Secondary metabolite profiles and biological activity of extracts from various isolates fungi *Alternaria sonchi* depending on the composition of the liquid nutrient medium

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Key message. Phytopathogenic fungus *A. sonchi* is able to produce metabolites with insecticidal properties. The composition of the culture media affected the metabolite profiles of the extracts. The results of the assessment of biological activity allowed to divide the working isolates with phytotoxic and insecticidal activity.

Keywords: *Alternaria sonchi*, insecticidal properties, chloromonilinic acids B and C, 4-chloropinenselin, tentoxin

Phytopathogenic fungi from the genus *Alternaria* are often considered as producers of herbicidal compounds, mycotoxins and antibiotic substances. There are few works aimed at the search for secondary metabolites with insecticidal properties in fungi of this genus. The object of research is the species *Alternaria sonchi*, a pathogen of weeds from the genus *Sonchus*. The aim of the work is to study the effect of various liquid nutrient media on the variability of the composition and biological activity of extracts obtained from *A. sonchi* cultures. Research objectives: extraction of fungal metabolites with solvents of different polarity; assessment of the biological activity of the obtained extracts against a wide set of test organisms; analysis and preliminary characterization of the composition of the extracts. We used four geographically distant isolates of *A. sonchi* (S-102, S-145, Ger 8.2, I. 5.4). Fungi were cultured for 3 weeks at a constant temperature (24 °C) and under variable lighting on synthetic (M1D and Czapek) and semi-synthetic (YMG and Sabouraud) media that differ in carbon and nitrogen sources. Secondary metabolites were extracted from the culture filtrate (CF) with methylene chloride at neutral pH and ethyl acetate after acidification to pH 3. The metabolite profiles of the extracts were analyzed by HPLC-MS. The obtained extracts were evaluated for phytotoxic, insecticidal, antibiotic and zootoxic activity. The yield of secondary metabolites, depending on the isolate, varied weakly, but significantly depended on the composition of the liquid nutrient medium and on the extractant. The yield of extractives from CF was several times higher upon extraction with ethyl acetate than upon extraction of secondary metabolites with methylene chloride. Among the chloromethylene (107 mg/L) and ethyl acetate (325 mg/L) extracts, the maximum yield of extractives was noted when culturing in Sabouraud liquid medium from the culture of isolate S-102 *A. sonchi*. Ethyl acetate extracts obtained by culturing isolates S-102, I. 5.4 on M1D medium showed the highest phytotoxic activity against the sowthistle and wheat leaves. The highest antibacterial activity against *Bacillus subtilis* and *Candida tropicalis* was observed in ethyl acetate and chloromethylene extracts of isolate S-102 obtained on M1D medium (the radius of inhibition zone 15 mm and 8 mm, respectively). Ethyl acetate extracts from CF isolate Ger 8.2 from YMG medium and isolate S-145 from M1D medium possessed insecticidal properties (efficacy at 85–90%) against *Schizaphis graminum* aphids. None of the obtained extracts at a concentration of 100 µg/ml showed acute toxicity to *Paramecium caudatum*. An analysis of metabolite profiles by HPLC-MS showed the presence of previously unknown substances in the extracts, in addition to the already known compounds of *Alternaria* spp.. Chloromonilinic acids B and C, 4-chloropinenselin were identified in the phytotoxic extracts. The latter substance is also present in extracts with antimicrobial properties. The ethyl acetate extract from the S-102 culture from YMG presumably contained tentoxin by HPLC-MS data. Thus, the qualitative composition of extracts from liquid cultures of *A. sonchi* depends on the selected nutrient substrates. The most favorable media for the production of biologically active extracts were M1D and YMG. The results of evaluating the biological activity of the extracts allowed to divide the working isolates into 2 groups: with phytotoxic (S-102, I. 5.4) and insecticidal (Ger 8.2, S-145) activity. The data of the study suggest that *A. sonchi*, the potential producer of mycoherbicide against sowthistle, is able to produce natural products with insecticidal properties. Further study of these metabolites is necessary for more complex toxicological characterization of this fungus, and for the detection of new natural insecticidal substances.

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