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EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 12 No. 2 (2020)

Egypt. Acad. J. Biolog. Sci., 12(2): 219-227 (2020)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Toxicity of Ten Native Edible and Essential Plant Oils Against the Granary Weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae)

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ARTICLE INFO
Article History
Received:11/7/2020
Accepted:9/11/2020
Keywords:
Sitophilus granarius, oils, Insecticidal effects, stored products.

ABSTRACT

The comparative toxicity of ten selected native essential and edible oils was assessed against *S. granarius*. The oils used are black seed oil (*Nigella sativa*), Sesame oil (*Sesamum indicum*), olive oil (*Olea europapea*), Peppermint oil (*Mentha piperita*), Basil Oil (*Ocimum basilicum*), orange oil (*Citrus sinensis*), Rosemary oil (*Rosmarinus officinalis*), Clove oil (*Dianthus caryophyllus*), Garlic oil (*Allium sativum*), and Cinnamon oil (*Cinnamomum zeylanicum*). Different concentrations of oils were mixed with wheat grains and provided for adults in test tubes. Mortalities were recorded for 3 days post-treatment. The overall results showed that Garlic oil was the most toxic and Rosemary was the least toxic among both edible and essential oils. A direct correlation was found between oil dose and percent mortality of adults under all exposure periods. The recorded values of LC_{50} and the toxicity index showed that Sesame oil was the most toxic edible oil followed by Black seed and Olive oils. The essential oils used can be arranged in descending order according to their toxicities to *S. granarius* adults as follows: Garlic > clove > Cinnamon > Basil > Orange > Peppermint> Rosemary. All the fixed and essential oils used, demonstrated satisfactory activity and proved to be promising as control agents of *S. granaries* and consequently other similar stored product insects. Some oils were highly toxic at low concentration and short exposure time; whereas some oils might be required in higher concentrations and longer exposure time to achieve satisfactory control of the insects.

INTRODUCTION

The use of pesticides, for the protection of stored products, in developed countries is increasingly organized by strict laws and systems to minimize deleterious side effects. In most underdeveloped countries such procedures are still largely lacking or not enforced.

In these countries, pesticides are frequently used as if their benefits always overshadow their adverse side effects. In addition, most developing countries cannot pay for importing the newer, less-environmentally but more expensive damaging pesticides. It is sensible, therefore, to search for alternative native pesticides. In this respect, botanicals could play a key role.

Mixing plant oils from locally available plants with stored grains was in fact an ancient Indian and African method of protection against insect attack (Periera, 1983) and most of the published studies with plant oils have been concerned with their use against stored grain insect pests which usually cause a huge economic loss.

Some reviews as Campolo *et al.* (2018) showed that large numbers of plant oils have been screened for preventing post-harvest losses due to insects in many countries. It is not surprising that oils from different geographic areas may cause different responses in the same insect species, because oils are extracted from cultivated and wild plants, and their composition is strongly subjected to variations according to their geographic origin. Thus, a full screening of the efficiency of such plant oils against stored product insect pests in each country is therefore required.

This study aims to screen the comparative toxicity of ten selected commercially available essential and edible oils against *S. granarius* (L.), and to explore the possibility of using these oils as effective, economical, and environmentally safe commercial insecticides for controlling the granary weevil *S. granarius* which is a major pest of wheat grains in Egypt.

MATERIALS AND METHODS

Test Insect:

Adults of the granary weevil, *Sitophilus granarius* (Coleoptera: Curculionidae) were used in the present work. Insects were maintained in the laboratories of Zoology Department, Faculty of Science, Sohag University, Egypt. The stock culture of the granary weevil, *S. granarius*, was kindly obtained from a colony reared for several years in laboratories of the Egyptian Atomic Energy Authority. The initial population of *S. granarius* was reared in one-liter wide-mouthed glass jars containing wheat grains with 12.5% - 13% moisture. Mouths of the jars were covered with muslin by rubber bands for ventilation and to prevent the escape of the weevil's populations. Cultures were maintained in incubators at 30 ± 1°C and 70 ± 5% RH. The wheat, before being used, was disinfected by freezing for one week and then kept in a clean tight glass container until used. The stock culture was set up by introducing {100 – 200} adults. The adults were permitted to oviposit on the grains and then removed after one week, leaving the egg plugs on the wheat. Adults (5-7) days old were collected and used in all experiments.

