INSECTICIDAL ACTIVITY OF THE ESSENTIAL OIL ISOLATED FROM *Azilia eryngioides* (PAU) HEDGE ET LAMOND AGAINST TWO BEETLE PESTS

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A large number of plant essential oils have been used against diverse insect pests since they, unlike conventional pesticides, present no risk to humans and the environment. This study was conducted to determine the toxicity of *Azilia eryngioides* (Pau) Hedge et Lamond (Apiaceae) essential oil against 1- to 7-d-old *Sitophilus granarius* (L.) (Curculionidae) and *Tribolium castaneum* (Herbst) (Tenebrionidae) adults. The essential oil was obtained from aerial parts of the plant using a Clevenger apparatus and analyzed by gas chromatography and mass spectrometry. The major constituents of the oil were α-Pinene and bornyl acetate. Fumigation bioassays revealed that *A. eryngioides* oil had a strong insecticidal activity on adult test insects that were exposed to 37.03, 74.07, 111.11, and 148.14 µL L⁻¹ to estimate mean lethal time (LT₅₀) values. Mortality increased as concentration and exposure time increased, and reached 100% at the 39-h exposure time and concentrations higher than 111.11 µL L⁻¹. Another experiment was designed to determine the mean lethal concentration at the 24-h exposure time (LC₅₀), and these values indicated that *S. granarius* was more susceptible than *T. castaneum*. It can be concluded that the essential oil of *A. eryngioides* has potential against two stored-product pests, *S. granarius* and *T. castaneum*.

**Key words**: Botanical insecticide, Apiaceae, fumigant toxicity, *Sitophilus granarius*, *Tribolium castaneum*.

Although stored grains can be destroyed by insects, fungi, and vertebrate pests, insect pests are often the most important because of the favorable environmental conditions that promote their development. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), can be a major pest in stored grains. This species has been found associated with a wide range of commodities including grain, flour, peas, beans, cacao, nuts, dried fruits, and spices, but milled grain products such as flour appear to be their preferred food (Campbell and Runnion, 2003). The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), is another important stored-grain pest. This species feeds on a variety of cereals or grains, particularly wheat and barley, which are among the most frequent sources of nutrition (Schwartz and Burkholder, 1991; Kucerova et al., 2003).

Fumigants are mostly used against stored-grain insect pests, not only because of their broad activity spectrum, but also because of their penetrating power resulting in minimal or no residues on the treated products. Although effective fumigants (e.g. methyl bromide and phosphine) are available, there is global concern about their negative effects, such as ozone depletion, environmental pollution, toxicity to non-target organisms, pest resistance, and pesticide residues (Hansen and Jensen, 2002; Benhalima et al., 2004; Bughio and Wilkins, 2004). Thus, there is an urgent need to develop safe alternative fumigants for stored-grain pest management. Herbal products are one potentially important source. Essential oils are secondary metabolism products in plants. These oils have strong aromatic components that give a plant its distinctive odor, flavor, or scent (Koul et al., 2008). Essential oils are complex mixtures of a large number of constituents in variable ratios (Van Zyl et al., 2006), their components and quality varying with geographical distribution, harvesting time, growing conditions, and extraction method (Yang et al., 2005). These oils are typically liquid at room temperature and are easily transform from a liquid to a gaseous state at room temperature or a slightly higher temperature without decomposing (Koul et al., 2008). Presently, essential oils are most often used in the food industry for flavoring, the cosmetic industry for fragrances, and the pharmaceutical industry for their functional properties. However, dozens of plant essential oils have been screened for fumigant toxicity against a variety of insect pests primarily for agricultural and food storage (Wang et al., 2006; Ayvaz et al., 2008; Benzi et al., 2009; Ebadollahi et al., 2010).

*Azilia eryngioides* (Pau) Hedge et Lamond (*Prangos eryngioides* Pau) is an Apiaceae (Umbelliferae), the only member of the genus *Azilia* endemic to Iran. The insecticidal activity of some Apiaceae essential oils has been evaluated against a number of stored-product insects.

