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Toxic and Repellent Effects of Four Plant Oils against the Red Flour Beetle, *Tribolium castaneum* (Herbst)

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ABSTRACT

The present study was carried out to evaluate the toxic and the repellent effects of four essential plant oils Coconut oil (*Cocus nucifera* L.), Ginger oil (*Zingiber officinale* Rosc.), Jojoba oil (*Simmondsia chinensis* L.) and spearmint oil (*Mentha spicata* L.) against the adult of red flour beetle. Three concentrations of each plant oils were used, 0.5, 1 and 2%. The LC_{50}, LC_{90} values were also calculated using Probit analysis technique. LC_{50}, LC_{90} values of coconut, ginger, jojoba and spearmint oils after 24, 48, 72 and 96 hrs intervals were determined. Results revealed that ginger oil was more effective against *Tribolium castaneum*. Data of 96 hrs showed that ginger still the most effective oil against the pest (LC_{50} = 0.37 and LC_{90} = 0.86%). The LC_{50} values were 0.56, 0.66 and 1.17% and LC_{90} values were1.09, 1.19 and 2.76% for coconut, spearmint and jojoba, respectively. Repellency test was conducted at 2% concentration for each oil. Data were recorded at 1, 3, 6, 12 and 24 hrs after insect release. The results showed that the highest repellent rate (45.08) was recorded for ginger and spearmint oil, while the lowest repellent rate (15.01) was recorded for jojoba oil after 24 hrs.

Keywords: Toxicity, Repellency, *Tribolium castaneum*, plant oils.

INTRODUCTION

The red flour beetle, *Tribolium castaneum* Herbst (Coleoptera Tenebrionidae) is a main pest of stored nutrition grains and is global in distribution (Good, 1933). It is a prevalent and the most injurious pest between the important stored product insect pests all over the world (Pranoto *et al*., 1991). Its existence in the stored products results in both pollution and economic damage causing loss of the product's market value and lower food value (Burkholder and Faustini, 1991). These insects are linked to stored goods that people feed, but grain products such as flour is the food of their choice., It has been found to infect a wide range of nutrients including cereals, flour, peas, beans, cocoa, nuts, fruits dried (Okonkwo and Okoye, 1996; Weston and Rattlingourd, 2000; Rahman *et al*., 2011; Sagheer *et al*., 2011 and Mishra *et al*., 2012a; 2012b).

Due to the problems associated with the indiscriminate use of synthetic insecticides, many scientists have given an extra impetus to search alternative ways to control this pest (Dyte and Blackman, 1972). It is needful to keep the stored food grains reserves so that the food stay all time available and the prices of nutriment grains and products stay stable (Talukder, 2006; Nadeem *et al*., 2012; Ukeh *et al*., 2012 and Jahromi *et al*., 2014). It has been proposed that the plant essential oils are less dangerous than artificial compounds, quickly degraded in the environment and they do not persist in soil and water and easily extractable (Isman, 2000, 2006 and Moretti *et al*., 2002).

Repellents from plant sources are considered safe in pest control operations as they minimize pesticide residues in environment and wildlife, and provide safety for people (Khan, 1982; Talukder and Howse, 1995; Talukder *et al*., 2004). Abundant plant products have been evaluated for their repellent and insecticidal activities against various stored grain pest insects (Ahmed *et al*., 1988; Su, 1990; Mukherjee and Joseph, 2000) especially in the form of plant oils, which have newly received much interest due to their multi-functions as insecticidal and antimicrobial agents (De Souza *et al*., 2005 and Shaaya *et al*., 1991).

Therefore, the aim of the present study is to evaluate the toxicity and repellency of some plant oils (coconut, ginger, jojoba and spearmint) against the red flour beetle, *T. castaneum*, under the laboratory conditions.

