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A Comparative Study of Three Plant Oils against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst): Toxic and Antifeedant Effects

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ABSTRACT

The aim of this study was to evaluate the effects of three essential oils extracted from *Matricaria chamomilla* flowers, *Pimpinella anisum* seeds and *Cuminum cyminum* seeds as biopesticide against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). Wherefore, the tested oils were obtained by steam distillation and evaporation of the selected plant parts and then treated at concentrations of 25000 and 50000 ppm.The weights of grains and insects were estimated before experiments taken place. After application, the mortality rates of insects, weight of grains and weight of a live insects were determined after four days. Concerning; toxic effects assay, LC_{50} of the oil of *P. anisum* (LC_{50} = 21534.82ppm) was the most potent toxicant, followed by C. *cyminum* (LC_{50} = 108310.64 ppm), and M .chamomilla (LC_{50} =148175.77 ppm) in case of *S. oryzae*. Also, in case of *T. castaneum*, the oil of *P. anisum* (LC_{50} = 24079.99 ppm) was the most potent toxicant, followed by *C. cyminum* (LC_{50} = 29980.19 ppm), and *M .chamomilla* (LC_{50} = 55621.91 ppm).In nutritional indices assay, all the tested oils had negative effects on feeding activity of *S.oryzae* and *T. castaneum,* particularly with the highest concentration (50000 ppm) of all tested oils. Ultimately, the three tested oils showed effects (toxic and antifeedant) against both insect species. This study may suggest using of such essential oils as potential biopesticides to protect stored grains from infestation by the examined insects.

Keywords: Biopesticides, Essential Oils, Toxicity, Stored Grains, Nutritional Indices.

INTRODUCTION

Plant oils and their constituents are known to potentially effect on insect pests, including stored product insects (Negahban and Moharramipour 2007; Bedini *et al.* 2015; Park *et al.* 2016). Plant oils are mixtures of unstable auxiliary metabolites acquired from plants by steam distillation. The term "essential" gets from "essence," which implies smell or taste, and identifies with the property of these substances of giving particular flavors and scents to many plants. (Enan 2001; Isman 2006). Much exertion has, accordingly, been focused on plant determined materials for conceivably valuable products as commercial insect control agents (Rajendran and Sriranjini 2008).

Sitophilus oryzae L. *(*Coleoptera: Curculionidae) is the serious primary insects of economically important crop products such as wheat, maize, rice, grain oats and other products (Bougherra *et al.* 2014). The larvae must complete development inside the grain and feed on the endosperm then emerged when emptying the grain of its starchy content. While, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most widespread destructive insects associated with food products and stored grain (Rees 1995), which consequently leads to damage of its food products. This insect has shown resistance to enormous range of chemical pesticides: making its control difficult to achieve. Both insect pests can cause multiple damages, either directly by feeding on the grain materials causing loss of quantity, low germination rate and/or indirectly by raising the moisture content of the grain that provides environment suitable for microbial growth and proliferation: all lead to a reduction in quantity and quality of grains (Boxall *et al.* 2002; Da Silva *et al.* 2012).

The propose of this study is to assess the potential of some oil extracts, as antifeedents, on *S. oryzae* and *T. castaneum* for the detection of nutritional indices activity and toxic effects.

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.

The yield of crude essential oils was estimated according to the following equation.

Dry weight of steam distillation / Dry weight of test plant

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* on whole wheat grains in incubator at 25 ± 1 °C and 60 ± 3 r.h. %, and a 10 L :14 D photoperiod. Adults, not separated sexually, were used in the experiments were 7-14 days old.

Toxic effects assay

Three replicates were prepared for each treatment and control. Mortality was recorded after 4 days from treatments and corrected according to Abbott's formula (Abbott. 1925). The 50% lethal concentration (LC_{50}) and its confidential limits were determined by Probit analysis (Finney 1971).

Nutritional indices assay.

