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Repellency of Ten Edible and Essential Native Plant Oils to The Granary Weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae)

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ABSTRACT

The comparative repellency of ten selected native essential and edible oils was assessed against adults of *S. granarius*. The oils used are black seed oil (*Nigella sativa*), Sesame oil (*Sesamum indicum*), olive oil (*Olea europaea*), 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*). The repellent activity of different concentrations of each oil was investigated using the area preference method. All essential oils used showed much higher repellent activity to this insect than edible oils. The used oils can be arranged in descending order according to their average mean repellent activity as follows: Cinnamon (96.19%), Garlic (91.27%), Clove (90.43%), Basil (87.04%), Peppermint (79.31%), Rosemary (74.49%), Orange peel (53.54%), Sesame (13.08%), Black seed (11.18%), whereas, Olive oil which was attractive to the insects (- 4.49%). In spite of Orange peel oil, no direct logarithmic correlation was found between the repellent activity of oils and doses used. Also, results showed that the repellent activity of oils fluctuated between decrease and increase as the time of exposure increased. The possibility of using oils as repellents for stored product pests was discussed.

INTRODUCTION

Egypt is the most populous country in the Arab World and is also by far the largest importer of wheat globally. For centuries, wheat has been a central component of the typical diet of the country's inhabitants (FAO, 2015). The annual report of the Egyptian Ministry of Agriculture and Land Reclamation in 2007 estimated the loss in wheat due to stored insects in Egypt as equivalent to half a million tons of which 12% is caused by the granary weevil alone, *Sitophilus granarius* (Massoud *et al.*, 2018). This weevil is one of the most destructive insects of stored grains worldwide, which is able to cause considerable economic losses (Hamza *et al.*, 2016; Karakas, 2016; Plata-Rueda *et al.*, 2018; Martynov *et al.*, 2019).

The traditional methods to control grain weevil and other pests of stored products have serious drawbacks, such as environmental pollution, insect's resistance, high

mammalian toxicity, and increasing cost of application. This had led to a search for more safe and less expensive alternative chemicals such as plant oils. Oils are safe to mammals, easily obtained, can be integrated with other pest management measures, and eliminate the risk associated with hand mixing of insecticides. Thus, in recent years, many authors concluded that oils can play an important role in the protection and control strategies of stored products as Alkan and Erturk, (2019; Baccari *et al.* (2020); Chenni *et al.* (2020); Ebrahimifar *et al.* (2020); Kheloul *et al.* (2020); Najem *et al.* (2020); Shafaie *et al.* (2019); Wang *et al.* (2020) and Yang *et al.* (2020).

The present work aims to screen the possibility of using certain native edible and essential plant oils for the protection of wheat grains by repelling stored product pests as *S. granarius* (*L.*), 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 a one-liter wide-mouthed glass jar 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 $30 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ 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, basil oil, and cinnamon oil) (0.15ml, 0.25ml, 0.5ml, 0.75ml, 1ml), more doses were used for garlic oil (0.05ml, 0.1ml, 0.15ml, 0.25ml, 0.5ml, 0.75ml, 1ml), 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.

Repellency of Oils:

The repellent activity of the selected plant oils (both fixed and essential) against *S. granarius* adults was investigated using the area preference method (Tapondjou *et al.*, 2005). In case of fixed oils (Black seed, olive, sesame oils) four doses were used (0.25ml, 0.5ml, 0.75ml, 1ml/ 100g of grains), whereas Five doses (0.15ml, 0.25ml, 0.5ml,

0.75ml, and 1ml/per 100g of grains) were used for volatile oils (orange, rosemary, clove, peppermint, basil and cinnamon oils). In case of Garlic oil which showed high repellent activity, two lower doses (0.05 and 0.1) were used. Acetone was used as a solvent. A filter paper (8 cm in diameter) was cut into two equal parts and was placed in a plastic petri dish (8cm in diameter). Wheat grains treated with oil were placed on one half of the filter paper. Wheat grains treated with acetone were placed on the other half and were used as control. Twenty adult insects were released at the centre of the dish. Three replicates were run for each tested dose so that 60 adults were assayed per dose. The test was carried out and kept in an incubator ($30 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH.). The numbers of insects on each side of the filter paper (treated and control sides) were recorded after 2, 4, and 6 hours from the beginning of the test.

