FUMIGANT TOXICITY OF *CARUM COPTICUM* L. OIL AGAINST *TRIBOLIUM CONFUSUM* DU VAL, *RHYZOPERTHA DOMINICA* F. AND *ORYZAPHILUS SURINAMENSIS* L.

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ABSTRACT: Plant secondary metabolites play an important role in the plant-insect interactions. Some compounds extracted from plants have insecticidal activity against insects. The chemical composition of the essential oil from seeds of *Carum copticum* grown in mashhad, Iran, was studied by gas chromatography mass spectrometry (GC-MS). Thymol (43%), γ-terpinen (15.85%) and β-cymene (21.67%) were found to be the major constituents of the oil. Fumigant toxicity test of above-mentioned plant essential oil against adults of *Tribolium confusum* du Val, *Rhyzopertha dominica* F. and *Oryzaephilus surinamensis* L. were carried out at 27±2°C and 60±5% RH. Results demonstrated that *O. surinamensis* (LC_{50} = 1.69 µl/l air) was significantly more susceptible than *R. dominica* (LC_{50} = 19.01 µl/l air) and *T. confusum* (LC_{50} = 58.70 µl/l air) at 24 h length of exposure to this essential oil. In all cases, considerable differences were observed in mortality of insects with essential oil vapor in different concentrations and times. As expected, mortality was increased by increasing of doses and exposure time after 72 h fumigation.

KEY WORDS: *Carum copticum*, Essential oil, *Tribolium confusum*, *Rhyzopertha dominica, Oryzaephilus surinamensis,* fumigant toxicity.

In many storage systems, fumigants are the most economical and convenient tools for managing stored-grain insect pests, not only because of their ability to kill a broad spectrum of pests but also because of their easy penetration into the commodity while leaving minimal residues (Mueller, 1990). Currently, methyl bromide and phosphine fumigants are widely used for insect pest control in stored products. However, because of its ozone depletion potential, methyl bromide is being phased out the world over. Additionally, it has been reported that some stored-product insects are found to have developed resistance to phosphine in many countries (Subramanyam & Hagstrum, 1995). Hence, there is a need to develop new types of selective insect-control alternatives with fumigant action. In fact, management of stored product pests, using substances of natural origin, is nowadays the subject of many studies (Isman, 2006).

Carum copticum is popularly known as ajowan; it is an annual herbaceous plant bearing feathery leaves and white flowers grow in compound umbels. When the seeds are ripe, they are dried and threshed. Ajowan is widely distributed plant throughout the world and especially in India (Thangam & Dhananjayan., 2003). It is indigenous to southern India and is cultivated in various areas such as Europe, Egypt, Pakistan, Afghanistan and Iran (Gersbach & Reddy, 2002). Ajowan fruits are an important commercial product for the food/flavoring industry and they accumulate up to 5% essential oil in compartments referred to as canals or vita (Minija & Thoppil, 2002). Composition of the essential oil has been analyzed extensively (Gersbach & Reddy, 2002; Minija & Thoppil., 2002). The essential oil is considered for its antimicrobial and insecticidal activity. Rani and Khullar (2004) showed antimicrobial activity of ajowan essential oil against multi-drug resistant *Salmonella typhi* (Schroeter).

In the present study, chemical constituents of the essential oil from *C. copticum* were analyzed by GC and GC-MS. Moreover, fumigant toxicity of the experimented material was assessed against Confused flour beetle *T. confusum* du val, Lesser grain borer *R. dominica* and Saw-toothed grain Beetle *O. surinamensis*.

MATERIALS AND METHODS

Insects

Preliminary population was obtained from laboratory stock cultures maintained at the Entomology Department, Urmia University, Iran. *T. confusum*, *R. dominica* and *O. surinamensis* were reared in glass containers (1 litter) containing wheat flour mixed with yeast (10:1 w/w), whole wheat grains and raisin, respectively, that was covered by a fine mesh cloth for ventilation. The cultures were maintained in the darkness in a growth chamber set at 27±2°C and 60%±5% RH.

Adult insects, 1-7 days old, were used for fumigant toxicity tests. All experimental procedures were carried out under the same environmental conditions as the cultures.

Extraction of the essential oil

Seeds of *C. copticum* were collected from mashhad, Iran. Essential oil was extracted from dried seeds with a Clevenger-type apparatus to hydrodistillation. For extraction of mentioned essential oil, 100 g of air-dried and grinded seeds were put in the Clevenger then some sterile water was added. After 2 h distillation, extracted essential oil was collected.

