

Research Article

The effects of topical application of two essential oils against *Sitophilus granarius* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae)

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Abstract: Bioassays were conducted to assess the contact toxicity of two plant essential oils against adults of *Sitophilus granarius* (L.) and *Tribolium confusum* Jacquelin du Val. Essential oils were extracted from seeds of *Carum copticum* L. and *Cuminum cyminum* L. Contact toxicity was assessed by topical application and adult mortality was counted 1, 2, 6 and 24 h after exposure. Bioassays were carried out at 27 ± 1 °C and $65 \pm 5\%$ RH in continuous darkness. Results indicated the two essential oils tested were topically toxic to both species. The mortality increased with increase in the oils dose and time exposed to each dose. Adults of *S. granarius* were more sensitive to *C. copticum* oil ($LD_{50} = 0.009$ µg/mg body wt) than *C. cyminum* essential oil ($LD_{50} = 0.016$ µg/mg body wt). In general, *T. confusum* showed similar sensitivity to both essential oils. However, adults of *T. confusum* were more resistant than *S. granarius*. *C. copticum* and *C. cyminum* oils have potential for use in stored-product pest management.

Keywords: Essential oil, stored products, topical application

Introduction

Protection of agricultural products from pest infestations is in the concern of government, farmers, and those involved in this matter. From earlier times, synthetic pesticides have been used for pest control. However, hazardous effects of synthetic pesticides tend to the use of safer pesticides (Conway and Pretty, 2013). Pesticides based on plant essential oils are considered relatively safe and their efficacy has been demonstrated against different insect species. Essential oils have volatile compounds with insecticidal, antifungal and antimicrobial activity (George *et al.*, 2014). Therefore, plant

essential oils or their constituents are one of the most promising alternatives to pesticides. They are of natural origin, eco-friendly and have low mammalian toxicity (Ebadollahi, 2011).

Ajwain, *Carum copticum* L. (Apiaceae) and cumin, *Cuminum cyminum* L. (Apiaceae) are two medicinal plants that their essential oils proved to have insecticidal activity against a number of stored product pests (Sahaf *et al.*, 2007). The insecticidal efficacy of *C. copticum* essential oil has been investigated against *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *Plodia interpunctella* (Hubner) (Sahaf and Moharramipour, 2008; Sahaf *et al.*, 2007; Shojaaddini *et al.*, 2008). Cumin is used as a food additive with white or pink flowers and small green seeds. It is an aromatic, annual plant that grows in Iran, Egypt, Saudi-Arabia, and some other part of the world (Boskabady *et al.*, 2006). The fumigant toxicity of *C. cyminum*

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essential oil has been evaluated on *C. maculatus*, *C. chinensis*, *Acanthoscelides obtectus* Say, *T. castaneum*, *S. oryzae* and *S. granarius* (Arabi *et al.*, 2007; Chaubey, 2007, 2008, 2011; Karakoc *et al.*, 2006).

The confused flour beetle, *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae) is categorized as a secondary pest feeding on cereal grains that are damaged, or going out of condition, flour and related products. The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), is known as a primary pest; these two are able to feed on whole and undamaged cereal grains. These pests are probably two of the most destructive stored-product insect pests throughout the world affecting the quantity as well as quality of the grains (Athanasassiou *et al.*, 2005).

The aim of the present study was to investigate contact toxicity of *C. copticum* and *C. cyminum* essential oils against adults of *T. confusum* and *S. granarius*.

Materials and Methods

Insects

Adults of *T. confusum* and *S. granarius* were used in the experiments. *T. confusum* was reared on wheat flour plus 5% brewer yeast and *S. granarius* on wheat at 27 ± 1 °C and $65 \pm 5\%$ RH in continuous darkness. All adults used in the experiments were 7-14 days old and of mixed sex.

Plant materials

Seeds of *C. copticum* and *C. cyminum* were purchased from a research farm in Ferdowsi University-Mashhad-Iran on June 2012. Seeds were packed in bags and kept in the refrigerator at 4 °C until they were needed.

Essential oil extraction

Oil extraction was performed using a Clevenger type apparatus (Made by Karimpour, Tehran University). Conditions of extraction were: 40 g of seeds; 600 mL distilled water and 4 h distillation. Anhydrous sodium sulphate (Merck Co.) was used to remove water after extraction.

Essential oil was stored in self standing screw cap microtubes covered with foil at 4°C until beginning of the experiment. Density of *C. copticum* essential oil was measured 0.9472 mg/ml and for *C. cyminum* oil density was 0.9574 mg/ml.