The percent repellency (PR) of oil was determined by the equation followed by (Ibrahim and Sahar, 2011).

$$\text{PR} = [(\text{Nc} - \text{Nt}) / (\text{Nc} + \text{Nt})] \times 100$$

Where Nt is the no of insects on the treated half and the Nc is that on the control half.

Statistical Analysis:

All experiments were repeated thrice and data are the mean \pm standard error subjected to Independent-Sample T-Test. The Differences between means were considered significant when $p < 0.05$. Analysis of data was performed with the SPSS program version 16.0.

RESULTS

Results tabulated in table (1) and graphically illustrated in figures (1 and 2) show the repellent activity of different fixed and essential oils against adults of the granary weevil *S. granarius*. Generally, all essential oils used showed much higher repellent activity to this insect than edible oils (Fig.2).

The selected oils can be arranged in descending order according to their average mean repellent activities as follows (fig.2): Cinnamon (96.19%), Garlic (91.27%), Clove (90.43%), Basil (87.04%), Peppermint (79.31%), Rosemary (74.49%), Orange peel (53.54%), Sesame (13.08%), Black seed (11.18%), and at last Olive oil which attracted the insects (- 4.49%)

In case of edible oils, no significant direct correlation was found between the dose and repellent activity of all oils used. In case of Black seed oil, the lowest dose, 0.25 ml/100ml grains, exhibited the strongest and highly significant repellent effect to *S. granarius* at 2h post-treatment (83.3%), and then the repellency suddenly decreased to (27.57%) 4h post-treatment. On the contrary, at a higher dose of the oil (1 ml/100gm), insects were attracted to the grains (mean negative repellency percentage -62.23%), and the power of attractiveness significantly increased by time (70 and 86.7% at 4 and 6 h of exposure, respectively). High doses of Olive oil, 0.75 and 1ml/100gm grains, showed moderate repellent activity (MPR=17.19 and 22.61, respectively), whereas at lower doses the oil attracted more insects than control (MPR= -36.67 and -21.10 at 0.25 and 0.5 ml, respectively). No significant correlation was found between the repellency of Olive oil and exposure time. Sesame oil showed a weak repellent activity against the insect. No significant difference was found between the repellency of grains treated with Sesame oil and control at all doses used except at 0.25 and 0.5 ml at 4 h post exposures. All essential oils used showed strong repellent activity against adults of *S. granarius*. The mean repellency index showed that Cinnamon, Garlic, Basil oils had the highest repellent class activity to the insect (80-100% repellency) at all tested doses and for 6 h post-exposure, followed by Clove, Peppermint, Rosemary and orange oils (Table, 1).

Table 1: Repellency % of *S. granarius* adults exposed for different periods to wheat grains treated with 6 essential oils at 30°C.

Plant oil	Doses (ml/100gm)	Repellency %±SE			Rep. % mean	Rep. class
		Exposure time				
		2hr	4hrs	6hrs		
Clove	0.15	96.46±0.33**	60±0.58**	46.7±2.6	67.72	IV
	0.25	96.70±0.33**	93.30±0.67**	86.7±0.67**	92.23	V
	0.5	100±0.33**	90±1.00**	100±0.33**	96.67	V
	0.75	96.70±0.33**	100±0.33**	93.30±0.33**	96.67	V
	1	96.70±0.33**	100±0.33**	100±0.33**	98.90	V
Rosemary	0.15	62.05±1.33*	60±1.52*	80±1.00**	67.35	IV
	0.25	76.7±1.20*	33.3±0.33**	66.7±1.20*	58.9	III
	0.5	92.56±.67**	96.7±0.33**	73.33±0.33**	87.53	V
	0.75	96.46±1.67**	100±0.33**	83.3±1.20**	93.25	V
	1	72.85±1.50**	86.7±0.88**	36.7±1.45**	65.42	IV
Cinnamon	0.15	96.7±0.33**	100±0.33**	100±0.33**	98.9	V
	0.25	93.33±0.33**	89.65±1.20**	93.19±0.58**	92.06	V
	0.5	100±0.33**	96.64±0.67**	93.3±0.67**	96.65	V
	0.75	100±0.33**	96.59±0.58**	93.3±0.33**	96.63	V
	1	96.7±0.33**	96.7±0.33**	96.7±0.33**	96.70	V
Basil	0.15	73.3±1.76**	93.3±0.67**	90±1.00**	85.53	V
	0.25	96.64±0.33**	90±0.58**	86.7±0.33**	91.11	V
	0.5	80±0.58**	89.83±0.67**	86.7±0.67**	85.51	V
	0.75	86.47±0.88**	76.7±0.33**	96.7±0.33**	86.62	V
	1	82.73±1.20**	93.3±0.67**	83.3±0.88**	86.44	V
Peppermint	0.15	80±0.58**	46.7±0.88**	70±0.58**	65.57	IV
	0.25	86.69±1.33**	96.64±0.33**	66.7±0.88**	83.34	V
	0.5	68.42±0.58**	92.95±0.33**	50±2.00*	70.46	IV
	0.75	93.19±0.58**	96.7±0.33**	72.39±0.88**	87.43	V
	1	79.31±0.33**	100±0.33**	90±0.33**	89.77	V
Orange	0.15	9.11±1.52	5.45±1.80	10.72±3.4	8.43	I
	0.25	19.26±0.88*	42.35±1.00**	60±0.58**	40.54	III
	0.5	62.68±1.00**	76.3±1.33**	45.78±3.17	61.59	IV
	0.75	75.89±1.15**	83.3±1.67**	68.96±2.08*	76.05	IV
	1	79.66±1.20**	86.47±1.67**	78.58±1.33**	81.57	V

