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Selective effects of the extract from *Angelica archangelica* L. against *Harmonia axyridis* (Pallas)—An important predator of aphids

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ABSTRACT

Effects of the extract from *Angelica archangelica* seeds on *Acyrthosiphon pisum*, pea aphid mortality was tested in laboratory conditions. The total yield of the extract was 12.5%, and the undertaken analysis demonstrated a high content of furanocoumarins with a major compounds imperatorin (50.4%), bergapten (17.9%) and phellopterin (12.2%). The extract proved to be highly toxic for the aphids and LC_{50} was estimated as 1.1, 0.8 and 0.4 mg L^{-1}, and LC_{90} as 8.7, 5.6 and 3.3 mg L^{-1} for 24, 48 and 72 h from application, respectively.

The effect of extract application on *Harmonia axyridis* was studied. Topical application was found to cause only low mortality (15.2%) in the 2nd–3rd instar larvae. No significant negative effect of the extract on food intake, mortality or fertility of adults was recorded. Based on present knowledge, we can recommend extracts from *A. archangelica* seeds for the development and production of new botanical insecticides.

1. Introduction

The pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphidiae) is an important cosmopolitan pest that feeds on a wide range of legume plants, including many agricultural crops in large parts of the world. In addition, it can act as a vector for more than 30 plant virus diseases (Blackman and Eastop, 2000; Nauen and Denholm, 2005).

Chemical insecticides are the major tool for combating aphids on crop plants. Extensive use of insecticides in crop systems, however, may cause resurgence of primary pests, replacement by secondary pests, environmental contamination, adverse effects on nontarget organisms, and development of pest resistance (Nauen and Denholm, 2005; Blackman and Eastop, 2000; Sharma et al., 2004). Therefore, alternatives to chemically intensive pest management are necessary.

Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health (Isman, 2006).

Currently, several dozen commercially produced botanical insecticides are used. Most commonly, these are products based on extracts from *Azadirachta indica*, *Chrysanthemum coccineum* and aromatic plants. However, the present production of botanical insecticides is often insufficient to cover the rising demand for plant protection products which are safe for both the environment and health. In addition, in order to prevent the emergence of resistant pest populations, it is important to continue finding highly active substances with a novel mechanism of effect. For these reasons, such plant species should be found whose extracts show not only good insecticidal characteristics, but also low toxicity for warm-blooded animals, while at the same time being friendly to natural enemies of the pests (Prakash and Rao, 1997; Isman, 2006; Dubey, 2011).

Such prospective plant species can be found particularly among medicinal and aromatic plants whose extracts have been studied in respect of their effects on human health. Based on these studies, the potential health safety of botanical insecticides made from them can be estimated.

Angelica archangelica L. (Apiaceae), an important medicinal plant, can also be considered one such prospective plant (Bruneton and Hatton, 1999). The pulpy roots and seeds (fruits) are used to obtain extracts or essential oils, which find their application in many fields of industry. For example, the essential oils of seeds and roots of *A. archangelica* are used as a spice and fragrance component in perfumery and cosmetics (Lawrence, 1996; Frater et al., 1998) and have also found a use in medicine on account of their important antispasmodic, stimulative, carminative, diuretic, nervine and tonic effects, as well as some other activities that have been found in them (Bruneton and Hatton, 1999). The extracts are reported to possess

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antimutagenic, antiulcerogenic, hepatoprotective, antiproliferative antitumor, and cytotoxic effects (Salikhova and Poroshenko, 1995; Khayyal et al., 2001; Yeh et al., 2003).

Besides having medicinal properties, extracts from seeds or roots of *A. archangelica* also provide significant pesticidal effects. In particular, extracts from the roots and seeds of *A. archangelica* showed important fungicidal (Zabka et al., 2011) and insecticidal (Wawrzyniak and Lamparski, 2006; Pavela, 2010, 2011) effects; it is therefore expected that the extracts from *A. archangelica* will be used in the development of new botanical pesticides.

Although some effects on mortality, antifeedancy and larval growth inhibition of insects have been published (Wawrzyniak and Lamparski, 2006; Pavela, 2010, 2011; Pavela and Vrchotova, 2013), effects of the extracts on natural enemies of the pests such as important predators of the Coccinellidae family have not been described.

This study is therefore new in focusing on efficacy of the extracts against the target pest *A. pisum* and its important predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). The purpose of the study was to estimate the potential selectivity of botanical insecticides based on extracts from *A. archangelica* with respect to this important representative of the natural predators of aphids. *H. axyridis* has been introduced to many European countries, including Belgium (1997), the Czech Republic (2003), France (1982), Germany (1998), and Greece (1994) (Roy and Wajnberg, 2008) as a biological control agent of aphids and other hemipteran pests. *H. axyridis* is indigenous to many regions of Asia, such as Taiwan, China, South Korea, Japan, Southern Siberia, the Ryukyu Islands and Bonin Island. It is a generalist predator that feeds primarily on several aphid species (Seo and Youn, 2000) and has been recognized for its potential contribution to the integrated management of various crop aphids, including *A. pisum*. It has been used successfully in greenhouses, orchards, gardens, and outdoor crops for aphid management (Majerus, 1994). However, *H. axyridis* often fails to achieve sufficient dynamics in natural agroecosystems in order to provide timely prevention of damage caused by aphids. Therefore, additional precautions are needed, such as the application of botanical insecticides; however, the insecticides should not exhibit a negative impact on *H. axiridis*.

