Isolation of Dalmatian chamomile extracts - environmentally friendly natural compounds with insecticidal action

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Abstract. Extracts of Dalmatian chamomile (*Pyrethrum cinerariaefolium* Trev) grown in the greenhouse complex of the Institute of Phytopathology were obtained. The resulting extracts were isolated from the dried inflorescences of this plant, by an initial stepwise extraction of lipid fractions with a mixture of dichloroethane / petroleum ether, methanol and a mixture of methanol / petroleum ether, followed by purification on activated carbon. By further separation of the obtained extracts by preparative column chromatography, biologically active fractions with different contents of natural insecticides from the pyrethrin group were isolated. The content of biologically active components in various fractions was determined by the methods of chromatomass-spectrometry. It was found that low-polarity eluents (hexane / chloroform) recover pyrethrin I, cinerin I, and jasmolin I most fully, and the maximum recovery of pyrethrin II and cinerin II is observed when using relatively polar eluents (hexane / acetone). The insecticidal effect of the end products on the great cereal aphid (*Sitobion avenae*) was studied. It was found that the maximum insecticidal effect (100% mortality of insects) was exhibited by the pyrethrine fraction isolated during extraction with low-polarity eluents (hexane / chloroform). It was found that the pyrethrine fraction isolated during extraction with relatively polar eluents (hexane / acetone) exhibited a moderate insecticidal effect (77–81% of insect mortality). It has been shown that Dalmatian chamomile extracts have a high insecticidal effect against such a pest of cereal crops as the large cereal aphid and can be successfully used as effective, environmentally friendly natural insecticides, including in the form of biologically active additives to commercial insecticidal preparations.

1 Introduction

It is known that the inflorescences of perennial grasses of the *Asteraceae* family from the genera *Pyrethrum*, *Chrysanthemum* and *Tanacetum* contain natural insecticides from the pyrethrins group [1–10], which are optically active esters of cyclic ketalcohols (pyrethrolone, cinerolone, and 3-substituted jasmolone) 2,2-dimethyl-cyclopropanecarboxylic acids (*trans*-chrysanthemum and *trans*-pyrethrinic) [11-14]. Chrysanthemum esters are referred to as pyrethrine I (PYI), pyrethrinic acid esters as pyrethrine II (PYII) [15–19], Figure 1.

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Fig. 1. Pyrethrine structural formulas.

Pyrethrine contains six esters (pyrethrin I and II, cinerin I and II, jasmolin I and II), the ratio of which can vary depending on the plant variety, growing and harvesting conditions. The share of pyrethrine I, II accounts for the main content of insecticides in plant inflorescences, however, pyrethrins are also less resistant to oxidative degradation under the action of atmospheric oxygen than minor cinerins and jasmolines, which should be taken into account when choosing methods for their isolation, storage and conditions of use as insecticides [20]. Pyrethrine is a non-volatile compound (at room temperature), practically insoluble in water, but soluble in many organic solvents (primarily in non-polar and low-polar ones - benzene, hexane, petroleum ether, as well as in methanol, ethanol, acetone). The most commonly used extractants for extracting pyrethrine are hexane, petroleum ether, or mixtures based on them. The priority in using these solvents is associated with their low boiling point, which allows the extraction of pyrethrine at a lower temperature, minimizing the dissolution of various impurities in the extract.

The aim of the study was to develop new methods for the isolation of pyrethrine from plant materials, which are necessary for the production of environmentally friendly natural precursors of drugs and biologically active insecticidal compositions intended to protect agricultural plants from pests.

2 Methods

2.1 Growing conditions Dalmatian chamomile

The research was carried out at the Institute of Phytopathology greenhouse complex. Dalmatian chamomile (Pyrethrum cinerariaefolium Trev) was grown in a greenhouse complex, in the amount of 48 plants, on sod-podzolic soil with clay texture, pH 5.8, with a humus content of 2.2–2.4%, exchangeable sodium 6.0–6.7%, exchangeable potassium 14.3
–14.4 mg, calcium 1.0 mg, mobile phosphorus 2.2–2.3 mg per 100 g of soil. Chamomile sowing was carried out at a soil temperature of 10 °C and an air temperature in a greenhouse box of 15–16 °C in mid-March 2018. General cultivation practices were followed during the growing season. The inflorescences were harvested in the second year at the end of June 2019 at optimal maturity. The collected inflorescences were spread in a thin layer on wooden pallets and dried for two months in the dark at a temperature of 18–20 °C. Dried inflorescences containing 88–90% dry matter were crushed by grinding in a mechanical mill to a fine powder, stored in dark glass bottles (to avoid oxidative degradation of pyrethrins in the light) at a temperature of 5–7 °C.

