Annual Review & Research in Biology
3(4): 649-658, 2013

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Toxicity and Physiological Effects of Essential Oil from *Agastache foeniculum* (Pursh) Kuntze Against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) Larvae

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Authors' contributions

This work was carried out in collaboration between all authors. Author AE designed the study, wrote the protocol, wrote the first draft of the manuscript and performed all experiments. Author RK performed the statistical analysis and bioassays. Author JJS supervised on the study. Author PH managed the literature searches and reared the insect and Author RMA Collected of plant material and extracted of essential oil. All authors read and approved the final manuscript.

Research Article

Received 5th April 2013
Accepted 17th June 2013
Published 28th June 2013

ABSTRACT

Aims: It is acknowledged that many essential oils isolated from various plants can exert toxic activity against insect species. In the present study, the essential oil from aerial parts of *Agastache foeniculum* (Pursh) Kuntze (Lamiaceae) evaluated for its larvicidal and physiological effects against the larvae of *Tribolium castaneum* Herbst.
Place and Duration of Study: Place – Department of plant protection, Faculty of agriculture, Guilan university, Rasht, Iran. Duration – May, 2012 to January, 2013.
Methodology: The essential oil was isolated from aerial parts of *A. foeniculum* by hydrodistillation method with a Clevengertype apparatus. In Larvicidal bioassay, five concentrations of the essential oil were prepared with acetone as solvent. Control samples were treated only with pure acetone. Ten same-aged instars were randomly selected,

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placed with treated diets and kept at 27 ± 2ºC and 60 ± 5% RH. The experiment was repeated four times and insect mortalities were recorded after 24 h. The effects of essential oil on total carbohydrate, lipid and protein contents on the surviving larvae were assessed. Also, the responses of general esterase and glutathione *S*-transferase as two detoxifying enzymes to essential oil were investigated.
Results: *A. foeniculum* essential oil caused high mortality and the mortality was dose dependent i.e. with increasing of essential oil concentrations more mortality achieved. Furthermore, study on the effect of essential oil on total carbohydrate, lipid and protein contents demonstrated that all of them were decreased with increasing of concentrations. When *A. foeniculum* essential oil was applied, inhibition of esterase and glutathione Stransferase activities was observed.
Conclusion: It has been found that the essential oil of *A. foeniculum* may produce a great range of biological effects on *T. castaneum* larvae and can be a potent candidate for such insect pest management.

Keywords: Agastache foeniculum; essential oil; biological effects; larvicidal; Tribolium castaneum.

1. INTRODUCTION

For the control of insect pests many synthetic insecticides are used, but their widespread use led to some serious problems including development of insect resistance to insecticides, toxic residues in food, toxicity to consumers, environmental pollution and increasing cost of application [1,2,3]. It has been estimated that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually [4]. All over the world, there is a need to find alternatives to synthetic insecticides. From this point of view, botanical pesticides are promising since they are effective, environmentally friendly, easily biodegradable, and often inexpensive [5].

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. The aromatic characteristics of essential oils provide various functions for the plants including attracting or repelling insects, protecting themselves from heat or cold; and utilizing chemical constituents in the oil as defense materials [4]. In the search for alternatives to conventional insecticides, essential oils extracted from aromatic plants have been widely investigated. Plant essential oils show wide and varied bioactivities against both agricultural pests and medically important insect species, ranging from toxicity with ovicidal, larvicidal, pupicidal and adulticidal activities to sub-lethal effects including oviposition deterrence, antifeedant activity and repellent actions as well as they may affect on biological parameters such as growth rate, life span and reproduction [6,7,8,9,10].

It has been reported that most xenobiotics are subject to enzymatic modification after penetration through protein binding and transportation in a biological interaction. There is an enzyme present in insects, glutathione S-transferase (GST) that is of interest as it functions in the detoxification mechanism due to its involvement in tolerance to pesticides [11,12]. Furthermore, it has been clearly demonstrated that esterases (EST) can play a vital role in the detoxification of xenobiotics to nontoxic materials [13].

