	Sub lethal concentration LC ₅₀ (ppm)	Confidence limits		Slope value (SV)	Toxicity index (TI)
		Lower	Upper		
24 h post treatment					
Basil	4227.54	2131.42	9238.64	1.57	55.50
Fennel	10082.00	7700.42	13417.22	2.04	23.27
Geranium	2346.27	1648.00	3386.00	1.57	100.00
48 h post treatment					
Basil	2983.72	1527.00	4072.00	1.45	36.06
Fennel	6062.75	3837.03	9578.75	1.26	17.75
Geranium	1076.16	273.06	1613.31	1.42	100.00
72 h post treatment					
Basil oil	1548.15	573.02	2541.19	1.22	45.54
Fennel oil	3848.03	1891.30	6059.93	1.02	18.32
Geranium oil	705.08	305.53	102.19	1.52	100.00

Each essential oil at concentrations of (20000, 10000, 5000, 2500, 1250, and 625 ppm) was tested.

Contact toxicity of essential oils using grains treatment:

Toxicity to *S. oryzae* adults:

In contrast to the data obtained in the thin film residue method, fennel essential oil exhibited the lowest LC₅₀ values to *S. oryzae* (66577.73, 61665.87, and 10956.43 ppm) after 1, 2, and 6 days post exposure, respectively

(Table 3). On the other hand, both basil, and geranium oils induced no toxic effect on *S. oryzae* adults after 1 or 2 days post exposure (Table 3). However, 6 days post exposure very low toxicity rate was recorded with geranium oil. Moreover, basil oil was not toxic after the same time of exposure.

Table 3. Insecticidal effect of basil, *Ocimum basilicum*, fennel, *Foeniculum vulgare* and geranium, *Pelargonium graveolens* oils on *Sitophilus oryzae* adults using grain treatment method 1, 2, and 6 days post-treatment

Essential oils	Sub lethal concentration LC ₅₀ (ppm)	Confidence limits		Slope value (SV)	Toxicity index (TI)
		Lower	Upper		
1 day post treatment					
Basil oil	-	-	-	-	-
Fennel oil	66577.73	34990.59	524330	1.51	100.00
Geranium oil	-	-	-	-	-
2 days post treatment					
Basil oil	-	-	-	-	-
Fennel oil	61665.87	34887.17	215850	1.40	100.00
Geranium oil	-	-	-	-	-
6 days post treatment					
Basil oil	-	-	-	-	-
Fennel oil	10956.43	19259.32	44496.79	2.58	100.00
Geranium oil	20666000	7395489	70651322	0.46	0.05

Each essential oil at concentrations of (20000, 10000, 5000, 2500, 1250, and 625 ppm) was mixed with 20 g of rice grains.

Toxicity to *C. maculatus* adults:

In contrast to *S. oryzae*, toxicity data reported against *C. maculatus* after 1 day post-exposure to the three tested oils revealed that geranium oil induced the highest mortality rate with LC₅₀ values of (3958.56 ppm) followed by basil and fennel

oils (4128.98 and 6402.43 ppm), respectively (Table 4). However, after 5 days post exposure, fennel oil achieved the lowest LC₅₀ value (125 ppm) followed by geranium and basil oils (485.61, and 2641.43 ppm), respectively (Table 4).

Table 4. Insecticidal effect of basil, *Ocimum basilicum*, fennel, *Foeniculum vulgare* and geranium, *Pelargonium graveolens* oils on *Callosobruchus maculatus* adults using seed treatment method 1, 2, and 5 days post-treatment

Essential oils	Sub lethal concentration LC ₅₀ (ppm)	Confidence limits		Slope value (SV)	Toxicity index (TI)
		Lower	Upper		
1 day post treatment					
Basil oil	4128.98	2426.53	7517.42	0.46	95.87
Fennel oil	6402.43	4474.92	10230.12	0.67	61.82
Geranium oil	3958.56	2286.36	6960.52	2.60	100.00
2 days post treatment					
Basil oil	3103	2398.53	3973.42	0.98	94.32
Fennel oil	2928	2274.92	3717.12	1.02	100.00
Geranium oil	3550	3041.36	4144.52	1.72	82.46
6 days post treatment					
Basil oil	2641.43	1427.10	4347.38	0.47	4.72
Fennel oil	125	35.4	251.7	1.01	100.00
Geranium oil	485.61	300.82	680.52	1.25	25.71

Each essential oil at concentrations of (20000, 10000, 5000, 2500, 1250, and 625 ppm) was mixed with 20 g of cowpea seeds.