Bioassays

In order to assess the contact toxicity of essential oils by topical application, preliminary dose setting experiment was carried out to determine a range of doses that would cause a range of 20–80% mortality. For each treatment doses were selected in the symmetric dose design (Robertson *et al.*, 1984) and measured in micrograms per mg body weight ($\mu\text{g}/\text{mg}$ body wt) (based on oils density). The doses are presented in Tables 1-4. Six replicates of 10 adults were prepared and untreated beetles served as a control. Adults weighing 1.54 ± 0.01 mg for *S. granarius* and 2.5 ± 0.01 mg in case of *T. confusum* were selected for the bioassays. Doses were topically applied to the ventral surface of the thoracic segments of the insects with a Hamilton microsyringe (Hamilton Company). Treated and untreated insects were introduced into petri dishes in incubator set at 27 ± 1 °C and $65 \pm 5\%$ RH in continuous darkness. The mortality was counted 1, 2, 6 and 24 h after exposure to the essential oils.

Statistical analysis

There were no mortality in control groups; so, there was no need to correct the mortality data. Mortality percentages were transformed to square root of arcsine to normalize the data. The data were subjected to one-way analysis of variances to determine significant differences between exposure time and dose levels. To estimate LD₅₀ values, data were subjected to Probit analysis (Finney, 1971). Statistical analysis were performed using SPSS software version 16.0 (SPSS, 2007).

Results

The toxicity of essential oils increased with increasing dose level and time exposed to each dose. The two essential oils tested were topically

effective to *S. granarius*. The dose of 0.06 µg/mg body wt of *C. copticum* oil was significantly more effective to control *S. granarius*, and caused 95% mortality after 2 h of exposure (Table 1). While for *C. cyminum* essential oil, this dose was effective after 6 h and lead to more than 92% mortality (Table 2). *T. confusum* treated with 0.152 µg/mg body wt of *C. copticum* oil caused 91.66% mortality after 1 h exposure which exceeded to 100% after 24 h (Table 3). The same results were observed for *C. cyminum* oil. The

dose of 0.152 µg/mg body wt caused 93.3% mortality after 1 h and reached to 100% after 24 h of exposure (Table 4); indicating the essential oils were more toxic at longer durations.

Based on LD₅₀ values adults of *S. granarius* were more sensitive to *C. copticum* oil (Table 5); while the toxicity of both essential oils were the same on *T. confusum* (Table 6). Also according to results *T. confusum* adults are more resistant than *S. granarius*.

Table 1 Mortality (%) ± SE of *Sitophilus granarius* exposed topically to different concentrations of *Carum copticum* essential oil.

Concentration (µg/mg body wt)	Exposure time (h)			
	1	2	6	24
0.006	0.0 ± 0.00h	21.7 ± 1.66g	26.3 ± 2.71fg	36.4 ± 3.35ef
0.019	3.3 ± 2.10h	58.3 ± 3.07d	63.2 ± 3.59cd	70.9 ± 3.63bcd
0.031	41.7 ± 3.07e	65.0 ± 4.28cd	70.2 ± 3.23bcd	80.0 ± 3.35b
0.060	73.3 ± 2.10bc	95.0 ± 2.23a	96.5 ± 2.21a	98.2 ± 1.81a
0.124	96.7 ± 2.10a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a

Means followed by the same letter are not significantly different; Tukey Multiple Range Test at $P = 0.05$.

Table 2 Mortality (%) ± SE of *Sitophilus granarius* exposed topically to different concentrations of *Cuminum cyminum* essential oil.

Concentration (µg/mg body wt)	Exposure time (h)			
	1	2	6	24
0.006	0.0 ± 0.00h	21.7 ± 3.07fg	28.1 ± 5.02f	32.7 ± 4.37ef
0.019	0.0 ± 0.00h	35.0 ± 3.41ef	36.8 ± 2.71def	45.5 ± 4.87cde
0.031	46.7 ± 2.10cde	51.7 ± 3.07cd	52.6 ± 2.35c	58.2 ± 3.35c
0.060	76.7 ± 3.33b	85.0 ± 4.28ab	93.0 ± 3.50a	94.5 ± 3.72a
0.124	95.0 ± 2.23a	98.3 ± 1.66a	100 ± 0.00a	100 ± 0.00a

Means followed by the same letter are not significantly different; Tukey Multiple Range Test at $P = 0.05$.

