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INHIBITION EFFECTS OF CYCLOHEXANESPIRO-5-HYDANTOIN AND 1-AMINOCYCLOHEXANECARBOXYLIC ACID TOWARDS SOUR CHERRY POLLEN

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***Abstract**: Possible deleterious effects of cyclohexanespiro-5-hydantoin and 1-aminocyclohexanecarboxylic acid to sour cherry pollen were examined with the current study. Dose-response modeling was carried out by R language for Statistical Computing, drc package.*

***Key words:** cyclohexanespiro-5-hydantoin, 1-aminocyclohexanecarboxylic acid, sour cherry pollen, drc, R language*

I. Introduction

The interest in studying cyclohexanespiro-5-hydantoin and 1-aminocyclohexanecarboxylic acid is caused by their biological activity.

Certain cyclohexanespiro-5-hydantoins have analgesic and anti-inflammatory activity [1].

Regional transport of l-aminocyclohexanecarboxylic acid, a nonmetabolizable amino acid, across the blood-brain barrier is studied in pentobarbital-anesthetized rats using an in situ brain perfusion technique [2].

Different derivatives of cyclohexanespiro-5-hydantoin are obtained in order to investigate their possible biological activity.

Some examples of such compounds include monotiho- and dithio- analogues of cyclohexanespiro-5-hydantoin, as well as copper complexes of the latter and its dithio- analogue [3, 4].

For ecotoxicological characterization and determination of eventual deleterious effects of biocides onto plants, pollen germination tests are from significant importance, due to the major impact of the pollen on plants' lives.

Quantification of pollen tube growth will allow this inhibitory effect to be expressed in a numerical

value: ED_{50}, ED_{25} (LOAEL), ED_{05} (NOAEL). In the past two decades, pollen grains and pollen tubes of various plant species have been used to determine the cytotoxic effects of environmental pollutants [5].

Sour cherry (*Cerasus vulgaris*, *Prunus cerausus*, tart cherry) is a very popular and important orchard culture for the food industry. The sour cherry is cultivated in all parts of Bulgaria, especially near the towns of Sofia, Pazardzik and Plovdiv district [6].

We are examining the possible deleterious effects of cyclohexanespiro-5-hydantoin and 1-aminocyclohexanecarboxylic acid to sour cherry pollen in the current study.

II. Materials and methods

II.1. Synthetic compounds

All chemicals used were purchased from Merck and Sigma-Aldrich.

The cyclohexanespiro-5-hydantoin (Fig. 1, a) was synthesized *via* the Bucherer-Lieb method [7].

The 1-aminocyclohexanecarboxylic acid (Fig. 1, b) was obtained in accordance with Stoyanov and Marinov [8].

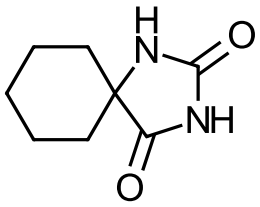
Melting points were determined with a Koffler apparatus and with a digital melting point apparatus SMP 10.

Elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106.

IR spectra were taken on spectrometers Bruker-113 and Perkin-Elmer FTIR-1600 in KBr discs.

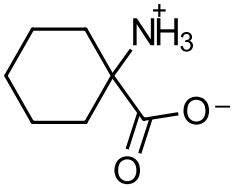
NMR spectra were taken on a Bruker DRX-250 spectrometer, operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C, respectively, and on a Bruker Avance II + 600 MHz spectrometer, operating at 600.130 and 150.903 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. Chemical shifts were referenced to tetramethylsilane (TMS). Measurements were carried out at ambient temperature.

a)



cyclohexanespiro-5-hydantoin
(chsh)

b)



1-aminocyclohexane-carboxylic acid
(achca)

Fig. 1. *Structures of the compounds*

The products obtained were characterized by physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical with the previously published in the literature [3, 8].

II.2. Ecotoxicological tests

Fresh collected pollen from a 5 year old sour cherry tree of the Oblacinska variety (which is the most popular sour cherry variety in

Bulgaria) was used. In order to stimulate pollen germination, 20 % (m/v) of sugar added to distilled water was used for dilution and preparation of the solution of tested compounds. Pollen suspensions with tested compounds in various concentrations were prepared with pollen grains density 2*10e-04 grains per ml determined with haemocydometer.

After a 24 h stay in a thermostat (22 °C), the germination of grains and length of elongation tube were measured with a light inverter microscope (20x magnification). Based on the germ tube germination, percents were calculated *via* Abbott's formula [9] with regard to the control variant. The dose-response modeling was conducted for each test variant for determination of ED_{50}, ED_{25} (LOAEL) and ED_{05} (NOAEL) using a drc package [10, 11].