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 [14].

Red flour beetle, *Tribolium castaneum* Herbst, can be a major pest in storage of grain-based products as it cause severe damage to cereal grains throughout the world. This species has a long association with human stored food and has been found in association with a wide range of commodities including grain, flour, peas, beans, nuts, dried fruits and spices [15].

The aim of present study is to identify the toxicity of essential oil from *A. foeniculum* against *T. castaneum* larvae and its effects on total protein, lipid and carbohydrate contents. Also, the responses of general esterase and GST to essential oil were investigated.

2. MATERIALS AND METHODS

2.1 Insect Culture

T. castaneum was obtained from a colony maintained at Department of Plant Protection, University of Tehran, Iran. It was reared in plastic containers (20 cm length × 14 cm width × 9 cm height) containing a wheat flour and barn mixture (2:8 w/w). The tops of the containers were covered with a fine mesh cloth for ventilation and to prevent the beetles from escaping. Larvae 7-14 days old were used for tests. They were maintained in darkness at 27 ± 1ºC and 60 ± 5% RH. All experimental procedures were carried out under the same environmental conditions as the cultures.

2.2 Plant Material and Essential Oil Preparation

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 27ºC for 14 d. The essential oil was isolated from dried plant samples by hydrodistillation with a Clevengertype apparatus. Extraction conditions were: 50 g of airdried 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 4ºC.

2.3 Larvicidal Bioassay

Five concentrations of the essential oil (1.5%, 2.5%, 5%, 10% and 15%) were prepared for bioassay with acetone as solvent. 1000 µl of each concentration was uniformly admixed with 0.5 g of wheat bran in a 7-cm diameter Petri dish. The wheat bran was left to dry at room temperature for 5 minutes. Control samples were treated only with pure acetone and dried in the same way. Ten same-aged instars (7-14 old days) were randomly selected, placed with treated diets and kept at 27 ± 2ºC and 60 ± 5% RH. The experiment was repeated four times and insect mortalities were recorded after 24 h. Toxicity of the larvicidal oil was calculated based on the 50% mortality of subjected insects (LC_{50}) after 24 h using the SPSS version 16.0 software package. For the following experiments the surviving larvae for each concentration were randomly selected.

2.4 Total Carbohydrate, Lipid and Protein Determination

For determination of carbohydrates and lipids the method presented by Yuval et al. [16] and Van Handel and Day [17] were adopted. Each larva was individually homogenized in 100 µl sodium sulphate (2%) and 750 µl chloroform: methanol (2: 1). Then, they were centrifuged for 10 m in 8 000 rpm under 4ºC. The supernatant (250 µl) was dried at 40ºC. Then 500 µl H_2SO_4 was added and were placed in a hot bath for 10 m at 90ºC. Briefly, 30 µl of sample was mixed with 270 µl of vanillin solution (600 mg of vanillin in 100 µl distilled water and 400 ml 85% H_3PO_4) and was placed in an Elisa plate. After 30 minutes the absorbance was read at 545 nm. Total lipid was calculated based on the standard curve. In the case of carbohydrate to 150 µl of supernatant, 100 µl distilled water was added along with 500 µl of anthrone (500 mg of anthrone dissolved in 500 ml H_2SO_4). The samples were placed at 90°C for reaction to take place, and after that 250 µl of sample were placed in the Elisa plate. The absorbance was read at 630 nm and carbohydrate level was calculated by a standard curve.

For determination of total protein, the method of Bradford [18] was adopted. Six treated larvae were homogenized with 350 µl of distilled water and then were centrifuged for 5 m in 10 000 rpm under 4ºC. 10 µl of the supernatant was mixed with 90 µl of distilled water and 2 500 µl dye. The absorbance was read at 630 nm in an Elisa reader (Awareness Technology INC, USA). Protein was quantified using BSA (Bovine Serum Albumin) (Bio-Rad, Munchen, Germany) as the standard.

