

**INHIBITION EFFECT OF CYCLOPENTANESPIRO-5-HYDANTOIN AND
CYCLOPENTANESPIRO-5-(2,4-DITHIOHYDANTOIN) TOWARDS APPLE TREE POLLEN**

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Abstract

Due to the major impact of pollen on plants' lives the pollen germination tests are of significant importance for ecotoxicological characterization and determination of eventual deleterious effects of biocides on plants. Possible deleterious effects of two synthetic organic compounds – cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin) to apple tree pollen have been examined in the present study. It has been found out that the titled compounds have an inhibitory effect on apple tree pollen. Dose-response modeling was carried out by R language for Statistical Computing, drc package.

Key words: *cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), apple tree pollen, drc, R language*

1. INTRODUCTION

The study interest in cyclopentanespiro-5-hydantoin and its dithioanalogue – cyclopentanespiro-5-(2,4-dithiohydantoin) is mainly due to the biological activity reported for these compounds. Oldfield and Cashin synthesized and tested a number of different cycloalkanespiro-5-hydantoins for toxicity, anticonvulsant and analgesic activity on mice. A low toxicity and low sedative activity were confirmed for cyclopentanespiro-5-hydantoins (Oldfield and Cashin 1965).

Cyclopentanespiro-5-(2,4-dithiohydantoin) is a compound with a strong insecticidal activity against *Cladius pectinicornis* (Marinov et al. 2012a).

Various ecotoxicological tests with the title compounds were performed in our previous studies. Their acute toxicity towards *Planorbis planorbis* (Ram's Horn Snail) was researched. It was found out that cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin) manifest a toxic effect on snails (Marinov et al. 2012b).

We also performed an ecotoxicological study of the above mentioned compounds on Black Sea Mussel (*Mytilus galloprovincialis*). Toxicity manifestation on mussels was observed only at cyclopentanespiro-5-(2,4-dithiohydantoin) (Ganchev et al. 2012a).

Acute toxicity deleterious effect of cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin) towards Sea lettuce (*Ulva lactuca*) conducted in accordance with international standards adopted by the Organisation for Economic Co-operation and Development (OECD) was evaluated. It was found out that relatively high levels of cyclopentanespiro-5-hydantoin concentration

will be able to produce acute deleterious effect on populations of Sea lettuce ($LD_{50} = 0.750 \%$) (Marinov et al. 2012c).

In vivo phytotoxicological study of cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin) towards some cultural and non-cultural plants was performed. The tests were conducted with cultural plants as Oil yielding rose (*Rosa damascena*), Plum tree (*Prunus cerasifera*) and Grape (*Vitis vinifera*) variety Muller-Thurgau, as well as with non-cultural plants as Oval-leaved privet (*Ligustrum ovalifolium*), Lime-tree (*Tilia platyphyllos*) and Chestnut (*Aesculus hippocastanum*). The cyclopentanespiro-5-hydantoin had a deleterious effect on Chestnut (*Aesculus hippocastanum*) and Lime-tree (*Tilia platyphyllos*) (Ganchev et al. 2012b).

On the other hand, cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin) are compounds with good coordination properties. These substances have been used as ligands for synthesis of complexes with ions of transitional metals (Enchev et al. 1999, Ahmedova et al. 2008, Marinova et al. 2012).

Due to the major impact of pollen on plants' lives pollen germination tests are of significant importance for ecotoxicological characterization and determination of eventual deleterious effects of biocides on plants. Such tests are principal for evaluation of pesticide compounds which have inevitable effect on plant pollen of cultural crops and non-cultural adjacent plants.

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

- ED_{50} ;
- ED_{25} (LOAEL – Lowest Observed Adverse Effect Level) – the lowest dose / concentration at which observable toxic effect onto test object can be detected;
- ED_{05} (NOAEL – No Observed Adverse Effect Level) – the highest dose / concentration at which no observable toxic effect onto test object can be detected.

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 (Kristen and Kappler 1995).

Apple tree (*Malus domestica*) is the most important orchard culture for Bulgaria in present days, cultivated in all parts of the country especially near the towns of Plovdiv, Sofia and Kyustendil (Mitov et al. 1996).

The aim of the current study is an examination of the possible deleterious effects of cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin) to apple tree pollen.

2. MATERIALS AND METHODS

2.1. Synthetic compounds

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

The cyclopentanespiro-5-hydantoin (Fig. 1) was synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb 1934). The cyclopentanespiro-5-(2,4-dithiohydantoin) (Fig. 2) was obtained in accordance with Marinov et. al (Marinov et al. 2012a).

Melting points were determined 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 ^1H and ^{13}C , respectively, and on a Bruker Avance II + 600 MHz spectrometer, operating at 600.130 and 150.903 MHz for ^1H and ^{13}C , respectively, using the standard Bruker software. Chemical shifts were referenced to tetramethylsilane (TMS). Measurements were carried out at an ambient temperature.