MATERIALS AND METHODS

1. Insect collection

The adults of red flour beetle, *T. castaneum*, used in this study were obtained from naturally infested wheat flour in the market in Assuit Governorate and reared in the laboratory to develop a homogenous population.

2. Insect rearing

This study carried in the Laboratory of the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Assiut Governorate during 2018, the red flour beetle were reared on the sterilized wheat flour kept in 500 ml plastic jars. Adult beetles were released for laid of eggs and after 5 days beetles were removed from the wheat flour through their sieving. The eggs were allowed to hatch and develop under uniform conditions in incubator at a constant temperature of 27 ± 3°C and 70 ± 5% R.H. The plastic jars were covered with muslin cloth tightened with rubber bands to avoid the escape of beetles. The homogenous population was achieved after 28-35 days as given by Islam and Talukder (2005).

3. Plant essential oil

Four plant oils (Coconut, ginger, jojoba, and spearmint) were used in the experiments. Plant oils were purchased from local market, and tested at the rate of 0.5, 1 and 2 % against the red flour beetle.

Mortality Bioassay:

The insecticidal efficacies of different plant oils (Table 1) were tested by residual film technique against the *T. castaneum* beetle. The residual film was done directly on petri dish (9 cm) without any food medium according to Qi and Burkholder (1981). Oil concentrations were 0.5, 1 and 2%, which were prepared in acetone. One ml of each concentration was pipetted in petri dish. The petri dishes were then kept open for sometimes to evaporate the solvents fully. After evaporation of acetone, twenty adults were placed in each petri dish. Only acetone solvent was used for the control treatment. Three replications were made for each concentration of all the treatments. The treatments were then incubated at 27 ± 3°C and 70 ± 5% R.H. Adult mortality was recorded at 24, 48, 72 and 96 hours after treatments (HAT). The Percentage of mortality was corrected according to Abbott's formula (1925).

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

Repellency Bioassay:

Repellency test was conducted following the method described by Conti *et al*. (2010). Half of filter paper disk (12 cm diameter, Whatman No. 1) was treated with 0.5 ml of 2% concentration for each tested oil (v:v) in acetone and dried under a fan. Half the bottom of a Petri dish was covered with treated filter paper, while the other half was covered with a half filter paper disk treated with acetone only. Twenty mixed-sex adults were released at the center of each Petri dish. The test was carried out in incubator at temperature of 27 ± 3°C and 70 ± 5% R.H. Three replicates were used for each tested oil. The observations were started after 1, 3, 6, 12 and 24 hrs. On each half of the filter paper, the number of insects was recorded, also the Kay square test (with Yates correction) was performed to compare the number of adults according to Sokal and Rohlf (1981). The following is Yates' corrected version of Pearson's chi-squared statistic:

$$\chi^2_{\text{Yates}} = \sum_{i=1}^{N} \frac{(|O_i - E_i| - 0.5)^2}{E_i}$$

Where:

Oi = an observed frequency

Ei = an expected (theoretical) frequency, asserted by the null hypothesis

N = number of distinct events

4- Statistical analysis:

Probit analysis was done to reckon either LC_{50} or LC_{90}, and confidence limits using SPSS V. 10 system software (SPSS Inc., 1999).

RESULTS AND DISCUSSION

Evaluation of four plant oils against the red flour beetle, *T. castaneum*:

Direct toxicity:

Data in Table (1) show the LC_{50} and LC_{90} and their confidence limit values and slope values of LCP lines of four plant oils applied against red flour beetle adults at three concentrations.

Results after 24 hrs exposure showed the LC_{50} values of the tested oils in an ascending order as following ginger (0.72%) > spearmint (0.93%) > coconut (1.08%) > jojoba (3.14%). The order of the efficacy of plant oils against adult beetle on the basis of LC_{90} took the same trend. Comparing the slope values of the LCP lines of the tested plant oils, the adults of the red flour beetles showed relatively high homogeneity response to spearmint oil (5.25) and coconut (4.62), followed by ginger (4.15) then jojoba (2.81) (Table 2).

