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Chemical Composition and Bioactivity of Three Plant Essential Oils against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.)

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ABSTRACT

Three essential oils extracted from German chamomile, *Matricaria chamomilla*, Anise, *Pimpinella anisum* and cumin, *Cuminum cyminum* were assessed against two of the major stored grain insect species, *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). The chemical composition of the essential oils was determined by gas chromatography (GC) and GC/mass spectrometry. The bioactivity of the oils determined was assessed against both species. A concentration of 100000 ppm, essential oils caused 100% mortality of *S.oryzae* after 96 h. of exposure, whereas, *T. castaneum* were more tolerant to the oils than *S. oryzae*. At this concentration, the values of F_1 progeny production, progeny reduction (%) and grain weight loss (%) of both insect species were lower than other concentrations. Eventually, all oils exhibited a negative impact on some biological aspects of both insects. These results highlight the potential of these oils as grain protectants and antifeedants, and consequently could be suitable for the management of insect pests in grain stores.

Keywords: Bioactivity, inhibitors, progeny production, tolerant.

INTRODUCTION

During storage, grains are exposed to the attack of numerous by insects, fungi and vertebrate pests from time of harvest to consumption (Manickavasagan *et al*. 2008).Insect pests, mainly weevils and beetles, play a significant role in reducing the quality and quantity of stored grain and causing yield losses of about 75% in developing countries (Nakakita. 1998 ; Papachristos and Stamopoulos. 2002). Additionally, consumption of food commodities, contaminate the grains with shed skins, feces and toxins (Arthur *et al*. 2006).

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Cucurlionidae) and red flour beetle, *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae) are of the most destructive insect pests of stored grains worldwide (Yoon *et al*. 2007) causing higher reduction in quality and quantity of food items. From the medical and allergic point of view, both species have great importance.

Currently, control of these insect pests is mainly based on synthetic insecticides (Sousa *et al.* 2009). Nevertheless, the huge use of these chemicals has caused several problems such as destruction of beneficial fauna, health hazards, accumulative residues of pesticides, contamination of underground water and rivers and development of insect resistance and pest resurgence. Therefore, there is a fundamental need to develop new types of safe, easily prepared and environmental, convenient and low cost alternatives (Ebadollahi 2011). Bioinsecticides, especially essential oils offer one of the most successful preservation materials suitable for a wide range of agricultural and food products (George *et al.* 2014). Essential oils and their constituents have different modes of action that can be associated with their potential for insect mortality (Copping and Menn 2000). It has been proven that essential oils surpassed the synthetic insecticides as inhibitors for oviposition, feeding, development and adult emergence for stored grain insect pests. As a part of future applications for stored product insect control, essential oils must be evaluated to determine their feasibility as alternative control methods rather than the traditional insecticides.

Therefore, this study aimed to identify the chemical composition and bioactivity of the essential oils that extracted from German chamomile, *Matricaria chamomilla* (Chompositae); Anise, *Pimpinella anisum* (Umbelliferae) and cumin, *Cuminum cyminum* (Apiaceae) against rice weevil *Sitophilus oryzae* L. and red flour beetle *Tribolium castaneum* Herbst.

MATERIALS AND METHODS

Plant oils

The dry flowers of Matricaria chamomilla and seeds of Pimpinella anisum and Cuminum cyminum were collected from different areas of Egypt. Direct steam distillation technique described by Hashem Brothers Company for Essential Oils and Aromatic Products (Kafr-Elsohby, Kalyoubeya, Egypt) was used for obtaining crude essential oils. In which, the targeted plant parts were placed in a container equipped with condenser at which steam was passed through it carrying essential oils which condensed the vapor. Then, condensed vapor was received in a receptor separation of oil from water. Extraction time and background information about the plant species are summarized in Table 1. The excess water was removed out by adding anhydrous sodium sulphate. The obtained oil was filtered twice and stored in dark glass bottles in a refrigerator at 4 °C till their application for bioassays.