2.2 Pyrethrine extraction
The extraction of pyrethrine was carried out by extracting the obtained dry powder of crushed inflorescences of Dalmatian chamomile in 1 g portions with a mixture of dichloroethane and petroleum ether (1: 3) with a volume of 100 ml three times. Organic extracts were combined, filtered off, and the solvent was distilled off on a rotary evaporator to obtain a 30–35% concentrate. The resulting concentrate was passed through activated carbon three times, successively extracted with methanol and a mixture of methanol and petroleum ether (1: 10), the resulting extract was purified again with activated carbon, the solvent was distilled off on a rotary evaporator.

2.3 Preparative extract chromatography
The final product in the form of a yellow oil was separated on a preparative chromatographic column (Silica gel 60 sorbent 0.04-0.063 mm, 230–400 mesh), successively eluting with hexane / acetone mixtures (10: 1, fraction I), hexane / acetone (7 : 2, fraction II), hexane / acetone (5: 3, fraction III), hexane / chloroform (15: 3, fraction IV), followed by distillation of the eluents. The completeness of the separation of the components was monitored by TLC methods on Sorbfil plates PTSKh-P-A-UV, PTSKh-AF-A-UV (silica gel, UF 254/365).

2.4 Chromatomass spectrometric analysis
Fractions I – IV were analyzed on a MaXis Impact HD quadrupole-time-of-flight chromatomass spectrometer (Bruker Daltonik GmbH) with a temperature range of 60-300 °C in the electrospray ionization (ESI) mode for solutions in acetonitrile at a sample feed rate of 240 μL / h with parameters default in small molecule infusion method. Mass calibration - external improved quadratic method using G1969-85000 calibration solution (Agilent Technologies). Ultrahigh-resolution mass spectra were obtained, registering the positive ions of the compounds contained in fractions I – IV.

2.5 Biotesting of fractions
The insecticidal effect of end products I – IV (diluted at the rate of 0.1 g of the product per 99 ml of a mixture of methanol and water 30/70) was tested by spraying wheat infected with a large cereal aphid (Sitobion avenae).

2.6 Mass-spectra of selected components
Pyrethrin I. Found: m/z 329.2113 [M+H]+. C21H29O3+. Calculated: 329.2111. Pyrethrin II. Found: m/z 329.2110 [M+H]+. C21H29O3+. Calculated: 329.2111. Cinerin I. Found: m/z
317.2114 [M+H]+. C_{20}H_{29}O_{3}. Calculated: 317.2111. Cinerin II. Found: m/z 317.2112 [M+H]+. C_{20}H_{29}O_{3}. Calculated: 317.2111. Jasmolin I. Found: m/z 331.2268 [M+H]+. C_{21}H_{31}O_{3}. Calculated: 331.2268.

3 Results and Discussion

The results of experimental studies on the chromatographic extraction of pyrethrine from extracts of Dalmatian chamomile and their content in various fractions are presented in Table 1.

| Pyrethrine | Fraction I | Fraction II | Fraction III | Fraction IV |
|------------|------------|-------------|--------------|-------------|
| Pyrethrin I| 34         | 37          | 39           | 56          |
| Cinerin I  | 10         | 11          | 13           | 15          |
| Jasmolin I | 3          | 4           | 7            | 9           |
| Pyrethrin II| 31        | 30          | 27           | 10          |
| Cinerin II | 17         | 12          | 7            | 4           |
| Jasmolin II| 5          | 6           | 7            | 6           |

Experimental data show that the extraction of pyrethrin I, cinerin I, and jasmolin I (fraction IV) was most fully carried out with the least polar eluent hexane / chloroform (15: 3), while the maximum recovery of pyrethrin II and cinerin II (fractions I and II) was observed at using relatively polar eluents hexane / acetone (10: 1) and hexane / acetone (7: 2). For the rest of pyrethrine, we did not find such regularities. Fraction IV showed the maximum insecticidal effect (100% mortality of insects), while the insecticidal effect of fractions II and III was only 77% and 81% of insect mortality, respectively. Fraction I showed the minimum insecticidal effect. The presented data indicate that fraction four, containing the maximum amount of pyrethrin I, cinerin I and the minimum amount of pyrethrin II, has the strongest effect on the large cereal aphid. The content of jasmoline I and II in all fractions is comparable, which indicates an insignificant role of these pyrethrine in the insecticidal action on the large cereal aphid.

4 Conclusions

It is shown that as a result of the research, new methods have been developed for the isolation of pyrethrine from plant materials, which are necessary for the production of environmentally safe natural insecticides. It has been shown that Dalmatian chamomile extracts have a high insecticidal effect against such a pest of cereal crops as the large cereal aphid and can be successfully used as effective, environmentally friendly natural insecticides, including in the form of biologically active additives to commercial insecticidal preparations.

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