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For example, Sahaf et al. (2007) found a strong insecticidal activity of the Carum copticum C.B. Clarke (Apiaceae) essential oil on Sitophilus oryzae (L.) (Curculionidae) and T. castaneum. Mortality of all these species reached 100% at concentrations higher than 185.2 µL L⁻¹ and 12-h exposure time. In another experiment, Chaubey (2008) studied the fumigant activity of from Anethum graveolens L. and Cuminum cyminum L. essential oils on Callosobruchus chinensis (L.) (Bruchidae). The 24-h LC₅₀ values against adults of this insect were 10.8 and 11.0 µL, respectively. Lopez et al. (2008) reported that Carum carvi L. and Coriandrum sativum L. were toxic against Rhyzopertha dominica (F.) (Bostrichidae) and S. oryzae. No study has yet reported the activity of A. eryngioides essential oil as an insecticide against insect pests. In the present study, the chemical constituents of A. eryngioides essential oil were determined, and its insecticidal activity was tested against S. granarius and T. castaneum adults.

**MATERIALS AND METHODS**

**Insect cultures**

Test insects were obtained from a colony maintained at the Entomology Department, University of Urmia, West Azerbaijan, Iran. Tribolium castaneum was reared in plastic containers (20 cm length × 14 cm width × 9 cm height) containing a wheat flour and yeast mixture (10:1 w/w). Sitophilus granarius was reared in wide-mouthed 1-L glass jars containing wheat grains. The tops of the containers and jars were covered with a fine mesh cloth for ventilation and to prevent the beetles from escaping. They were maintained in darkness at 27 ± 1 °C and 60 ± 5% RH. The insects for these experiments were 1- to 7-d-old adults.

**Plant material, extraction, and analysis of the essential oil**

The aerial parts of A. eryngioides were collected at the beginning of flowering in Aligoodarz (Shool Abad). The species were identified by the Agricultural Department of JundiShapour, Kashan, Iran where a voucher specimen was deposited with the number 170-1. Air-dried A. eryngioides aerial parts were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h. The oil was isolated and dried on anhydrous sulfate.

Oil analysis was carried out by gas chromatography (GC) and GC/MS (mass spectrometry) with the HP 6890 Series GC system (Agilent Technology, Santa Clara, California, USA), and an HP-5MS capillary column (60 m × 0.25 mm, film thickness 0.25 µm). The oven temperature program was initiated at 40 °C for 1 min then raised to 230 °C at a rate of 3 °C min⁻¹ for 10 min. Helium was used as the carrier gas at a 1.0 mL min⁻¹ flow rate. Detector and injector temperatures were 250 and 230 °C, respectively. GC/MS analysis was conducted by a HP6890 GC system coupled with a 5973 network mass selective detector with a capillary column as abovementioned, gas helium with a 1 mL min⁻¹ flow rate as a carrier with a split ratio equal to 1/50, and programmed injector and oven temperature was identical to GC. The oil compounds were identified by comparing their retention indices, mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, and NIST (National Institute of Standards and Technology) (Adams, 2001).

**Fumigant toxicity**

To determine the time required for 50% mortality (LT₅₀) at different concentrations, as described by Taghizadeh-Saroukolai et al. (2010), 3-cm diameter pieces of Whatman Nº 1 filter paper were impregnated with oil at a concentration calculated to give equivalent fumigant concentrations from 37.03 to 148.14 µL L⁻¹. Then, the impregnated filter paper was attached to the bottom of the screw caps of a gastight glass jars (280 mL). Caps were screwed tightly on the jars containing 10 adults (1- to 7-d-old) of each species of insect taken separately. The insects had no contact with the impregnated filter paper and stayed at the bottom of the jars throughout the experiments. Control insects were kept under the same conditions without any essential oil. Mortality was assessed by the same interval observations to estimate the lethal time required to achieve 50% kill (LT₅₀). Four replicates were performed for each dose and the untreated control.

Another experiment was designed to determine 50% lethal concentration (LC₅₀) as described by Negahban et al. (2007). Different concentrations were prepared to evaluate insect mortality after an initial dose-setting experiment. The essential oil concentrations used for T. castaneum were 14.97, 22.76, 34.60, 52.61, and 80 µL L⁻¹, and 4.98, 8.40, 14.03, 23.77, and 40 µL L⁻¹ for S. granarius. Control insects were maintained under the same conditions without any essential oil. The number of dead and live insects in each jar was counted at the end of a 24-h exposure period. Each concentration was replicated five times. Insects were considered dead when no leg or antennal movements were observed. Percentage insect mortality was calculated using Abbott’s (1925) correction formula for natural mortality in untreated controls. Experiments were arranged in a completely randomized design and data were analyzed by ANOVA. The LC₅₀ and LT₅₀ values with their fiducial limits were calculated by probit analysis with the SPSS version 16.0 software package.

