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FUMIGANT TOXICITY OF SOME PLANT VOLATILE OILS AGAINST STORED GRAIN WEEVILS

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ABSTRACT: Cereal grains considered one of the most important crops in Egypt and all over the world, these stored grains are infested with many insects that reduce their economic value and cause a significant problem for food security. The current study aimed to protect stored grains from infestation of different weevil species using plant oils as fumigants. Six plant volatile oils i.e., Garlic, Clove, Peppermint, Orange, Onion, and Camphor were used to determine the fumigant toxicity of oils the adults of *Sitophilus granaries*, *Sitophilus zeamais* and *Sitophilus oryzae*, as well as, percentages of total protein and total carbohydrates were determined in both healthy and infested seeds. The results indicated that Camphor 2.0 %, Orang 2% and Peppermint 2.0 %, respectively, were the most effective oils against the tested insects. The number of died *Sitophilus granaries* per 80 insects reached 74, 73 and 70 insect for Camphor 2.0 %, Orang 2% and Peppermint 2.0 %, respectively, after 10 day of application, while in *S. zeamais* were 72, 73 and 70 insect, respectively. The number of died *S. oryzae* per 80 insects recorded 78, 77 and 74 insect, respectively. The results also showed that the total protein and total carbohydrates were greatly affected by the infestation of insects. Finally, the fumigation with plant essential oils could be used as safe insecticide against stored product insects alternative to chemical pesticides.

Key words: Stored grains, Grain weevils, Essential oils, Fumigant toxicity

INTRODUCTION

Stored grains and legumes are attacked by various insects that can infest the stored grains with fungi and bacteria, which in turn led to damage incurred by stored grain and devaluation in the final products. Moreover, the feeding of these pests causes qualitative and quantitative losses in grain weight, nutritional value and germination capacity of seeds (Scheepens et al., 2011 and Jayakumar et al., 2017).

The granary weevil, *Sitophilus granarius* (L.), *S. zeamais* (L.) and *S. oryzae* (L.) are the most widespread and destructive insect pests attacking stored grains. Control of these insects around the world is dependent upon the synthetic insecticides and fumigants, which resulted in hazard effects on non-target organisms, environment, human health, as well as development of insect resistance and increasing the level of pesticide residues in food (White and Leesch, 1995, Bughio and Wilkins, 2004). Recently, scientists has paid attention towards plant products as novel and effective materials in plant protection as it consider non phytotoxicity, easy biodegradability and ecofriendly. The plant products and the botanical pesticides are being used globally as Fumigants and green pesticides (Dubey et al., 2008; Jayakumar et al., 2017; Tu et al., 2018 and Adesina 2022), they are biologically active, not absorbed by grain, not flammable, high volatility and toxicity to stored-grain insect pests (Shaaya et al., 1997 and Fouad et al., 2021). The fumigation with traditional insecticides, methyl bromide phosphine has been argued to be genotoxic to human and animals (Garry et al., 1989; WMO, 1991; Chaudhry, 1995 and Fields and White, 2002).

From the previous points, this study was conducted to protect stored grains from infestation of different weevil species using plant oils as fumigants.

MATERIALS AND METHODS

Target Insects:

The investigated species of stored grain insects were: the granary weevil, *Sitophilus granarius* (L.), the maize weevil, *S. zeamais* (L.), and the rice weevil, *S. oryzae* (L.).

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Pure cultures of each weevil were reared under laboratory conditions of 25 ± 5 ºC, and 56 ± 5 % Relative humidity where it was reared in glass boxes 60 ×40 × 30 cm covered with muslin material on the three hosts, wheat, maize, and rice seeds.

Tested grains:

The tested grains in the current study were: Wheat (*Triticum aestivum*) variety Sakha 93 and Maize (*Zea mays*) Variety High Tech 2031, and Rice *(Oryza sativa)* Giza 179. Tested grains were dried very well by exposing it to the solar heat for three days and stored for one year in jute bags under laboratory conditions 25 ± 5 ºC, and 56 ± 5 % Relative humidity.

