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FUMIGANT TOXICITY OF TWO MEDICINAL PLANT ESSENTIAL OILS ON *TRIBOLIUM CASTANEUM* (HERBST.)

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ABSTRACT

In recent years, interests in researches of medicinal plant essential oils have been growing. In this study, the fumigant activity of essential oils from *Thymus kotschyanus* Boiss and Hohen. and *Mentha longifolia* L. were tested on *Tribolium castaneum* (Herbst.) The flowering dried parts of the plants were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. Experiment was carried out at 26±1 ◦C and 65±5 r.h.% in dark condition on 1-7-day-old adult insects. The mortality of insects was tested in different concentrations. The mortality was increased with concentration from 384 - 769 µL/L air for *T. kotschyanus* and 0.2-1 µL/L air for *M. longifolia*. Data analysis demonstrated that lethal concentration to kill 50% of the population (LC_{50}) of *T. kotschyanus* and *M. longifolia* were 573.94 and 1.46 µL/L air respectively and lethal time to kill 50% of the population (LT_{50}) of *T. kotschyanus* at 615-1076 µL/L air were estimated for 7-4 h. In case of *M. longifolia*, LT_{50} value at 18-46 µL/L air were estimatedfrom 0.73 to 2 h. The present study showed that essential oil of *M. longifolia* was more toxic than *T. kotschyanus*. Also these medicinal plants can use as a safe insecticidal to control of stored product pests.

Key words: *Thymus kotschyanus*, *Mentha longifolia*, *Tribolium castaneum*, essential oil, fumigant toxicity

INTRODUCTION

Insects cause a significant nutritional and economical problem to products in most countries. The red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) is a serious and cosmopolitan pest (Via, 1999; Weston and Rattlingourd 2000). Many synthetic chemicals are being used to control of this insect can adversely affect non-target animals (Jamber et al., 1995; Jovanovic et al., 2007; Kamali Hatil Hashim, 2009). Resistance to synthetic insecticides, economic costs to conform with pesticide laws, consumer expectations and preferences, are important considerations for the management of *T. castaneum* and other stored-product pests. The imminent loss of the fumigant methyl bromide through compliance with the Montreal Protocol (Anonymous, 2004) will certainly further affect management programs for *T. castaneum*, accelerating the demand for new control strategies (Zettler et al., 1973; Phillips et al., 2000; Lee et al. 2001). Therefore, studies on insecticides that are safe and ecologically acceptable seem to be necessary.

In recent decades, global research has focused on plant secondary metabolites, especially essential oils, in protection of stored agricultural products. Essential oils and their major components have attracted research attention as potential alternatives to classical fumigants (Ogendoet al., 2010). More than 100,000 secondary metabolites from about 200,000 plant species have been identified, which include alkaloids, terpenoids, flavonoids and others which can have insecticidal properties (Vendramim and Castiglioni, 2000; Potenza et al., 2004). *Thymus kotschyanus* Boiss. and Hohen. (Labiatae) and *Mentha longifolia* L. (Lamiaceae) are medicinal plants that grow throughout the Iran. In the present research, toxicity of essential oils from these medicinal plants has been studied against *T. castaneum*.

MATERIALS AND METHODS

Plant materials

Aerial parts of *T. kotschyanus* and *M. longifolia* were collected at full flowering stage from Tehran, Iran in June 2007. The collected plants were dried naturally on laboratory benches at room temperature 23-27°C for a week until crisp dry. The dried parts of the plant were separately hydrodistilled in a Clevenger-type apparatus for 4 h. The resulting oil was dried over anhydrous sodium sulfate. The oil was kept at 4°C in the sealed brown vials until required. Oil yield for *T. kotschyanus* (1.66 ± 0.13% w/w) and for *M. longifolia* (2.08 ± 0.17% w/w) was calculated on a dry weight basis.

Insect rearing

Tribolium castaneum was used in this study and was reared on wheat flour mixed with yeast (10:1, w/w). The insect was obtained from laboratory cultures maintained in the dark in incubators set at 26 ± 1°C and 65 ± 5% r.h. All experiments were carried out under the same environmental conditions as the cultures.

Fumigant toxicity

The method used to determine the fumigant activity of extracted compounds was based as Negahban et al. (2006). Ten adult insects (1-7 days old) were put into glass bottle. Filter papers (2.0 cm diameter) were impregnated with an appropriate concentration of the oil with dichloromethane as a solvent. After evaporating the solvent for 2 min, each filter paper was placed on the underside of the screw cap of a glass vial. The cap was then screwed on tightly. Control insects kept in the same conditions without any essential oil. Each concentration and control was replicated five times. After 24 h from commencement of exposure to the essential oil, the insects were transferred to another glass bottle without any essential oil and the number of dead and live insects in each bottle was counted. When no leg or antennal movements were observed, insects were considered dead. Probit analysis (Finney, 1971) was used to estimate LC_{50} and LC_{90} values, SAS program (SAS Institute, 1997).