2.5 Assay of Esterase and Glutathione S-Transferase

Esterase activity was determined using the method described by Han et al. [19]. Initially six larvae were homogenized with 1000 µl 0.1 mol phosphate (pH = 7), then the homogenized solution was centrifuged at 10000 g for 10 minutes at 4ºC. Seventy-five microliters of α-naphthyl acetate (10 mm) and 75 µl fast blue RR salt (1 mm) were added into each tube. The reaction was initiated by addition of 50 µl of enzyme solution. Optical density (OD) at 450 nm was recorded at intervals of 10 s for 1 min using microplate reader.

For determining activity of glutathion S-transferase (GST) the method of Habing et al. [20] was used. In this study 1-chloro-2, 4-dinitrobenzene (CDNB) (20 mM) was used as substrate. First six larvae were homogenized in 20 µl distilled water, then the homogenized solution was centrifuged at 12500 g for 10 minutes at 4ºC. Fifteen µl of supernatant was mixed with 135 µl of phosphate buffer (pH = 7), 50 µl of CDNB and 100 µl of GST. The absorbance at 340 nm was read at intervals of 9s in 1 min. All data were analyzed in a completely randomized design using SAS software and Tukey's studentized test was used for comparisons of means at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Larvicidal

A. foeniculum oil revealed a strong toxicity against the *T. castaneum* larvae. Results showed that the mortality of larvae was dose-dependent and increasing concentrations of the essential oil exacerbate mortality. Probit analysis showed that LC_{50} and LC_{90} values were calculated as 8.022% and 80.152%, respectively (Table 1).

Table 1. Results of probit analysis of the toxicity of the essential oil from *A. foeniculum* against *T. castaneum* larvae

LC_{50} (%)	LC_{90} (%)	df	χ^2	Sig.	Slope	Intercept
8.022 (5.719 – 13.20)[a]	80.152 (34.744 – 156.309)[a]	3	1.225	0.747[b]	1.282	3.841

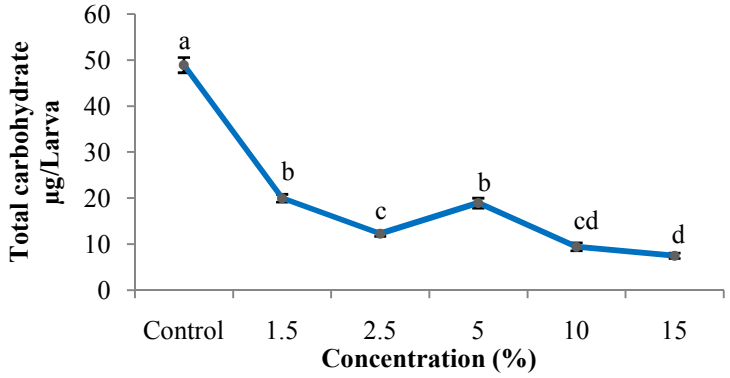
[a] *95% lower and upper fiducial limits are shown in parenthesis.*

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

In our recently studies, the fumigant toxicity of *A. foeniculum* essential oil were demonstrated against the adults of *Callosobruchus maculatus* F., *Lasioderma serricorne* F., *Oryzaephilus surinamensis* L., *Rhyzopertha dominica* F. and *T. castaneum* [21,22,23] and there isn't any other study in this context that revealed the effects of this essential oil on insects and in the present study toxicological effects of *A. foeniculum* essential oil were investigated on *T. castaneum* larvae for first time. Previous studies have shown that the toxicity of plant essential oils against insect pests is related to their major components [24,25]. Estragole (94.003%) and 1,8-cineole (3.334%) were detected as major constituents in *A. foeniculum* essential oil [22]. Lopez et al. [26] reported estragole as an example of toxic compounds that are active against insect pests. Another major constituent, 1,8-cineole, was reported as a toxic agent against some insect pests [27,28,29]. It can be conclude that constituents of *A. foeniculum* essential oil such as estragole and 1,8-cineole are main reasons of its insecticidal activities.

