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Susceptibility of Two Sitophilus species (Coleoptera: Curculionidae) to Essential Oils from Foeniculum vulgare and Satureja hortensis

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Abstract. This study was conducted to determine the insecticidal activity of essential oils from Fennel, *Foeniculum vulgare* (Apiaceae), and Summer savory, *Satureja hortensis* (Lamiaceae), against two stored-product insects. Essential oils from two species of plants were obtained by Clevenger-type water distillation and their fumigant toxicities were tested against adults of the wheat weevil, *Sitophilus granarius* and rice weevil, *Sitophilus oryzae* (Curculionidae). The mortality was determined after 24 and 48 hrs from beginning of exposure. LC_{50} values of each essential oil were estimated for each insect species. Fumigation bioassays revealed that essential oils from two plants had strong insecticidal activity on experimental insects. LC_{50} values indicated that *S. granarius* was more susceptible than *S. oryzae* to essential oils at the exposure time 24 and 48 hrs. The mortality effect of *S. hortensis* oil was lower than *F. vulgare* oil. The LC_{50} values decreased with the duration of exposure to the essential oil concentrations. In all case, responses varied according to plant material, concentration, and exposure time. These results indicated that essential oils from *S. hortensis* and *F. vulgare* could be applicable to the management of stored product insects to decrease ecologically detrimental effects of utilization synthetic insecticides.

Key words: Essential oil, *Foeniculum vulgare*, *Satureja hortensis*, Fumigant toxicity, *Sitophilus granarius*, *Sitophilus oryzae*.

Introduction

To preserve the quantity and quality of stored-product foodstuff particularly cereals it is necessary to reduce the population of the insect pests such as *Sitophilus* species. The rice weevil, *Sitophilus oryzae* (L.) and the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) are two of the most widespread and destructive insect pests of stored cereals. These pests are internal feeders and cause considerable loss to cereals affecting the quantity as well as quality of the grains (KUCEROVA *et al.*, 2003; PARK *et al.*, 2003).

Synthetic pesticides have been considered the most effective and accessible means to control insect pests of stored products (HUANG & SUBRAMANYAM, 2005). These chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for mammalian health (BENHALIMA *et al.*, 2004; ISMAN, 2006; HALDER *et al.*, 2010). The adverse effects of synthetic pesticides have amplified the need for effective and biodegradable pesticides. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. Among various kinds of natural substances that have received

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particular attention as natural agents for insect management are essential oils from aromatic plants. Essential oils are renewable, non-persistent in the environment and relatively safe to natural enemies, non-target organisms and human beings (HALDER *et al.*, 2010). Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavor or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites (KOUL *et al.*, 2008). Because of the intensity of plant-insect interactions, the plants there have well-developed defense mechanisms against pests and are excellent sources of new insecticidal substances. Their components and quality vary with geographical distribution, harvesting time, growing conditions and method of extraction (YANG *et al.*, 2005). Effects of essential oils on stored-product insect pests have been extensively reported (OGENDO *et al.*, 2008; PARK *et al.*, 2008; BENZI *et al.*, 2009; AYVAZ *et al.*, 2010; NYAMADOR *et al.*, 2010; TAGHIZADEH-SAROUKOLAI *et al.*, 2010). Iran is a country comprised largely of arid and semiarid areas, and contains many indigenous aromatic plants such as Fennel and Summer savory. Fennel, *Foeniculum vulgare* Gaertner is a species of flowering plant in the Apiaceae (Umbelliferae). The insecticidal activity of some essential oils from Apiaceae has been evaluated against a number of stored product insects. For example, SAHAF *et al.* (2007) found the strong insecticidal activity of essential oil from *Carum copticum* (Apiaceae) on *S. oryzae* and *Tribolium castaneum* (Tenebrionidae). The mortalities of the insect species reached 100% at concentrations higher than 185.2 μl/l air and 12 hrs exposure times. In another experiment, CHAUBEY (2008) studied fumigant activity of the essential oils from *Anethum graveolens* and *Cuminum cyminum* (Apiaceae) on *Callosobruchus chinensis* (Bruchidae). The 24-hrs LC_{50} values against the adults of the insect were 10.8 and 11.0 μl oils, respectively. Summer savory, *Satureja hortensis* L., is a species in the mint family (Lamiaceae). There are numerous investigations on the insecticidal activity of essential oils from Lamiaceae family (RAJENDRAN & SRIRANJINI 2008; TUNAZ *et al.*, 2009; AYVAZ *et al.*, 2010). Furthermore, our earlier studies indicated that the essential oil from *Agastache foeniculum* (Lamiaceae) had strong fumigant toxicity on the adults of *Oryzaephilus surinamensis* (Silvanidae) and *Lasioderma serricorne* (Anobiidae) (EBADOLLAHI *et al.*, 2010a). In the other study, we found that *Lavandula stoechas* (Lamiaceae) was very toxic against *L. serricorne* and *Rhyzopertha dominica* (Bostrichidae**)** (EBADOLLAHI *et al.*, 2010b).