Control Experiments:

Six plant volatile oils: Garlic, Clove, Peppermint, Orange, Onion, and Camphor (Table 1) were used to determine their fumigant toxicity on the one week old adults of *Sitophilus granaries*, *S. zeamais* and *S. oryzae.*

Preparation of plant oil extracts:

Garlic, Clove, Peppermint, Orange, Onion, and Camphor vegetative parts, were collected from the experimental farm of Faculty of Agriculture, Menoufia University, Shebin Elkom, Egypt and dried under shade at room temperature (26±2 ºC) for about 20 days. The dried parts were powdered using blender. About 100 g of each dried parts were soaked in methanol (1 L) and ethanol (1 L), respectively for 7 days, then filtered through Whatman filter paper No.1. The obtained filtrates concentrated under vacuum on a rotary evaporator at 40 ºC and stored at 4 ºC until use as described in Sahile et al., (2012).

Fumigation toxicity:

Glass jars of 500 ml capacity, provided with their screw lids were used as exposure chambers. Concentrations were conducted by releasing the required amounts of the pure oils (Garlic, Clove, Peppermint, Orange, Onion, and Camphor) from an automatic micropipette onto a circular filter paper equal to the internal diameter of the lid jar, which lined with aluminum foil. Two concentrations of volatile oils (1 and 2 %) were used for exposure periods (10 days). All experiments were performed in glass vials (10 ml capacity) secured with perforated aluminum foil cover to permit vapor to penetrate. Eighty unsexed newly emerged adults (one week old) were counted into the glass bottles with 5 g of each seeds wheat, maize and rice covered with a piece of gauzed aluminum foil, then into larger fumigation chamber and placed inverted on clean perforated trays. Control treatment consisted of identical jars (500 ml capacity) containing 80 adults, in four replicates, without any volatile oil. Daily mortality was recorded for 10 day as described in Mahmoudvand et al., (2011).

Seed analysis:

Percentages of total protein and total carbohydrates were determined according to AOAC (2005).

Statistical analysis:

Collected data were subjected to statistical analysis of variance (ANOVA) at 5 % probability using CoStat version 6.400 Copyright © 1998-2008 Cohort Software.798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA. Mortality percentages were calculated according Abbot Formula (1925).

Table (1): Volatile oils used in the experiments

English name	Scientific name	Used part
Garlic	*Allium sativum*	Bulbs
Clove	*Syzygium aromaticum*	Bud
Peppermint	*Mentha piperita*	Leaves
Orange	*Citrus sinensis* (Naval)	Peels
Onion	*Allium cepa*	Bulbs
Camphor	*Cinnamomum camphora*	Leaves

RESULTS

Data presented in Table (2) show the numbers of *Sitophilus granarius* insects as influenced by two concentrations of some botanical oils applied on wheat seeds as fumigant treatment. The obtained results indicate that the highest numbers of dead weevils, after 10 days of treatment, were recorded with the treatment of Camphor 2.0 % (74 insects), Orange 2.0 % (73 insects) and Peppermint 2.0 % (70 insects), while the least numbers of dead weevils were recorded with the treatments of Clove 1.0 % (38 insects), Onion 0.1% (41 insects), Clove 2.0 % (43 insects) and Onion 0.2% (46 insects). The rest treatments occupied intermediate status.

The obtained results indicated that the highest reduction percentages, after 10 days of treatment, were recorded with the treatment of Camphor 2.0% (92.5 %), Orange 2.0 % (91.25 %), Peppermint 2.0 % (87.5 %), Orange 1.0 % (83.75%) and Garlic 2.0 % (80 %), while the least reduction percentages after 10 days of treatment, were recorded with the treatment of Clove 1.0 % (47.5 %), Onion 0.1% (51.25 %), Clove 2.0 % (53.75 %) and Onion 0.2% (57.5%). The rest treatments occupied intermediate status.