Analysis of the essential oil

The oil composition was analyzed by gas chromatography-mass spectrometry (GC–MS). GC–MS analysis was performed using a Thermofinnigan Trace GC 2000 equipped with a MS fused silica capillary column (30 m × 0.25 mmi.d, film thickness 0.25 μm). For GC–MS detection, an electron ionization system with ionization energy of 200 eV was used. Carrier gas was helium at a flow rate of 1.5 ml/min; injection speed 1ml/min; split flow 15 ml/min. The oven temperature was programmed from 120 to 260°C at 5°C/min, then held isothermal for 12 min and finally raised to 350°C at 10°C/min. A relative percentages of the oil constituents was expressed as percentages by peak area normalization. The identification of individual compounds of essential oils was based on comparison of their relative retention index with those of authentic samples on DB-225 capillary column, and by matching of their mass spectra and peaks with those obtained from authentic samples.

Fumigant toxicity

To determine the fumigant toxicity of the *C. copticum* oil, filter papers (Whatman No. 1, cut into 2 cm diameter pieces) were impregnated with oil at doses calculated to give equivalent fumigant concentrations. Then the impregnated filter paper was attached to the under-surface of the screw cap of a plastic tube (35 mL). The caps were screwed tightly on the plastic tube containing twenty adults (1-7 days old) of each insect, separately. Each concentration and control was replicated three times. Mortality was determined 24, 48 and 72 h after exposure. Those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead.

Statistical analysis

The experiment was arranged by factorial experiment and the data were analyzed with analysis of variance (ANOVA) by using the SPSS 16.0 software. Differences between means were tested using Tukey test and values with $p < 0.05$ were considered significantly different. Probit analysis was used to estimate LC_{50} and LC_{95} values with their fiducial limits by SPSS 16.0 software package.

RESULTS

Chemical constituents of essential oil

Nine compounds were identified in the oil of *C. copticum* representing 96.1% of the total oil, with thymol (43%), γ-terpinen (15.85%) and β-cymene (21.67%) as the major constituents (Table 1).

Fumigant toxicity

Carum copticum oil showed potential fumigant activity against adults of *O. surinamensis*, *R. dominica* and *T. confusum* at different concentrations and exposure times. LC_{50} values were obtained 1.69, 19.01 and 58.70 μl/l air after 24 h exposure for *O. surinamensis*, *R. dominica* and *T. confusum,* respectively. LC_{50} values were resulted 0.80, 15.12 and 51.96 μl/l air, for *O. surinamensis*, *R. dominica* and *T. confusum,* respectively after 48 h of exposure. These values were obtained for *O. surinamensis, R. dominica* and *T. confusum* 0.43 μl/l , 12.83 μl/l and 47.05 μl/l air, respectively after 72 h length of exposure (Table. 2 A).As expected, in all of three tested insects results showed that the increasing of exposure time was caused to decreasing LC_{50} and LC_{95}. Results of LC_{50} and LC_{95} values demonstrated that *O. surinamensis* is more susceptible than *R. dominica* and *T. confusum*. Therefore, *T. confusum* is more resistance than two experimented pest in three times of experiments.

Table. 2 B showed that LT_{50} and LT_{95} values for each three experimented insect. For obtaining LT_{50} values highest concentration of essential oil was used in each insect. The LT_{50} value was obtained 9.74, 11.85 and 5.19 h and LT_{95} was resulted 43.81, 33.8 and 62.25 h for *O. surinamensis R. dominica* and *T. confusum*, respectively.

Figure A demonstrated the mortality percentages of five different concentrations in three times on *T. cofusum*. Figure B showed the percentage of mortality in five concentrations of essential oil for *R. dominica* in three times. Also figure C showed these values for *O. surinamensis*.

DISCUSSION

Results of this study showed that essential oil of *C. copticum* had potent fumigant toxicity against adults of *O. surinamensis*, *R. dominica* and *T. confusum*. The insecticidal activity varied with insect species, concentrations of the oil and extension of exposure times. To the best of our knowledge, no studies have been reported previously concerning the activity of *C. copticum* as fumigant on *O. surinamensis*, *R. dominica* and *T. confusum*.