Identification of oil components (GC-MS analysis)

Chemical composition of essential oils was identified with gas chromatography-mass spectrometry (GC/MS) using HP5890 system with a HP column (60 meter X 0.25 millimeter, 0.25 µm film thickess). The oils were detected using flame ionization detector (FID). Nitrogen and hydrogen were the stationary phase. Initial temperature was 60 °C and maximum temperature was 250 °C. The injector temperature was 240 °C. Relative amounts of oils were calculated from peaks total area by apparatus software. The compounds were identified by matching the mass spectra data with those held in a computer library (Wiley 275 L). All steps of sample preparation, extraction and analysis procedures were carried out in the laboratory of Hashem Brothers Company (Abdel Moneim Riad St. Giza, Egypt).

Table 1. Plant species used and their oil information.

Plant species Information	*Matricaria chamomilla*	*Pimpinella anisum*	*Cuminum cyminum*
Family	Chompositae	Umbelliferae	Apiaceae
Common Name	German chamomile	Anise	Cumin
Tissue collected	Dry flowers	Seeds	Seeds
Extraction time	Direct steam for 12 h.	Direct steam for 8 h.	Direct steam for 12 h.

Culture of insects

Adults of *S. oryzae* and *T. castaneum* were used in the laboratory experiments. *T. castaneum* was reared on broken wheat grains mixed with 5% dried yeast and *S. oryzae* was reared on whole wheat grains in incubator at 25 ± 1 °C and 60 ± 3 r.h. %, and 10 L :14 D photoperiod. Unsexed adults used in the experiments were 7-14 days old.

Bioassays

All bioassay experiments were conducted at 27±2 °C, 65±5 % RH and 12L: 12D photoperiod until new adult emergence.

A direct contact assay was applied to evaluate the activity of the tested oils against the adults of *S. oryzae* and *T. castaneum*. Series of concentrations were prepared in acetone, and completely mixed with 20 g of (whole wheat or broken wheat) grains in 50 ml plastic containers to give the concentrations of 25000, 50000, 75000 and 100000 ppm, beside the control treatment which treated with acetone alone. The solvent was allowed to evaporate for 2 min prior to the introduction of insects. Adults of the tested insects were released in groups of twenty unsexed into 50 ml plastic containers containing the one of the prepared concentrations.

All the treatments were replicated three times. Mortality was recorded after 48 h, 72 h and 96 h from treatment and corrected according to Abbott's formula (Abbott. 1925). After 96 h, all the survival insects were separated from the grains and discarded. The grains were left inside the jars until the emergence of F_1 progeny and % Weight grain losses were counted (Nukenine *et al.* 2007). The F_1 progeny were recorded after 60 days from infestation for insects.

Statistical analysis

The data of mortality, loss grain weight, and antifeedant were analyzed by one-way analysis of variance (ANOVA) using SigmaPlot 12.0 software. In case of significant means were separated using Fisher LSD test at 0.05 probability level.

RESULTS

Chemical components of essential oils

The results of GC/MS analysis of the essential oils obtained by hydrodistillation are summarized in Table 2. The main compounds (>1%) of essential oils were identified by matching their spectra with available one in the library. Total yield of the identified essential oils gave 86.66 %, 92.50 % and 51.35 % of *M. chamomilla*, *P. anisum,* and *C. cyminum*, respectively. Bisabolol oxide A (40.54%), 7, 11-dimethyl-3-methylene (17.01%), and Bisabolol oxide B (7.43%) were the major components in *M. chamomilla.* For oil of *P. anisum* oil, more than three quarters of the oil was identified as Transanisole (86.74%).Whilst, γ-Terpinene (15.76%) and Benzene methanol (11.32%) followed by Beta Pinene (10.37%) were the major components of *C. cyminum.*

Table 2. Main chemical constituents of the extracted oils

Main Components	Retention time (min)	Concentration(%)
German Chamomile Flowers (*Matricaria chamomilla*)		
7,11-dimethyl-3-methylene	28.58	17.01
Germacrene-D	29.35	1.90
Germacrene-B	29.94	1.26
3,7,11-Trimethyle	30.45	1.14
5,8-Dimethylisoquinoline	30.64	1.11
Alpha-bisabolol	36.52	6.43
Bisabolol oxide B	35.28	7.43
Chamazulen	39.23	3.52
Bisabolol oxide A	40.52	40.54
Lend-in-dicycloether	44.87	6.32
Anise seeds (*Pimpinella anisum*)		
Methyl chavicol	14.61	1.68
Transanisole	18.94	86.74
Estragol	25.68	4.08
Cumin seeds (*Cuminum cyminum*)		
Benzene methanol	10.94	11.32
γ-Terpinene	11.23	15.76
Beta Pinene	8.56	10.37
P- cymine	12.05	7.45
1-phenil-1-butanol	8.25	6.45