Data presented in Table (3) show the numbers of *S. zeamais* insects as influenced by two concentrations of some botanical oils applied on maize seeds as fumigant treatment. The obtained results indicate that the highest numbers of dead weevils, after 10 days of treatment, were recorded with the treatment of Camphor 2.0 % (72 insects), Orange 2.0 % (73 insects) and Peppermint 2.0 % (70 insects), while the least numbers of dead weevils were recorded with the treatments of Onion 0.1% (39 insects), Clove 1.0% (41 insects), Onion 0.2% (45 insects), Garlic 1.0 % (47 insects) and Clove 2.0 % (49 insects). The rest treatments occupied intermediate status.

Table (2): Number of dead insects of *Sitophilus granarius* as influenced by botanical oils applied as fumigant treatment on wheat seeds

Oil extracts	No of dead insects days after treatment										Total mortality	Total reduction %
	1	2	3	4	5	6	7	8	9	10		
Garlic 1%	0	0	2	2	3	6	10	10	10	14	57	70.0
Garlic 2%	0	0	3	4	3	7	7	10	13	17	64	80.0
Clove 1%	0	0	0	1	1	2	4	5	10	15	38	47.5
Clove 2%	0	1	1	1	2	2	3	5	11	17	43	53.75
Peppermint 1%	0	0	1	2	2	5	6	10	15	20	61	76.25
Peppermint 2%	1	2	2	3	5	5	6	9	14	23	70	87.5
Orange 1%	0	0	1	4	4	7	11	11	12	17	67	83.75
Orange 2%	0	1	2	3	6	10	9	10	14	18	73	91.25
Onion 1%	0	0	0	1	0	2	5	9	11	13	41	51.25
Onion 2%	0	0	1	2	2	4	5	6	11	15	46	57.5
Camphor 1%	0	0	2	2	4	5	7	8	16	19	63	78.75
Camphor 2%	1	1	2	3	3	6	9	10	16	23	74	92.5
Control	0	0	0	0	0	0	0	0	0	0	0	0.0

Each treatment contains 80 adult weevils divided to four replicates (Before treatment)

Table (3): Number of dead insects of *Sitophilus zeamais* as influenced by botanical oils applied as fumigant treatment on maize seeds

Oil extracts	No of dead insects days after treatment										Total mortality	Total reduction %
	1	2	3	4	5	6	7	8	9	10		
Garlic 1%	0	0	2	3	3	5	7	8	9	10	47	57.5
Garlic 2%	0	1	1	3	3	6	8	8	11	16	57	71.25
Clove 1%	0	0	0	1	0	2	4	5	13	16	41	51.25
Clove 2%	0	1	2	1	3	2	5	8	10	17	49	61.25
Peppermint 1%	0	1	3	2	3	3	8	8	14	19	61	76.25
Peppermint 2%	1	2	2	4	4	5	6	10	12	24	70	86.25
Orange 1%	0	3	2	2	6	6	8	10	10	15	62	77.5
Orange 2%	1	2	2	3	6	10	9	9	13	18	73	91.25
Onion 1%	0	0	1	1	1	3	5	8	10	10	39	84.75
Onion 2%	0	1	1	2	3	2	3	5	11	17	45	56.25
Camphor 1%	0	0	1	3	4	6	6	8	13	19	60	75.0
Camphor 2%	1	0	2	1	3	7	10	9	16	23	72	90.0
Control	0	0	0	0	0	0	0	0	0	0	-	0

Each treatment contains 80 adult weevils divided to four replicates (Before treatment)

The obtained results indicated that the highest reduction percentages, after 10 days of treatment, were recorded with the treatment of Orange 2.0% (91.25 %), Camphor 2.0 % (90 %), Peppermint 2.0 % (86.25 %), and Onion 1.0 % (84.75 %), while the least reduction percentages after 10 days of treatment, were recorded with the treatment of Clove 1.0 % (51.25 %), Onion 0.2% (56.25 %), and Garlic 0.1% (57.5 %). The rest treatments occupied intermediate status.

