RESEARCH

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CHEMICAL CONSTITUENTS AND TOXICITY OF *Agastache foeniculum* (PURSH) KUNTZE ESSENTIAL OIL AGAINST TWO STORED-PRODUCT INSECT PESTS

Asgar Ebadollahi[1*]

ABSTRACT

The uncontrolled use of synthetic insecticides is a great hazard for the environment and consumers. Essential oils were introduced as low toxic agents against mammals and non-targeted insects. In this study, essential oil from aerial parts of blue giant hyssop (*Agastache foeniculum* [Pursh] Kuntze) (Lamiaceae) was isolated by the water steam distillation method with a Clevenger apparatus, and its chemical composition was studied by gas chromatography mass spectrometry. The toxicity of *A. foeniculum* essential oil against red flour beetle, *Tribolium castaneum* (Herbst), and lesser grain borer *Rhyzopertha dominica* (F.) was evaluated by fumigation at 24, 48, and 72 h exposure times. Estragole and 1,8-cineole were identified as major constituents of the *A. foeniculum* oil. Fumigation bioassays revealed that *A. foeniculum* oil had strong insecticidal activity on experimental insects. *Rhyzopertha dominica* was more susceptible than *T. castaneum* for all exposure times. Insecticidal activity varied with essential oil concentration and exposure time. Probit analysis showed that increased exposure time and essential oil concentration increased mortality. These results indicated that *A. foeniculum* essential oil can be applied in the management of stored-product insects to decrease the detrimental effects of synthetic insecticides.

Key words: Blue giant hyssop, fumigation, *Rhyzopertha dominica*, *Tribolium castaneum*.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), has long been associated with stored food for human consumption and with a wide range of commodities including grain, flour, peas, beans, cacao, nuts, dried fruits, and spices, although milled grain products such as flour appear to be its preferred food. Its presence in stored foods directly affects both the quantity and quality of the commodity (Campbell and Runnion, 2003). The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a destructive insect pest of stored grains. Both larvae and adults of this insect feed on whole, sound grains and cause extensive damage (Dowdy and McGaughey, 1992).

Controlling these insects and other stored-product pests relies heavily on gaseous fumigants. Although effective synthetic insecticides such as methyl bromide or phosphine are available, there is a global concern about their negative effects causing ozone depletion, environmental pollution, toxicity to non-target organisms, and pesticide residues (Lee *et al*., 2004; Isman, 2006). There is an urgent need to develop safe alternatives with the potential to replace toxic fumigants, and still be effective, economical, and convenient. Natural compounds of plant origin are biodegradable, often of low mammalian toxicity, and pose a low danger to the environment if used in small amounts (Papachristos and Stamopoulos, 2002; Ayvaz *et al*., 2008). There has recently been a growing interest in research as to possibility of using plant extracts as alternatives to synthetic insecticides. Essential oils are complex mixtures comprised of a large number of constituents in variable ratios (Van Zyl *et al*., 2006). Furthermore, they are volatile and can act as fumigants for stored-product protection. Plant essential oils have had insecticidal (Tapondjou *et al*., 2002; Negahban *et al*., 2007; Park *et al*., 2008), antifungal (Razzaghi-Abyaneh *et al*., 2008), nematicidal (Oka *et al*., 2000), virucidal (Schuhmacher *et al*., 2003), and anti-bacterial (Kotan *et al*., 2008) effects. This is mainly because essential oils are easily extractable, eco-friendly i.e., biodegradable, easily catabolized in the environment, and do not persist in soil and water (Isman, 2000; 2006). All these properties of essential oils permit their use even in sensitive areas such as schools, restaurants, hospitals, and homes. In spite of the widespread recognition that many plants possess

[1]Young Researchers Club, Islamic Azad University, Ardabil branch, P.O. BOX: 467, Ardabil, Iran.
[*]Corresponding author (Asgar.ebadollahi@gmail.com).
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insecticidal properties, only a handful of pest control products directly obtained from plants are used because the commercialization of new botanicals can be hindered by a number of issues (Isman, 1997).