Therefore, the main goal of the present study was to evaluate the insecticidal activities of essential oils from *F. vulgare* and *S. hortensis* grown in Iran in the control of two stored-grain insects, *S. granarius* and *S. oryzae*.

Material and methods

Plant materials and extraction of essential oils. The ripe seeds of *Foeniculum vulgare* and aerial parts from 1.5 cm of top of *Satureja hortensis* at flowering stage were harvested from plants grown in the experimental farm of the Department of Horticultural, University of Urmia, West Azerbaijan, Iran. These materials were air dried in the shade at room temperature (26-28 °C) for 20 days and stored in darkness until distillation. The essential oils were isolated from dried plant samples by hydrodistillation using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1:10 plant material/water volume ratio, 3 hrs distillation. The essential oils were collected, dried over anhydrous sodium sulfate and stored at 4 °C until use.

Insect cultures and experimental conditions. *Sitophilus granarius* (L.) and *Sitophilus oryzae* (L.) were reared in a 1 L wide-mouthed glass jars containing wheat grains. Mouth of the jars was covered with a fine mesh cloth for ventilation and to prevent escape of the weevils. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. The cultures were maintained in an incubator at 27 ± 2 °C and 60 ± 5% RH (Relative Humidity). Insects used in all experiments were 1 to 7 day old adults. All experimental procedures were carried out under the same environmental conditions as the cultures.

Bioassays. The fumigant bioassays were conducted as described by NEGAHBAN *et al.* (2007), with slight modifications. In this study, tested insects were confined in the plastic tubes. Thus, there was no direct contact between the oil and the insects. Different concentrations were prepared to evaluate mortality of insects after an initial dose-setting experiment. Concentrations of *Foeniculum vulgare* oil tested on *S. granarius* were 10, 14.92, 22.32, 33.41 and 50 µl/ l air and on *S. oryzae* were 25, 31.13, 38.74, 48.21 and 60 µl/ l air. *S. granarius* were exposed to the essential oil of *Satureja hortensis* at 20, 26.85, 36.85, 36.05, 48.41 and 70 µl/ l air and *S. oryzae* at 30, 37.08, 45.83, 56.64 and 70 µl/ l air. Each concentration was applied to filter paper stripe (4 × 5 cm, Whatman N° 1) and the impregnated filter papers were then attached to the screw caps of glass jars with volumes of 1 L. Twenty insect adults were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with clothes mesh and each tube were placed at the bottom of glass jar. Caps of glass jars were screwed tightly. The jars were kept in the incubator and mortality was determined after 24 and 48 hrs from beginning of exposure. Each experiment was replicated for five times at each concentration. The control insects were maintained under the same conditions without any essential oil. When no leg or antennal movements were observed, insects were considered dead.

The experiments were arranged in a completely randomize design and the data were analyzed with ANOVA. The means were separated using the Duncan's test at the 5% level. The LC_{50} values with their fiducial limits were calculated by probit analysis using the SPSS version 16.0 software package.