* Significant $p < 0.05$, **highly significant $p < .001$ (T-test)

0: R % >0.01 to 10

I: R % from 10.1 to 20

II: R % from 20.1 to 40

III: R% from 40.1 to 60

IV: R % from 60.1 to 80

V: R% from 80.1 to 100

A significant increase in the repellency of essential oil with the increase of used doses was only recorded with Orange peel oil after 2 and 4 h of exposure. The mean repellency% of the oil at the doses 0.15, 0.25, 0.5, 0.75 and 1 mg/100g were 8.43, 40.54, 61.59, 76.05 and 81.57%, respectively. In spite of Orange peel oil, it is clear from the data in table 1 that there is no direct logarithmic correlation between the repellent activity of oils and doses used. Also, our results showed that the repellent activity fluctuated between decrease and increase as the time of exposure increased.

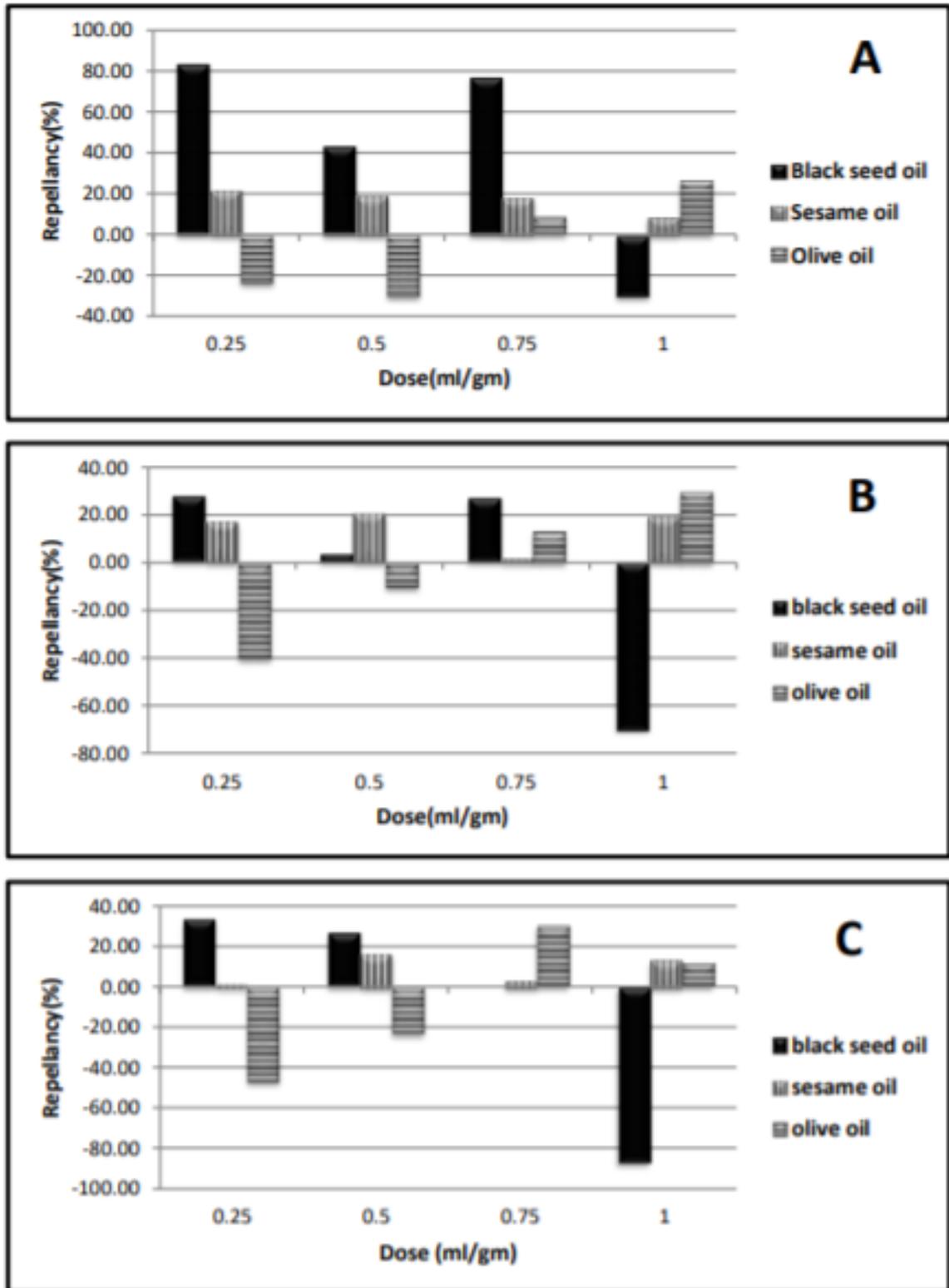