**RESULTS AND DISCUSSION**

Results of the chemical analysis are shown in Table 1. The major components were α-pinene (63.8%) and bornyl acetate (18.9%) followed by β-pinene (2.6%), linalool (2.1%), Z-citral (1.3%), p-cymene-8-ol (1.1%), and trans-α-bergamotene (1.0%) (Table 1).
Azilia eryngioides essential oil was toxic for *S. granarius* and *T. castaneum* adults at several concentrations and exposure times (Figure 1). An exposure time > 39 h for *S. granarius* was enough to obtain 100% kill of the insects in space tests. The 37.03 µL L⁻¹ concentration and 48-h exposure time was enough to attain 100% mortality of all the insects. At the highest concentration (148.14 µL L⁻¹), 100% mortality of *T. castaneum* was observed after a 3-h exposure; however, 18 h of exposure time was enough to obtain complete mortality of *S. granarius* adults.

The time needed for the essential oil to cause LT₅₀ for *T. castaneum* ranged from 24.96 h [95% lower and upper fiducial limits (FL) = 23.39 to 26.57 h] for the lowest dose (37.03 µL L⁻¹) to 15.31 h (95% FL = 13.88 to 16.65 h) for the highest dose (148.14 µL L⁻¹). The LT₅₀ values for *S. granarius* ranged from 21.04 h (95% FL = 19.22 to 22.93 h) to 10.38 h (95% FL = 9.39 to 11.17 h) for the lowest and highest doses, respectively (Table 2). Generally, LT₅₀ values decreased when the essential oil concentration increased. In all cases, increased susceptibility of both insects was directly associated with oil concentration and exposure time.

The essential oil concentration to cause LC₅₀ in *S. granarius* was 20.05 µL L⁻¹ (95% FL = 15.71 to 27.68 µL L⁻¹), whereas it was 46.48 µL L⁻¹ (95% FL = 36.92 - 64.55 µL L⁻¹) in *T. castaneum* after a 24-h treatment (Table 3). Therefore, *T. castaneum* was more resistant than *S. granarius*.

The failure to discover a significant new class of insecticides has led many researchers back to biodiscovery studies in the search for new and economically viable alternatives. Diverse types of aromatic plant preparations, such as powders, solvent extracts, and essential oils, are being investigated for their insecticide activity (Tapondjou et al., 2002; Kim et al., 2003; Han et al., 2006; Rajendran and Sriranjini, 2008; Ayvaz et al., 2008). *Azilia eryngioides* is an aromatic grass annual Apiaceae that grows in the central areas of Iran. There is no report of insecticide activity of *A. eryngioides* against insect pests. In this, *A. eryngioides* essential oil had strong toxicity

### Table 1. Chemical composition and relative proportions of *Azilia eryngioides* essential oil.

| Compound          | Retention index | Composition |
|-------------------|----------------|-------------|
| α-Pinene          | 867            | 63.8%       |
| Camphene          | 872            | 0.66%       |
| Verbenene         | 875            | 0.15%       |
| β-Pinene          | 899            | 2.6%        |
| β-Myrcene         | 913            | 0.34%       |
| 1,5,8-π-Menthatriene | 921         | 0.12%       |
| Cymene            | 937            | 0.13%       |
| Eucalyptol        | 943            | 0.2%        |
| 1-Limone          | 946            | 0.5%        |
| cis-Oxime         | 955            | 0.1%        |
| Dehydro-p-cymene  | 997            | 0.2%        |
| 2-Cyclopent-1-one | 1004           | 0.26%       |
| Linalool          | 1011           | 2.1%        |
| α-Campholen aldehyde | 1022        | 0.28%       |
| Trans-pinocarveol | 1038           | 0.5%        |
| Z-Citral          | 1046           | 1.3%        |
| Pinocarvone       | 1050           | 0.17%       |
| Borneol           | 1062           | 0.3%        |
| Methyl-p-totyl ketone | 1066         | 0.22%       |
| 2,3,4,6-Tetramethyl phenol | 1078 | 0.5% |
| p-Cymen-8-ol      | 1080           | 1.1%        |
| Verbenone         | 1090           | 0.54%       |
| Myrtanol          | 1092           | 0.28%       |
| Trans carveol     | 1112           | 0.19%       |
| Geraniol          | 1150           | 0.14%       |
| Bornyl acetate    | 1177           | 18.9%       |
| Trans-α-bergamotene | 1184         | 1.0%        |
| 2-Methylboronane  | 1344           | 0.15%       |
| α-Cadinol         | 1499           | 0.17%       |
| Dibutyl phthalate | 1758           | 0.14%       |
| Total             | 97.04          |             |