Results of 48 hrs after application of the plant oils against the red flour beetle (Table 3) showed that the values of LC_{50} and LC_{90} were less than that obtained after 24 hrs. Ginger oil was the highest effective one (LC_{50} and LC_{90} were 0.53 and 1.17%) followed by coconut oil (0.77 and 1.55%), whereas spearmint (0.80 and 1.55%) and jojoba (1.97 and 3.23%) were less effective.

According to the slope values of LCP line of the four plant oils, adult of red flour beetle showed relatively high homogeneity response to spearmint (5.42) followed by coconut and ginger (4.40 and 4.40), then jojoba (3.74).

After 72 hrs exposure of red flour beetle to the four plant oils, the LC_{50} values of ginger and coconut were comparable to that obtained after 48 hrs (0.45 and 0.64%) but for spearmint and jojoba, the values decreased to be 0.75 and 1.32%, respectively For LC_{90}, the values decreased for all tested oils. The homogeneity response of the adult beetle according to slope value may be arranged as follows spearmint (5.84) > coconut (4.71) > jojoba (3.91) > ginger (2.93) (Table 4).

Data of 96 hrs (Table 5) show that ginger still the most effective oil against the insect pest (LC_{50} = 0.37 and LC_{90} = 0.86%). Other oils were comparable, the LC_{50} values were 0.56, 0.66 and 1.17% and LC_{90} values were 1.09, 1.19 and 2.76% for coconut, spearmint and jojoba, respectively.

The homogeneity response of the adult beetles was comparable for spearmint, coconut, ginger and jojoba as the slope values were 5.56, 4.51, 3.68 and 3.51, respectively.

Table 1. List of plant common and scientific names, family name and the plant parts used for extraction of oil

Common name	Scientific name	Family	The plant parts used for extraction of oil
Coconut	*Cocus nucifera*	Arecaceae	Fruits
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizomes
Jojoba	*Simmondsia chinensis*	Simmondsiaceae	Seeds
Spearmint	*Mentha spicata*	Lamiaceae	Leaves

Table 2. LC_{50}, LC_{90} , their confidence limit values and the slope of LCP lines for four plant oils tested against *T. castaneum* after 24 hours.

Plant oils	Mean ± SE						
	Slope	LC_{50} %	Confidence Limits		LC_{90} %	Confidence Limits	
			Lower	Upper		Lower	Upper
Coconut	4.62± 0.50	1.08 ± 0.08	0.87 ± 0.07	1.36 ± 0.11	2.08 ± 0.21	1.58 ± 0.12	3.71 ± 0.66
Ginger	4.15 ± 0.48	0.72 ± 0.02	0.53 ± 0.04	0.90 ± 0.01	1.51 ± 0.10	1.15 ± 0.04	2.83 ± 0.57
Jojoba	2.81 ± 0.57	3.14 ± 0.44	1.91 ± 0.09	66.10 ± 1.89	7.21 ± 1.35	3.84 ± 0.53	76.93 ± 3.72
Spearmint	5.25 ± 1.37	0.93 ± 0.02	0.73 ± 0.06	1.19 ± 0.05	1.98 ± 0.49	1.43 ± 0.21	2.77 ± 0.53

Table 3. LC_{50}, LC_{90}, their confidence limit values and the slope of LCP lines for four plant oils tested against *T. castaneum* after 48 hours.