Bioassay

The results given in Table 3 and 4 described the mortality, F_1 progeny production, reduction percentage in progeny and weight grain loss of *S.oryzae* and *T. castaneum* exposed to different concentrations of *M. chamomilla*, *P. anisum,* and *C. cyminum* essential oils mixed with grain diet. Generally, results showed that all concentrations were less toxic against *T.castaneum* than *S.oryzae*. The mortality caused by these essential oils varied among concentrations. Mortality tended to increase with ascending concentrations as time of exposure multiplied. The oil of *C. cyminum* was most effective against *S.oryzae* than oils of *M. chamomilla* and *P. anisum* , whereas, the oil of *P. anisum* was more effective against *T.castaneum* than oils of *M. chamomilla* and *C. cyminum*. The complete mortality occurred at 100000 ppm after 72 h exposure to *C. cyminum* and *P. anisum* for *S.oryzae* and *T.castaneum*, respectively, with significant differences with control.

All essential oils significantly reduced F_1 progeny of *S.oryzae* and *T.castaneum* (Table 3 and 4). The oils of *P. anisum* at concentrations of 75000 and 100000 ppm, *M. chamomilla* at 75000 ppm, and *C. cyminum* at 75000 and 100000 ppm completely diminished the population increase of *S.oryzae* and achieved 100% reduction in adult progeny at the same concentrations. Whereas, *T.castaneum* exhibited more tolerance to all essential oils than *S.oryzae*. Further, higher numbers of *T.castaneum* adults were recorded alive in treatments with *C. cyminum* oil followed by those of *M. chamomilla* at all concentration compared with the control. No *T.castaneum* progeny was produced at 100000 ppm of *P. anisum* oil. Subsequently, the rate of increase of F_1 progeny production of both insects was significantly reduced (Table 3 and 4). Moreover, the weight grain loss was positively associated with the decrease of the mortality percentages and the increase in F_1 progeny.

Table 3. Mortality, F_1 progeny production, reduction and weight grain loss of *Sitophilus oryzae* exposed to essential oils mixed with wheat or grains at different concentrations

Source of oil	Con. (ppm)	Mean % Adult Mortality (±SE)			No. F_1 progeny (mean ± SE)	Reduction in progeny number %	Grain weight loss %
		48 h	72 h	96 h			
P. anisum	25000	26.67± 0.28c	46.67± 0.27 b	60.12±0.47 b	10.67±0.27 b	94.12 b	1.15 b
	50000	56.67±0.67b	83.33±0.33 b	86.67±0.33 b	2.67±0.23 c	98.53 a	0.62 b
	75000	73.33±0.33b	90.00±0.57 a	100.00±0.00 a	0.00±0.00 d	100.00 a	0.24 b
	100000	83.33±0.88a	95.66±0.22 a	100.00±0.00 a	0.00±0.00 d	100.00 a	0.13 b
	Control	0.00±0.00 d	0.00±0.00 c	0.00±0.00 c	181.43 a	00.00 c	24.85 a
M. chamomilla	25000	16.67±0.27b	63.33±0.27 bc	73.33±0.28 b	19.67±0.34 b	89.16 c	2.16 b
	50000	20.33±0.03b	60.00±0.57 c	76.67±0.33 b	11.33±0.67 c	93.75 b	1.43 b
	75000	36.67±0.33a	73.33±0.33 b	90.0±0.57 a	7.67±0.41 d	95.77 b	0.89 b
	100000	40.33±0.57a	90.33±0.57 a	100.00±0.00 a	0.00±0.00 e	100.00 a	0.02 b
	Control	0.00±0.00 c	0.00±0.00 d	0.00±0.00 c	181.43 a	00.00 d	24.85 a
C. cyminum	25000	23.67±0.25d	46.67±0.27 c	66.66±0.27 b	7.67±0.88 b	95.78 b	0.84 b
	50000	43.33±0.33c	83.33±0.33 b	96.67±0.33 a	1.67±0.57 c	99.08 a	0.42 b
	75000	56.67±0.33b	93.33±0.33 a	100.00±0.00 a	0.00±0.00 d	100.00 a	0.14 b
	100000	80.33±0.03a	100.00±0.00 a	100.00±0.00 a	0.00±0.00 d	100.00 a	0.01 b
	Control	0.00±0.00 e	0.00±0.00 d	0.00±0.00 c	181.43 a	00.00 c	24.85 a