Data presented in Table (4) show the numbers of *S. oryzae* insects as influenced by two concentrations of some botanical oils applied on rice seeds as fumigant treatment.

The obtained results indicate that the highest numbers of dead weevils, after 10 days of treatment, were recorded with the treatment of Camphor 2.0 % (78 insects), Orange 2.0 % (77 insects), Peppermint 2.0 % (74 insects), and Garlic 2.0 % (73 insects), and Orange 1.0 % (70 insects), while the least numbers of dead weevils were recorded with the treatments of Onion 0.1% (44 insects), Onion 0.2% (51 insects), and Clove 1.0 % (55 insects). The rest treatments occupied intermediate status.

The obtained results indicated that the highest reduction percentages, after 10 days of treatment, were recorded with the treatment of Camphor 2.0% (97.5 %), Orange 2.0% (96.25 %), Peppermint 2.0 % (92.5 %), Orange 1.0 % (87.5%) and Peppermint 1.0 % (86.25 %), while the least reduction percentages after 10 days of treatment, were recorded with the treatment of Onion 0.1% (55 %), Clove 1.0 % (62.5 %), and Onion 0.2% (63.75 %) and Garlic 1.0 % (68.75%). The rest treatments occupied intermediate status.

Table (4): Number of dead insects *of Sitophilus oryzae* as influenced by botanical oils applied as fumigant treatment on rice seeds

Oil extracts	No of dead insects days after treatment										Total mortality	Total reduction %
	1	2	3	4	5	6	7	8	9	10		
Garlic 1%	0	0	2	5	4	5	9	10	12	14	61	68.75
Garlic 2%	0	0	3	3	5	9	8	14	14	17	73	80.0
Clove 1%	0	0	1	0	5	3	5	8	12	16	55	62.5
Clove 2%	0	1	2	2	3	4	4	11	13	19	59	73.75
Peppermint 1%	0	1	3	3	5	6	8	7	14	22	69	86.25
Peppermint 2%	1	2	2	4	6	5	6	10	13	25	74	92.5
Orange 1%	0	3	2	3	6	8	8	12	11	17	70	87.5
Orange 2%	1	1	3	2	6	10	11	10	13	20	77	96.25
Onion 1%	0	0	0	1	1	3	5	7	12	15	44	55.0
Onion 2%	0	1	2	2	3	3	3	9	10	18	51	63.75
Camphor 1%	0	1	1	3	4	6	8	8	15	21	67	83.75
Camphor 2%	1	1	2	2	4	7	10	10	17	24	78	97.5
Control	0	0	0	0	0	0	0	0	0	0	0	0.0

Each treatment contains 80 adult weevils divided to four replicates (Before treatment)

Chemical composition of infested and healthy grains with grain weevils:

Wheat grains:

The obtained results in Table (5) show the chemical composition of infested and healthy stored grains with the three granary weevils, stored for one year under laboratory conditions.

Total protein in healthy wheat grains was 15.13 % compared with 4.55% at infested grains with loss percentages of 69.93 %. As for total carbohydrates, it was 21.7 % in healthy grains compared with 0.95 % at infested wheat grains with loss percentages of 95.62 %. Regarding to other components, it was 1.5 % in healthy grains compared with 0.55 % at infested wheat grains with loss percentages of 63.33 %.

Maize grains:

Total protein in healthy maize grains was 7.98 % compared with 2.9 % at infested grains with loss percentages of 63.4 %. Total carbohydrates percentage was 63.85 % in healthy grains compared with 19.45 % at infested maize grains with loss percentages of 69.54 %. Regarding to other components, it was 1.3 % in healthy maize grains compared with 0.67 % at infested wheat grains with loss percentages of 48.46 %.