The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility they have fumigant action that might be of great importance for stored product insects (Lee *et al.*, 1997; Ahn *et al.*, 1998). Thymol, a monoterpenoid is the major component in *C. copticum* essential oil. There are numerous reports on insecticidal activity of thymol. Regnault-Roger and Hamraoui (1995) tested 22 essential oils for their fumigant toxicity to the bean weevil, *Acanthoscelides obtectus* (Say), and found that thymol, carvacrol and terpineol to be effective in inhibiting beetle reproduction. Erler (2005) reported the fumigant activity of thymol against adults and eggs of *Tribolium confusum* Jacquelin Du Val, and larvae and eggs of *Ephestia kuehniella* Zeller. *β*-cymene (21.67%), γ-terpinene (15.85%), α-pinene (4.86%), β-pinene (3.62%), 4- terpineol (1.65%) are the other components of *C. copticum* oil that have insecticidal activity. For instance, among the major monoterpenoids of essential oil, it was reported that γ-terpinene and thymol were the most active constituents against adults of the rice weevil, *S. oryzae* (Erler, 2007). α-pinene was reported to be toxic to *T. confusum* (Ojimelukwe & Alder, 1999). Suya et al. (1998) showed the fumigant activity of thymol against *Rhyzopertha dominica* after its isolation from the essential oil of *Elsholtzia* spp. So the toxic effects of *C. copticum* oil could be attributed to thymol and other components.

As major constituents of *C. copticum* are monoterpenoids, they are typically volatile and can penetrate into insect commodity rapidly (Lee et al., 2003). There are several reports indicating that *Tribolium* sp. is relatively tolerant to essential oils of different plants (Xie *et al.*, 1995; Huang et al., 1997; Liu & Ho, 1999). Sahaf et al. (2007) studied chemical constituents and fumigant toxicity of essential oil from *C. copticum* against *S. oryzae* and *T. castaneum*. They observed that *S. oryzae* (LC_{50} = 0.91 μL/L) were significantly susceptible than *T. castaneum* (LC_{50} = 33.14 μL/L). Obtained LC_{50} and LC_{95} values of current study showed that these results is more different from than Sahaf's *et al.* results. Probably, these differences is due to condition of hydrodistillation, plant species, the used plant part in these studies, the season and the method of harvesting of target plant and geographical zone and pedological conditions for the plant growth.

Our observations indicated that *T. confusum* is more tolerant than *R. dominica* and *O. surinamensis*.

The insecticidal activity of some essential oils from Apiaceae has been evaluated against a number of stored product insects. Also, the essential oil of *C. copticum* had activity against growth stages of Indian meal moth, *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) (Shojaaddini et al., 2008). Chaubey (2007) investigated insecticidal activity of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) essential oils against stored product beetle *T. castaneum*. The death of adults of *T. castaneum* was caused by fumigation with these essential oils. In an other experiment using extracts of *Foeniculum vulgare* Gaetner (Apiaceae) fruit, over 90% mortality was achieved in adults of *S. oryzae* and *C. chinensis* at 3 or 4 days after treatment (Kim et al., 2003). Ogendo et al. (2008) evaluated bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five stored product pests. The oil caused 98%, 99% and 100% mortality of *R. dominica*, *O. surinamensis* and *C. chinensis*, respectively, 24 h after treatment, whereas eugenol achieved 79%, 61% and 100% mortality of the same insects except for *T. castaneum* which was more tolerant. Kordali et al. (2008) tested insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* on two stored product pests. *O. acutidens* oil caused 68.3% and 36.7% mortality of *S. granarius* and *T. confusum* adults, respectively, after 96 h of exposure. Results showed that the oil was more toxic against *S. granarius* as compared with its toxicity against *T. confusum*. Ebadollahi et al. (2010) tested toxicity of essential oil of *Agastache foeniculum* (Pursh) Kuntze to *Oryzaephilus surinamensis* L. and

Lasioderma sericorne F. LC_{50} data's for adults showed that *O. surinamensis* was more susceptible than *L. serricorne* at the exposure time 24 h. Our experiments demonstrated that *O. surinamensis* adults were more susceptible than *R. dominica* and *T. confusum*. Results of the present and earlier studies demonstrated that the essential oil from *C. copticum* have had toxic properties on the insect pests. The use of plant materials in pest control could become important supplements or alternatives to imported synthetic pesticides. Therefore, it is important that appropriate technology is developed to promote a direct preparation of traditional pesticides at the extensive level for those poor farmers who have no access to commercial pesticides or cannot afford.

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Table 1. Chemical constituents of the essential oil from *Carum copticum*.