Plant oils	Mean ± SE						
	Slope	LC_{50} %	Confidence Limits		LC_{90} %	Confidence Limits	
			Lower	Upper		Lower	Upper
Coconut	4.40± 0.41	0.77 ± 0.06	0.60 ± 0.05	0.96 ± 0.08	1.55 ± 0.21	1.18 ± 0.13	2.84 ± 0.57
Ginger	4.40 ± 1.34	0.53 ± 0.05	0.31 ± 0.12	0.69 ± 0.04	1.17 ± 0.09	0.88 ± 0.02	2.74 ± 0.77
Jojoba	3.74 ± 1.41	1.97 ± 0.50	1.35 ± 0.21	2.31 ± 0.25	3.23 ± 0.46	2.54 ± 0.35	30.02 ± 3.69
Spearmint	5.42 ± 1.51	0.80 ± 0.01	0.63 ± 0.02	0.99 ± 0.04	1.55 ± 0.28	1.20 ± 0.15	2.38 ± 0.46

Generally, regardless the exposure period, Comparing the four plant oils on the basis of LC_{50} and LC_{90} values, ginger oil was comparatively the highest effective one, whereas coconut, spearmint and jojoba were of lowest effectiveness, respectively .

Rpellency rate:

According to results obtained in Table (6) the highest repellency rate of ginger oil had the most repellent effect on *T. castaneum* where the repellency rate ranged from 13.08 to 45.08 through the time of exposure, 1 to 24 hrs, respectively. Also, results showed that the repellent effect increased after 24 hrs time of exposure. The repellency rates were 38.41, 45.08, 15.01 and 45.08 for coconut, ginger, jojoba and spearmint oils, respectively.

The essential oils of different spices like mint (*Mentha piperita* L.) have fumigant toxicity to *T. castaneum* (Shaaya *et al*., 1991). Also, Magdy and Abdelgaleil (2008) reported that the essential oils of eight plant species : *Achillea santolina* L., *Artemisia judaica* L., *Citrus reticulate* Balanco, *Schinus terebenthifolius* Raddi, *Mentha microphylla* Koch., *Lantana camara* L., *Majorana hortensis* Moench and *Eucalyptus camaldulensis* Dehnh. showed a promising insecticidal activity against *T. castaneum*. Ngamo *et al*. (2007) used three oils of aromatic plants *(Hyptis spicigera* L. (Lamiaceae), *Annona senegalensis* Pers. (Annonaceae) and *Lippia rugosa* L. (Verbenaceae)) and found that their natural chemicals caused significant mortality against four major of stored grain pests, *T. castaneum*, *Sitophilus oryzae* L., *Sitophilus zeamais* Motsch., and *Callosobruchus maculatus* F. Mundal and Khalqzaman (2006) tested three essential oils (cardamom (*Elletaria cardamomum* Maton), Clove (*Syzygium aromaticum* L.) and cinnamon (*Cinnamomum aromaticum* nees) against the red beetle, *T. castaneum* larvae and adults. Tapondjou *et al*. (2005) stated that the oils from the *Eucalyptus saligna* and the *Cupressus sempervirens* showed a significant proportion of mortality against *S. zeamais* and *Tribolium confusum* Duval. Ajayi and Olonisakin (2011) evaluated essential oils extracted from black pepper in West Africa, *Piper guineense* (Schum and Thonn), cloves, *S. aromaticum* and Ethiopian pepper, *Xylopia aethiopica* (Dunal) as an repellent and toxic compounds against *T. Castanem.* The results of Manzoor *et al*. (2011) showed repellent and lethal effects of the ethanolic extract of plant leaves viz., bakain, *Melia azedaracha* L., datura, *Datura stramonium* L.; lemongrass, *Cymbopogon citratus*; habulas, *Myrtus communis* and mint, *M. longifolia* compared to untreated against *T. castaneum*, *Callosobruchus chinensis* L. and *Oryzaephilus surinamensis* L. .

Table 4. LC_{50}, LC_{90}, their confidence limit values and the slope of LCP lines for four plant oils tested against *T. castaneum* after 72 hours.