Rice seeds:

Total protein in healthy rice grains was 9.4 % compared with 2.92 % at infested grains with loss percentages of 46.94 %. As for total carbohydrates, it was 79.9 % in healthy grains compared with 25.47 % at infested maize grains with loss percentages of 68.12 %. The other components percentage was 1.9 % in healthy maize grains compared with 0.57 % at infested wheat grains with loss percentages of 70.0 %.

Table (5): Chemical composition of infested and healthy stored grains with the three granary weevils, stored for one year under laboratory conditions

Grains		Total protein		Total carbohydrates		Others	
		Content %	Loss %	Content %	Loss %	Content %	Loss %
Wheat grains	Infested	4.55	69.93	0.95	95.62	0.55	63.33
	Healthy	15.13	-	21.70	-	1.50	-
Maize grains	Infested	2.90	63.40	19.45	69.54	0.67	48.46
	Healthy	7.98	-	63.85	-	1.30	-
Rice grains	Infested	2.92	46.94	25.47	68.12	0.57	70.00
	Healthy	9.40	-	79.90	-	1.90	-

DISCUSSION

The grain weevils are major pests attaching the stored grain in Egypt and all over the world causing significant economic losses and negative impact on food security. Therefore, there is an urgent need to find new pesticides that are effective and safe to the human, animals and the environment.

The present results show that the volatile oils of Garlic, Clove, Peppermint, Orange and Camphor have varying degrees of insecticidal activity against the tested insects. The insecticidal activity increased with increasing concentration and exposure times. These findings are agree with, Jayakumar et al. (2017) who studied the repellent activity and fumigant toxicity of plant oils, Camphor, Citronella, Eucalyptus, Geranium, Aniseed, Lavender, Rosemary, Vetiver, Lemon and Wintergreen at concentrations of 10 and 50µL against of *Sitophilus oryzae* adults under the laboratory conditions and the maximum repellency was observed with camphor oil. Ebadollahi et al. (2010) stated that the essential oil from leaves and flowers of *Lavandula stoechas* has strong insecticidal impact against adults of *Rhyzopertha dominica* F. *Tribolium castaneum* Herbst, and *Lasioderma serricorne* F. as the mortality increased with increasing both essential oils dose and exposure time.

Teke and Mutlu (2021) tested the insecticidal, repellent and fumigant activities of Rosemary, Daphne, Echinacea, Marjoram, Basil and Fennel extracts against *S. granarius* and *T. castaneum*, all tested essential oils showed repellent effects against both insects. furthermore, Fennel oil caused 100% fumigant toxicity against *T. castaneum*. Also, Patiño-Bayona et al. (2021) determined the bioactive effects of various essential oils extracted from aromatic plants on the maize weevil, the tested oils observed strong repellent effect by both contact and fumigant methods and the most active oils were *L. stoechas* and *L. alba*. Mahmoudvand et al. (2011) found that the essential oils extracted from *Zataria multiflora*, *Mentha pulegium* L., *Rosmarinus officinalis* L., and *Citrus sinensis* (L.) have good fumigant toxicity on stored-product pests, *Plodia interpunctella*, *S. granarius*, *Callosobruchus maculatus*, and *T. castaneum*. Moreover, Keita et al., (2001) reported that *C. maculates* was affected by the essential oil of *Ocimum basilicum* L. and *O. gratissimum* L. as the mortality of insects were increased as the essential oil concentration increased after12 h of fumigation.

El-Nahal et al. (1989) reported that the period of exposure was the most important factor caused mortality to *C. chinensis* when exposed to fumigant of *Acorus calamus* (L.) essential oil.

