ИЗСЛЕДВАНЕ НА ФИТОТОКСИЧНОСТТА НА ЦИКЛОПЕНТАНСПИРО-5-ХИДАНТОИН И НЕГОВИ ПРОИЗВОДНИ СПРЯМО ПШЕНИЦА
PHYTOTOXICITY STUDY OF CYCLOPENTANESPIRO-5-HYDANTOIN AND ITS DERIVATIVES TOWARDS WHEAT

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Резюме

Пшеницата е икономически най-важната селскостопанска култура за района на България с ключова роля за аграрния бизнес. В предишно наше изследване колективът установи, че циклопентанспиро-5-хидантоин (CPSH), циклопентанспиро-5-(2,4-дитиохидантоин) (CPSDTH) и 1-аминоциклопентанкарбоксилната киселина (ACPCA) проявяват силна антифунгална активност срещу един от най-важните фитопатогени по пшеницата - *Blumeria graminis f. sp. tritici* (брашнеста мана по пшеницата).

В настоящото проучване се изследва наличието на евентуално фитотоксично действие на тези вещества с цел определяне на тяхното безопасно приложение в бъдещата растителнозащитна практика като синтетични фунгициди.

Abstract

Wheat is the most important culture in economic terms for Bulgaria, playing a key role in agricultural business. In a previous investigation we found that the cyclppentanspiro-5-hydantoin (CPSH) cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH) and 1-aminocyclopentanecarboxylic acid (ACPA) show strong antifungal activity against one of the most important wheat phytophatogens - *Blumeria graminis f. sp. tritici* (wheat powdery mildew).

The present study investigates the eventual phytotoxic action of those compounds on wheat plants in order to determine their safe applications in future practices as promising synthetic fungicides.

Ключови думи: фитотоксичност, пшеница, циклопентанспиро-5-хидантоин, циклопентанспиро-5-(2,4-дитиохидантоин), 1-аминоциклопентанкарбоксилна киселина.
Key words: phytotoxicity, wheat, cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), 1-aminocyclopentanecarboxylic acid.

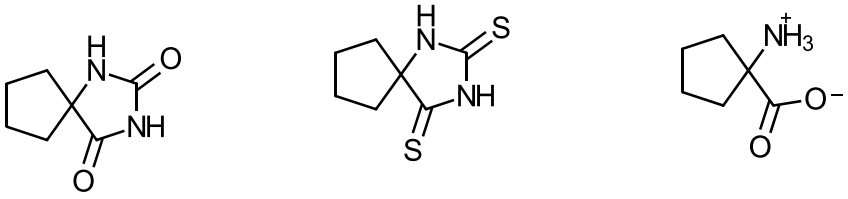
INTRODUCTION

The investigations for phytotoxic action of toxicants and plant protection products under development have a key role for the ecotoxicological characterization of compounds. These studies ensure the products safety according to non-target plant organisms (treated plants) and prevent the eventual damages on crops as result of treatments with pesticides.

The compounds used for this investigation are presented in Figure 1.

MATERIALS AND METHODS

All used chemicals were purchased from Merck and Sigma-Aldrich. The melting points were determined with a Koffler apparatus and with a digital melting point apparatus SMP 10. The 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 +



cyclopentanespiro-5-hydantoin (CPSH) | cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH) | 1-aminiocyclopentanecarboxylic acid (ACPCA)

Fig. 1

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 (300 K). The cyclopentanespiro-5-hydantoin (CPSH, Figure 1) was synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb, 1934). The cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH, Figure 1) was synthesized in accordance to Marinov et al. (Marinov et al., 2012). The 1-aminocyclopentanecarboxylic acid (ACPCA, Figure 1) was obtained according to Stoyanov and Marinov (Stoyanov and Marinov, 2012). All 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 (Marinov et. al., 2012; Stoyanov and Marinov, 2012; Marinov et al., 2005; Enchev et al., 1999).

Standard phytotoxicity tests were conducted with accordance of OECD Guide 227 - Terrestrial Plant Test: Vegetative Vigour Test (OECD Test No. 227, 2006) and Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD Test No. 208, 2006).

Plants from wheat variety "Kristi" were grown from seeds to the 75 growth stage (Meier, 2001) using plotting soil with organic carbon content 1.5% and pH=7.0 Test substances were sprayed on the plant and leaf surfaces at tested ten concentrations to the point of run off. Each test variant was set in five replicates. The test period was 21 days. The plants were weekly observed for visual phytotoxicity and mortality manifestations. At the end of the test, measurement of biomass, surviving plants, shoots and roots height were conducted.

Untreated seeds from wheat variety "Kristi" were placed on wet filter paper discs in the standard 15 cm diameter Petri dishes – 10 seeds per container.

Filter paper discs were wetted with test substances in ten concentrations. Each Petri dishes represent one test variant. Each test variant was set in five replicates. Duration of test was 21 days. After test period the plants were observed and measurement as presence of visual damages on shoots and roots, percent seed germination, fresh biomass, length of shoots and roots were conducted.

