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Original Contribution

IN VIVO PHYTOTOXICOLOGICAL STUDY OF CYCLOPENTANESPIRO-5-HYDANTOIN AND ITS DERIVATIVES TOWARDS SOME CULTURAL AND NON-CULTURAL PLANTS

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Abstract: *This paper represents ecotoxicoilogical study about phytotoxic action of cylopentanespiro-5-hydantion, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards cultural and non-cultural plants conducted in accordance of Organisation for Economic Co-operation and Development standards for such kind investigation. The dose-response modeling was conducted with R language for Statistical Computing and drc package.*

Key words: *spirohydantoins, phytotoxic, drc, R language*

I. Introduction

Ecotoxicological research of phytotoxic action of toxicants has a key role in their characterization and adoption in practice. Studies of this type are used to reveal eventual deleterious action of toxicants with regard to aquatic and terrestrial plants and to prevent possible damages on cultural and non-cultural plants.

In the present study, examination of acute phytotoxic action of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid was conducted towards cultural and non-cultural terrestrial plants in accordance with international standards for such studies.

II. Materials and methods

II.1. Synthetic compounds

All chemicals used were purchased from Merck and Sigma-Aldrich. The cyclopentanespiro-5-hydantoin (CPSH, Fig. 1) was synthesized *via* the Bucherer-Lieb method [1]. The cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH, Fig. 2) was synthesized in accordance with Marinov et. al. [2]. The 1-aminocyclopentanecarboxylic acid (ACPCA, Fig. 3) was obtained in accordance with Stoyanov and Marinov [3]. 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.

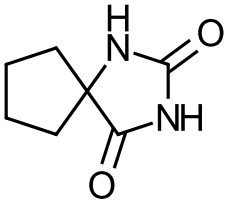

Fig. 1. *Cyclopentanespiro-5-hydantoin (CPSH)*

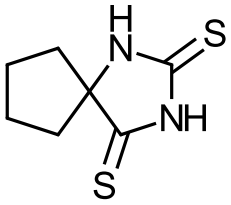

Fig. 2. *Cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH)*

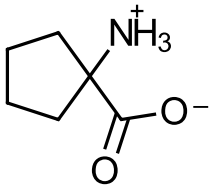

Fig. 3. *1-Aminocyclopentane-carboxylic acid (ACPCA)*

All 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 [2-4].

II.2. Phytotoxicological tests

Three cultural and three non-cultural plants and their growth stages [5] were used as follows:

• Cultural plants:

- Oil yielding rose (*Rosa damascena*), BBCH growth stage 19;
- Plum tree (*Prunus cerasifera*), BBCH growth stage 75;
- Grape (*Vitis vinifera*) variety Muller-Thurgau, BBCH growth stage 19.

• Non-cultural plants:

- Lime-tree (*Tilia platyphyllos*), BBCH growth stage 19;
- Chestnut (*Aesculus hippocastanum*), BBCH growth stage 19;

- Oval-leaved privet (*Ligustrum ovalifolium*), BBCH growth stage 19.

Standard phytotoxicity tests were conducted in accordance with OECD Guide 227 - Terrestrial Plant Test: Vegetative Vigour Test [6]. Test substances were sprayed on the plant and leaf surfaces with ten tested concentrations to the point of runoff. Ten concentrations of each compound were tested, with the saturated concentration of the given compound in distilled water.

Saturated concentrations of the compounds in water were as follows:

- CPSH – 1 %;
- CPSDTH – 0.025 %;
- ACPCA – 0.1 %.

Each test variant was set in five replicates. The test period was 21 days. The plants were weekly observed for visual phytotoxicity and mortality manifestation and Percentage Disease Indexes (PDIs) were calculated based on a 5-grade scale [7]. Based on PDI values, dose-response modeling was conducted by using R language for statistical computing [8] and R language drc package [9] in order to describe phytotoxic action of the compounds. Physical/chemical properties of tested chemicals, required for such ecotoxicological studies, were estimated by using The EPI (Estimation Programs Interface) Suite™ - Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by the EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC) [10].

III. Results and Discussion

The test results revealed that at the saturated concentration of the compounds in distilled water they do not cause any phytotoxic manifestations on Oil yielding rose (*Rosa damascena*), Plum tree (*Prunus cerasifera*), Grape (*Vitis vinifera*) variety Muller-Thurgau, i.e. examined cultural plants, as well as Oval-leaved privet (*Ligustrum ovalifolium*) from the non-cultural species.