3.2 Total Carbohydrate, Lipid and Protein Determination

The results of the total carbohydrates, lipid and protein in 7-14 days' old larvae of *T. castaneum* after treatment with essential oil from *A. foeniculum* are displayed in Fig. 1. The mean total carbohydrate, lipid and protein contents in all treatments in the comparison with that of the controls significantly reduced.



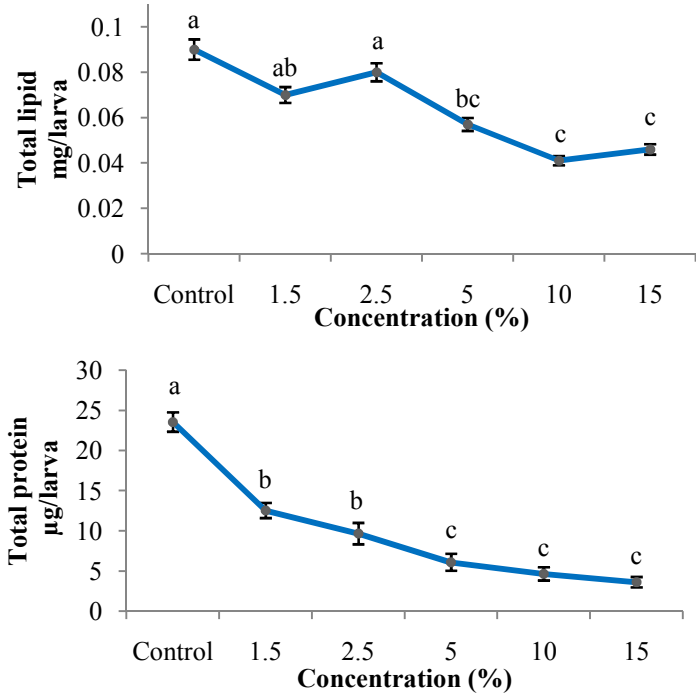


Fig. 1. Amount of carbohydrate, lipid and protein contents in *T. castaneum* larvae exposed to different concentrations of *A. foeniculum* essential oil after 24 h. Different letters indicate significant differences according to Tukey test at p = 0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±)

Decrease in protein content was observed in the larvae fed on treated wheat barn than the larvae fed on control. Reduction in protein content is a common phenomenon in insects after treatment with toxic compounds [30]. It is likely that the insect degrades proteins to resultant amino acids in order to let them enter into the TCA cycle as a keto acid for compensation for the lower energy caused by stress [31]. Our results are supported by a number of reports where toxicity of essential oil lead to reduced protein content of insects [32,33,34]. Lipids are an important source of energy and are reserved in fat bodies. The reserve of lipids during the feeding period increases but is reduced in the non-feeding stage and their amount can vary with growth stage and feeding condition [35]. Khosravi *et al*. [36] found similar results in the reduction of lipid and carbohydrate rates on *Glyphodes pyloalis* Walker with extract of *Artemisia annua* L. Reduction in lipid and carbohydrate rates could be related to a strong deterrence effect of *A. foeniculum* essential oil.

3.3 Esterase and Glutathione S-Transferase Activities

Esterases constitute a widely distributed family of enzymes in insects. They hydrolyze carboxyl ester, amide and thioester bonds in a variety of compounds and they are important enzymes involved in resistance to many insecticides [37]. Also, esterase is one of the enzymes showing the strongest reaction to environmental stimulation [38]. According to Fig. 2, essential oil of *A. foeniculum* caused that esterase activity of *T. castaneum* larvae was

decreased after 24 h exposure time. In the two low concentrations (1.5% and 2.5%), essential oil stimulated the expression of EST to increase the detoxification ability. In other concentration (5%, 10% and 15%), because of a toxic effect EST activity was suppressed (Fig. 2).