Fig 1: Repellency (%) of 3 edible oils to *S. granarius* adults, using treated wheat grains at 2 h (A), 4 h (B) and 6 h (c) after exposure.

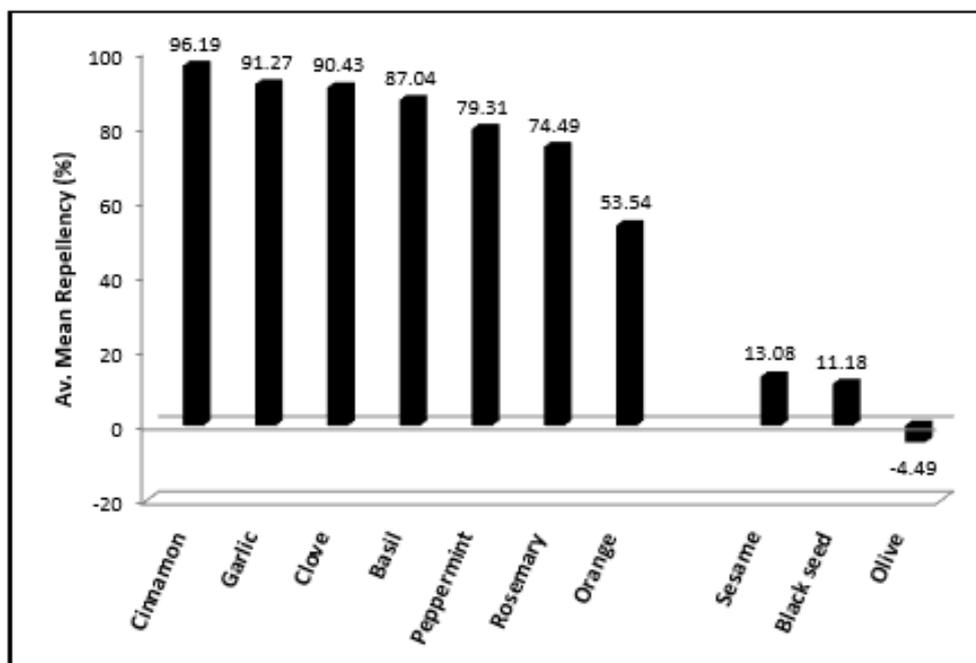


Fig 2: Average mean Repellency % of *S. granarius* adults exposed to wheat grains treated with 7 essential oils and 3 edibles

The statistical analysis of data indicated that Low doses of Garlic oil, repelled significantly ($p < .001$) more insects than higher doses. Garlic oil repelled 100% of *S. granarius* at all exposure times at a dose of 0.1 ml/100 gm. grains. The average recorded repellency for the subsequent doses was 81.12, 88.89, 90.00, 93.33, 94.44% (Table, 2). This decrease in repellency was accompanied by an increase in mortality. Similarly, the highest mean repellency of Rosemary oil (93.25%) was recorded at 0.75ml, whereas at a higher dose (1.0 ml) the average mean repellency decreased to 65.42%.

