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FUMIGANT TOXICITY OF *LAVANDULA STOECHAS* L. OIL AGAINST THREE INSECT PESTS ATTACKING STORED PRODUCTS

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Abstract: Plant secondary metabolites play an important role in plant-insect interactions and therefore such compounds may have insecticidal activity against insects. The chemical composition of the essential oil from leaves and flowers of *Lavandula stoechas* grown in Kashan, Iran, was studied by gas chromatography mass spectrometry (GC-MS). 1,8-Cineole (7.02%), γ-Cadinene (5.33%), T-Cadinol (5.07%), p-Mentha-1-en-8-ol (5.02%) and Caryophyllene (5.01%) were found to be the major constituents of the oil. In fumigant toxicity tests with the essential oil against adults of *Tribolium castaneum* Herbst, *Lasioderma serricorne* F. and *Rhyzopertha dominica* F. at 27±1°C and 60±5% RH, it was observed that *L. serricorne* (LC_{50} = 3.835 μl/l) were significantly more susceptible than *R. dominica* (LC_{50} = 5.66 μl/l) and *T. castaneum* (LC_{50} = 39.685 μl/l) 24 h after treatment. In all cases, considerable differences in mortality of insects to essential oil vapor were observed with different concentrations and times. Mortality increased as the doses of essential oils and exposure period increased and after 72 h fumigations, greatest percentages of mortality were obtained. The findings indicate the strong insecticidal activity of *L. stoechas* oil and it may be used in grain storage against insects *L. serricorne, R. dominica* and *T. castaneum*.

Key words: *Lavandula stoechas*, essential oil, *Tribolium castaneum, Lasioderma serricorne, Rhyzopertha dominica*, fumigant toxicity

INTRODUCTION

Of 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 a 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 over the world. Additionally, it was reported that some stored product insects developed resistance to phosphine in many countries (Taylor 1989; MBTOC 2002). 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). In this regard plant-based insecticides (PBIs) as described by Rosenthal (1986) can be less toxic to man, readily biodegradable, suitable for the use by small scale farmers and yet capable of protecting crops from attack by a wide range of insect pests. The use of plant derived insecticides played important role in traditional method of storage pest control in Africa and Asia (Hassanali *et al.* 1990; Niber 1994; Bekele and Hassanali 2001).

Iran is a country comprised largely of arid and semi-arid areas, and possess many aromatic plants from different families. *Lavandula stoechas* L. is one of these medicinal herbs, and grows in some of areas in Iran. A medicinal importance of the *L. stoechas* is well documented (Poucher 1974; Khazaeli *et al.* 2009).

In the present paper, laboratory studies are reported on the fumigant toxicity of *L. stoechas* L. essential oil against wheat flour pest *T. castaneum*, lesser grain borer *R. dominica* and cigarette beetle *L. serricorne*.

MATERIALS AND METHODS

Insects

T. castaneum and *L. serricorne* were reared in glass containers (1 litter) containing wheat flour that was covered by a fine mesh cloth for ventilation. The cultures were maintained in the dark in a growth chamber set at 27±2°C and 60%±5% RH. *R. dominica* was reared in a container containing whole wheat grains. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. Adult insects, 7–14 days old, were used for fumigant toxicity tests. All experimental procedures were carried out under the same environmental conditions as the cultures.

Plant materials

Essential oil of leaves and flowers of *L. stoechas* L. was purchased from company of Barij Essens (Kashan, Iran). Primary plant material was collected from Kashan, Iran.

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Gas chromatography/mass spectrometry

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 x 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. Injector and MS transfer line temperatures were set at 250 and 200°C, respectively. The oven temperature was programmed from 80 to 250°C at 8°C/min, then held isothermal for 10 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 times 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

Concentrations of 14.28 µl to 60.1 µl, 1.76 µl to 14.23 µl and 0.9 µl to 8 µl of the oil of *T. castaneum, R. dominica* and *L. serricorne* respectively, were dissolved in 200 µl acetone and applied to Whatman No. 1 filter paper stripes (4x5 cm), and dried in air for 2 min. Treated filter paper stripes were placed at the bottom of 280 ml glass jars. Twenty adults (7 to 14 days old) insects were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical centre of the glass jars, which were then sealed with air- tight lids. Mortality was determined after 24, 48 and 72 h from the start of exposure. When no leg or antennal movements were observed, insects were considered dead. Percentage of insect mortality was calculated using the Abbott correction formula for natural mortality in untreated control (Abbott 1925).