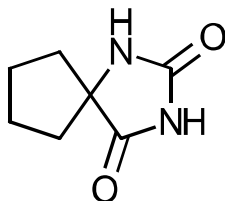


Fig. 1. Structural formula of cyclopentanespiro-5-hydantoin
(Systematic name: 1,3-diazaspiro[4.4]nonane-2,4-dione)

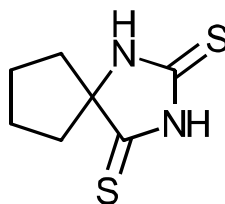


Fig. 2. Structural formula of cyclopentanespiro-5-(2,4-dithiohydantoin)
(Systematic name: 1,3-diazaspiro[4.5]decane-2,4-dione)

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 (Enchev et al. 1999; Marinov et al. 2005).

2.2. Ecotoxicological tests

Freshly collected pollen from a 5 year old apple tree of the *Golden Delicious* variety (which is the most popular apple variety in Bulgaria) was used. In order to stimulate pollen germination, a solution of 20 % (m/v) sugar in distilled water was used for dilution and preparation of the solution of tested compounds.

Saturated concentrations of the compounds in water were as follows:

- cyclopentanespiro-5-hydantoin – 1 %;
- cyclopentanespiro-5-(2,4-dithiohydantoin) – 0.025 %.

Pollen suspensions with tested compounds in various concentrations were prepared with pollen grains density $2 \cdot 10^{-4}$ grains per ml determined with haemocytometer. 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, percentage was calculated via Abbott's formula (Abbott 1925) with regard to the control variant.

The dose-response modeling was conducted for each test variant for determination of LD_{50} , LD_{25} (LOAEL) and LD_{05} (NOAEL) using a drc package (R Foundation for Statistical Computing, <http://www.R-project.org/>, Ritz and Streibig 2005).

3. RESULTS AND DISCUSSION

The initial cyclopentanespiro-5-hydantoin was obtained with an application of the Bucherer-Lieb method (Bucherer and Lieb 1934). This technique is the main method for hydantoin and spirohydantoin synthesis. It is based on the interaction between the cyclopentanone, sodium cyanide, ammonium carbonate and ethanol.

The cyclopentanespiro-5-hydantoin obtained was subjected to a thionation with the reagent combination of P_4S_{10} and hexamethyldisiloxane, following a procedure of Curphey (Curphey 2002), modified by Marinov et. al (Marinov et al. 2012a). The advantage of this technique is in obtaining of product of higher yield and shorter reaction time as compared to previously used thionation methods (Marinov et al. 2005).

Conducted trials reveal that cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin) have a significant deleterious effect on the apple tree pollen (especially the spirodithiohydantoin).

Fig. 3 and Fig. 4 represent the dose-response curves (models) of the cyclopentanespiro-5-hydantoin and cyclopentanespiro-5-(2,4-dithiohydantoin).

The abbreviations used on the graphics depicted below are as follows:

- CPSH for cyclopentanespiro-5-hydantoin;
- CPSDTH for cyclopentanespiro-5-(2,4-dithiohydantoin).