Plant oils	Mean ± SE						
	Slope	LC_{50} %	Confidence Limits		LC_{90} %	Confidence Limits	
			Lower	Upper		Lower	Upper
Coconut	4.71± 0.42	0.64 ± 0.04	0.48 ± 0.04	0.79 ± 0.05	1.22 ± 0.13	0.95 ± 0.09	2.19 ± 0.31
Ginger	2.93 ± 0.98	0.45 ± 0.04	0.20 ± 0.08	0.59 ± 0.02	0.98 ± 0.09	0.74 ± 0.04	2.63 ± 0.77
Jojoba	3.91 ± 0.77	1.32 ± 0.05	1.04 ± 0.05	1.84 ± 0.13	3.01 ± 0.37	2.07 ± 0.13	10.98 ± 1.42
Spearmint	5.84 ± 0.94	0.75 ± 0.01	0.61 ± 0.00	0.91 ± 0.02	1.29 ± 0.11	1.03 ± 0.06	1.99 ± 0.25

Table 5. LC_{50}, LC_{90}, their confidence limit values and the slope of LCP lines for four plant oils tested against *T. castaneum* after 96 hours.

Plant oils	Mean ± SE						
	Slope	LC_{50} %	Confidence Limits		LC_{90} %	Confidence Limits	
			Lower	Upper		Lower	Upper
Coconut	4.51± 0.43	0.56 ± 0.06	0.37 ± 0.09	0.70 ± 0.07	1.09 ± 0.08	0.85 ± 0.06	2.08 ± 0.16
Ginger	3.68 ± 0.72	0.37 ± 0.05	0.09 ± 0.02	0.52 ± 0.02	0.86 ± 0.01	0.62 ± 0.03	2.26 ± 0.39
Jojoba	3.51 ± 0.34	1.17 ± 0.07	0.91 ± 0.07	1.59 ± 0.09	2.76 ± 0.13	1.92 ± 0.06	6.97 ± 1.01
Spearmint	5.56 ± 1.11	0.66 ± 0.01	0.52 ± 0.03	0.81 ± 0.01	1.19 ± 0.10	0.95 ± 0.05	1.95 ± 0.29

Table 6. Repellency assay using four plant oils at 2 % treatment after different exposure times against *T. castaneum*

Plant oils	Trial	N. of beetles on each half after each exposure time																	
		1h				3h				6h				12h				24h			
		Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r
Coconut	1	6	14	3.25		3	17	9.85		4	16	7.25	13.08**	16	4	7.25		0	20	20.05	
	2	6	14	3.25	9.61**	5	15	5.05	21.61**	5	15	5.05		18	2	12.85	29.41**	4	16	7.25	38.41**
	3	6	14	3.25		4	16	7.25		7	13	1.85		17	3	9.85		2	18	12.85	
Ginger	1	4	16	7.25		1	19	16.25		3	17	9.85	15.01**	3	17	9.85		0	20	20.05	
	2	3	17	9.85	13.08**	5	15	5.05	19.28**	2	18	12.85		2	18	12.85	24.08**	1	19	16.25	45.08**
	3	9	11	0.25		7	13	1.85		10	10	0.05		6	14	3.25		3	17	9.85	
Jojoba	1	7	13	1.85		2	18	12.85		4	16	7.25		8	12	0.85		4	16	7.25	
	2	7	13	1.85	4.28*	3	17	9.85	21.61**	7	13	1.85	8.08**	7	13	1.85	1.68 n.s.	8	12	0.85	15.01**
	3	8	12	0.85		7	13	1.85		8	12	0.85		10	10	0.05		3	17	9.85	
Spearmint	1	6	14	3.25		3	17	9.85		5	15	5.05	13.08**	4	16	7.25		0	20	20.05	
	2	6	14	3.25	11.28**	5	15	5.05	19.28**	5	15	5.05		3	17	9.85	21.61**	2	18	12.85	45.08**
	3	5	15	5.05		5	15	5.05		6	14	3.25		5	15	5.05		2	18	12.85	

Tr = treated half Un = untreated half χ^2 r = overall χ^2* = significant ** = highly significant n.s. = not significant

