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Insecticidal effect of furanocoumarins from fruits of *Angelica archangelica* L. against larvae *Spodoptera littoralis* Boisd.

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ABSTRACT

The essential oil (EO) and extracts (EXs) obtained from the seeds of *Angelica archangelica* were used to determine the efficacy in terms of chronic and acute toxicity, antifeedancy and growth inhibition of *Spodoptera littoralis* larvae. The EO and the EXs were analyzed by GC/MS and HPLC. The EO contained β-phellandrene as the major substance (60%). Furanocoumarins were identified in the EXs obtained using organic solvents, with the majority content of imperatorin (50–56%). Their varying efficacy was caused by the different composition of the EO and EXs obtained. Significant acute toxicity was caused only by the EO (LD_{50} 96 μg/larva). Significantly higher chronic toxicity was found for the EXs obtained using organic solvents (LD_{50} was estimated at 0.32, 0.82 and 0.52 mg g^{-1} for benzene, acetone and methanol, respectively), compared to the EO (LD_{50} 7.53 mg g^{-1}). All the tested EXs and the EO caused growth inhibition. The highest larval growth inhibition was caused by the benzene extract, with ED_{50} estimated at 2.4 μg g^{-1}. All EXs and the EO also showed antifeedant activity. However, the benzene extract showed the highest efficacy (ED_{50} 0.31 μg cm^{-2}), while the least efficacy was shown by the water extract (ED_{50} 1.92 μg cm^{-2}). In order to determine the dependence of biological efficacy of the EXs, LD_{50} and ED_{50} values were subjected to regression and correlation analysis. The analysis showed dependence between the total content of furanocoumarins in the extracts and chronic toxicity ($R^2 = 0.8451$; $P < 0.01$) or larval growth inhibition ($R^2 = 0.8941$; $P < 0.01$). This dependence was also confirmed for all individual furanocoumarins except bergapten, which showed no dependence.

Based on a comparison of the determined efficacy and yield of the extracts, extracts from *A. archangelica* seeds obtained using methanol can be recommended as suitable for the development of botanical insecticides against *S. littoralis* larvae.

1. Introduction

Angelica archangelica L. (*A. archangelica*) is an aromatic and herbaceous plant (Bruneton, 1999). The pulpy roots and seeds (fruits) are used to obtain extracts (EXs) or essential oils (EOs), which find their application in many fields of industry. For example, the EOs of seeds and roots of *A. archangelica* are used as a spice and fragrance component in perfumery and cosmetics (Fritter et al., 1998; Lawrence, 1996). In addition, the EOs have 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, 1999). The EXs are reported to possess antimutagenic, antiulcerogenic, hepatoprotective, antiproliferative antitumor, and cytotoxic effects (Salikhova and Poroshenko, 1995; Khayyal et al., 2001; Yeh et al., 2003; Sigurdsson et al., 2005a,b).

Besides the possible uses mentioned above, the EOs and EXs could potentially be used in plant protection. In particular, EXs from the roots and seeds of *A. archangelica* showed important fungicidal (Žabka et al., 2009, 2011) and insecticidal (Wawrzyniak and Lamparski, 2006; Pavela, 2010, 2011) effects; it is therefore expected that the EXs or EOs from *A. archangelica* will be used in the development of new botanical pesticides.

The development of botanical insecticides is important given that the number of pest populations resistant against active substances of synthetic insecticides (Baldwin and Graves, 1991; Saleem et al., 2008; Ahmad et al., 2009) has been continually rising. Use of botanical insecticides is one of the plant protection alternatives, generally considered safe for the environment and health (Isman, 2000; Pavela, 2007; Rattan, 2010).

Insecticidal effects of the EXs and EOs obtained from *A. archangelica* seeds depend on the qualitative and quantitative contents of the active substances themselves. As described in several

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papers from other authors, the fruits (seeds) contain 0.3–5% of the essential oil, with the major component being the cyclic monoterpene phellandrene (≤87%), usually present in two isomeric forms as α- and β-phellandrenes, with higher contents of β-phellandrenes (Wolski et al., 2003; Nivinskiene et al., 2007). Besides aromatic terpenes, the fruits contain a relatively high percentage of furanocoumarins such as umbelliferone, xanthotoxin, isopimpinellin, bergapten, imperatorin and phellopterin (Harkara et al., 1984; Murphy et al., 2004). Furanocoumarins play the role of phytoalexins in plants, which can be synthetized as a result of elicitation by microorganisms, insects and fungi, as well as abiotic elicitors such as UV radiation, environmental pollutants and physical damage. The defensive activity of furanocoumarins consists in their toxicity against phytopathogens (Konno, 2011).

Based on previous studies, the insecticidal effect can thus be attributed to aromatic monoterpenes in the EOs and furanocoumarins in the EXs (Isman, 2000; Konno, 2011). However, two important steps to be done in the further development of botanical insecticides are: (1) understanding the mechanism of action of the EOs and EXs obtained from *A. archangelica*; and (2) developing a simple method of isolation a maximum amount of substances with the highest biological effect.