Results and Discussion

All the treatments with the essential oils showed significant level of toxicity to the insects. *Foeniculum vulgare* essential oil showed strong fumigant activity against *S. oryzae* and *S. granarius* adults. Compare means showed that there were significant differences in the mortality of *S. oryzae* and *S. granarius* exposed to different concentrations of *F. vulgare* oil for 24 and 48 hrs (P ≤ 0.05, Duncan’s test) (Fig. 1).

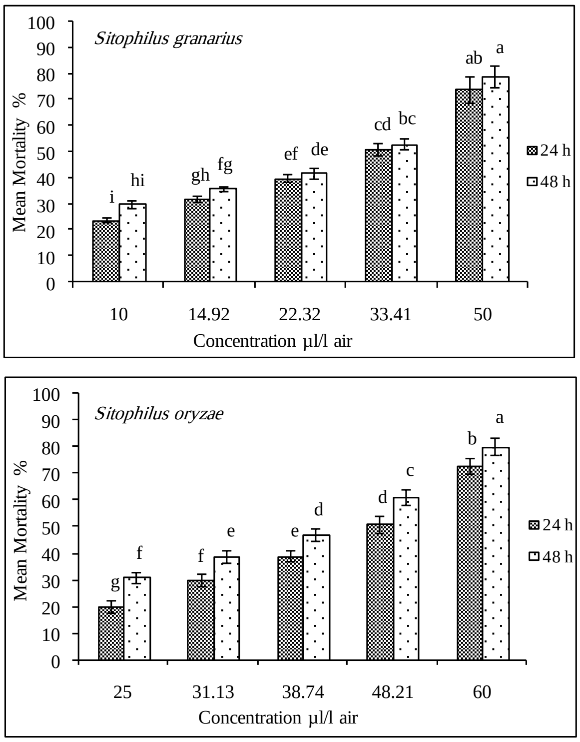

Fig. 1. Mean mortality (%) of *Sitophilus granarius* and *Sitophilus oryzae* exposed to different concentrations of the essential oil from *Foeniculum vulgare*. Different letters on top of columns indicate significant differences according to Duncan's test at p ≤ 0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±).

The graphs in Fig. 2 display that *Satureja hortensis* essential oil was very toxic on *S. oryzae* and *S. granarius* and there were significant differences in percentage mortality of insects exposed to different concentrations for 24 and 48 hrs (P ≤ 0.05, Duncan’s test).

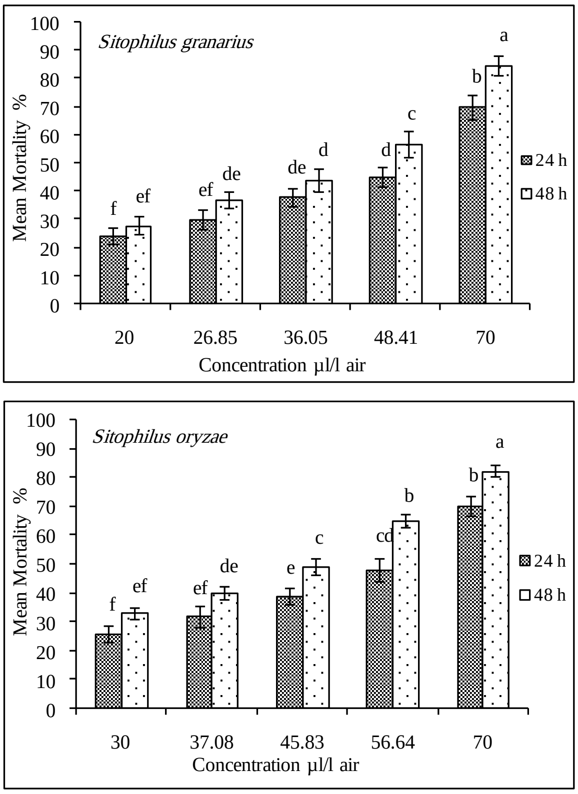

Fig. 2. Mean mortality (%) of *Sitophilus granarius* and *Sitophilus oryzae* exposed to different concentrations of the essential oil from *Satureja hortensis*. Different letters on top of columns indicate significant differences according to Duncan's test at $p \leq 0.05$. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±).