Table 2: Repellency % of *S. granarius* adults exposed for different periods to wheat grains treated with Garlic oil at 30°C.

doses (ml/100gm)	Repellency %			Rep %mean	Rep. class
	Exposure time				
	2hr	4hrs	6hrs		
0.05	73.33±0.67**	100±0.33**	100±0.33**	91.11	V
0.1	100±0.33**	100±0.33**	100±0.33**	100	V
0.15	76.7±0.33**	83.33±0.33**	83.33±0.33**	81.12	V
0.25	90.00±0.58**	83.33±0.88**	93.33±0.33**	88.89	V
0.5	80.00±0.58**	93.33±0.33**	96.67±0.33**	90.00	V
0.75	90.00±0.58**	93.33±0.33**	96.67±0.33**	93.33	V
1	96.67±0.33**	93.33±0.33**	93.33±0.33**	94.44	V

*significant $p < 0.05$, **highly significant $p < .001$ (T-test)

Percentages of repellency were categorized according to the following scale

Class Repellency Rate (R %)

0: R % >0.01 to 10

I: R % from 10.1 to 20

II: R % from 20.1 to 40

III: R% from 40.1 to 60

IV: R % from 60.1 to 80

V: R% from 80.1 to 100

DISCUSSION

Generally, all essential oils used showed much higher repellent activity to *S. granarius* than edible oils. This may be due to the structure of essential oils which contain a complex combination of volatile organic compounds formed as secondary metabolites in plants; containing hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, ethers, esters, aldehydes, ketones, phenols, lactones, and phenol esters (Guenther, 1972).

Although edible oils are safer, cheaper and more available than essential oils, the weak repellent activity of all edible oils used and the fluctuation in their repellent activities at different doses and exposure time may point that these oils are not practical for use as repellents for this insect pest. A similar assumption was reported by Hori, (2004). The author concluded that any materials that attract insects at lower doses should not be used as a repellent. Also, Dethier *et al.*, (1960) reported that the insect repellents must have a significant effect on the pests that causes them to move away from the source of the substance.

All essential oils used showed strong repellent activity against adults of *S. granarius*. The mean repellency index showed that Cinnamon, Garlic and Basil oils had the highest repellent activity to the insect (80-100% repellency) and for 6 h post-exposure, followed by Clove, Peppermint, Rosemary, and Orange oils. The difference in repellency of essential oils might be clarified with the insects' reactions to the compounds existing in the essential oils (Teke and Mutlu, 2020). The outstanding repellent activity of Cinnamon and Clove oils against *S. granarius* was also reported by Plata-Rueda *et al.* (2018) and Yang *et al.*, (2020) against *S. zeamais*. Teke and Mutlu (2020) concluded that the essential oils of Rosemary and Basil could potentially be used for the control of *S. granarius* L. and *Tribolium castaneum*. The strong repellent activity of Clove oil was also reported by Sabbour and Abd Elaziz (2011) against the faba bean beetle *Bruchidius incarnatus* (Coleoptera: Bruchidae).

Low doses of Garlic oil showed high repellent activity against *S. granarius* (PR=100%) at 4 and 6 h. This activity may be attributed to its chemical composition. Garlic essential oil has a high percentage of diallyl disulfide as the main volatile compound and repellent properties to *S. zeamais* and *T. castaneum* (Huang *et al.*, 2000). The strong repellent activity of Garlic oil was also reported by Plata-Rueda *et al.* (2017) who stated that the repellency index for adults of *T. molitor* differed with the concentration. The recorded decrease in repellency at higher doses may be due to a reversed response behavior of the insect or inhibition of certain receptors at higher doses.

Orange peel oils exhibited moderate repellency against *S. granarius* with a mean repellent activity of 53% activity. This repellent activity could be attributed to limonene. The moderate repellent activity of this oil was also reported by Kim and Lee (2014) against *S. zeamais* and *T. castaneum* adults.