In our paper, we determined the efficacy of hydrodistillate and the EXs obtained using solvents of various polarity in terms of acute and chronic toxicity, antifeedant efficacy and larval growth inhibition of *Spodoptera littoralis* Boisd. (*S. littoralis*). The larvae of *S. littoralis* were chosen for the tests since they are an important polyphagous pest, widely distributed in Africa and Mediterranean Europe (Baldwin and Graves, 1991). Larvae of this pest can feed on 90 economically important plant species belonging to 40 families, and the rate of development has a strong nutritional component (Azab et al., 2001). Commonly, the control of this pest has largely been dependent on the use of neurotoxic insecticides, including chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids. However, the control achieved has not been successful because of the insect's high capacity to develop resistance toward the majority of these compounds (Abo Elghar et al., 2005; Ahmad et al., 2009).

2. Materials and methods

2.1. Plant material

A. archangelica L. (Family: Apiaceae Lindl.; Syn.: *Archangelica officinalis* (Moench) Hoffm., *Angelica officinalis* Moench) – herbarium items are stored under registration number 1047 in Crop Research Institute, The seeds of *A. archangelica* were collected manually in October, 2010 from plants grown in the field of Crop Research Institute. Impurities were removed from the collected materials and the materials were dried in the dryer at 40 °C (48 h).

2.1.1. Essential oil extraction and analysis

The dried seeds were powdered mechanically using a commercial electrical stainless steel blender. The dried seeds were subjected to hydrodistillation for 1 h using a Clevenger-type apparatus. The oil obtained was separated from the water and dried over anhydrous Na_2SO_4.

The identification of the chemical components of the oil samples was done in a complete HP 6890 gas chromatograph using a mass selective detector HP 5973, equipped with Chemstation software and Wiley 275 spectra data. An HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The chromatographic conditions were: column temperature 60 °C (8 min), 60–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), interface 180 °C, split ratio 1:100, carrier gas, He (55.4 kPa), flow rate 1.0 ml/min, ionization energy 70 eV, mass range 40–350, volume injected 0.5 μl, solvent cut, 3.5 min.

GC analysis was performed on an HP 5973 gas chromatograph with FID detector using an HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.50 μm film thickness). The chromatographic conditions were: column temperature 40 °C (8 min), 40–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), injector temperature 250 °C, split ratio 1:50, detector temperature 250 °C, carrier gas hydrogen (34 kPa), flow rate 1.0 ml/min, volume injected 0.2 μl.

2.1.2. Preparation of plant extracts and analysis

The powdered seeds (50 g) were extracted using 100% pure benzene (1000 ml), acetone (1000 ml), methanol (1000 ml) and distilled water (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.

Analyses were performed on an HPLC Hewlett Packard (HP1050) with DAD detector (Agilent G1315B). Separation was carried out on a Luna C18(2) (150 mm × 2 mm, 3 μm) in a gradient of acetonitrile (Merck), water and phosphoric acid.

Mobile phase A: 5% acetonitrile + 0.1% o-phosphoric acid, mobile phase B: 80% acetonitrile + 0.1% o-phosphoric acid. Gradient for separation: gradient from 10% to 80% of mobile phase B within 50 min was used 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 records were scan in range 190–600 nm and compounds were detected at 200 and 220 nm.

The determined compounds were identified by comparing the standards, retention time and UV–VIS spectrum (Härmälä and Vuorela, 1990; Kamiński et al., 2003; Vogl et al., 2011). The content of caffeic acid (Sigma Aldrich) and furanocoumarins such as psolaren (Extrasynthese) and bergapten (Extrasynthese) was determined according to the calibration curve of corresponding standards; the content of others furanocoumarins was determined according to the calibration curve for bergapten.

2.2. Insects

S. littoralis Boisd. (Lepidoptera: Noctuidae): bioassays were conducted using larvae of the tobacco cutworm, *S. littoralis*, obtained from an established laboratory colony (>20 generations; outcrossed once). The larvae fed on an artificial insect diet (Stonefly Industries, Bryan, TX, USA); adults fed on a 10% honey solution and were able to oviposit on filter paper. The colonies were reared at 25 ± 1 °C and a 16:8 (L:D) photoperiod. This experiment was performed with pre-weighed, newly-moulted (0–6 h after ecdysis) 3rd instar larvae.

2.3. Bioassays

2.3.1. Toxicity

2.3.1.1. Acute toxicity. The acute toxicity, measured as mortality after 24 h of exposure, was determined by topical application to early 3rd instar larvae of *S. littoralis*.

The stock solutions of essential oils or extracts were dissolved in acetone as a carrier, and each larva received 1 μl of the solution per treatment, with acetone alone as the control treatment. A range of six doses (300, 150, 100, 60, 25 and 10 μg) for larvae were used to establish the lethal doses. Four replications of 20 larvae were tested per dose. The doses were applied to the dorsum of each larva's body using a repeating topical dispenser attached to 100 μl syringes. All treated larvae from each replicate were transferred to the relevant diet in plastic boxes (10 cm × 10 cm × 7 cm), which were closed using perforated caps to make sure that the experiment was not affected by the fumigation effect of the EOs. The