On the base of obtained results, chemotherapeutic indexes as ratio between NOAEL and minimum effective concentration according to wheat powdery mildew were calculated for each compound.

The required for such kind ecotoxicological investigations physical/chemical properties of tested chemicals were estimated 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) (Environmental Protection Agency for EPI Suite™, 2011).

RESULTS AND DISCUSION

The results from "Vegetative Vigour Test" show that at the most saturated concentration of the compounds in water – 0.01% for CPSH, 0.025% for CPSDTH and 0.1% for ACPCA (according to the active substances), tested compounds show strong phytotoxic effect. At these concentrations, at the end of the test (21 days after treatment) they were able to completely destroy the plants. However, CPSH at 0.03 %, CPSDTH at 0.00075 % and ACPCA at 0.003 % did no manifest any visual phytotoxic signs. The conducted ANOVA analysis with R language for statistical computing (R Development Core Team, 2011) show no significant differences in plant biomass, shoots height and roots height ($p < 0.05$) of tested compounds at these concentrations.

Calculated chemotherapeutic coefficients were as follows:

- For CPSH – 3
- For CPSDTH – 241.93
- For ACPCA – 60

On figures below are presented the dose-response models according to the biomass of tested plants received from conducted "Seedling Emergence and Seedling Growth Test". The modeling and establishing the NOAEL (LD_{05}), LOAEL (LD_{25}) and LD_{50} was carried out with R language