Fumigant toxicity of essential oils against *S. oryzae* adults:

One day post treatment, basil oil vapor caused the highest mortality of *S. oryzae* adults at LC₅₀ level of 53644.12 ppm followed by geranium oil with LC₅₀ value of 173640 ppm (Table 3). Fennel oil evoked no fumigant toxicity to *S. oryzae* adults. Two and three days post treatment, basil and geranium oil treatments induced the highest fumigant effect

on *S. oryzae* adults with LC₅₀ values of 42437.88 and 11175.07 ppm after 48 h and 42569.55 and 18496.07 ppm after 72 h, respectively (Table 5). Fennel was the least toxic oil, with LC₅₀ values of 45967.32 and 28675.45 ppm after 48, and 72h, respectively (Table 5). Three days post exposure, basil oil was the most toxic agent followed by

geranium oil with toxicity index values of 100% and 60%, respectively. Fennel oil was the least toxic to *S. oryzae* with toxicity index ranged between 38.97% and 92.32%. Both fennel and geranium oils caused moderate fumigant toxic actions against *S. oryzae* compared with basil oil especially 2 and 3 days post treatment.

Table 5. Fumigant toxicity of basil, *Ocimum basilicum*, fennel, *Foeniculum vulgare* and geranium, *Pelargonium graveolens* essential oils to *Sitophilus oryzae* adults 24, 48, and 72 h post-treatment

Essential oils	Sub lethal concentration LC ₅₀ (ppm)	Confidence limits		Slope value (SV)	Toxicity index (TI)
		Lower	Upper		
24 post treatment					
Basil	53644.12	50448.49	57381.38	11.81	100.00
Fennel	-	-	-	-	-
Geranium	173640	143090	236190	2.47	30.89
48 post treatment					
Basil	42437.88	42026.24	52275.90	5.21	100.00
Fennel	45967.32	41648.00	59973.00	2.99	92.32
Geranium	42569.55	25925.92	65816.00	3.98	99.69
72 post treatment					
Basil	1175.07	9416.49	16819.45	2.50	100.00
Fennel	28675.45	23895.00	34410.00	2.55	38.97
Geranium	18496.07	14496.34	21895.09	2.88	60.41

Each essential oil at concentrations of (20000, 10000, 5000, 2500, 1250, and 625 ppm) was tested.

Fumigant toxicity to *C. aculates* adults:

Obtained data in table 6 prove that basil vapor caused the highest mortality to *C. aculates* followed by geranium oil with LC₅₀ values of 53017.28 and 64272.50 ppm, respectively (Table 6). In contrast, fennel oil evoked no mortality to *C. aculates*. Two and three days post exposure, geranium oil caused the highest toxic effect followed by basil and fennel oils with LC₅₀ values of 29355.69, 43303.39, and 52344.57 ppm and 21492.04, 35009.82, and 50681.86 ppm, respectively (Table 6). Three days post exposure, geranium oil was the most toxic oil followed by basil with toxicity indices values of 100 and 61%, respectively. The fumigant toxicity of the oils increased with increasing the time of exposure.

Table 6. Fumigant toxicity of basil, *Ocimum basilicum*, fennel, *Foeniculum vulgare* and geranium, *Pelargonium graveolens* essential oils to *Callosobruchus aculates* adults 24, 48, and 72 h post-treatment

Essential oils	Sub lethal concentration LC ₅₀ (ppm)	Confidence limits		Slope value (SV)	Toxicity index (TI)
		Lower	Upper		
24 h post treatment					
Basil	53017.28	43293	65905	2.71	100.00
Fennel	87189.45	72788	105169	3.09	60.80
Geranium	54272.50	42204	73378	1.96	97.68
48 h post treatment					
Basil	43303.39	35286	54326	2.63	67.79
Fennel	52344.57	40184	69064	2.09	56.08
Geranium	29355.69	22157	39886	1.94	100.00
72 h post treatment					
Basil	35009.82	27751	45673	2.28	61.38
Fennel	50681.86	38106	67881	1.97	42.40
Geranium	21492.04	16855	28179	2.21	100.00

Each essential oil at concentrations of (20000, 10000, 5000, 2500, 1250, and 625 ppm) was tested.