Determination of antifeedant activity of the tested oils against *S. oryzae* and *T. castaneum* was performed following the method given by Shukla *et al.* (2011), with slight modifications. For each type of oil, one milliliter (prepared in acetone) was mixed with 20 ± 0.0001 g of (whole or broken) grains in glass jars thoroughly rotary shaker for 15 min to give the concentrations of 25000 and 50000, then left at room temperature for 15 min to allow the solvent to evaporate. A control treatment (acetone only) was applied. Twenty adults of each insect previously weighed were placed into each jar (the insects used were starved for 24 h before beginning the experiments to standardize their levels of hunger). Three replicates were applied. The weights of grains, the weight of insects alive and mortality were determined after four days. The nutritional indices were calculated by the formulae of Farrar *et al.* (1989) as follows:

The relative growth rate (RGR): RGR = (A – B)/B x day.

The relative consumption rate (RCR): RCR = D / B x day.

The efficiency of conversion of ingested food (ECI %): ECI % = (RGR / RCR) x 100.

A feeding deterrence index (FDI %): FDI % = (C – T) / C x 100.

Where, A is the weight of live insects, B is the original weight of the insects, D is the ingested biomass (mg), C is the consumption of checked diets, and T is the consumption of treated diets.

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 separated using Fisher LSD test at 0.05 probability level. The LC_{50} was separated based on their 95% CI and deemed significantly different if CIs did not overlap (*e.g*., Mahdian *et al*. 2007).

Table 1. Plant species used.

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

RESULTS

Extraction yields and physical properties of tested oils extracts

Percentages of yield oils were 0.4, 1.5 and 5 %, from *M. chamomilla*, *P. anisum* and *C cyminum*, respectively (Table 2). While, the appearance was dark blue in *M. chamomilla*, yellow in *P. anisum* and Bright yellow in *C cyminum*. With regard to Specific Gravity (Density) at 20 °C; registered that, (0.96, 0.98 and 0.93) of *M. chamomilla, P. anisum* and *C cyminum*, respectively.

Table 2. Yield and Physical Properties of tested oils

Tested oils	Yield (%)[a] (w/w)	Appearance	Density
Matricaria chamomilla	0.4	Dark blue	0.96
Pimpinella anisum	1.5	Yellow	0.98
Cuminum cyminum	5	Bright yellow	0.93

[a] Yield of crude essential oils = [Dry weight of steam distillation / Dry weight of test plant] x 100

Antifeedant assay

It is known that essential oils can modify nutritional indices and provoke feeding deterrence in stored insect pest. The antifeedant activity of essential oils increased with the increasing concentration (Table 3 and 4). All the essential oils had negative effects on nutritional indices of *S.oryzae* and *T. castaneum*. Statistical analysis showed that the most active was *M. chamomilla* oil that inhibited RGR (-0.19976713) and ECI (-85.214578), reduce significantly ($p< 0.05$) the RCR at 50000 ppm and caused a high antifeedant effect (98.61%) on *S.oryzae* (Table 3). On the other hand, the most active was *C.cyminum* oil that inhibited RGR (0.115446515) and ECI (15.30572809), reduce significantly ($p< 0.05$) the RCR at 50000 ppm and caused a high antifeedant effect (84.57 %) on *T. castaneum* (Table 4).

Based on 95% CIs, the analysis revealed that the LC_{50} was significantly differed among the three essential oils on both insect examined (Table 5). The oil extracted from *M. chamomilla* recorded the highest LC_{50}, wheres that of *P. anisum* recorded the lowest ones.

The LC_{50} values decreased with increasing concentration of essential oils. Based on LC_{50} significant differences were observed between *S.oryzae* and *T. castaneum*. In case of *S. oryzae*, the oil of *P. anisum* (LC_{50} = 21534.82ppm) was the most potent toxicant, followed by *C. cyminum* (LC_{50} = 108310.64 ppm), and *M .chamomilla* (LC_{50} =148175.77 ppm).Also, in case of *T. castaneum*, the oil of *P. anisum* (LC_{50} = 24079.99 ppm) was the most potent toxicant, followed by *C. cyminum* (LC_{50} = 29980.19 ppm), and *M .chamomilla* (LC_{50} = 55621.91 ppm).