Compound	Retention index	Composition (%)
Acetic acid	819	4.76
α- pinene	948	4.86
4- carene	919	0.45
Β- pinene	987	3.62
Γ- terpinene	998	15.85
Β- cymene	1025	21.67
4- terpinneol	1182	1.65
Thymol	1262	43.00
Phenol	1620	0.24
Other components		3.9

Table 2. Results of probit analysis from fumigant toxicity of *Carum copticum* oil against *O. surinamensis*, *R. dominica* and *T. confusum*

A. LC_{50} and LC_{95} values within 3 day

Insects	Time [h]	LC_{50} [μl/l]	LC_{95} [μl/l]	X^2	Slope ± SE	Intercept [a]
	24	*1.69*	*31.94*	*1.30*	*1.291 ± 0.157*	*4.703*
O. surinsmensis	*48*	*0.80*	*16.30*	*1.35*	*1.256 ± 0.162*	*5.122*
	72	*0.43*	*4.78*	*4.22*	*1.585 ± 0.204*	*5.567*
	24	*19.01*	*49.80*	*0.114*	*3.933 ± 0.475*	*-0.031*
R. dominica	*48*	*15.12*	*34.13*	*0.980*	*4.653 ± 0.553*	*-0.489*
	72	*12.83*	*27.44*	*0.913*	*4.985 ± 0.654*	*-0.526*
	24	*58.70*	*105.70*	*0.045*	*6.439 ± 0.799*	*-6.389*
T. confusum	*48*	*51.96*	*92.91*	*0.069*	*6.517 ± 0.838*	*-6.18*
	72	*47.05*	*84.24*	*0.54*	*6.503 ± 0.907*	*-5.878*

B. LT_{50} and LT_{95} values at the highest dose (85.71 μl/l air for *T. confusum*, 37.14 μl/l air for *R. dominica* and 8.57 μl/l air for *O. surinamensis)*

Insects	LT_{50}[h]	LT_{95}[h]	X^2	Slope ± SE	Intercept[a]
O.surinsmensis	9.74	43.81	1.99	2.519 ± 0.843	2.51
R. dominica	11.85	33.8	0.169	3.614 ± 1.264	1.22
T. confusum	5.19	62.25	0.252	1.525 ± 0.692	3.91

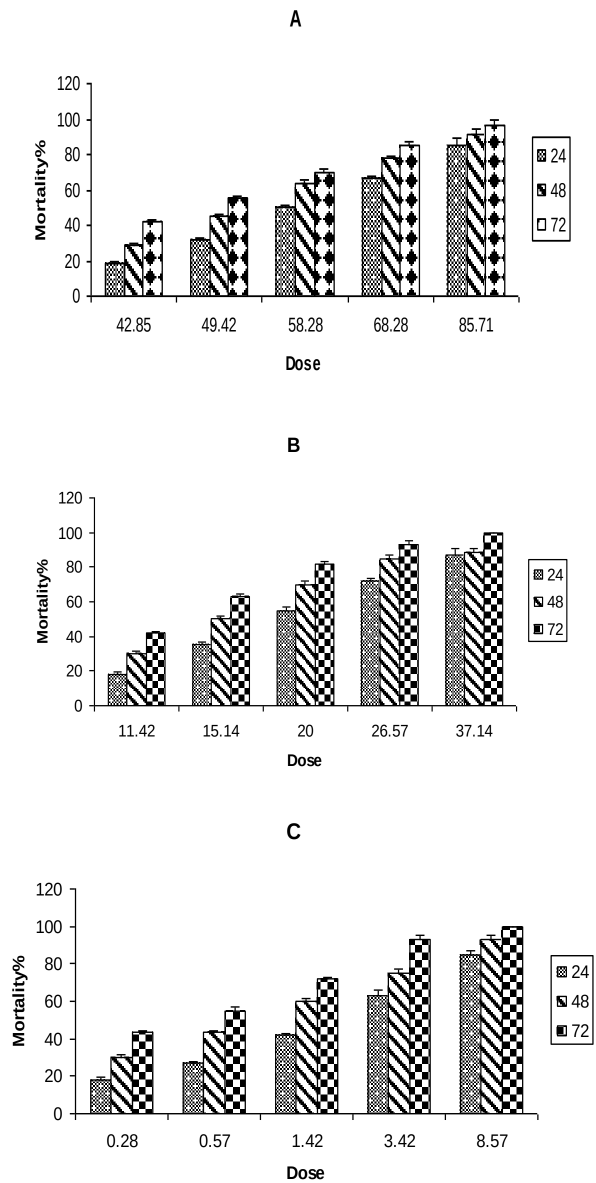

Figure 1. Fumigant toxicity of essential oil from *C. copticum* against adults of *T. confusum* (A), *R. dominica* (B) and *O. surinamensis* (C), after 24, 48 and 72 h.