DISCUSSION:

In the present study laboratory experiments were carried out to evaluate the lethal effect of three of available naturally occurring plant oils (geranium, fennel, and basil) against two of the most common stored grain pests *S. oryzae* and *C. maculatus*. Three methods of application (thin film residue, mixing with the feeding grains, and fumigation) were used in this evaluation. It is an attempt to control these stored grain pests and avoid the environmental risks and mammalian toxicity arose from chemical insecticides. Essential oils obtained from basil, fennel, and geranium demonstrated contact and fumigant toxicity on *S. oryzae* and

C. maculatus adults. The results indicated that the insecticidal activity of the essential oils varied depending on the application methods, the type of essentials oils, and the insect species. In direct contact toxicity method in absence of rice grains (thin film residue), the results showed that adults of both *S. oryzae* and *C. maculatus* were susceptible to all oils and lower doses were required to achieve 50% mortality. The toxic properties of oils were more rapid against adult insects within 24 h post treatment. This might be due to the accessibility of the tested oils by direct taste and olfaction by exposed insects, which caused rapid lethal effect.

Moreover, geranium oil showed a higher toxicity against adult insects than fennel or basil oils 3 days post exposure. This might be due to the chemical composition of geranium oil and presence of active chemical groups which were easily received by direct contact with insect and causing rapid suffocation and contact toxicity to adult insects.

The insecticidal activity of many plant essential oils might be attributed to monoterpenoids (Waliwitiya *et al.*, 2005; Tong, 2010). Due to the high volatility they have fumigant activity that might be of importance for controlling stored product insects (Konstantopoulou *et al.*, 1992; Koul, 2004). Monoterpenoids were reported earlier as fumigants and contact toxicants on various insect pests (Rice and Coats, 1994; Tsao *et al.*, 1995). The toxicity of essential oils to stored product insects is influenced by the chemical composition of the oil and plant part to be used (Don-Pedro, 1966; Lee *et al.*, 2001). From the previous reports on the insecticidal and repellent properties of monoterpenoids and phenolic acids, it can be stated that common chemicals found in essential oils such as monoterpenoids, 1,8-cineole, alpha pinene, carvone, linalool, etc., and phenolic acids were responsible for the insecticidal activity of the essential oils (Rani, 2012). Tapondjou *et al.* (2005) and other researchers demonstrated that essential oils consisting of 1, 8-cineole, terpineol and alpha-pinene as major constituents show toxic and repellent properties. Lee *et al.* (2004) indicated that 1, 8-cineole induced fumigant toxicity against major stored grain insects. Obeng-Ofori *et al.* (1997) found 8-cineole to be highly repellent and toxic to *S. granaries*, *S. zeamais*, *Tribolium confusum*. Moreover, bioactivity of essential oil was directly related to its chemical composition, which can vary dramatically, even within the same species sources of compositional variability include the plant part extracted. Angioni *et al.* (2006) and Isman *et al.* (2000) reported that the bioactivity of essential oil was related to the phenological state of the plant and time of year, as well growth environmental conditions. Bioactivity of essential oils is also affected by interactions among their structural compounds (Sampson *et al.*, 2005; Angioni *et al.*, 2006; Bakkali *et al.*, 2008).

In contact toxicity bioassay in the presence of rice grains, essential oils showed lower mortality rates to weevils at higher dosage 5, and 6 days post treatment. However, fennel oil was the most toxic oil against *S. oryzae* and *C. maculatus* adults than basil or geranium oils. However, higher doses of oils and longer exposure time were required to achieve high toxicity of *S. oryzae* adults. In contrast, *C. maculatus* adults were more susceptible to fennel oil than *S. oryzae* with lower dose of fennel oil and shorter exposure time to achieve higher toxicity. In

general, mixing essential oils with feeding grains may reduce their toxic effect due to antagonistic effect between oil constituents and grain semiochemicals; the chemical substances of feeding grains which carry information to the weevils, after absorption of oils by rice grains. If so, fennel oil would be the most resistant to this antagonism and caused considerable mortality to adult insects compared to basil, and geranium oil.