Blue giant hyssop, *Agastache foeniculum* (Pursh) Kuntze, is a species of perennial plant of the mint family (Lamiaceae). It is native to the southwestern and eastern US and central Asia. This flowering plant is very attractive to bees and butterflies and commonly used as garnish for fruit salads, iced tea, desserts, and anise-flavored spices. This species is a candidate for large-scale, domestic cultivation as an aromatic plant with a wide variation in essential oil composition and content (Ayers and Widrlechner, 1994). The *Agastache* genus has received considerable attention for its variation in essential oil content and composition (Charles *et al*., 1991).

This paper describes a laboratory study carried out to assess the potential of essential oil as an insecticide. This study investigates fumigant toxicity of the essential oil from aerial parts of *A. foeniculum* against *T. castaneum* and *R. dominica* under laboratory conditions.

MATERIALS AND METHODS

Plant material, extraction, and analysis of essential oil

Aerial parts from 1.5 cm from the top of *A. foeniculum* were collected at flowering stage from plants grown on the experimental farm of the Department of Horticulture, University of Urmia, Urmia, Iran. This material was air-dried in the shade at room temperature (26 to 28 °C) for 14 d. The essential oil was isolated from dried plant samples by hydrodistillation with a Clevenger-type apparatus. Extraction conditions were: 50 g of air-dried sample, 1:10 plant material/water volume ratio, 4-h distillation. Anhydrous sodium sulfate was employed to remove water after extraction. Extracted oils were stored in a refrigerator at 4 ºC.

The constituents of *A. foeniculum* essential oil were analyzed by gas chromatography mass spectrometry (GC-MS) (Thermo-UFM, Italy). The GC-MS conditions were as follows: capillary column pH-5 (10 m × 0.1 mm, film thickness 0.4 μm); helium as carrier gas (0.5 mL min^{-1}); oven temperature program initially at 60 °C rising to 285 °C; and injector and detector temperatures of 280 °C. The identification of individual compounds was based on the comparison of their relative retention index with those of original samples on a capillary column (Davies, 1990).

Insects

Tribolium castaneum was bred in glass containers (1 L) containing wheat (*Triticum aestivum* L.) flour. The mouth of the containers was covered with a fine mesh cloth for ventilation and to prevent the beetles from escaping. *Rhyzopertha dominica* was bred on whole-wheat in similar containers. Cultures were maintained in an incubator at 27 ± 2 °C and 60 ± 5% RH in the dark. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. Adult insects, 1 to 7 d old, were used for fumigant toxicity tests. All experimental procedures were carried out under the same environmental conditions as the cultures.

Fumigant bioassay

The fumigant bioassays were conducted as described by Negahban *et al*. (2007) with only slight modifications. Concentrations of 10 to 48 μL L^{-1} and 4 to 32 μL L^{-1} of the oil were used for *T. castaneum* and *R. dominica*, respectively. Each concentration was dissolved in 200 μL acetone (solvent) and applied to filter paper strips (4 × 5 cm, Whatman N° 1), which were air-dried for 2 min. Treated filter papers were placed at the bottom of 1-L glass jars. Twenty insect adults were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. Tubes were hung at the geometrical centre of the glass jars and then sealed with air-tight lids. Thus, there was no direct contact between the oil and the insects. In the control jars, only acetone was applied on the filter papers. Jars were kept in the incubator and mortality was determined after 24, 48, and 72 h after exposure began. Each experiment was replicated three times for each concentration. Insects were considered dead when no leg or antennal movements were observed.

Statistical analysis

Mortality percentages were calculated by the Abbott correction formula for natural mortality in the untreated control (Abbott, 1925). To equalize variances, insect mortality percentages were transformed by the squared root of the arcsin. Experiments were arranged in a completely randomized design and data were analyzed by ANOVA. Lethal concentration (LC_{50} and LC_{95}) was estimated by probit analysis whereas lethal time (LT_{50} and LT_{95}) values were obtained with SPSS software (SPSS, 2001). The means were separated by the Tukey test at the 5% level.