Oils Used:

Ten oils, three fixed, and seven essential, plant oils were selected and used during the present investigation. The fixed oils used are black seed oil (*Nigella sativa*), Sesame oil (*Sesamum indicum*) and olive oil (*Olea europaea*). The essential oils used are Peppermint oil (*Mentha piperita*), Basil oil (*Ocimum basilicum*), orange oil (*Citrus sinensis*), Rosemary oil (*Rosmarinus officinalis*), Clove oil (*Dianthus caryophyllus*), Garlic oil (*Allium sativum*), and Cinnamon oil (*Cinnamomum zeylanicum*). All oils used were obtained from Nefertari Company, Cairo, Egypt with purity higher than 98%.

The weight of 100 g of clean and un-infested wheat grains, which had not been pre-treated with weevil repellents, was treated with four different doses of fixed oils (black seed oil, olive oil and sesame oil) (0.25ml, 0.5ml, 0.75ml, 1ml) and five doses of volatile oils (orange oil, rosemary oil, clove oil, peppermint oil and basil oil) (0.15ml, 0.25ml, 0.5ml, 0.75ml, 1ml), more doses were used for garlic oil and cinnamon oil (0.05ml, 0.1ml, 0.15ml, 0.25ml, 0.5ml, 0.75ml), acetone was used as a solvent. After treatment, wheat grains were mixed manually for 5 minutes to ensure an even coating of grains and then left for another 10 minutes to allow evaporation of acetone. Untreated wheat grains were mixed with acetone only and were used as control.

Toxicity of Oils:

In order to test the toxicity of oils against the pest adults, three replicates, were made for each dose of the tested oils, and also for the control group. All bioassays were carried out in test tubes of 10 ml volume containing the treated grains. 10 unsexed adults, 5 to 7 days old, were transferred to each test tube, then each tube was covered with muslin cloth fixed with a rubber band to prevent insects escaping and kept in an incubator (30 ± 1°C and 70 ± 5% RH.).

Mortality counts were recorded 24h, 48h, and 72h post-exposure for *S. granarius*. Insects were considered dead if they did not move away when touched gently or tilted.

Statistical Analysis:

The toxicity data were analyzed using probit analysis to estimate lethal concentrations LC_{50} with their 95% fiducial limits (LDP line). All experiments were repeated thrice and data are the mean ± standard error subjected to one-way ANOVA (Analysis of variance). The Differences between means were considered significant when $p < 0.05$. Analysis of data was performed with the SPSS program version 16.0.

RESULTS

Toxicity of Edible Oils to *S. granaries*:

The toxicity of the three edible oils on adults of *S. granarius* is presented in table 1 and table 3. The results showed that the three oils are toxic to adults of *S. granarius* (Table 1). It was obvious that there was a direct correlation between oil dose and percent mortality of adults under all exposure periods. For example; the percentage mortalities after 24h exposure to black seed oils were 06.67, 13.33, 23.33, and 76.67% at dose levels of 0.25, 0.5, 0.75, 1ml/gm grains, respectively. The same trend was true in all cases regardless of exposure duration. After 24h of exposure, and in most cases, succeeding doses do not result in a significant increase in mortalities. A significant increase in mortalities was found between the effects of the lowest and highest doses used.

Table 1 : Mortality % of *Sitophilus granarius* adults exposed for different periods to wheat grains treated with 3 edible oils at 30 ^{0}C.