Statistical analysis

The experiment was arranged by randomized complete block design and the data were analysed with analysis of variance (ANOVA) by using the MSTAT C software. Differences between means were tested using Duncan 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

Chemical analysis of essential oil of *L. stoechas* revealed 40 components in which 1,8-Cineole (7.02%), γ-Cadinene (5.33%), T-Cadinol (5.07%), p-Mentha-1-en-8-ol (5.02%) and Caryophyllene (5.01) were the major constituents (Table 1).

Table 1. Major components of the essential oil from *L. stoechas* leaves and flowers collected from Kashan in Iran

Components	RT [min]	[%]
7,7-Dichloro-2-heptanone	1.69	1.01
(+)-α-Pinene	4.17	2.71
(-)-β-Pinene	4.87	2.35
1,8-Cineole	6.59	7.02
β-Terpineol	6.79	4.11
Alcanfor	8.32	4.16
Borneol	9.11	4.87
p-Mentha-1-en-8-ol	9.28	5.02
2-Ethenylidene-6,6-dimethylbicyclo [3.1.1] heptane	9.62	4.98
1,3,3-Trimethyl-2-vinyl-1-cyclohexene	9.94	4.76
Cuminyl alcohol	10.03	2.53
Geranial acetate	10.92	3.64
Caryophyllene	11.58	5.01
Cedrene	11.62	2.91
Cubenol	11.78	2.85
Isoledene	11.98	1.57
Nerolidyl acetate	12.42	2.92
γ-Cadinene	12.71	5.33
Caryophyllene oxide	13.12	2.80
T-Cadinol	14.26	5.07
α-Cadinol	14.35	1.44
α-Bisabolol	14.55	3.23
2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-Butenal	14.79	2.22
2-methylene-5α-Cholestan-3α-ol	16.02	1.47
2,6,10-Trimethyltetradecane	16.54	2.31

RT – retention time on the DB-225 column

Fumigant toxicity

L. stoechas oil showed strong fumigant activity against *L. serricorne*, *R. dominica* and *T. Castaneum* adults at different concentration and exposure times. As the time of exposure increased, LC_{50} values decreased at 72 h, for *L. serricorne*, *R. dominica* and *T. castaneum* to 2.345 µl/l, 2.360 µl/l and 26.772 µl/l, respectively. Based on LC_{50} and LC_{95} values, the adults of insects were significantly more susceptible to *L. stoechas* oil (Table 2.A).

Probit analysis showed that after 24 h exposure, *L. serricorne* (LC_{50} = 3.855 µl/l) was more susceptible than *R. dominica* (LC_{50} = 5.660 µl/l) and *T. castaneum* (LC_{50} = 39.665 µl/l). With the increase of exposure time, LC_{50} values were decreased and after at 72 h, they were for *L. serricorne*, *R. dominica* and *T. castaneum* 2.345 µl/l, 2.360 µl/l and 26.772 µl/l, respectively (Table 2 A). On the basis of the LT_{50} and LT_{95} values, insecticidal effect of *L. stoechas* oil on *R. dominica* and *T. castaneum*, as increase times of exposure for the highest dose (60.71 µl/l for *T. castaneum*, 14.23 µl/l for *R. dominica* and 8 µl/l for *L. serricorne*) were increased (Table 2.B). Susceptibility of insects, as increase of concentrations of oil was increased. Furthermore, as increase of time exposure of insect with different concentrations of oil, susceptibility of insects was increased. On the other hand, the increase of susceptibility of three insect pests was associated with increase of different concentrations of oil and time of exposure (Fig. 1).