Another experiment was designed to determine the median effective time causing mortality of 50% of the test insects (LT_{50} values) at 615.4, 769.2, 923.1 and 1076.9 µL/L air of *T. kotschyanus*, and at 18.5, 27.7, 36.9 and 46.2 µL/L air of *M. longifolia* essential oil. The mortality was assessed by direct observation of the insects over the exposure time. Time-mortality data for each experiment was analyzed by the method of Finney (1971) with time as the explanatory variable to estimated the number of hours required for 50% mortality. Estimates were compared to see if there was overlap of the 95% fiducial limits.

RESULTS

The results showed that *T. kotschyanus* and *M. longifolia* essential oils were toxic to *T. castaneum* adults. LC_{50} values for the essential oils were 574 and 0.56 µL/L air for *T. kotschyanus* and *M. longifolia*, respectively. Also the probit analysis showed that the LC_{90} values of *T. kotschyanus* and *M. longifolia* oils were 908 and 1.46 µL/L air, respectively (Table 1). Therefore the *M. longifolia* oil was clearly more toxic to *T. castaneum* than that of *T. kotschyanus*.

Table 1. Fumigant toxicity of *Thymus kotschyanus* and *Mentha longifolia* essential oils on adults of *Tribolium castaneum*

Essential oil	χ^2 (df)	P-Value	Intercept ± SE	Slope ± SE	LC_{50} (µL/L air)[1]	LC_{90} (µL/L air)[1]
T. kotschyanus	3.486(4)	0.480	-17.74±2.27	6.43±0.82	573.94 (542.65 -607.69)	908.15 (816.98 -1075)
M. longifolia	0.78(3)	0.854	0.78±0.13	3.06±0.40	0.56 (0.49-0.63)	1.46 (1.16-2.10)

1) 95% lower and upper fiducial limits are shown in parenthesis

Median effective time to cause mortality of 50% of the test insects again showed that *T. castaneum* was more sensitive to *M. longifolia* than *T. kotschyanus*. LT_{50} and LT_{90} values for the highest dose of *T. kotschyanus* (1076.9 µL/L air) were 4.14 and 13.92 h, and for the lowest dose (615.38 µL/L air) were 7.56 and 17.62 h respectively. Also, LT_{50} and LT_{90} values for the highest dose of *M. longifolia* (46.2 µL/L air) were 0.73 and 2.97 h, and for the lowest dose (18.5 µL/L air) were 1.95 and 4.68 h respectively (Table 2).

DISCUSSION

In this initial exploratory work, the essential oil of *T. kotschyanus* and *M. longifolia* demonstrated fumigant toxicity against the red flour beetle *T. castaneum* within 24 h. The insecticidal activity depends on oil concentration. Other researches have shown that essential oils and their constituents may potentially be used as alternative compounds to synthetic fumigants in present usage (Shaaya et al., 1997; Regnault- Roger et al., 1993; Shakarami et al., 2005). It has been shown that *Artemisia tridentata* and *Artemisia vulgaris* essential oils have significant fumigant activity against adults, larvae and eggs of *T. castaneum* (Dunkel, and Sears, 1998; Wang, et al., 2006). The essential oil of *Artemisia annua* showed fumigant activity against *T. castaneum* (Tripathi, et al., 2000). The present results indicate that the essential oil of *M. longifolia* may be more effective than the other essential oils tested.

Rise of problems relating to the use of modem synthetic chemical insecticides, such as persistence of residues, environmental concerns and insect populations becoming resistant to conventional chemicals have generated attention towards naturally occurring products. The outcome of the results presented in this study indicate that *M. longifolia* oil or its major constituents could provide an effective fumigant and be considered for integration with other pest management procedures. More studies are needed to evaluate the fumigant activity of these essential oils and their constituents to other insects. Also, the mechanism of action of essential oils in target pests could be an attractive area of research.

Table 2. Regression analysis and $LT_{50/90}$ values of *T. kotschyanus* and *Mentha longifolia* essential oils on *Tribolium castaneum*

Essential oil	Conc. (µL/L air)	χ^2 (df)	P-Value	Intercept ± SE	Slope ± SE	LT_{50} (h)[1]	LT_{90} (h)[1]
T. kotschyanus	615.38	2.50(4)	0.648	-9.26±1.25	3.49±0.47	7.56 (6.80-8.37)	17.62 (14.52-24.17)
	769.23	0.72(4)	0.948	-8.05±1.11	3.10±0.43	6.64 (5.92 -7.47)	17.22 (13.66-21.35)
	923.08	1.76(4)	0.781	-7.32±0.98	2.92±0.39	5.35 (4.71-6.04)	14.70 (11.74-18.13)
	1076.9	2.84(4)	0.585	-5.83±0.86	2.43±0.35	4.14 (3.47-4.78)	13.92 (10.76-15.42)
M. longifolia	18.5	0.74(4)	0.947	-6.99±0.94	3.38±0.45	1.95 (1.75-2.17)	4.68 (3.87-6.36)
	27.7	1.00(4)	0.910	-4.90±0.67	2.60±0.35	1.28 (1.11-1.47)	3.99 (3.11-6.00)
	36.9	3.10(4)	0.541	-4.16±0.57	2.38±0.32	0.93 (0.79-1.08)	3.23 (2.46-5.02)
	46.2	6.19(4)	0.186	-3.45±0.46	2.10±0.28	0.73 (0.62-0.87)	2.97 (2.12-5.12)

1) 95% lower and upper fiducial limits are shown in parenthesis

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