Plant oil	Dose (ml/100gm)	Mortality %± SE at different exposure time		
		24h	48h	72h
Black seed	0.25	06.67^{a} ±0.34	6.67^{a}±0.34	13.33^{a}±0.67
	0.5	13.33^{ab}±0.34	43.33^{b}±0.34	80.00^{b}±0.00
	0.75	23.33^{b}±0.34	76.67^{c}±0.1.40	96.67^{bc}±0.34
	1	76.67^{c}±0.34	96.67^{c}±0.00	100.00^{c}±0.00
Sesame	0.25	23.33^{a}±0.34	50.00^{a}±0.58	56.67^{a}±0.67
	0.5	33.33^{ab}±0.34	53.33^{a}±0.67	70.00^{ab}±1.00
	0.75	40.00^{bc}±0.00	70.00^{ab}±0.00	83.33^{ab}±0.67
	1	50.00^{c}±0.00	93.33^{b}±0.67	100.00^{b}±0.00
Olive	0.25	3.33^{a}±0.34	20.00^{a}±0.58	26.67^{a}±0.67
	0.5	26.67^{b}±0.67	40.00^{ab}±0.58	53.33^{b}±0.67
	0.75	26.67^{b}±0.34	63.33^{bc}±0.67	70.00^{b}±0.00
	1	40.00^{b}±0.00	73.33^{c}±0.67	83.33^{b}±0.67

For each oil, means in the same column followed by the same letters do not differ significantly ($P \leq 0.05$) as determined by Tuckey's multiple comparison test.

The recorded values of LC_{50} and the toxicity index showed that black seed oil was the most toxic oils followed by Sesame oil and then olive oil after 24h exposure. After 48 and 72h of exposure, Sesame oil was the most toxic edible oil followed by black seed oil and olive oil (Table, 3). The LC_{50s} of Sesame oil, black seed oil and olive oil at the end of the experiment were 0.23, 0.39 and 0.45 and the toxicity indexes were 4.35, 2.56, and 2.22, respectively. According to the aforementioned results, Sesame oil was the most toxic to *S granarius* adults followed by black seed oil and olive oil.

Table 2 : Mortality % of *Sitophilus granarius* adults exposed for different periods to wheat grains treated with 7 essential oils at 30 ^{0}C.

Plant oil	Dose (ml/100gm)	Mortality % after		
		24h Exposure	48h Exposure	72h Exposure
Rosemary	0.15	$0.00^a±0.00$	$0.00^a±0.00$	$0.00^a±0.00$
	0.25	$3.33^a±0.34$	$6.67^a±0.34$	$6.67^a±0.34$
	0.5	$3.33^a±0.34$	$10.00^a±0.00$	$16.67^a±0.67$
	0.75	$33.33^b±0.34$	$53.33^b±0.34$	$63.33^b±0.34$
	1	$90.00^c±0.00$	$100.00^c±0.00$	$100.00^c±0.00$
Clove	0.15	$16.67^a±0.34$	$43.33^a±0.88$	$60.00^a±0.58$
	0.25	$20.00^a±0.00$	$46.67^a±0.67$	$76.67^a±0.67$
	0.5	$23.33^a±0.88$	$66.67^{ab}±0.34$	$83.33^b±0.67$
	0.75	$43.33^a±0.34$	$63.33^{ab}±0.34$	$100.00^b±0.00$
	1	$40.00^a±0.00$	$80.00^b±0.00$	$100.00^b±0.00$
Garlic	0.05	$50.00^a±0.58$	$60.00^a±0.58$	$80.00^a±1.00$
	0.1	$86.67^b±0.34$	$96.67^b±0.34$	$100.00^b±0.00$
	0.15	$100.00^c±0.00$	$100.00^b±0.00$	$100.00^b±0.00$
Peppermint	0.15	$0.00^a±0.00$	$3.33^a±0.34$	$13.33^a±0.34$
	0.25	$6.67^a±0.67$	$20.00^b±0.67$	$23.33^b±0.58$
	0.5	$33.33^b±0.34$	$56.67^c±0.34$	$93.33^c±0.34$
	0.75	$43.33^b±0.34$	$63.33^d±0.34$	$100.00^c±0.00$
	1	$50.00^b±0.00$	$76.67^e±0.34$	$100.00^c±0.00$
Cinnamon	0.1	$6.67^a±0.34$	$10.00^a±0.00$	$10.00^a±0.00$
	0.15	$53.33^b±0.34$	$60.00^b±0.00$	$86.67^b±0.34$
	0.25	$96.67^c±0.88$	$100.00^c±0.00$	$100.00^c±0.00$
	0.5	$100.00^c±0.00$	$100.00^c±0.00$	$100.00^c±0.00$
Basil	0.15	$3.33^a±0.34$	$13.33^a±0.34$	$43.33^a±0.67$
	0.25	$26.67^b±0.34$	$30.00^b±0.00$	$53.33^a±0.67$
	0. 5	$100.00^c±0.00$	$100.00^c±0.00$	$100.00^b±0.00$
Orange peel	0.15	$3.33^a±0.34$	$26.67^a±0.34$	$26.67^a±0.34$
	0.25	$20.00^b±0.00$	$46.67^b±0.00$	$50.00^b±0.34$
	0.5	$43.33^c±0.34$	$56.67^b±0.34$	$70.00^b±0.34$
	0.75	$50.00^c±0.00$	$70.00^c±0.00$	$86.67^c±0.34$
	1	$93.33^d±0.34$	$100.00^d±0.00$	$100.00^c±0.00$