RESULTS AND DISCUSSION

Chemical analysis of the essential oil determined that estragole (94.003%) and 1,8-cineole (3.334%) were the predominant components (Table 1). *Agastache foeniculum* oil revealed a strong toxicity against the insects. A 50% lethal concentration for *T. castaneum* and *R. dominica* at 24 h exposure time were 22 and 14 μL L^{-1}, respectively. *Rhyzopertha dominica* was most susceptible and *T. castaneum* was most tolerant for all exposure times (Table 2A). LT_{50} values (the time needed to kill 50% of the

Table 1. Major chemical constituents of blue giant hyssop (*Agastache foeniculum*) essential oil and its relative proportions.

Component	Retention index	Percentage (%)
1-Octen-3-ol	977	0.461
3-Octanone	985	0.407
1,8-Cineole	1058	3.334
Octen-3-yl-acetate	1108	0.386
Estragole	1200	94.003
α-Copaene	1375	0.029
β-Boarbonene	1386	0.084
E-Caryophyllene	1418	0.058
Germacrene D	1485	0.430
Bicyclogermacrene	1500	0.020
Spathulenol	1570	0.039
β-Eudesmol	1650	0.015
Total		99.266

population) were 12.47 h for *T. castaneum* and 10.05 h for *R. dominica* at the highest concentrations (42 μL L^{-1} for *T. castaneum* and 32 μL L^{-1} for *R. dominica*) (Table 2B). The susceptibility of both insects increased with exposure time and concentration, and LC_{50} values decreased within 72 h (Table 2 and Figure 1). On the other hand, the increased susceptibility of the two insects was associated with the increase of the different oil concentrations and exposure time. For example, *T. castaneum* showed LC_{50} = 22 μL L^{-1} 24 h after fumigation, and this value decreased to 13 μL L^{-1} within 72 h.

The most promising botanical groups are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Lamiaceae (e.g. *A. foeniculum*), Aristolochiaceae, and Malvaceae (Regnault-Roger, 1997). Purple giant hyssop, *Agastache rugosa* (Fisch. & C.A. Mey.) Kuntze, essential oil has been evaluated for insecticidal and nematicidal activity (Kim *et al*., 2003; Choi *et al*., 2007), and *A. foeniculum* essential oil indicated strong fumigant toxicity against *T. castaneum* and *R. dominica* in the present study. These insects are from different insect families, thus confirming the wide toxicity range of this essence.