Table 2. Results of probit analysis from fumigant toxicity of *L. stoechas* oil against *L. serricorne*, *R. dominica* and *T. castaneum*

A. LC50 and LC95 values within 3 days
NS – no significant difference
* indicate significant difference at p < 0.05

Insects	Time [h]	LC50 [µl/l]	LC95 [µl/l]	X^2 [df = 3]	Intercept [a]	Slope [b]
L. serricorne	24	3.835	52.807	1.616*	–3.43	1.444
	48	3.011	25.476	4.335*	–3.49	1.773
	72	2.345	17.356	6.480*	4.3	1.892
R. dominica	24	5.660	80.565	0.372*	3.926	1.426
	48	4.275	28.868	4.027*	3.74	1.983
	72	2.360	21.367	11.803 NS	–1.41	1.719
T. castaneum	24	39.685	216.855	4.294*	1.435	2.230
	48	29.418	112.843	3.010*	0.863	2.817
	72	26.772	95.275	3.502*	0.74	2.984

B. LT50 and LT95 values at the highest dose (60.71 µl/l for *T. castaneum*, 14.23 µl/l for *R. dominica* and 8 µl/l for *L. serricorne*)

Insects	LT50 [h]	LT95 [h]	X^2	DF	P	Intercept [a]	Slope [b]
L. serricorne	10.887	114.393	0.218	1	0.640	3.33	1.610
R. dominica	15.779	52.566	2.528	1	0.112	3.142	1.229
T. castaneum	7.427	147.274	0.260	1	0.610	3.896	1.288

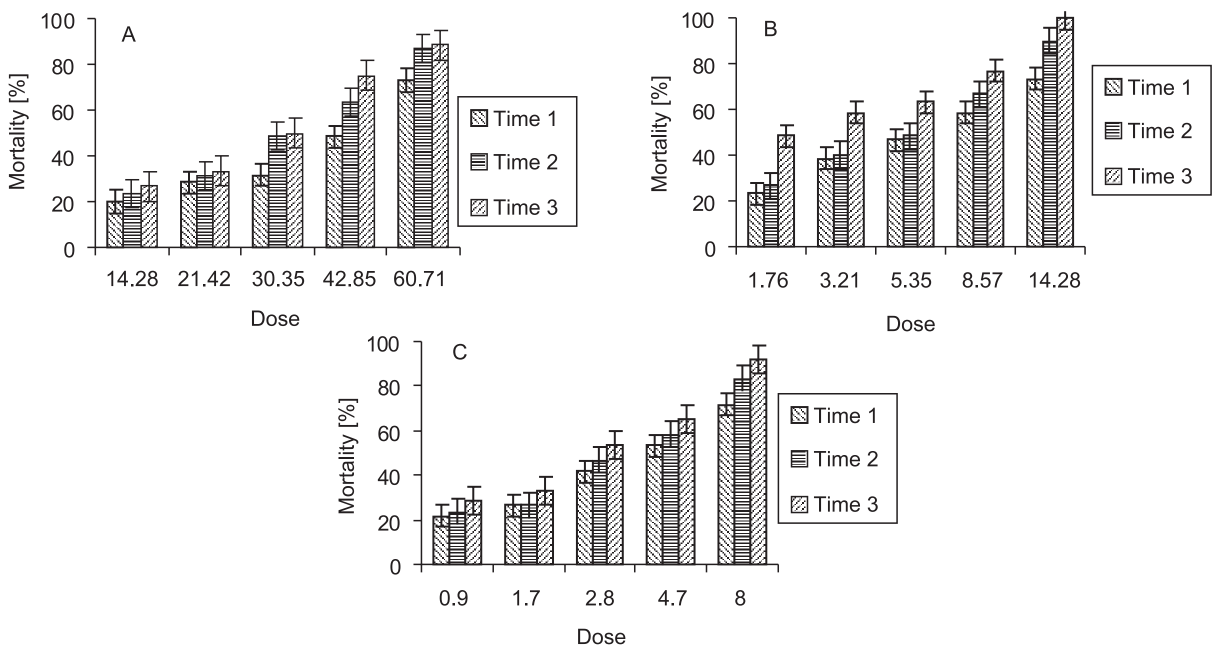

Fig. 1. Fumigant toxicity of essential oil from *L. stoechas* against adults of *T. castaneum* (A), *R. dominica* (B) and *L. serricorne* (C), after 24, 48 and 72 h fumigation