In contrast, in fumigation bioassay, it was observed that basil oil induced the most toxic effect as a fumigant agent against *S. oryzae* adults at lower dosage ($LC_{50} = 1175$ ppm) than when mixed to rice grains after the same time of exposure (3 days). However, in the two methods feeding grains were present but it was blinded with grains in mixing method, and in fumigation method the grains were in the bottom of the jar and oils were on the ceiling of it. This result confirms our previous suggestion of antagonistic effect of mixing oils with rice grains in contrast of using it as fumigants in a distance out of grains. So far, basil oil might be containing more active volatile groups that were easily received by insect olfaction and caused rapid suffocation to them in comparison with fennel and geranium oils.

Moreover, these differences in the type of the most toxic oil within different application method might be due to differences in the active chemical constituents of each oil that has different mode of action within different application method. Data also revealed that, geranium oil was the most toxic against *C. maculatus* adults as a fumigant agent followed by basil and fennel oils. However, higher doses of oils were required to achieve higher mortality rate of *C. maculatus* compared with those with *S. oryzae*. These differences might be due to the feeding habit of the weevil, or difficulties in air flow cross of oils volatiles into *C. maculatus* respiratory structures which made them less toxic than with *S. oryzae*. Generally, fumigant toxicity of the essential oils tested varied with season, insect species, oil concentration and exposure time Jemaa *et al.* (2012).

Rani (2012) found that *S. oryzae* adults showed high susceptibility to coriander and eucalyptus essential oils, even at low concentrations and less exposure period, in fumigation method than in direct contact with these oils. In fumigation test with adult beetles, insecticidal activity of fruit extract of *F. vulgare* was much more effective in closed cups than in open ones, indicating that the insecticidal activity of these materials was largely attributable to fumigant action (Kim *et al.*, 2003a).

Several studies reported the toxicity and protectant potential of essential oils extracted from different plants against major stored

product insects (Talukder *et al.*, 2004; Islam and Talukder, 2005; Isman, 2006). For instance, basil oil Abdel-Aziz and El-Sayed (2009) found that basil oil was toxic to adults and last larval instars of the confused flour beetle *T. confusum*. Moreover, Lopez *et al.* (2008) found that the essential oils from leaves of five different varieties of *O. basilicum*, were toxic against three stored rice pests, *S. oryzae*, *R. dominica* and *C. pusillus*. The basil chemo-types with both linalool and estragole produced greater volatile toxicity against *R. dominica* and *C. pusillus* (Lopez *et al.*, 2008). Essential oil extracted from basil proved to be very toxic towards *C. maculatus* adults (Liboudo *et al.*, 2010). However, there were different essential oils that have been used as insecticidal agents against various stored grain pests. For example, the oil of *Mintha piperita* L. (peppermint oil) was evaluated for its insecticidal activity against several stored grain insects (El-Nagar *et al.*, 2012). Furthermore, Kim *et al.* (2003b) reported that the cinnamon oil, horseradish oil, and mustard oil induced 100% mortality of *S. oryzae* adults after 1 day of application. Tapondjou *et al.* (2002) found that the essential oil obtained from dry ground leaves of *Chenopodium ambrosioides* caused high mortality to *C. chinensis*, *C. maculatus*, *Acanthoscelides obtectus*, *S. aculatus*, *S. zeamais* and *Prostephanus truncatus*. The essential oils of west African plant white basil *Ocimum canum* (Family: Labiatae), and sweet basil, *O. basilicum* after twenty four hours of fumigation, achieved 99% adult mortality (Keita *et al.*, 2000). Whereas, Negahban *et al.* (2007) indicated that fumigant of some species of *Artemisia* were insecticidal, against adults of *C. maculatus*, *S. oryzae*, and *T. castaneum*; the toxicity increased with concentration and exposure time which were sufficient to obtain 100% kill of the insects.

Moreover, Mikhael (2011) showed that basil gave 100% mortality for *E. kuehniella* adults within 24 h and for *T. castaneum* adults by fumigation. Furthermore, Suthisut *et al.* (2011) proved the fumigant toxicity of essential oils from *Alpinia conchigera*, *Zinger zerumbet*, *Curcuma zedoaria* and their major compounds; camphene, camphor, α -pinene and terpinen to adults of *S. zeamais* and *T. castaneum*; terpinen was the most toxic to both insects. There were different oils that have toxic activity by fumigation on insects for example, vapors of essential oil distilled from anise *Pimpinella anisum*, cumin *Cuminum cyminum*, eucalyptus *Eucalyptus camaldulensis*, oregano *Organum syriacum* were toxic to *T. confusum*, *Ephestia kuehniella* and *Rosmarinus officinalis* (Tunc *et al.*, 2000).

Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes. However, in some cases, these chemicals can work synergistically, improving their effectiveness. Some of these plant oils affect on acetyl choline esterase that means that these oils have an action on the nervous system (Mikhael, 2011).

The plant essential oils may explored as a potential natural insecticides for stored product insect pests because of their toxicity, repellency, and fumigant activity, inhibition activity on fecundity, their residual activity and its chemical composition which contain many different chemicals that have different modes of action on target pests.

The results obtained suggest good potential for the use of essential oils as both fumigant and contact toxic agents against *S. oryzae* and *C. maculatus* adults.

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فعالية ثلاثة من الزيوت العطرية المصرية المحلية ضد سوسة الارز، سايتوفيلاس اوريزا (غمدية الأجنحة: كوركليونيدي) وسوسة اللوبيا، كالوسوبراكس ماكبولانيس (غمدية الأجنحة: براكيدي)

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جزء في الالف / 6 أيام). ولم يظهر زيت إبرة الراعي أي وفيات يمكن تسجيلها. بالنسبة لسوسة اللوبيا، وجد ان زيت الشمر يسبب أيضا أعلى معدل وفيات وهي (125 جزء في المليون / 5 أيام)، و يليه زيت إبرة الراعي، وزيت الريحان (485.61 و 2641.43 جزء في المليون / 5 أيام، على التوالي). في عملية التبخير، حقق زيت الريحان أدنى LC50 وهي (1175 جزء في المليون / 3 أيام) ضد سوسة الارز. يليه زيت إبرة الراعي، وزيت الشمر (28675 و 18496 جزء في المليون / 3 أيام) على التوالي. بالنسبة لسوسة اللوبيا، حقق زيت إبرة الراعي أدنى LC50 وهي (21492 جزء في المليون / 3 أيام)، يليه زيت الريحان (35009 جزء في المليون / 3 أيام) وزيت الشمر (50681 جزء في المليون / 3 أيام). تمت مناقشة البيانات في ضوء سمية هذه الزيوت والقابلية للتسمم بهم لدى النوعين المختلفين من السوس. النتائج تشير بقوة إلى إمكانية استخدام الزيوت الأساسية كمواد وإبخرة سامة ضد كل من سوسة الارز وسوسة اللوبيا في ظروف التخزين في مصر.

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المخاطر البيئية والصحية من الإستخدام المفرط للمبيدات الكيميائية تسلط الضوء على الحاجة إلى استراتيجيات جديدة لحماية الحبوب المخزونة من الآفات الحشرية في مصر. لذا تم دراسة السمية المباشرة بالملامسة والتبخير لثلاثة من الزيوت العطرية المحلية وهي زيوت عشبية إبرة الراعي، *Pelargonium graveolens*، وبنور الشمر، وعشبة الريحان *Ocimum basilicum*، وبنور الشمر، *Foeniculum vulgare* ضد البالغين من سوسة الارز سايتوفيلاس اوريزا (غمدية الأجنحة: كوركليونيدي) وسوسة اللوبيا، كالوسوبراكس ماكبولانيس (غمدية الأجنحة: براكيدي). اعتمدت الدراسة علي ثلاث طرق مختلفة من التطبيقات وهي التعرض لطبقة رقيقة من الزيت (بدون الحبوب)، والاختلاط مع الحبوب، والتبخير. في غياب الحبوب وجد أن زيت إبرة الراعي هو الأكثر فعالية ضد سوسة الارز وسوسة اللوبيا وذلك لصغر قيم LC50 وهي 710 و 705 جزء في المليون على التوالي بعد التعرض لمدة ثلاثة أيام، تليها كل من زيوت الريحان والشمر (1305، 3848 جزء في المليون، و3030، و1548 جزء في المليون) على التوالي. في وجود الحبوب، خلط التركيزات نفسها مع الحبوب يتطلب وقتا أطول وتركيزا أعلى من الزيوت لتحقيق نسبة وفيات 100% من الحشرات البالغة. ومع ذلك، أظهر فقط زيت الشمر أدنى قيمة من LC50 ضد سوسة الارز وهي (1095 جزء في المليون / أيام)، يليه زيت الريحان (20