However, all tested compounds were able to cause phytotoxic damages to Lime-tree (*Tilia platyphyllos*) in different concentration. CPSH substance showed deleterious effect on Chestnut (*Aesculus hippocastanum*). The rest of the compounds were not toxic at the saturated concentration in distilled water with regard to this plant species.

Fig. 4 shows acute phytotoxic manifestation of Lime-tree caused by CPSH compound at 1 % concentration with regard to the active substance.

Fig. 4. *Lime-tree, CPSH treated variant (1 % active substance)*

Fig. 5 shows the action of the same compound at 1 % concentration with regard to the active substance but on Chestnut tree.

Fig. 5. *Chestnut tree, CPSH treated variant (1 % active substance)*

The results from dose-response modeling, conducted with regard to the Lime-Tree, are as follows:

CPSH compound:

- NOAEC (LD_{05}) = 0.73 %;
- LOAEC (LD_{25}) = 0.85 %;
- LD_{50} = 0.94 %.

CPSDTH compound:

- NOAEC (LD_{05}) = 0.02 %;
- LOAEC (LD_{25}) = 0.05 %;
- LD_{50} = 0.10 %.

ACPCA compound:

- NOAEC (LD_{05}) = 0.060 %;
- LOAEC (LD_{25}) = 0.073 %;
- LD_{50} = 0.082 %.

Dose-response curves, created by drc package, are presented on Fig. 6, Fig. 7 and Fig. 8.

With regard to the Chestnut tree, only CPSH compound revealed an insignificant phytotoxic effect (Fig. 5) at the saturated concentration in distilled water (1%).

Values calculated by the drc package of NOAEC (LD_{05}), LOAEC (LD_{25}) and LD_{50} are as follows:

- NOAEC (LD_{05}) = 0.90 %;
- LOAEC (LD_{25}) = 2.56 %;
- LD_{50} = 4.56 %.

It is obvious from the dose-response modeling results presented that, regarding its phytotoxic action towards lime-tree and chestnut-tree, LD_{50} of CPSDTH compound is much higher than the saturated concentrations of substance in distilled water. This proves the relative safeness of these compounds towards selected terrestrial plants.

However, the CPSH and ACPCA substances revealed serious deleterious effect (especially CPSH) at the saturated concentrations in water which makes them potentially dangerous to plants.

CHSH compound did not manifest any phytotoxic signs on plants treated in saturated concentration in distilled water.

Physical/chemical and ecotoxicological properties of compounds, as calculated by EPI Suite software, are presented on Table 1 below.

Future research of CPSH and ACPCA compounds will be conducted in order to determine Predicted Environmental Concentrations, as well as to evaluate Toxicity Exposure Ratios (TERs) of chemicals towards terrestrial ecosystems and respective safeguards.