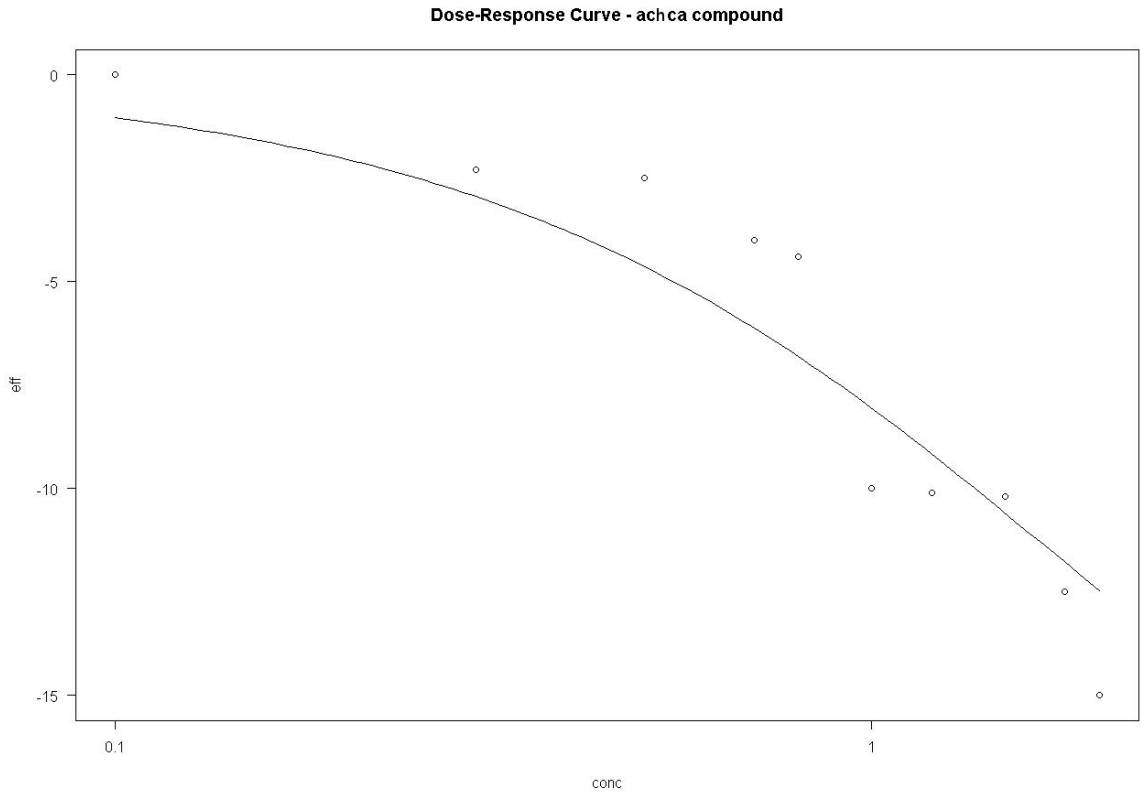


Fig. 2. *Dose-response curve of 1-aminocyclohexanecarboxylic acid*

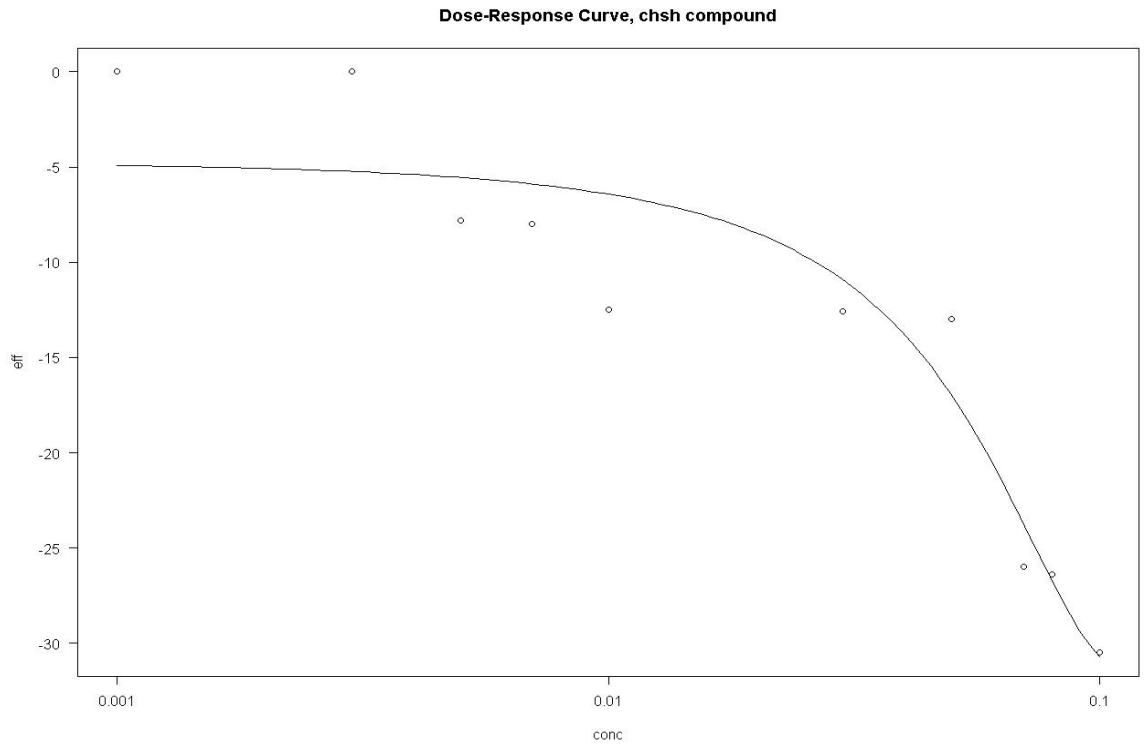


Fig. 3. *Dose-response curve of cyclohexanespiro-5-hydantoin*

III. Results and discussion

Conducted trials reveal that all tested compounds do not cause any deleterious effect on *Prunus cerausus* pollen. What is more, at the saturated concentrations in water – 2 % (m/v) for achca and 0.1 % (m/v) for chsh, they have a stimulating effect on pollen germination.

Fig. 2 and Fig. 3 represent the dose-response curves for each compound.

The calculated values of the NOEL and LOEL for achca compound are 0.08 % (v/v) and 0.47 % (v/v), respectively; for chsh compound – 0.004 % (v/v) and 0.014 % (v/v) for NOEL and LOEL, respectively.

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