Table 3 Mortality (%) ± SE of *Tribolium confusum* exposed topically to different concentrations of *Carum copticum* essential oil.

Concentration (µg/mg body wt)	Exposure time (h)			
	1	2	6	24
0.019	0.0 ± 0.00k	23.3 ± 2.10ij	31.6 ± 4.70hij	36.6 ± 4.20ghi
0.038	5.0 ± 3.41k	31.6 ± 1.60hij	35.0 ± 4.28hi	40.0 ± 3.65fgh
0.076	20.0 ± 2.58j	53.3 ± 2.10ef	63.3 ± 2.10de	73.3 ± 3.30cd
0.114	50.0 ± 2.58efg	61.6 ± 3.00de	70.0 ± 2.58cd	81.7 ± 1.66bc
0.152	91.7 ± 3.07ab	95.0 ± 2.23ab	98.3 ± 1.66a	100 ± 0.00a

Means followed by the same letter are not significantly different; Tukey Multiple Range Test at $P = 0.05$.

Table 4 Mortality (%) \pm SE of *Tribolium confusum* exposed topically to different concentrations of *Cuminum cyminum* essential oil.

Concentration ($\mu\text{g}/\text{mg}$ body wt)	Exposure time (h)			
	1	2	6	24
0.019	0.0 \pm 0.0l	20.0 \pm 2.58jk	30.0 \pm 2.58hij	36.7 \pm 2.10gh
0.038	10.0 \pm 3.65kl	23.3 \pm 2.10ij	33.3 \pm 2.10hi	40.0 \pm 2.58fgh
0.076	21.6 \pm 3.07j	30.0 \pm 3.65hij	45.0 \pm 2.23efg	48.3 \pm 3.07ef
0.114	55.0 \pm 2.23e	75.0 \pm 2.23d	83.3 \pm 2.10cd	88.3 \pm 3.07bc
0.152	93.3 \pm 2.10abc	96.6 \pm 2.10ab	100 \pm 0.00a	100 \pm 0.00a

Means followed by the same letter are not significantly different; Tukey Multiple Range Test at $P = 0.05$.

Table 5 LD₅₀ values of *Sitophilus granarius*, 24 h after topically exposing to *Carum copticum* and *Cuminum cyminum*.

Plants	LD ₅₀ ($\mu\text{g}/\text{mg}$ body wt)	95%Confidence limit		Slope	χ^2 (df = 2)	P value
		Lower	Upper			
<i>C. copticum</i>	0.009	0.006	0.011	1.94	2.44	0.29
<i>C. cyminum</i>	0.016	0.007	0.037	0.81	0.27	0.60

Table 6 LD₅₀ values of *Tribolium confusum*, 24 h after topically exposing to *Carum copticum* and *Cuminum cyminum*.

Plants	LD ₅₀ ($\mu\text{g}/\text{mg}$ body wt)	95%Confidence limits		Slope	χ^2 (df = 2)	P value
		Lower	Upper			
<i>C. copticum</i>	0.037	0.027	0.046	1.63	2.370	0.305
<i>C. cyminum</i>	0.039	0.029	0.049	1.66	4.185	0.123

Discussion

Fumigant toxicity of *C. copticum* and *C. cyminum* essential oils have been extensively studied (Chaubey, 2007; Sahaf and Moharrampour, 2008; Sahaf *et al.*, 2007; Shojaaddini *et al.*, 2008). The effectiveness of these plant powders have also been proved on *S. granarius* and *T. confusum* adults (Ziaee and Moharrampour, 2013). However, contact toxicity of the oils has not been evaluated. Results indicated that the longer exposure time to the oils, the lower dose is required to control the beetles. Essential oils can be toxic via the respiratory system (fumigant effect), cuticle (contact effect) and digestive system (ingestion effect) (Prates *et al.*, 1998). In direct contact, essential oils may penetrate 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). According to our results, 100% mortality was observed on both tested beetles when exposed for 24 h to *C. copticum* and *C. cyminum* essential oils and it could be concluded that topical application method could control insects effectively. Haouas *et al.* (2012) stated that topical application of *Chrysanthemum grandiflorum* (L.) Batt. essential oil caused a significant insect mortality (27%) on *T. confusum* after 7 days of exposure. Zapata and Smagghe (2010) evaluated contact toxicity of two essential oils by topical application. They claimed that LD₅₀ values by topical application of *Laurelia sempervirens* essential oil extracted from leaf was 44 $\mu\text{g}/\text{mg}$ insect; and for *Drimys winteri* leaf oil was 85 $\mu\text{g}/\text{mg}$ insect against *T. castaneum* after 24 h of exposure. On the basis of LD₅₀ values, in this study, *C. copticum* and *C. cyminum* essential oils were found to be more toxic than *L. sempervirens* and *D. winteri*