Probit analysis showed that the concentration for the essential oil of *F. vulgare* to cause 50% mortality (LC_{50}) in *S. granarius* was 27.30 µl/l air (95% lower and upper fiducial limit (FL) = 23.62 – 32.38), whereas in *S. oryzae* was 44.16 µl/l air (95% FL= 41.03 – 48.21) after 24 hrs of treatment (Table 1). Therefore, *S. oryzae* was noted to be more tolerant than *S. granarius* to *F. vulgare* essential oil.

The LC_{50} values of the *S. hortensis* oil on *S. oryzae* and *S. granarius* were 52.96 µl/l air (95% FL= 48.49 – 59.34) and 46.89 µl/l air (5% FL= 41.38 – 55.27), respectively, for 24 hrs exposure time (Table 1).

The mortality effect of essential oil from *S. hortensis* was lower than *F. vulgare* essential oil. LC_{50} values for two insects were decreased after 48 hrs from commencement of exposure time and *S. granarius* was more susceptible than *S. oryzae* to two essential oils and times (Table 1). In all case, the insecticidal activity varied with insect species, concentrations of the oil and extension of exposure times.

Discussion

Essential oils from various plants have shown promise as sources for insecticides. Earlier attempts to explore the toxicity of plant derivatives against *Sitophilus granarius*

Table1. LC_{50} values of essential oils from *Foeniculum vulgare* and *Satureja hortensis* against the adults of *S. granarius* and *S. oryzae*.

Essential oil	Insect (N: 100)	Time (h)	LC_{50} (Min – Max) (µl/l air)	χ^2 (df= 3)	Sig *	Intercept	Slope
F. vulgare	*S. granarius*	24	27.30 (23.62 – 32.38)	3.60	0.31	2.82	1.82
		48	22.00 (19.02 – 25.41)	3.05	0.38	2.48	1.87
	S. oryzae	24	44.16 (41.03 – 48.21)	2.26	0.52	- 0.99	3.64
		48	37.52 (34.53 – 40.63)	3.14	0.37	- 0.28	3.36
S. hortensis	*S. granarius*	24	46.89 (41.38 – 55.27)	3.47	0.33	1.39	2.16
		48	37.62 (33.70 – 42.17)	4.27	0.23	1.17	2.43
	S. oryzae	24	52.96 (48.49 – 59.34)	3.72	0.30	- 0.17	3.00
		48	42.99 (39.51 – 46.400	2.23	0.53	- 0.63	3.45

N: Number of the tested insects for each time

* Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits

and *Sitophilus oryzae* have been made by essential oils. ASLAN *et al*. (2005) evaluated essential oil from the plant species *Micromeria fruticosa*, *Nepata racemosa* and *Origanum vulgare* (Lamiaceae) for their toxicities against the adults of *Lasioderma serricorne* (Anobiidae) and *S. granarius* and larvae (third instar) of *Ephestia kuehniella* (Pyralidae). In that study, although insecticidal activities against these pests were achieved with essential oils of all three plant species, the oil of *O. vulgare* was found to be the most effective against *S. granarius.* KORDALI *et al*. (2006) studied the toxicity of essential oils isolated from three *Artemisia* species (*A. absinthium*, *A. santonicum* and *A. spicigera*) to *S. granarius*. All of the essential oils tested were found to be toxic to adults of *S. granarius.* The oils showed about 80–90% mortality of granary weevil, *S. granarius* at a dose of 9 µl/l air after 48 hrs of exposure. KORDALI *et al*. (2008) tested insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* on *S. granarius* and *Tribolium confusum*. *Origanum acutidens* oil caused 68.3% and 36.7% mortality of *S. granarius* and *T. confusum* adults, respectively, after 96 hrs of exposure. Results showed that the oil was more toxic against *S. granarius* as compared with its toxicity against *T. confusum*. BENZI *et al*. (2009) investigated the biological activity of essential oils from leaves and fruits of pepper tree, *Schinus molle*, against S. *oryzae*. Their study showed repellent, fumigant activity, nutritional indices, and feeding deterrent action of pepper tree oils on *S. oryzae* adults. With respect to fumigant activity, neither of the essential oils was found to be toxic. These findings are parallel with the results of present study for sensibility of *S. granarius* and *S. oryzae* to essential oils isolated from plants.