DISCUSSION

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 larvae and adults of *T. castaneum* was caused by fumigation with these essential oils. Kim *et al.* (2003) studied contact and fumigant toxicity of aromatic essential oils against *L. serricorne*. In a fumigation test with the beetle adults, insecticidal activity of horseradish oil and mustard oil was much more effective in closed cups than in open ones, indicating that the insecticidal activity of these materials was largely attributable to fumigant action. 1,8-cineole, a monoterpenoid is the major component in *L. stoechas* oil. There are numerous reports on insecticidal activity of 1,8-cineole. Aggarwal *et al.* (2001) tested toxicity of 1,8-cineole toward *Callosobruchus maculates* F., *R. dominica* and *Sitophilus oryzae* L. The component was more effective as a fumigant and gave 93–100% mortality of all the three pest species at the dose of 1.0 ml/l under empty jar conditions. LC50 values were found 0.28, 0.33 and 0.46 ml/l against *C. maculates*, *R. dominica* and *S. oryzae*, respectively. Pinene is reported to be toxic to *T. confusum* (Ojimelukwe and Alder 1999). So the toxic effects of *L. stoechas* oil could be attributed to 1,8-cineole and other components.

Jacobson (1989) demonstrated that the most promising botanical insect control agents are in the families *Annonaceae*, *Asteraceae*, *Canellaceae*, *Lamiaceae*, *Meliaceae*, and *Rutaceae*. Lavandula genus is an important member of family *Labiateae* (Lamiaceae). No previous studies were conducted regarding the activity of *L. stoechas* L. as a fumigant for the control of insect pests. However, the insecticidal effect of some *Lamiaceae* extracts has previously been evaluated against a number of stored product insects (Clemente *et al.* 2003).

The insecticidal activity varied with insect species, oil concentrations, and exposure time. It can be concluded that essential oil products are generally broad-spectrum, due to the presence of several active ingredients that operate via several modes of action (Chiasson *et al.* 2004).

Experiments showed that *T. castaneum* is more tolerant than *R. dominica* and *L. serricorne*. There are several reports indicating that *T. castaneum* is relatively tolerant to essential oils of different plants (Huang *et al.* 1997; Liu and Ho 1999; Sahaf *et al.* 2008).

The observed fumigant activity demonstrates that essential oils are a source of biologically active vapors which may potentially prove to be efficient insecticides. Furthermore, the essential oil evaluated in this study is considered to be less harmful to humans and the environment than the majority of conventional insecticides (Isman 2006). Consequently, a possibility of employing these natural fumigants to control insects in stored products may deserve further investigation.

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POLISH SUMMARY

TOKSYCZNOŚĆ ZADYMIANIA OLEJKIEM *LAVANDULA STOECHAS* L. PRZECIWKO TRZEM SZKODLIWYM OWADOM ATAKUJĄCYM PRZECHOWYWANE PRODUKTY

Wtórne metabolity roślinne odgrywają ważną rolę we wzajemnych stosunkach rośliny z owadem, wykazując aktywność owadobójczą. Badano chemiczny skład olejku eterycznego z liści i kwiatów *Lovandula stoechas* L., rośliny uprawianej w Kashan, w Iranie, wykorzystując gazową chromatografię spektrometrii masy (GC-MS). Wykazano, że głównymi składnikami olejku były: 1,8-Cineole (7,02%), γ-Cadinene (5,33%), T-Cadinol (5,07%), p-Mentha-1-en--8-ol (5,02%) i Caryophyllene (5,01%). W testach toksyczności fumigacyjnej z olejkiem przeciwko dorosłym osobnikom *Tribolium castaneum* Herbst., *Lasioderma serricorne* F. i *Rhyzopertha dominica* F., w 27±1°C i wilgotności względnej 60±5% *L. serricorne* (LC_{50} = 3,835 µl/l) były one bardziej istotnie wrażliwe niż *R. dominica* (LC_{50} = 5,66 µl/l) i *T. castaneum* (LC_{50} = 39,685 µl/l), 24 godziny po zabiegu. W przypadkach różnych stężeń i czasu ekspozycji owadów na opary olejku eterycznego, obserwowano znaczne różnice w śmiertelności owadów, która wzrastała w miarę wzrostu dawki olejków eterycznych i czasu ekspozycji. Po 72 godzinach fumigacji uzyskiwano wyższe procenty śmiertelności. Wyniki wskazują na silną aktywność owadobójczą *L. stoechas*, która może być wykorzystana w przechowalni nasion przeciwko owadom *L. serricorne, R. dominica* i *T. castaneum.*