There are many reports where inhibition of esterase activity by plant products has been recorded [30,33,37]. GSTs are multifunctional detoxifying enzymes that catalyze the conjugation of reduced glutathione and plays an essential role in detoxification of insecticides such as organophosphorus and organochlorine compounds, thereby rendering them less or non-toxic [39]. Other xenobiotics such as plant defense allelochemicals against phytophagous insects induce GST activity [40]. In this study, when *A. foeniculum* essential oil was applied, inhibition of GST activity was also observed. Its activity was dose-dependent and increased with increasing of essential oil concentration. The decline of the detoxification ability can be main reason for the insecticidal activities.

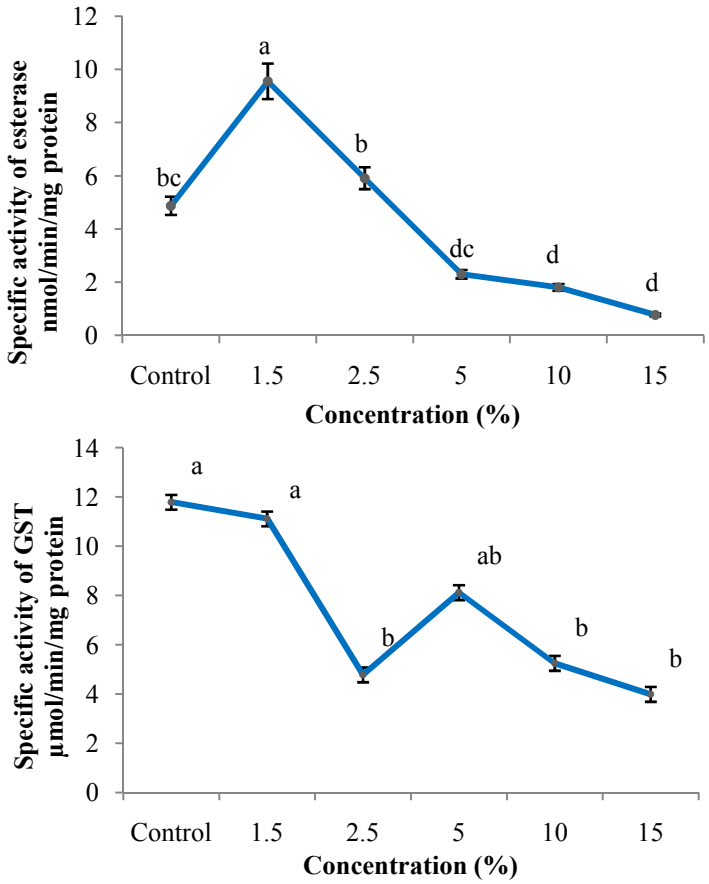


Fig. 2. Activity of esterase and glutathione–S–transferase in *T. castaneum* larvae exposed to different concentrations of *A. foeniculum* essential oil after 24 h. Different letters indicate significant differences according to Tukey test at p = 0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±)

4. CONCLUSION

The present study clearly demonstrated the strong larvicidal potential of essential oil from *A. foeniculum* as a natural product against *T. castaneum* larvae. The essential oil isolated from *A. foeniculum* not only showed high toxicity on *T. castaneum* larvae but also decreased the activity of esterase and GST besides reducing total carbohydrate, lipid and protein contents. This suggests that *A. foeniculum* essential oil may have multiple biological activities that contribute to its toxicity to the larvae. Moreover, it can be say that the mode of action of *A. foeniculum* essential oil against red flour beetle is inhibition of esterase and GST activities. Hence, this essential oil may serve as an alternative to conventional insecticide for the control of red flour beetle.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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