*Vertical bars indicate standard error (±).*

![Figure 1. Mortality over time of *Sitophilus granarius* and *Tribolium castaneum* exposed to several concentrations of *Azilia eryngioides* essential oil.](image)
Table 2. LT50 values of *Azilia eryngioides* essential oil against *Sitophilus granarius* and *Tribolium castaneum* at various concentrations.

| Insect (N = 40) | Concentration | LT50a | Slope ± SE | Chi-square | df | P value |
|----------------|---------------|-------|------------|------------|----|---------|
|                | µL L"⁻¹" | h     |            |            |    |         |
| *S. granarius* | 37.03         | 21.04 (19.22 - 22.93) | 3.41 ± 0.36 | 4.60' | 9 | 0.87    |
| 74.07          | 17.44 (15.95 - 18.87) | 4.05 ± 0.41 | 11.33' | 8 | 0.18    |
| 111.11         | 14.80 (13.31 - 16.16) | 4.06 ± 0.49 | 8.45' | 6 | 0.21    |
| *T. castaneum* | 148.14        | 10.38 (9.39 - 11.17) | 7.63 ± 1.20 | 2.43' | 2 | 0.30    |
|                | 24.96 (23.39 - 26.57) | 4.45 ± 0.35 | 11.20' | 12 | 0.51    |
|                | 21.87 (20.24 - 23.53) | 3.93 ± 0.35 | 12.83' | 10 | 0.23    |
|                | 19.04 (17.36 - 20.67) | 3.60 ± 0.36 | 13.23' | 9 | 0.16    |
|                | 15.31 (13.88 - 16.65) | 4.22 ± 0.49 | 9.37' | 6 | 0.16    |

1LT50 units were applied for different concentrations at 27 ± 1 °C and 60 ± 5% RH.
295% lower and upper fiducial limits are shown in parenthesis.
3Since Chi square goodness of fit is not significant (P > 0.15), no heterogeneity factor is used in the calculation of fiducial limits.

Table 3. LC50 values of *Azilia eryngioides* essential oil against *Sitophilus granarius* and *Tribolium castaneum*.

| Insect (N = 50) | 24-LC50a | Slope ± SE | Chi-square | df | P value |
|----------------|----------|------------|------------|----|---------|
| *S. granarius* | 20.05 (15.71 - 27.68) | 1.55 ± 0.27 | 2.10' | 3 | 0.55    |
| *T. castaneum* | 46.48 (36.92 - 64.55) | 1.62 ± 0.33 | 0.79' | 3 | 0.85    |

N: Number of tested insects.
495% lower and upper fiducial limits are shown in parenthesis.
5Since Chi square goodness of fit is not significant (P > 0.15), no heterogeneity factor is used in the calculation of fiducial limits.

against *S. granarius* and *T. castaneum*, and the findings indicate strong insecticidal activity of *A. eryngioides* essential oil as a fumigant for insects. In our observations, *A. eryngioides* was characterized by a rapid knockdown effect, convolution, paralysis, and death. Rapid kill is one of the main points because no progeny are produced if insects die quickly.

*Sitophilus granarius* was significantly more susceptible than *T. castaneum*. Several reports also indicate that *T. castaneum* is relatively tolerant to diverse plants (Naghaban et al., 2007; Sahaf et al., 2007; Ogendo et al., 2008; Sahaf et al., 2008; Taghizadeh-Saroukolai et al., 2010). These findings are comparable with the results of the present study where *T. castaneum* is more resistant than *S. granarius*.

The major components of *A. eryngioides* oil (from Aligoodarz, Iran) were α-pinene, bornyl acetate, β-pinene, and linalool. Masoudi et al. (2005) reported that the main component of *A. eryngioides* oil (from Chahar-Mahale Bakhtiari, Iran) was bornyl acetate, which was the second most abundant oil constituent in the present study.