Previous studies demonstrated that essential oils and extracts isolated from *F. vulgare* and *S. hortensis* have pesticides effects. For example, ASLAN *et al*. (2004) tested essential oils from *S. hortensis*, *Ocimum basilicum* and *Thymus vulgaris* (Lamiaceae) for their toxicities against the nymphs and adults of *Tetranychus urticae* (Acari: Tetranychidae) and adults of *Bemisia tabaci* (Aleyrodidae). Although desirable insecticidal and acaricidal activities against both of these pest species were achieved with essential oils of the three plant species, *S. hortensis* was found to be the most effective, compared with the other two species. In another experiment, *F. vulgare* fruit extract gave 67% and 100% mortality (contact action) in *Attagenus unicolor japonicus* larvae at 5.2 mg/cm^2, 21 and 28 days after treatment respectively (HAN *et al*., 2006). IBRAHIM *et al*. (2006) were disclosed effect of flower extract of *F. vulgare* on hatching, migration and mortality of the root-knot nematodes, *Meloidogyne incognita*. The acaricidal activities of components derived from *F. vulgare* seed oil were demonstrated against the stored product mite, *Tyrophagus putrescentiae* adults using direct contact application (LEE *et al*., 2006). IŞIK & GÖRÜR (2009) studied the aphidicidial activity of *F. vulgare* essential oil against cabbage aphid, *Brevicoryne brassicae* (Aphididae), under laboratory conditions. Applications of *F. vulgare* essential oil significantly reduced the reproduction potential of the cabbage aphid and resulted in higher mortality. These studies confirm results of present investigation related to insecticidal effects of *F. vulgare* and *S. hortensis*.

LEE *et al*., (2001b) suggested that the toxicity of essential oils to stored-product insects was influenced by the chemical composition of the oil. Previous studies indicated that Methyl Chavicol (= Estragole) and Limonene in the essential oil of *F. vulgare* (IBRAHIM *et al*., 2006; MIGUEL *et al*., 2010) and Carvacrol, Thymol, γ-Terpinene and p-Cymene in the *S. hortensis* essential oil (RAZZAGHI-ABYANEH *et al*., 2008; MIHAJILOV-KRSTEV *et al*., 2009) were the major components. LEE *et al*. (2001a) showed *p*-Cymene (LC_{50} = 25.0 µl/l air) was the most toxic fumigant on *S. oryzae*, followed by, α-Terpinene (LC_{50} = 71.2 µl/l air) and Carvacrol (LC_{50} = 79.4 µl/l air). PAPACHRISTOS & STAMOPOULOS (2004) investigated relationship between the chemical composition of the essential oils from *Lavandula hybrida*, *Rosmarinus officinalis* and *Eucalyptus globulus* and their insecticidal activity against *Acanthoscelides obtectus*. They found that p-Cymene, S(-)Limonene, R(+)Limonene, γ-Terpinene and α-Terpineol exhibited insecticidal activity against both male and female adults. LOPEZ *et al*., (2008) reported that Estragole is example of toxic fumigant compound in the essential oils from coriander (*Coriandrum sativum*), caraway (*Carum carvi*) and basil (*Ocimum basilicum*) that is active against insect pests. Therefore, the insecticidal activity of *F. vulgare* and *S. hortensis* essential oil could be related to these constituents. On the other hand, these results demonstrated that the essential oils isolated from different plants might have different toxicity, which can be attributed to their different chemical composition and different major or minor components.

Foeniculum vulgare and *Satureja hortensis* used as culinary and medicinal plants are considered to be less harmful than most conventional insecticides. Apart from a natural origin, the essential oils of *F. vulgare* and *S. hortensis*, like most of plant essential oils, can pose fewer or lesser risks to human health and the environment. However, further research is needed in order to evaluate the effectiveness of *F. vulgare* and *S. hortensis* essential oils, explore their mode of action and establish their utility as natural insecticidal agents.

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