A significant increase in the repellency of essential oil with the increase of used doses was only recorded with Orange peel oil. In spite of Orange peel oil, 2 and 4 h post-treatment, it is clear from data that there is no direct logarithmic correlation between the repellent activity of oils and doses used. The statistical analysis of data indicated that a low dose of Garlic oil (0.1 ml) repelled significantly more insects than higher doses. Similarly, the highest mean repellency of Rosemary oil (93.25%) was recorded at 0.75ml, whereas at a higher dose (1.0 ml) the average mean repellency decreased to 65.42%. The fluctuation of repellent-attraction of many edible and essential oils used was also reported by Khemira *et al.* (2013) who studied the repellent activity of the essential oil of *Eucalyptus astringens* against *Rhyzopertha dominica* and *Oryzaephilus surinamensis* and

according to their results the repellent activity fluctuated between decrease and increase as the time of exposure and concentration increased for both species. In contrast to our results, many authors as Yoon *et al.* (2007), Ibrahim and Sahar (2011), Sagheer *et al.* (2011) and 2013), Kedia *et al.* (2015), and Amini *et al.* (2019) mentioned that the maximum repellency was related to the highest concentration of essential oils.

Conclusion

All essential oils tested during the present investigation proved efficient repellent natural products for the management of the granary weevil. The selection of oils for use in repelling insect pests must consider the toxicity & biological activity of the pest, as well as the persistence, safety, and acceptance by consumers. For example, low doses of Garlic oil showed high repellent activity against *S. granarius*. However, the strong unaccepted essence of this oil may prevent or decrease the possibility of using it as a repellent. The higher toxicity of certain oils as Cinnamon, Clove, and Basil oils to *S. granarius* adults (Zohry *et al.*, 2020) and their high repellent activity reported in the present study may provide an advantage to their use for the management of this dangerous pest. Studies are needed to reveal the residue problem of these essential oils in the final products. Most of the essential oils tested in our study showed no statistical difference in their repellent activity at different exposure times. However, our observations declared that the persistence of the repellent activities of all essential oils greatly decreased the next day. This decrease may be attributed to the fact that most constituents of plant essential oils are highly volatile due to their low molecular weight (Regnault-Roger *et al.*, 2012), and the volatility of essential oils can be affected by the types and structure of the test surface and formulation. Possible explanations include the rapid absorption to the surface of wheat grains, degradation of active compounds by the metabolic process of grains, or evaporation of active constituents. To solve these problems, a safe formulation of essential oils could be initiated for the management of stored grain pests (Teke and Mutlu, 2020). It is known that the production of EOs insect repellent formulations must be suitable to the market wishes by employing low-cost small-scale casting or pilot-scale production that could be sustained by the manufacturer and meet the consumer's preference. Different processes of incorporating EOs in a polymer matrix may increase the capacities of holding the active agent in the treated products. Within a certain period of time, the active agent could be released slowly to the environment or the grains. Any EOs formulations for repelling insects should not be harmful to touch as well as do not release toxic particles into grains. In the future, researchers can also be undertaking into developing specialty of EO agents which not only could repel insects but also added value to the stored grains as antioxidant, antimicrobial, antifungal effects. All the requirements are vital to ensure that the insect repellent product can be widely used and acceptable to consumers and manufacturers (Marsin, *et al.*, 2020).

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ARABIC SUMMARY

الأثر الطارد لعشرة زيوت نباتية محلية، صالحة للأكل وعطرية ضد سوسة القمح (غمدية الأجنحة: السوسيات)

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تم مقارنة قدرة عشرة زيوت نباتية مختارة، عطرية وصالحة للأكل، على طرد سوسة القمح (غمدية الأجنحة: السوسيات) باستخدام طريقة تفضيل المنطقة. 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. وأظهرت جميع الزيوت الأساسية المستخدمة نشاطاً طارداً أعلى بكثير لهذه الحشرة من زيوت الطعام. ويمكن ترتيب الزيوت المستخدمة تنازلياً وفقاً لمتوسط نشاطها الطارد كما يلي: القرفة (96.19٪)، الثوم (91.27٪)، القرنفل (90.43٪)، الريحان (87.04٪)، النعناع (79.31٪)، الروزماري (74.49٪)، البرتقال (53.54٪)، السمسم (13.08٪)، الحبة السوداء (11.18٪)، بينما كان زيت الزيتون جاذباً للحشرات (-4.49٪). وباستثناء زيت قشر البرتقال، لم يتم العثور على ارتباط لوغاريتمي مباشر بين النشاط الطارد للزيوت والجرعات المستخدمة. كما أوضحت النتائج أن النشاط الطارد للزيوت يتأرجح بين النقص والزيادة مع زيادة وقت التعرض. كما تم مناقشة إمكانية استخدام الزيوت كمواد طاردة لأفات المنتجات المخزونة.

الكلمات المفتاحية: سوسة القمح، الزيوت النباتية، حماية الحبوب، المنتجات المخزونة، المواد الطاردة للحشرات