2. Materials and methods

2.1. Insect culture

A continuous culture of *A. pisum* is maintained under standard conditions (23 ± 2 °C, 68 ± 5% relative humidity, 16 h photoperiod) in the Laboratory of Entomology, Crop Research Institute, Czech Republic. The aphids are reared on faba bean plants (*Vicia faba* L., Fabaceae), and all bioassays were carried out using newborn adults of 0–12 h age.

Adult *H. axyridis* were collected from alfalfa fields during summer 2011 at the Crop Research Institute farm, Czech Republic. Egg-producing *H. axyridis* females were separated into individual Petri dishes (polystyrene, 100 × 15 mm) and were provided cotton soaked with 10% sucrose water and an ad libitum supply of *A. pisum*. Faba beans, *V. faba*, were used to rear *A. pisum*. Larvae used in this study were third-generation offspring from the field-collected females. Following egg eclosion, larvae were separated and placed individually within plastic Petri dishes (polystyrene, 60 × 15 mm) and provided with an ad libitum supply of *A. pisum* and cotton soaked with 10% sucrose water daily. Adults and larvae were maintained at 23 ± 2 °C, 68 ± 5% relative humidity, 16 h photoperiod.

2.2. Extract and analysis

A. archangelica L. (Family: Apiaceae Lindl.; Syn.: *Archangelica officinalis* (Moench) Hoffm., *Angelica officinalis* Moench) – herbarium items are stored under registration number 1208 in the Crop Research Institute. The seeds of *A. archangelica* were collected manually in October 2012, from plants grown in the field of the Crop Research Institute. Impurities were removed from the collected materials and the materials were dried in a dryer at 40 °C (48 h).

The powdered seeds (50 g) were extracted using 100% pure methanol (1000 ml). The extract was concentrated (after 48 h of maceration) under a reduced pressure of 22–26 mm Hg at 45 °C, and the residue obtained was stored at 4 °C.

A Hewlett Packard (HP1050) liquid chromatography instrument with DAD detector (Agilent G1315B) was used for the analyses, which were performed on Luna C18(2) (150 mm × 2 mm, 3 μm) in a gradient of acetonitrile (Merck), water and *o*-phosphoric acid. Mobile phase A: 5% acetonitrile + 0.1% *o*-phosphoric acid, mobile phase B: 80% acetonitrile + 0.1% *o*-phosphoric acid. The composition of the mobile phase during the analyses was the following: gradient from 10% to 80% of mobile phase B within 50 min and thereafter from 80% to 90% of B within 10 min; the flow rate was 0.25 ml/min. Column temperature was 25 °C. The chromatograms were recorded in the range of 190–600 nm, and the compounds were detected at wavelengths of 200 and 220 nm. The compounds were identified by comparing retention time and UV–vis spectra of the standards and measured compounds (Harmala et al., 1990; Kaminski et al., 2003; Vogl et al., 2011). Quantification of caffeic acid and furanocoumarins was based on standards from either Sigma Aldrich (caffeic acid) or Extrasynthese (furanocoumarins such as psolaren and bergapten) using the calibration curve of corresponding standards; the content of other furanocoumarins was determined according to the calibration curve for bergapten.

2.3. Bioassay

2.3.1. Aphids toxicity

Adult aptera females (1–3 days old) were used to estimate lethal concentrations. Always 50 adults were uniformly distributed over filtration paper in an open Petri dish (18 cm in diameter). Extract from *A. archangelica* was mixed in water, using a disintegrator (T 18 Basic Ultra-Turax), to achieve resulting solutions of the concentration series 10, 8, 5, 3, 2, 1, 0.5, 0.2 and 0.1 mg mL^{-1}. Individual concentrations were applied using an electrical applicator in the dose 40 mL m^{-2} (corresponding approximately to practical application of a potential botanical insecticide in the dose of 400 L of spray liquid per ha). Control aphids were treated only with water. After application, the aphids were placed onto small bean plants (*Vicia faba* L.) and located in an air-conditioned room maintained at a temperature of 21 ± 1 °C, at a humidity of 50–70% RH, and in a 16:8 photoperiod (L:D). The experiment was repeated four times.

Aphid mortality was assessed 24, 48, 72 and 96 h after application. Aphids that did not respond were considered dead.

2.3.2. Effect of extract application on Harmonia axyridis

In order to determine the effect of the extract on *H. axyridis* growth and development, the extract was applied identically as described in Section 2.3.1. Concentrations of 1 and 10 mg mL^{-1} were applied, which correspond approximately to the estimated LC values, causing 50% and 90% mortality in *A. pisum* aphids within 24 h from application (see Table 2). The control was treated only with water. After extract or water application, the eggs, newly moulted larvae of the 2nd and 4th instars, and adults were introduced separately in Petri dishes (5.0 cm in diameter). The number of hatched larvae was evaluated for the eggs. A defined number of aphids were offered ad libitum to the treated larvae and adults, and the amount