For each oil, means in the same column followed by the same letters do not differ significantly ($P \leq 0.05$) as determined by Tuckey's multiple comparison test

The results given in the tables (Table 2 and 3) showed that the essential oils used exhibited different levels of toxicity to *S. granarius* adults. Some oils as garlic showed very high toxic effects during the 1st 24h of exposure. A dose of 0.15 mg Garlic/100gm grains resulted in 100% mortality. The same level of toxicity was recorded for Cinnamon and basil oils, but at a higher dose (0.5 oil/100 gm. grains). In most cases, a positive correlation was found between mortalities and the dose of oil used. This correlation was clear and significant between the lowest and highest doses used for most oils. Unlike edible oils, it was quite clear that in most oils used, percent mortalities due to essential oils are dependent on dosage rather than the period of exposure. This can be seen in case of Garlic, Rosemary and Cinnamon oils, where no significant increase in mortalities was recorded with the increase in exposure time. Whereas, increasing exposure time to Clove and peppermint oils resulted in a significant increase in mortality. In case of Basil oil, mortality increased significantly when the exposure time was increased to 72h at 0.15 and 0.25 g/100 g grains, whereas in Orange peel oil the recorded increase in mortality was only significant when the exposure time was increased from 24 to 72h. Based on the LC_{50} values (Table 3) garlic oil had the highest toxicity against *S. granarius* adults. A dose of 0.04 was needed to kill 50% of the insect after 24 and 48 h of exposure, whereas, the recorded LC_{50} after 72 h of exposure was 0.01 mg. /100ml grains. So, garlic oil was selected as a reference for determining the toxicity index for other oils used during the present study. Clove oil was found to have a weakened delayed effect and was only efficient and more toxic after 48 h of exposure and became the 2nd most toxic oils to the insect after 72 h of exposure. The recorded LC_{50} values and the toxicity index after 24 h of exposure showed that the essential oils used can be arranged in descending order according to their toxicities to *S. granarius* adults as follows: Garlic > Cinnamon > Basil > Orange >Rosemary > Peppermint > clove. At the end of the experiments (72 h of exposure) this order was changed as follows: Garlic > clove > Cinnamon > Basil > Orange > Peppermint> Rosemary.

The overall results show that Garlic oil was the most toxic and Rosemary was the least toxic to adults of *S granarius* among both edible and essential oils used in the present investigation.