Table 3. Variation of nutritional indices of *Sitophilus oryzae* treated during 4 days with the tested oils at different concentrations

Source of oil	Con (ppm)	RGR	RCR	ECI %	FDI%
P. anisum	25000	-0.062 b	0.344 a	-19.840 a	73.895 a
	50000	-0.186 b	0.184 b	-78.889 a	93.800 a
Control		0.180a	0.419 a	42.602 a	0.00 b
M.chamomilla	25000	-0.132 b	0.123 b	-80.134 b	91.545 a
	50000	-0.199 b	0.037 b	-85.214 b	98.611 a
Control		0.180 a	0.419 a	42.602 a	0.00 b
C. cyminum	25000	-0.084 b	0.305 b	-27.859 c	92.208 b
	50000	-0.038 b	0.074 c	-58.333 b	94.444 a
Control		0.180 a	0.419 a	42.602 a	0.00 b

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

RGR relative growth rate, RCR relative consumption rate, ECI efficiency of conversion of ingested food, FDI feeding deterrence index

Table 4. Variation of nutritional indices of *Tribolium castaneum* treated during 4 days with the tested oils at different concentrations

Source of oil	Con (ppm)	RGR	RCR	ECI %	FDI%
P. anisum	25000	-0.133 b	0.664 b	-20.583 b	60.489 b
	50000	-0.589 c	0.022 c	-86.212 c	95.238 a
Control		0.149 a	0.984 a	36.873 a	00.00 c
M.chamomilla	25000	-0.165 b	0.526 b	-28.782 b	56.518 b
	50000	-0.236 b	0.323 b	-84.183 c	78.564 a
Control		0.149 a	0.984 a	36.873 a	00.00 c
C.cyminum	25000	0.087 a	0.881 b	10.109 c	48.402 b
	50000	0.115 a	0.269 a	15.305 b	84.579 a
Control		0.149 a	0.984 b	36.873 a	00.00 c

Different letters in the same column (for each oil) indicate significant differences at the 0.05 levels (Fisher LSD)
RGR relative growth rate, RCR relative consumption rate, ECI efficiency of conversion of ingested food, FDI feeding deterrence index

Thus, *P. anisum* NE had the lowest LC_{50} value and the highest toxicity ratio for this concentration and both insects. The linear regression model was the best fit to the results of the number of adult emergence, indicating that they were inversely proportional to increasing concentrations of nanoemulsions. The *p* value or probability value is the probability for a given statistical model that, when the invalid hypothesis is valid. The hypothesis is appropriate when p > .05 (Nuzzo 2014); this is agreement with the all results obtained.

Table 5. LC_{50} values of the tested oils against *Sitophilus oryzae* and *Tribolium castaneum*

Insect	Source of oil	LC_{50} ^{a} (ppm)	95 % confidence limits (ppm)	
			Lower	Upper
	P. anisum	21534.82 b	16991.13	25278.04
Sitophilus oryzae	*M .chamomilla*	148175.77 a	118540.62	185219.71
	C. cyminum	108310.64 b	86648.51	135388.31
	P. anisum	24079.99 b	20686.54	26935.72
Tribolium castaneum	*M. chamomilla*	55621.91 a	51358.38	59985.01
	C. cyminum	29980.19 b	19915.69	37728.01

[a]LC50 values are after 96 h, The same letters in a coulumn are not significantly differed among LC50 based on 95% CIs.

DISCUSSION

The oil yield is an important element to express some of its advantages and characteristics such as the quantity extracted from the quantity of a specific crop and its economic feasibility and commercial production (Vitti and Brito 1999). In the present investigation, the yield of P. anisum seeds was in the range of 1.5–3.5% similar to those previously reported (Olle and Bender. 2010; Habib Ullah *et al.* 2014). While, the yield of *M. chamomilla* dry flowers was in the range of 0.3-0.9% (Guzelmeric *et al.* 2017; Kawthar *et al*. 2017) and that of *C. cyminum* seeds was in the range of 3.5-5.5% (Hajlaoui *et al.* 2010; Khan *et al.* 2017). However, the yield concentrations for the tested oils were fluctuating and different, both increasing and decreasing. These differences might arise from oil extraction technique, the initial plant mass, and the used plant part (Atti-Santos *et al.* 2005).Besides, high polarity solvent (ethanol, methanol and aqueous acetone) always gives the highest yield of crude extract (Poonsri *et al.* 2015), in addition to, an environmental condition, processing technology and genetic constituents influences the yield of essential oil (Saxena *et al.* 2015).