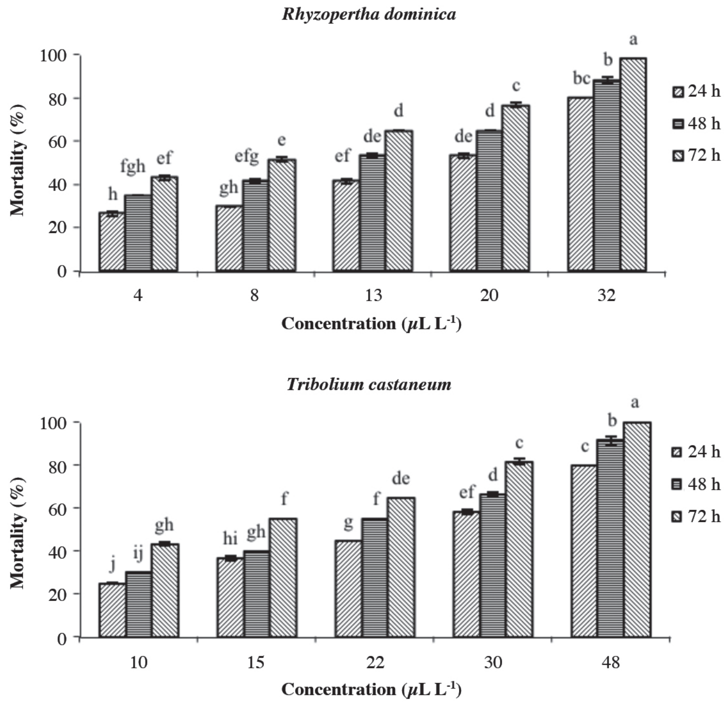
The effect of many essential oils used as insecticides to protect against *T. castaneum* and *R. dominica* infestation has been studied, and these beetles have shown susceptibility to some plant-derived chemicals. Experiments have shown that *T. castaneum* is more tolerant than *R. dominica*. Sahaf *et al*. (2007) studied fumigant toxicity of *Carum copticum* C.B. Clarke (Apiaceae) essential oil against *Sitophilus oryzae* (L.) (Curculionidae) and *T. castaneum* observing that *S. oryzae* (LC_{50} = 0.91 μL L^{-1}) was significantly more susceptible than *T. castaneum* (LC_{50} = 33.14 μL L^{-1}). Chaubey (2007) investigated insecticidal activity of *Trachyspermum ammi* (L.) Sprague ex Turrill (Apiaceae), *Anethum graveolens* L. (Apiaceae), and *Nigella sativa* L. (Ranunculaceae) essential oils against *T. castaneum*. The death of *T. castaneum* adults was caused by fumigation with these essential oils. Fumigant toxicity of *Vitex pseudonegundo* (Hausskn.) Hand.-Mazz. (Lamiaceae) essential oil against *T. castaneum* and *S. oryzae* was evaluated by Sahaf *et al*. (2008). They demonstrated that *S. oryzae* (LC_{50} = 31.96 μL L^{-1}) was more susceptible than *T. castaneum*

Table 2. Result of probit analysis to calculate LC_{50}, LC_{95} (A), and LT_{50}, LT_{95} (B) values. LT values and their corresponding information were calculated at the highest concentrations (42 μL L^{-1} for *Tribolium castaneum* and 32 μL L^{-1} for *Rhyzopertha dominica*).

A Insects	Time	LC_{50}	LC_{95}	χ^2 [df = 3]	*P*	Intercept	10
	h	μL L^{-1}					
T. castaneum	24	22.24	129.93	1.32[a]	0.72	3.89	2.15
	48	18.86	119.46	0.17[a]	0.98	3.62	2.05
	72	12.81	68.12	1.29[a]	0.73	3.51	2.27
R. dominica	24	14.17	166.01	6.63[b]	0.09	4.77	1.54
	48	9.62	163.79	1.94[a]	0.59	4.31	1.34
	72	6.24	82.36	1.77[a]	0.92	4.17	1.46

B Insects	LT_{50}	LT_{95}	χ^2 [df = 1]	*P*	Intercept	Slope
	h					
T. castaneum	12.47	48.66	2.34[b]	0.13	1.95	2.78
R. dominica	10.05	62.22	1.99[a]	0.16	2.92	2.08

[a]Since goodness-of-fit Chi square is not significant (P > 0.15), no heterogeneity factor is used.
[b]Since goodness-of-fit Chi square is significant (P < 0.15), a heterogeneity factor is used.



Different letters over columns indicate significant differences according to Tukey test at $p \leq 0.05$. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±); very small values are not represented.

Figure 1. Mean mortality of *Rhyzopertha dominica* and *Tribolium castaneum* exposed to different concentrations of *Agastache foeniculum* essential oil.