Table 3:LC_{50}s , slope values and % toxcity index of mortality of *Sitophilus granarius* adults exposed for different periods to selected edible and essential oils at (30 ^{0}C)

Plant oil	24h exposure			48h exposure			72h exposure		
	LC_{50}	Slope	Toxicity index (%)	LC_{50}	Slope	Toxicity index (%)	LC_{50}	Slope	Toxicity index (%)
Black seed	0.90	3.65	4.44	0.52	4.80	7.69	0.39	4.81	2.56
Olive	1.27	2.20	3.15	0.57	2.50	7.01	0.45	2.57	2.22
Sesame	1.11	1.16	3.60	0.31	1.90	12.9	0.23	2.18	4.35
Basil	0.30	6.14	13.33	0.28	4.98	14.29	0.19	3.25	5.26
Orange peel	0.54	3.13	7.41	0.30	2.04	13.33	0.26	2.50	3.85
Peppermint	0.94	1.82	4.26	0.50	2.65	8.0	0.29	4.34	3.45
Clove	2.43	0.85	1.65	0.24	1.16	16.67	0.08	1.33	12.5
Rosemary	0.78	6.51	5.13	0.66	5.51	6.06	0.62	5.48	1.61
Garlic	0.04	2.34	100	0.04	2.87	100	0.01	1.12	100
Cinnamon	0.15	7.47	26.7	0.14	7.95	28.57	0.13	9.28	7.69

DISCUSSION

Results of the present work showed that the three edible oils used are toxic to adults of *S. granaries*. Sesame oil was the most toxic followed by Black seed and Olive oil. A direct and significant correlation was found between oil concentration and insect mortality at different exposure times. This correlation was also observed by many investigators as Yildirim *et al*., (2011) and Darwish (2016).

The lower mortality rates recorded after 24 h exposure to edible oils may be due to the slow lethal action of oils by reducing respiratory activity or elimination of toxic metabolites or direct toxic effect of oil or oil constituents that possibly penetrate adult (Don-Perdo, 1989). A similar trend of activity was observed by Barakat *et al.,* (2000) who found that Soya beans and Maize had a low initial kill for *S. granaries* adult at one and two days of exposure, then the prolongation of the exposure time caused a high increase in percent mortality. Although the increase of exposure time the oils gave higher insect mortality, it also increases the time provided to the living insects, for feeding and egg lying which leads to a corresponding increase in insect population and damage of the stored grains. So, using more oil doses may be needed for fast killing.

Essential oils used were generally more toxic and killed insects within a short time. The high toxicity index and fast effect of most essential oils may be due to the penetration of oils via insect’s cuticle and contact the nerve endings in the invertebrate pest's trachea, and cause neurotoxic activity and more rapid death (Bessette *et al.*, 2013). Ryan and Byrne (1988) proposed that the toxic effect may be due to reversible competitive inhibition of acetyl cholinesterase by occupation, of the hydrophobic site of the enzyme’s active center.

Our results showed that the essential oils used exhibited different levels of toxicity to *S. granarius* adults. This difference in efficiency may be attributed to the differences in families of the tested plant species. Moreover, the soil and climatic conditions faced, physiological structure, and nature of the plant species also influence the composition and toxicity of essential oils (Teke and Mutlu, 2020).

Garlic oil was the most toxic against *S. granaries* adults followed by Clove oil. The high toxic activity of Garlic oil against *S. granarius* and other stored product pests was reported by many investigators as; Cardiet *et al.* (2012) and Chang *et al.* (2017) on *Sitophilus oryzae*; Plata-Rueda *et al. (*2017) on *Tenebrio molitor;* Isikber (2010) on *Ephestia Kuehniella*; Ho *et al.* (1997) on *Tribolium castaneum* and *Sitophilus zeamais*. The toxic effect of Garlic oil on this wide range of stored product pests may enhance the possibility of using this oil for protection from stored product pests. Insects exposed to the garlic essential oil and toxic compounds exhibited modification in locomotion activity and muscle contractions. The rapid toxicity of this oil and its constituents in insects may point to neurotoxic activity and rapid knock-down effect or immobilization. Acetyl cholinesterase is an enzyme that has been shown to be inhibited by garlic compounds (Plata-Rueda *et al.,* 2017).