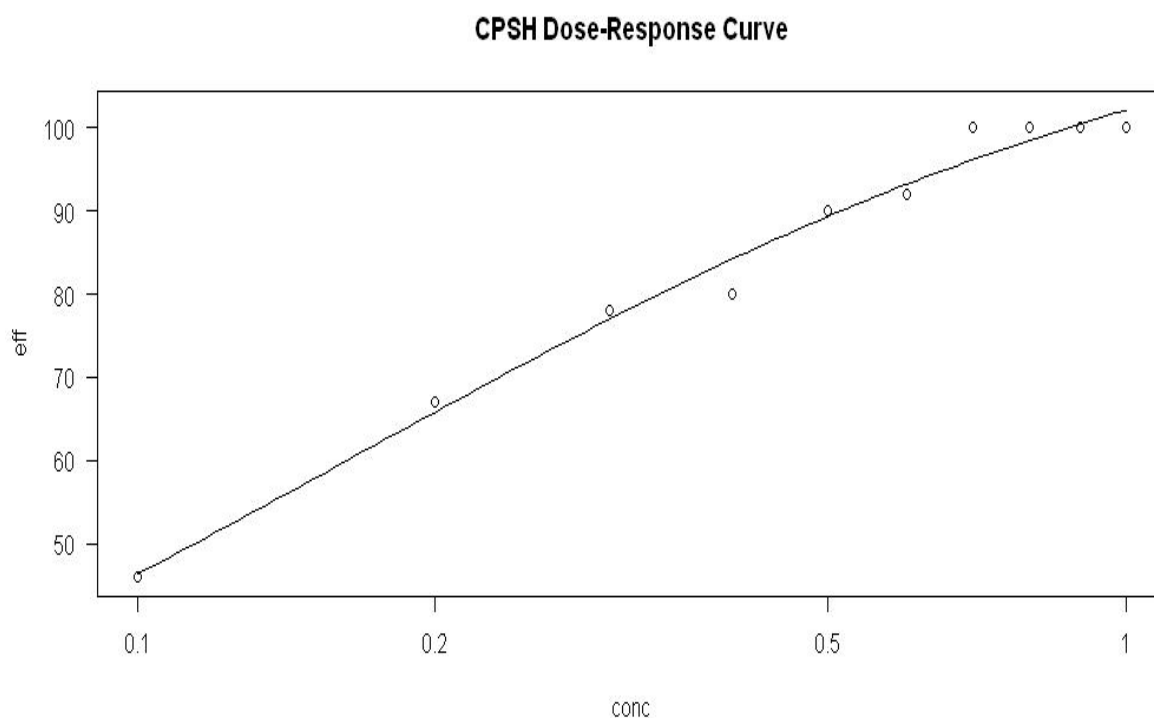


Fig. 3. Dose-response curve of cyclopentanespiro-5-hydantoin

The calculated values of the NOAEL (LD_{05}), LOAEL (LD_{25}) and LD_{50} for the products obtained are as follows:

For cyclopentanespiro-5-hydantoin:

- $LD_{05} = 0.0072 \%$;
- $LD_{25} = 0.051 \%$;
- $LD_{50} = 0.16 \%$.

For cyclopentanespiro-5-(2,4-dithiohydantoin):

- $LD_{05} = 0.003 \%$;
- $LD_{25} = 0.0054 \%$;
- $LD_{50} = 0.0077 \%$.

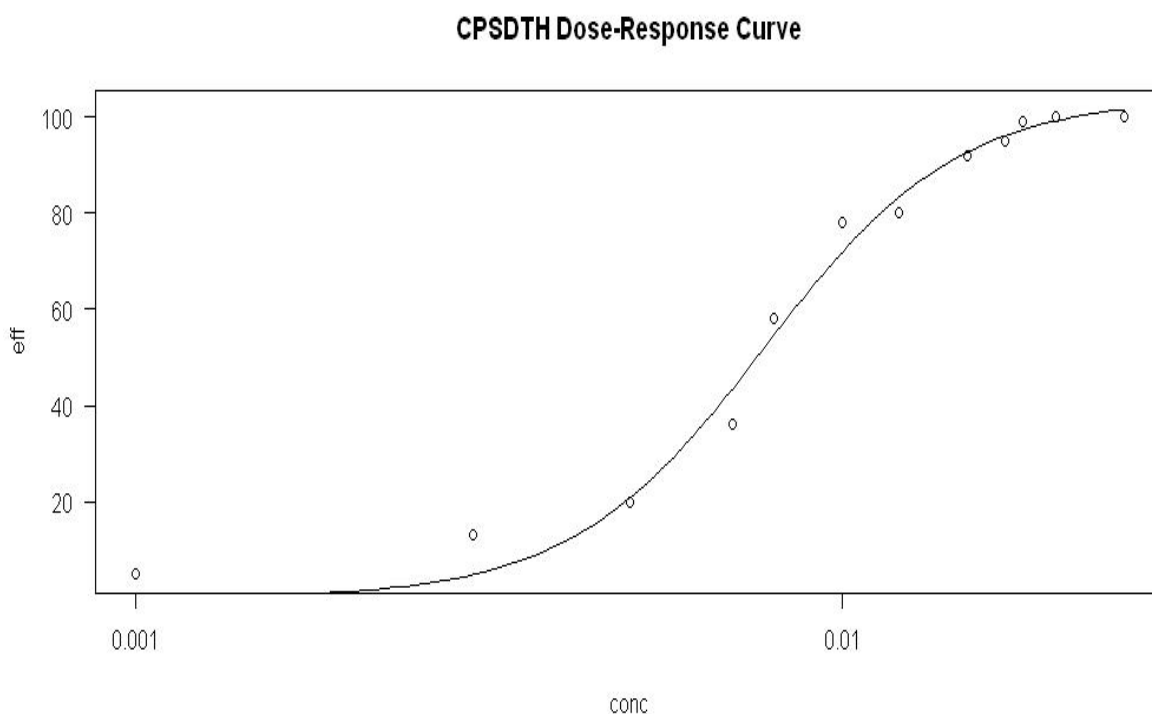


Fig. 4. Dose-response curve of cyclopentanespiro-5-(2,4-dithiohydantoin)

4. CONCLUSIONS

The tests and conducted dose-response analysis prove the extreme toxic effect of tested compounds onto plant apple pollen – $LD_{50} = 0.16 \%$ for cyclopentanespiro-5-hydantoin and $LD_{50} = 0.0077 \%$ for cyclopentanespiro-5-(2,4-dithiohydantoin). This should indicate a careful future use of the above mentioned compounds in case they show potential pesticidal properties.

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