oils. In addition, *S. granarius* adults indicated more sensitivity (overlap in 95% confidence limits) than *T. confusum*. Liu and Ho (1999) declared that adults of *S. zeamais* were more sensitive ($LD_{50} = 0.043 \mu\text{g}/\text{mg}$ insect) than *T. castaneum* ($LD_{50} = 0.118 \mu\text{g}/\text{mg}$ insect) to *Evodia rutaecarpa* Hook f. et Thomas essential oil applied topically. Chu *et al.* (2011) evaluated contact toxicity of some constituents of *Illicium difengpi* stem bark essential oil by topical application against *T. castaneum* and *Sitophilus zeamais* Motsch. They reported that safrole have more contact toxicity against both insect species with $LD_{50} = 4.67 \mu\text{g}/\text{adult}$ for *T. castaneum* and $8.54 \mu\text{g}/\text{adult}$ for *S. zeamais*. According to their findings linalool efficacy was less than safrole with $LD_{50} = 8.12 \mu\text{g}/\text{adult}$ for *T. castaneum* and $24.88 \mu\text{g}/\text{adult}$ for *S. zeamais*. Therefore, essential oils toxicity could be according to their chemical constituents.

Moreover, the method applied for the bioassays is also effective in causing mortality in stored-products insect pests. Aggarwal *et al.* (2001) evaluated toxicity of 1,8-cineole against *C. maculatus*, *S. oryzae* and *Rhyzopertha dominica* (F.). They stated that higher doses were required to achieve 100 % kill when using fumigant toxicity with filter paper discs than in topical application. This may be because impregnated filter paper provides less direct contact. Deletre *et al.* (2013) emphasized that essential oils easily penetrate insect cuticle, which increases their bioavailability which may be the reason for high toxicity of essential oils in direct contact.

Conclusion

It could be concluded that *T. confusum* adults were significantly more resistant than *S. granarius*. The insecticidal efficacy of both essential oils was more or less the same. Direct contact of essential oils cause more penetration of the oils via insect's cuticle causing fast knockdown and more mortality. Therefore, the essential oils of *C. copticum* and *C. cyminum* may be applied as an alternative to synthetic insecticides for stored-products insect pest management.

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اثر حشره‌کشی دو اسانس گیاهی علیه *Sitophilus granarius* (Coleoptera: Curculionidae) و *Tribolium confusum* (Coleoptera: Tenebrionidae) به روش موضعی

معصومه ضیائی

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چکیده: زیست‌سنجی‌ها به منظور ارزیابی اثر تماسی دو اسانس گیاهی علیه حشرات بالغ شپشه گندم (*Sitophilus granarius* (L.)) و شپشه آرد *Tribolium confusum* Jacquelin du Val. به روش موضعی صورت گرفت. اسانس از بذر دانه‌های زنیان *Carum copticum* L. و زیره سبز *Cuminum cyminum* L. استخراج شد. مرگ و میر حشرات کامل ۱، ۲، ۶ و ۲۴ ساعت پس از تیمار شدن بررسی شد. زیست‌سنجی‌ها در دمای 1 ± 27 درجه سلسیوس، رطوبت نسبی 5 ± 55 درصد و در تاریکی انجام شد. نتایج نشان داد هر دو اسانس مورد آزمایش به صورت موضعی روی دو گونه حشره سمی بودند. مرگ‌ومیر حشرات کامل با افزایش دز اسانس و زمان قرارگیری در مجاورت هر دز افزایش یافت. حشرات کامل شپشه گندم نسبت به اسانس زنیان ($LD_{50} = 0.09 \mu\text{g}/\text{mg body wt}$) حساس‌تر از اسانس زیره سبز ($LD_{50} = 0.16 \mu\text{g}/\text{mg body wt}$) بودند. به طور کلی، شپشه آرد حساسیت مشابهی به هر دو اسانس نشان داد. حشرات بالغ شپشه آرد مقاوم‌تر از شپشه گندم بودند. اسانس‌های زنیان و زیره سبز دارای پتانسیل استفاده در برنامه‌های مدیریت آفات محصولات انباری هستند.

واژگان کلیدی: اسانس گیاهان، محصولات انباری، کاربرد موضعی