Results of the present work also cleared that Clove, Cinnamon, and Basil oils are also promising and highly toxic against *S. granarius*. Similar findings were reached by other investigators as Plata-Rueda *et al.,* (2018) who stated that cinnamon and clove essential oils showed 100% mortalities in the adult of *S. granarius* 24 h after application. Also, Kim *et al*. (2003) found that cinnamon oil was effective (80% mortality) after 1 day after treatment of *Sitophilus oryzae* and gave 100% mortality at 2 days after treatment. The toxic effect of orange peel oil on *S. granarius* was also recorded by Magdalena (2009) and Ibrahim & Sahar (2011). Our results indicated that Rosemary oil was the least toxic oil against *S. granarius* adults. These findings contradict the results of Laznik *et al.,* (2012) who concluded that Rosemary oil is more effective against *S. granarius* than peppermint oil.

Also, our results agree with the results of Saad *et al.* (2017) who stated that Basil oil is more toxic than Clove oil. Our results confirmed what has been found by the authors who noticed that the clove showed its higher toxicity after 72 hrs. Also, our results run parallel to the results of Kim & Lee (2014) who reported that Basil oil was more toxic than Orange oil against *Sitophilus zeamais and Tribolium castaneum*.

All the fixed and essential oils used, during the present work, demonstrated satisfactory activity and proved to be promising as control agents of *S. granarius* and consequently other similar stored product insects. Some oils were highly toxic at low concentration and short exposure time; whereas some oils might be required in higher concentrations and longer exposure time to achieve satisfactory control of the insects. The big number of publications on the neurotoxic, cytotoxic, phyototoxic, and mutagenic activities of many edible and essential oils in different creatures (Bakkali *et al.*, 2008) make them a good, cheap, and safe alternative for protecting and controlling stored product pests.

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ARABIC SUMMARY

سمية عشرة زيوت نباتية محلية، صالحة للأكل وعطرية ضد سوسة القمح (غمدية الأجنحة: السوسيات)

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تم تقييم السمية النسبية لعشرة زيوت نباتية محلية، صالحة للأكل وعطرية ضد سوسة القمح (غمدية الأجنحة: السوسيات). والزيوت المستخدمة هي: زيت الحبة السوداء (حبة البركة)، زيت السمسم، زيت الزيتون، زيت النعناع، زيت الريحان، زيت البرتقال، زيت الروزماري، زيت القرنفل، زيت الثوم، وزيت القرفة. تم خلط تراكيز مختلفة من كل زيت مع حبوب القمح وتقديمها للحشرات البالغة في أنابيب اختبار. وتم تسجيل الوفيات لمدة 3 أيام بعد المعالجة. وقد أظهرت النتائج الإجمالية أن زيت الثوم هو الأكثر سمية وأن الروزماري كان الأقل سمية بين كل من الزيوت الأساسية والصالحة للأكل. وتبين وجود علاقة طردية مباشرة بين جرعة الزيت ونسبة وفيات الحشرة عند جميع فترات المعاملة. وأظهرت القيم المسجلة للجرعات القاتلة لنصف العشيرة (LC_{50}) ومؤشر السمية أن زيت السمسم كان أكثر زيوت الطعام سمية يليه زيت الحبة السوداء وزيت الزيتون. ويمكن ترتيب الزيوت الأساسية المستخدمة تنازليًا وفقًا لسميتها للحشرات البالغة على النحو التالي: ثوم> قرنفل> قرفة> ريحان> برتقال> نعناع> روزماري. وأظهرت جميع الزيوت الصالحة للأكل والأساسية المستخدمة نشاطًا مرضيًا وأنها واعدة كعناصر مكافحة الحشرة في مخازن الحبوب. وبالتالي غيرها من الحشرات المماثلة. وتبين أن بعض الزيوت كانت شديدة السمية عند التركيزات المنخفضة ووقت التعرض القصير، في حين أن بعض الزيوت ينبغي استخدامها بتركيزات أعلى ووقت تعرض أطول لتحقيق مكافحة مرضية للحشرة.