Jilani *et al.* (1988) announced that turmeric oil high as a repellent to different grain insects. While, plant oils of clove was toxic to *Rhyzopertha dominica* (F.) and *S. oryzae* (L.) (Sighamony *et al.* 1986). In the same context, the essential oil of anise (*P. anisum*.) and peppermint (*Mentha piperita* L.) have been found to have toxicity through fumigant to stored grain against four main stored product pests *T. castaneum* , *S. oryzae* Orzyaephilus surinamensis and *R. dominica*, (Shaaya *et al*. 1991). Huang *et al.* 1997, proved that, the essential oils of nutmeg seeds (*Myristica fragrans* Houtt) and cinnamon bark (*Cinnamomum aromaticum* Nees) were toxic and antifeedant of *T. castaneum* and *S. zeamais*. These previous studies are consistent with this results obtained.

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دراسة مقارنة لثلاثة زيوت نباتية ضد حشرتى سوسة الارز *Sitophilus oryzae* (L.) وخنفساء الدقيق الصدئية *Tribolium castaneum* (Herbst) : التاثيرات السامة ومانعات التغذية

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تهدف هذه الدراسة الى تقييم تاثير الزيوت الاساسية المستخلصة من البابونج الالمانى (زهور) ونبات اليانسون (بذور) ونبات الكمون (بذور) كمبيد حيوى ضد حشرتى سوسة الارز *Sitophilus oryzae* (L.) وخنفساء الدقيق الصدئية *Tribolium castaneum* (Herbst) , ولعمل هذا تم تحضير الاجزاء النباتية المختبر' فى الدراسة عن طريق التقطير بالبخار المباشر ثم التبخير للحصول على الزيوت النباتية الاساسية ومعاملة الحشرات بالتركيزات ٢٥٠٠٠ و ٥٠٠٠٠ جزء فى المليون وقبل المعاملة تم وزن الحبوب ووزن الحشرات قبل المعاملة ثم تم حساب نسبة الموت ووزن الحشرات ووزن الحبوب مره اخرى بعد المعاملة (٤ أيام من المعاملة) ,وأتضح من النتائج المتحصل عليها ان زيت بذور اليانسون هو الاكثر سمية مقارنة بالزيوت الاخرى حيث كان التركيز اللازم لقتل ٥٠% من الحشرات بعد أربعة ايام يساوى ٢١٥٣٤.٨٢ جزء فى المليون تلاه زيت بذور الكمون بتركيز ١٠٨٣١٠.٦٤ جزء فى المليون ثم زيت زهور البابونج الالمانى عند تركيز ١٤٨١٧٥.٧٧ جزء فى المليون فى حالة معاملة حشرة سوسة الارز *S. oryzae*, بالمثل عند معاملة خنفساء الدقيق الصدئية *T.castaneum* كان التركيز اللازم لقتل ٥٠% من الحشرات بعد أربعة ايام هو (٢٤٠٧٩.٩٩, ٢٩٩٨٠.١٩, ٥٥٦٢١.٩١) جزء فى المليون لزيوت بذور اليانسون وزيت بذور الكمون وزيت زهور البابونج الالمانى على التوالى, ومن جهة اخرى كان للزيوت النبايته المختبره تاثيرات سلبيه على المؤشرات الغذائية لكل من الحشرتين خاصة مع التركيز ٥٠٠٠٠ جزء فى المليون للزيوت المختبرة., ومن هنا يمكن أستنتاج ان الزيوت النباتية الثلاثة ضد أفات الحبوب المخزونه اظهرت تاثيرات سامة ومانعة للتغذية مما يتيح لنا استخدامها كاستيراتيجيات جديدة فى وقاية الحبوب المخزونة.