Different letters in the same column (for each oil) indicate significant differences at the 0.05 levels (Fisher LSD)

Table 4. Mortality, F_1 progeny production, reduction and weight grain loss of *Tribolium castaneum* exposed to essential oils mixed with wheat or grains at different concentrations

Source of oil	Con. (ppm)	Mean % Adult Mortality (±SE)			No. F_1 progeny (mean ± SE)	Reduction in progeny number %	Grain weight loss %
		48 h	72 h	96 h			
P. anisum	25000	40.33±0.33 b	44.23±0.31 b	50.66±0.07 b	43.33±0.46 b	64.25 c	2.47 b
	50000	90.33±0.33 a	92.67±0.08 a	96.67±0.33 a	25.33±0.46 c	79.10 b	1.82 b
	75000	93.33±0.14 a	97.33±0.14 a	100.00±0.00 a	2.67±0.57 d	97.80 a	0.95 b
	100000	95.33±0.29 a	99.12±0.05 a	100.00±0.00 a	0.00±0.00 e	100.00 a	0.46 b
	Control	0.00±0.00 c	0.00±0.00 c	0.00±0.00 c	121.23 a	00.00 d	17.53 a
M. chamomilla	25000	16.67±0.33 ab	30.33±0.03 b	46.67±0.33 b	99.33±0.46 b	18.06 d	3.49 b
	50000	17.67±0.34 ab	43.33±0.33 b	60.33±0.55 b	70.33±0.17 c	41.98 c	2.99 b
	75000	23.33±0.33 a	56.67±0.33 a	73.33±0.55 a	60.34±0.17 d	50.23 b	2.81 b
	100000	29.67±0.03 a	61.67±0.20 a	80.33±0.07 a	46.67±0.46 e	61.51 a	2.09 b
	Control	0.00±0.00 b	0.00±0.00 c	0.00±0.00	121.23 a	00.00 e	17.53 a
C. cyminum	25000	0.00±0.00 d	3.33±0.33 c	6.67±0.33 d	114.67±0.46 b	5.42 d	4.87 b
	50000	26.67±0.33 c	30.33±0.58 b	43.33±.88 c	75.67±0.46 c	37.58 c	3.65 b
	75000	36.67±0.33 b	39.33±0.06 b	66.33±0.32 b	49.67±0.62 d	59.03 b	2.77 b
	100000	90.33±0.03 a	93.00±0.05 a	97.67±0.15 a	31.34±0.46 e	74.15 a	1.79 b
	Control	0.00±0.00 d	0.00±0.00 c	0.00±0.00 d	121.23 a	00.00 e	17.53 a

Different letters in the same column (for each oil) indicate significant differences at the 0.05 levels (Fisher LSD)

DISCUSSION

Our results on main chemical constituents were similar to those previously reported on *P. anisum* (Hassan and Elhassan 2017), *M. chamomilla* (Roby *et al.*, 2013) and on *C. cyminum* (Ladan Moghadam 2016). However, our results disagree with those previously reported on *P. anisum* (Acimovic *et al.* 2015), *M. chamomilla* (Iordache *et al*., 2009) and on *C. cyminum* (Moghaddam *et al.*, 2015). Differences observed on both composition and main compounds of essential oils may be due to several factors, such as geographical conditions (Burt 2004), season, climatic, soil variations (Isman and Machial 2006), genetic differences , plant parts extracted and the nutritional status of the plants (Ozcan and Chalchat 2006).