Abid and Butt (2015) reported a repellent activity for three plant extracts, ginger (*Z. officinale*), cardamom (*E. cardamomum*), and nutmeg (*Myristica fragrans*) against the red flour beetle, *T. castaneum*. Al-Jabr (2006) found that *Cinnamomum camphora, Cymbopogon winterianus, Matricaria chamomilla, Mentha varidis, Prunus amygdalus var amara, Rosmarinus afficinalis* L. and *Simmondsia chinensis* essential oils possess different toxicities and

repellent effects against the adults of saw toothed grain beetle, *O. surinamensis* and the red flour beetle, *T. castaneum* in the laboratory. Rahdari and Hamzei (2017) reported the repellency effect of some essential oils such as *M. piperita*, *R. officinalis* and *Coriandrum sativum* against *T. confusum.* The results showed that essential oil of *R. officinalis* and *M. piperita* were more repellent than *C. sativum*. Khanzada *et al.* (2015) evaluated the repellency of plant oils such as Mustard (*Brassica campestris*), Coconut (*C. nucifera*), Sesame (*Sesamum indicum* L.) and Rocket seed oil (*Eurica sativa*) against red flour beetle, *T. castaneum* in wheat. Caballero-Gallardo *et al*. (2012) indicated that adult beetles of *T. castaneum* were repelled significantly by essential oils of *Cymbopogon martini*, *C. flexoisus* and *Lippia origanoides*. Mahmoodavand and Shakarami (2014) evaluated the repellent effect of four plant essential oils including *Artemisa haussknechtii, M. longifolia*, *Thymus daenensis* and *Achillea wilhelmisii* against *T. confusum* and *T. castaneum* and found that the essential oil of *M. longifolia* exhibited a significant strong repellent effect.

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التأثير السام والطارد لأربعة زيوت نباتية على خنفساء الدقيق الصدئية، تريبوليوم كاستانيوم (هيربست)

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أجريت هذه الدراسه لتقييم التاثيرات السامة والطاردة لأربعة زيوت نباتية أساسية، وهى زيت جوز الهند (*Cocus nucifera* L.)، زيت الزنجبيل (*Zingiber officinale* Rosc.)، زيت الجوجوبا (*Simmondsia chinensis* L.) ، وزيت النعناع (*Mentha spicata* L.) ضد خنفساء الدقيق الصدئية. تم استخدام ثلاثة تركيزات لكل زيت من هذه الزيوت (0.5 و 1 و 2 ٪). تم حساب قيم LC_{90} و LC_{50} أيضًا باستخدام تحليل Probit. تم تحديد قيم LC_{90} و LC_{50} لكل من زيت جوز الهند والزنجبيل والجوجوبا والنعناع بعد 24، 48 ، 72 و 96 ساعة. أظهرت النتائج أن زيت الزنجبيل كان أكثر فاعلية ضد تريبوليوم كاستانيوم. أيضا أظهرت النتائج أن زيت الزنجبيل ما زال هو الزيت الأكثر فعالية ضد الآفة بعد مرور 96 ساعة من المعاملة (LC_{90} = 0.86٪ و LC_{50} = 0.37) بلغت قيم LC_{50} لزيت جوز الهند، النعناع والجوجوبا 0.56 ، 0.66 و 1.17٪ بينما بلغت قيم LC_{90} 1.09 ، 1.19 و 2.76٪ لجوز الهند، النعناع والجوجوبا على التوالي. أجري اختبار الطرد بتركيز 2 ٪ لكل الزيوت. تم تسجيل البيانات بعد 1 و 3 و 6 و 12 و 24 ساعة من إطلاق الحشرات. وأظهرت النتائج أن زيت الزنجبيل والنعناع سجل أعلى معدل طرد (45.08) ، في حين كان أدنى معدل طرد (15.01) لزيت الجوجوبا بعد 24 ساعة