(LC_{50} = 47.27 μL L^{-1}). Ogendo *et al*. (2008) found that, except for the more tolerant *T. castaneum*, LC_{50} values for *S. oryzae*, *R. dominica*, *Oryzaephilus surinamensis* (L.) (Silvanidae), and *Callosobruchus chinensis* (L.) (Bruchidae) adults ranged from 0.20 to 14 mL L^{-1}, 0.01 to 17 mL L^{-1}, and 0.80 to 23 mL L^{-1} air 24 h after treatment with *Ocimum gratissimum* L. (Lamiaceae) essential oil, eugenol, and b-(Z)-ocimene, respectively. In the other experiment, it was found that *Lavandula stoechas* L. (Lamiaceae) essential oil had insecticidal effects on *Lasioderma serricorne* (F.) (Anobiidae), *R. dominica*, and *T. castaneum*. *Lasioderma serricorne* (LC_{50} = 3.835 μL L^{-1}) was significantly more susceptible than *R. dominica* (LC_{50} = 5.66 μL L^{-1}) and *T. castaneum* (LC_{50} = 39.685 μL L^{-1}) 24 h after treatment (Ebadollahi *et al*., 2010). These findings are similar to the results of this study for sensibility of *T. castaneum* and *R. dominica* to plant essential oils and *T. castaneum* is more tolerant than *R. dominica*.

Previous studies have shown that, in general, the toxicity of plant essential oils against stored-product pests is related to their major components (Isman *et al*., 2001; Tapondjou *et al*., 2002; Singh *et al*., 2003). Estragole (= methyl chavicol) is a major constituent of *A. foeniculum* essential oil (Charles *et al*., 1991; Mazza and Kiehn, 1992), fact which was confirmed in this study. Lopez *et al*. (2008) reported estragole as an example of toxic fumigant compounds in *Coriandrum sativum* L. (Apiaceae), *Carum carvi* L. (Apiaceae), and *Ocimum basilicum* L. (Lamiaceae) essential oils that are active against insect pests. Another major constituent of *A. foeniculum* oil, 1,8-cineole, is reported as a toxic agent against some insect pests (Tripathi *et al*., 2001; Yang *et al*., 2004; Kordali *et al*., 2006; Stamopoulos *et al*., 2007).

CONCLUSIONS

According to the results obtained from the current study and previous studies, it can be suggested that *A. foeniculum* essential oil, or probably its components, can be used to control stored-product insect pests. If cost-effective commercial problems are solved, essential oils obtained from plants can be used as part of integrated pest management strategies. Therefore, large quantities of plant material must be processed to obtain sufficient quantities of essential oils for commercial-scale tests, situation which also requires breeding these plants in great quantities.

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RESUMEN

Componentes químicos y toxicidad del aceite esencial de *Agastache foeniculum* (Pursh) Kuntze contra dos plagas de insectos de productos almacenados. El uso incontrolado de los insecticidas sintéticos causa gran peligro para el medio ambiente y los consumidores. Los aceites esenciales se presentan como agentes tóxicos leves contra mamíferos e insectos no objetivo. En el presente estudio, el aceite esencial de las partes aéreas del hisopo gigante azul (*Agastache Foeniculum* [Pursh] Kuntze) (Lamiaceae) se aisló por el método de destilación al vapor de agua, utilizando un aparato de Clevenger y se estudió su composición química mediante cromatografía de gases y espectrometría de masas. La toxicidad del aceite esencial de *A. foeniculum* se evaluó por métodos de fumigación a las 24, 48 y 72 h contra el escarabajo rojo de la harina (*Tribolium castaneum* (Herbst)) y el barrenador menor de granos (*Rhyzopertha dominica* (F.)). El estragol y 1,8-cineole se detectaron como componentes principales en el aceite *A. foeniculum*. Los bioensayos revelaron que el aceite de *A. foeniculum* tuvo una fuerte actividad insecticida sobre los insectos experimentales. *R. dominica* fue más susceptible que *T. castaneum* en todos los tiempos. La actividad insecticida varió con las concentraciones de aceite esencial y los tiempos de exposición. El análisis Probit mostró que la mortalidad aumenta con el incremento del tiempo de exposición y la concentración del aceite esencial. Estos resultados indican que el aceite esencial del *A. foeniculum* podría ser aplicable al manejo de insectos de productos almacenados con el fin de disminuir los efectos perjudiciales de la utilización de insecticidas sintéticos.

Palabras clave: hisopo gigante azul, fumigación, *Rhyzopertha dominica*, *Tribolium castaneum*.

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