On the other hand, the results of contact bioassay using essential oils showed a strong toxicity, i.e. 100 % mortality in the range of 75000 - 100000 ppm after 96 h of exposure compared to untreated controls against both insects. This study showed that *T. castaneum* is relatively more tolerant to the effect of essential oils than *S. oryzae*. The findings of the present investigation show that the oil of *P. anisum* plant has higher effect on *T.castaneum* reproduction than *M. chamomilla* and *C. cyminum* However, *C. cyminum* has found to cause a reduction in oviposition, development and an increase in development period of *T. castaneum* probably due to suffocation and inhibition of various biosynthetic processes of the insect (Chaubey 2007).

A significant and/or complete reduction (100% inhibition) of the F_1 progeny of both insects was obtained as a result of parental exposure to the tested botanicals, especially at the highest doses applied. To estimate of plant oils (chamomile, sweet almond and coconut) at 2.5, 3.5, 5.0, 7.0 and 10.0 mL/kg against *Rhyzopertha dominica* in wheat grain to prevent insect infestation, treatments with essential oils at high dose (10.0 mL/kg) achieved over 95% control within 24 h of exposure compared to untreated controls (Nikpay 2007). Fumigant toxicity of *Carum copticum* and *Cuminum cyminum* essential oils have been extensively studied against stored product pests (Shojaaddini *et al.* 2008). Moreover, the oils could also act as antifeedants, thereby discouraging insect penetration and feeding (Weaver and Subramanyam. 2000).Nenaah and Ibrahim (2011) found that dose of 1.50 ml cm^{-2} of the oils of *Cinnamomum camphora* and *Ocimum basilicum* completely controlled *Trogoderma granarium* insect, while 100% mortality of *T. castaneum* adults was recorded with *P. anisum* oil.

To understand the bioactivities of any essential oil, it is important to know the main chemical components of the target oil in the research. For example, all components of *C. cyminum* were monoterpenes. Monoterpenes have insecticidal toxicity including contact and antifeedant action on stored product insect pests (Ziaee *et al.,* 2014). Also, Hyung *et al.*, (2012) reported that chemical composition of *Origanum vulgare* L. including thymol and γ-Terpinene (Those in *C. cyminum*) showed good fumigant toxicity against *T. castaneum* (LC_{50} = 0.012-0.195 mg/cm3).

This suggests that different plant extracts does have toxic effects against variety of insects and variability can be attributed to the qualitative and quantitative variation in their chemical constitutions (Bashir *et al.* 2013; Kanda *et al*. 2017).

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التحليل الكيماوى والنشاط الحيوي لثلاثة من الزيوت الاساسية ضد حشرتى سوسة الارز *Sitophilus oryzae* (L.) وخنفساء الدقيق الصدئية *Tribolium castaneum* (Herbst)

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فى هذه الدراسة تم تقييم ثلاثة من الزيوت النباتية هم البابونج الالمانى (زهور) ونبات اليانسون (بذور) ونبات الكمون (بذور) لحماية الحبوب والمود المخزونة ضد أثنين من اهم حشرات الحبوب المخزونة هما حشرتى سوسة الارز (L.) *Sitophilus oryzae* وخنفساء الدقيق الصدئية (Herbst) *Tribolium castaneum*. كما تم تحليل الزيوت الكيماويه للتعرف على مركباتها الداخلية من خلال جهازى gas chromatography (GC) and GC/mass spectrometry. وجد فى حالة المعاملة المباشرة ان تقييم السمية عند تركيز ١٠٠٠٠ جزء فى المليون فى الزيوت الثلاثة سببت نسبة موت تامه لحشرة سوسة الارز بعد ٩٦ ساعه فى حين ان خنفساء الدقيق الصدئية كانت اكثر تسامحا للزيوت الثلاثة من سوسة الارز .ايضا على نفس التركيز وجد ان قيم انتاجية الخلفه للجيل الاول والنسبة المئوية للخفض فى الخلفة ووزن الحبوب قل بالمقارنه بالتركيزات الاخرى لكلا الحشرتين . فى النهاية نجد ان كل الزيوت المختبره اظهرت تاثيرات سلبيه على بعض القياسات البيولوجية لكلا الحشرتين . ايضا من خلال هذه النتائج يمكننا القول ان هذه الزيوت يمكن ان تستخدم كبديل للمبيدات الحشريه فى مكافحة أفات المخازن.