Similar results were observed by Su (1991) when *A. calamus* oil was used against *C. maculates*, *S. oryzae* and *L. serricorne*. adults. Also, Park et al. (2003) reported that the adult responses of *C. chinensis* and *S. oryzae* exposed to direct contact of *Chamaecyparis obtusa* leaves essential oil varied according to both insect spices and applied dose. Kim, et al. (2003) studied the responses of *S. oryzae* L. and *C. chinensis* L. to the fumigant of cinnamon, garlic, horse dish and mustard, they found that plant-derived material, insect species and exposure time were the main reasons of the variation in the insecticidal effect.

These results indicate that the insecticide mode of action of the used oils may be attributable to the fumigant action as they may cause toxic by rapidly penetrating into insect's body through the respiratory system and interfere with their physiological functions. Also these oils have high volatility, so their fumigant action might be importance for stored-product, which is supported by the authors Konstantopoulou et al., 1992; Regnault-Roger and Hamraoui, 1995; Ahn et al., 1998 and Lee et al., 2002, Ebadollahi et al., 2010 and Trivedi et al., 2017 conducted fumigation studies of different volatile oils and found the strong species-specific toxicity was highly dependent upon the concentration and time after treatment.

Finally, the effects of these volatile oils are not dangerous to consumers since they are commonly used in many pharmaceutical preparations (Bauer et al., 1990). Furthermore, the plant-derived insecticidal activity could be developed into products suitable for integrated pest management as they are selective to pests and ecofriendly. It can be applied to all crops in different agricultural systems (Arnason et al., 1989; Schmutterer, 1992; Burks et al., 2000; Enan, 2004; Prabakar and Jebanesan, 2004; Rani, 2012; Tu et al., 2018; Fouad et al., 2021 and Adesina, 2022).

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سمية بعض الزيوت النباتية كمدخنات على سوس الحبوب المخزونة

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تعتبر الحبوب من أهم المحاصيل في مصر وجميع أنحاء العالم ، وهذه الحبوب المخزنة تهاجم بالعديد من الحشرات التي تقلل من قيمتها الإقتصادية وتسبب مشكلة كبيرة للأمن الغذائي. وتهدف الدراسة الحالية إلى حماية الحبوب المخزونة بإستخدام التدخين بالزيوت النباتية وذلك للحد من إستخدام المبيدات الحشرية الكيميائية الضارة بصحة الإنسان والحيوان والبيئة.

تم إستخدام ستة زيوت نباتية طيارة هى الثوم والقرنفل والنعناع والبرتقال والبصل والكافور لدراسة تاثير سمية التدخين بهذه الزيوت على الحشرات الكاملة من سوسة القمح وسوسة الذرة وسوسة الأرز ، كما تم تقدير نسب البروتين والكربوهيدرات الكلية فى كلا من الحبوب السليمة والحبوب المصابة.

وأظهرت النتائج أن زيت الكافور والبرتقال والنعناع بتركيز ٢٪ كانوا الأفضل تأثيرا ضد الحشرات المختبرة على التوالى. حيث وصل عدد الحشرات الميتة من سوسة القمح لكل ٨٠ حشرة الي ٧٣ ، ٧٤ ، ٧٠ حشرة لكلا من زيت الكافور والبرتقال والنعناع بتركيز ٢٪ على التوالى بعد ١٠ أيام من المعاملة ، فى حين كانت أعداد سوسة الذرة الميتة لكل ٨٠ حشرة هو ٧٠ ، ٧٢ ، ٧٣ حشرة على التوالى. كما وصل عدد الحشرات الميتة لسوسة الأرزلكل ٨٠ حشرة هو ٧٨، ٧٧، ٧٤ حشرة على التوالى.

أظهرت النتائج أيضا أن نسب البروتين والكربوهيدرات الكلية قد تأثرت سلبيا فى الحبوب المصابة مقارنة بالحبوب السليمة.

فى النهاية يمكن التوصية بإستخدام التدخين بالزيوت النباتية كمبيدات حشرية آمنة ضد حشرات الحبوب المخزونة وكبديل عن المبيدات الكيمائية.