Lee et al. (2001) suggested that the toxicity of essential oils for stored-product insects was influenced by their chemical composition. The fumigant activity of *A. eryngioides* could be attributed to oil constituents, such as α-pinene, bornyl acetate, β-pinene, linalool, and other components. These constituents had insecticidal activity against several stored-product insect pests. For example, Ojimelukwe and Adler (1999) found that their results showed α-pinene and β-pinene as toxic for *Tribolium confusum* du Val. (Tenebrionidae). In another experiment, Choi et al. (2006) reported that α-pinene, β-pinene, and linalool were toxic components in thyme (*Thymus vulgaris* (L.), Lamiaceae) essential oil against mushroom sciarid (*Lycoriella mali* Fitch (Sciariidae)) adults. They indicated that α-pinene was the most toxic fumigant compound in thyme essential oil (LC50 = 9.85 µL L"⁻¹") followed by β-pinene (LC50 = 11.85 µL L"⁻¹"), and linalool (LD50 = 21.15 µL L"⁻¹"). The mixture of α- and β-pinene exhibited a stronger fumigant toxicity than α- or β-pinene against mushroom fly adults. Kounink et al. (2007) studied the toxicity of α-, β-pinene, δ-3-carene, and terpinen-4-ol, which are major components in the *Xylopya aethiopica* Dunal (Annonaceae) essential oil of *S. zea* Motschulsky. This study showed that all the components had strong toxicity and synergic effect, and when mixed, it was observed that the mortality percentage for crude oil was restored. Bornyl acetate is known to have antifeeding activity against *Hylobius pales* (Herbst) (Salom et al., 1994), larval growth-inhibiting effects against *Choristoneura occidentalis* (Freeman) (Zou and Cates, 1997), adulticidal effects on *C. chinensis*, *S. oryzae* and *S. granarius* (Park et al., 2003; Kordali et al., 2006), as well as bactericidal, viricidal, expectorant, sedative, and spasmylytic activity (Petropoulou et al. 2004). There is also evidence of linalool’s high toxicity against insect pests (Rozman et al., 2007; López et al., 2008). Moreover, the repellency of α-pinene, β-pinene, bornyl acetate, borneol, linalool, p-cymene, and camphene against *Myzus persicae* (Sulzer) (Aphididae) was proven (Masatoshi, 1998). Therefore, the insecticidal activity of *A. eryngioides* essential oil could be related to these constituents.

CONCLUSIONS

The *A. eryngioides* essential oil contains insecticidal compounds that are toxic for *S. granarius* and *T. castaneum*. If the cost-effective commercial problems can be solved, essential oils obtained from plants can be effectively used as part of integrated pest management strategies. Given the rapid volatilization and low persistence of essential oils in the environment, it is unlikely that they will be used in field crops; however, this property is conducive to using them to control stored-product pests in a controlled condition. The practical use of these essential oils as novel fumigants requires additional study to develop formulations to improve their effectiveness and stability.
Actividad insecticida del aceite esencial aislado de *Azilia eryngioides* (Pau) Hedge et Lamond contra dos escarabajos plaga. Una gran cantidad de aceites esenciales de plantas se han utilizado como agentes de control biológico contra diversos insectos plaga, ya que no presentan riesgo para los seres humanos y el medio ambiente, a diferencia de los pesticidas convencionales. Se determinó la toxicidad del aceite esencial de *Azilia eryngioides* (Pau) Hedge et Lamond (Apiaceae) contra adultos de 1 a 7 días de edad de *Sitophilus granarius* (L.) (Curculionidae) y *Tribolium castaneum* (Herbst) (Tenebrionidae). El aceite esencial se obtuvo de las partes aéreas de la planta utilizando un aparato de Clevenger y se analizó por cromatografía de gases por espectrometría de masas. Los principales componentes del aceite fueron α-pineno y acetato de bornilo. El aceite de *A. eryngioides* tuvo una fuerte actividad insecticida sobre los insectos experimentales. Los insectos adultos fueron expuestos a concentraciones de 37,03; 74,07; 111,11 y 148,14 µL L⁻¹ para estimar el tiempo letal medio (LT₅₀). La mortalidad aumentó con la concentración y el tiempo de exposición. La mortalidad de ambas especies alcanzó 100% a concentraciones superiores a 111,11 µL L⁻¹ y 39 h de exposición. Otro experimento, diseñado para determinar la concentración letal media en 24 h de exposición (LC₅₀), indicó que *S. granarius* es más susceptible que *T. castaneum*. Se puede concluir que el aceite esencial de *A. eryngioides* tiene potencial contra *S. granarius* y *T. castaneum*.

**Palabras clave**: Insecticidas botánicos, Apiaceae, toxicidad fumigante, *Sitophilus granarius*, *Tribolium castaneum*.

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