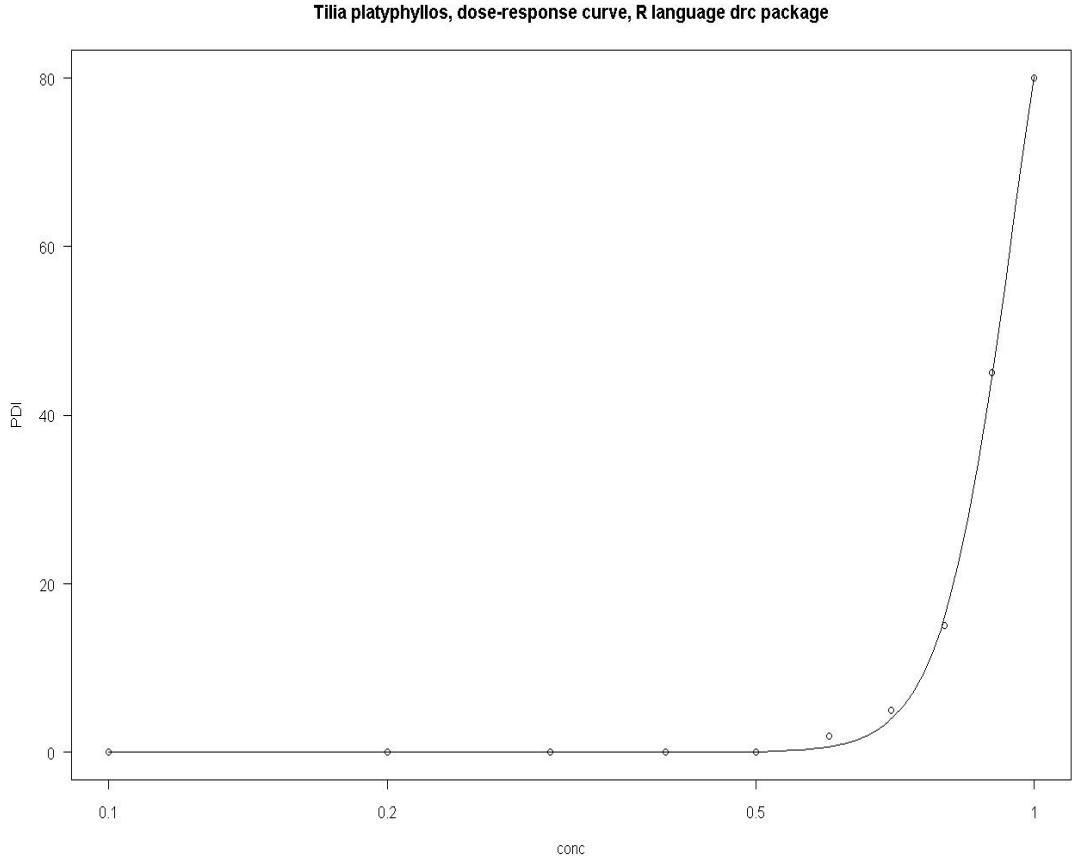

Fig. 6. *Tilia platyphyllos, CPSH compound Dose-Response Curve*

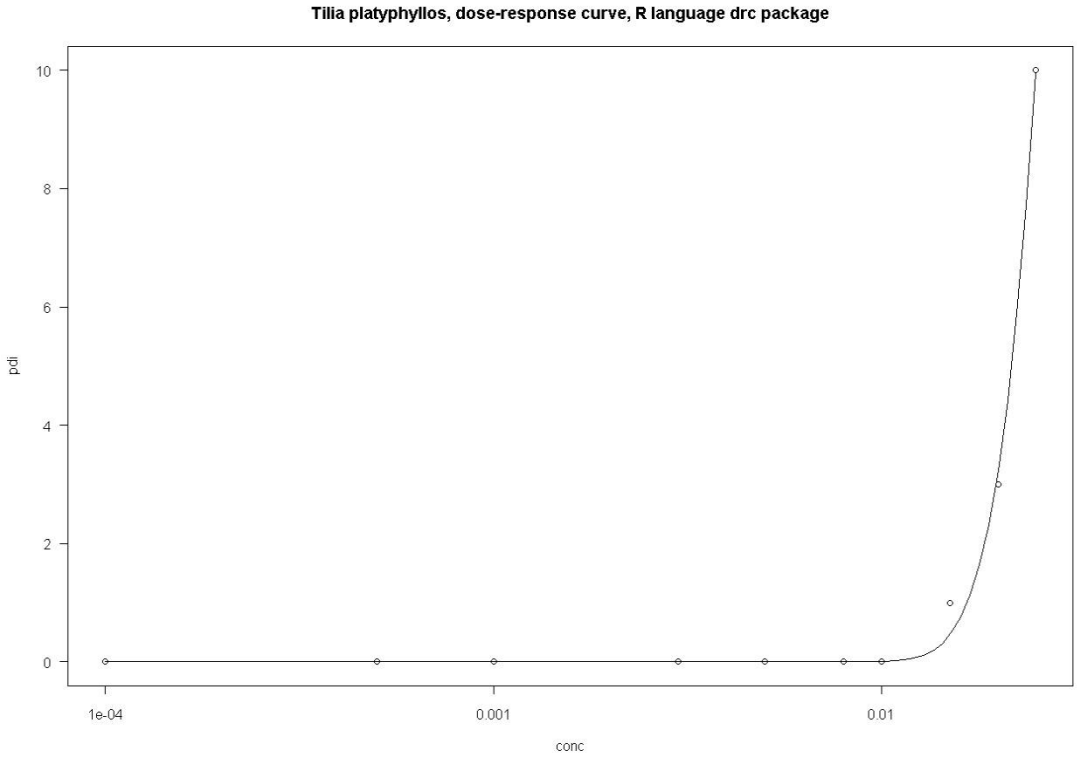

Fig.7. *Tilia platyphyllos, CPSDTH compound Dose-Response Curve*

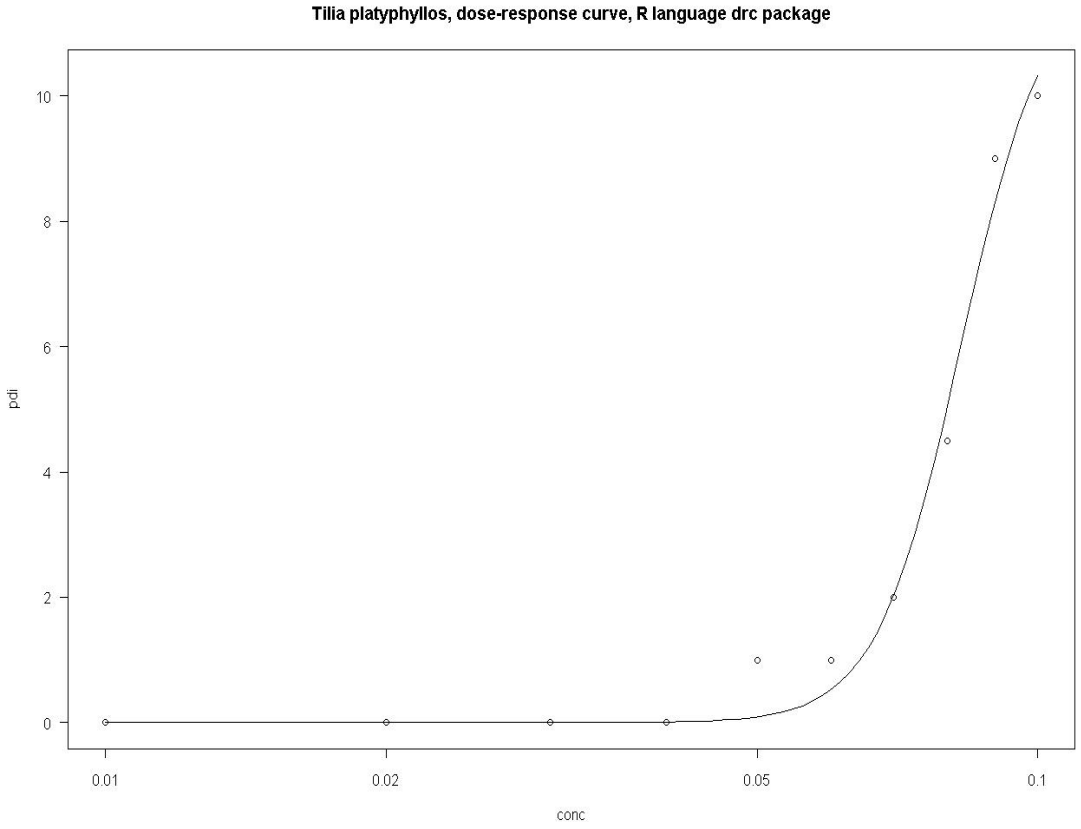

Fig. 8. *Tilia platyphyllos, ACPCAcompound Dose-respond curve*

Table 1. *Physical/chemical properties of tested compounds calculated by EPI Suite*

physical/chemical properties	CPSH	CPSDTH	ACPCA
Log Kow	0.60	0.12	-1.67
Vapour Pressure P (mm Hg,25 deg C)	3.78e-007	4.74e-007	9.49e-010
Melting Point (deg C):	164.17	158.29	330
Water Solubility at 25 deg C (mg/L)	2.245e+004	1987	6.982e+004
Henry's Law Constant (25 deg C) - atm-m^3/mole	2.15e-009	2.94e-007	1.54e-009
Soil Adsorption Coefficient (Koc)	10	10	1.808
DT_{50} Air (hours)	26.9	1.98	10.2
DT_{50} Water (hours)	900	900	360
DT_{50} Soil (hours)	1.8e+003	1.8e+003	720
DT_{50} Sediment (hours)	8.1e+003	8.1e+003	3.24e+003
Persistence Time (hours)	994	566	551

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