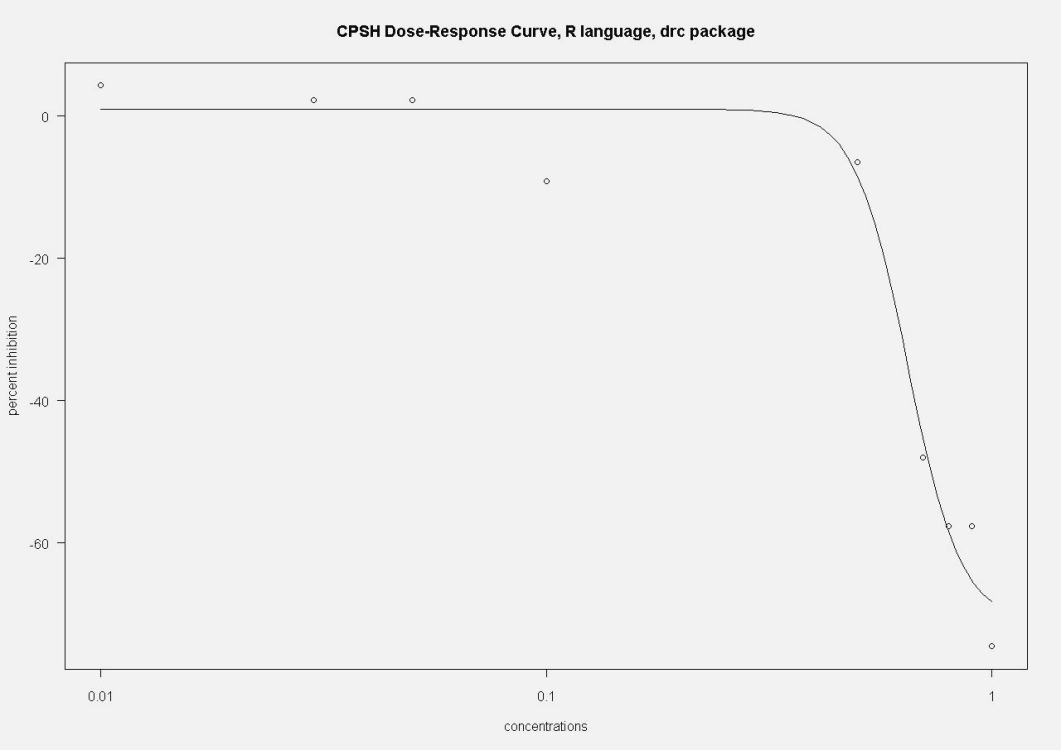


Fig. 2. *Dose – Response Curve – CPSH compound*

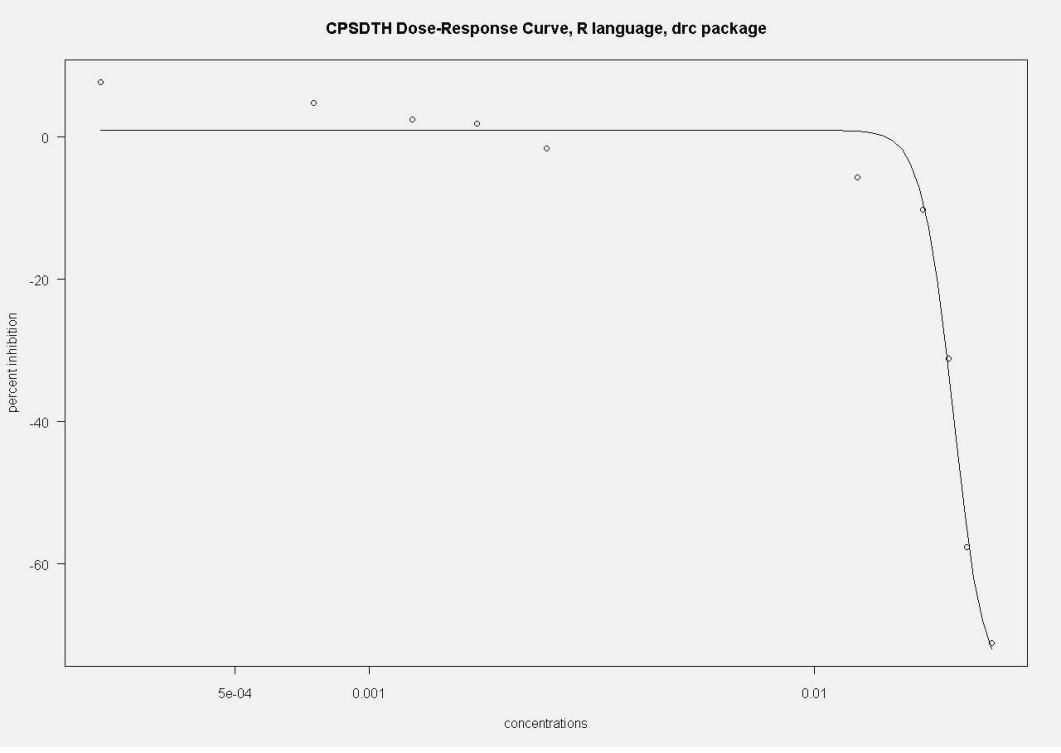


Fig. 3. *Dose – Response Curve – CPSDTH compound*

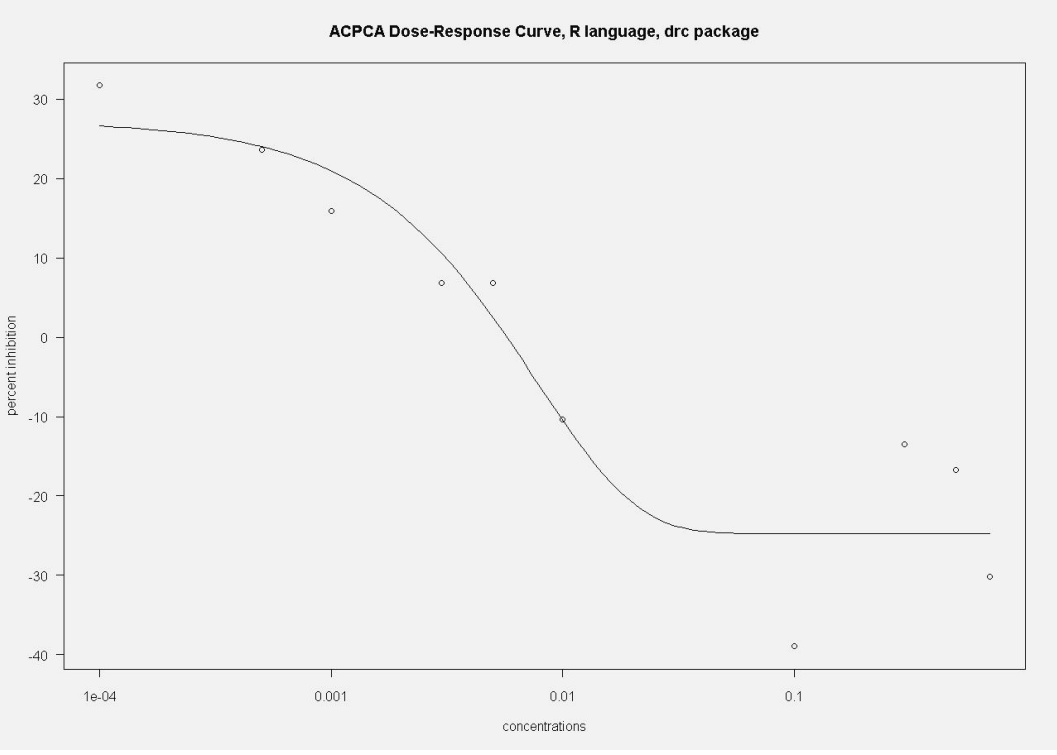


Fig. 4. *Dose – Response Curve – ACPCA compound*

for statistical computing and R language drc package (Ritz and Streibig, 2005).

For model fitting, R language, drc package, drm() function for general model fitting function for concentration/ dose/time-response models was used. The values of the NOAEL (LD_{05}), LOAEL (LD_{25}) and LD_{50} were calculated with ED() function for estimation of effective doses (ECp/EDp/ ICp) for given reponse levels. The Dose – Response Curves were created by function plot() with secondary argument – function drm() and it's parameters.

Figure 2 shows Dose – Response Curve of CPSH compound.

Chemotherapeutic coefficient for CPSH according to seedling emergence and seedling growth test is 43.3.

Figure 3 shows Dose – Response Curve of CPSDTH compound.

Chemotherapeutic index for CPSDTH according to seedling emergence and seedling growth test is 5193.5.

Figure 4 shows Dose – Response Curve of ACPCA compound.

Table 1

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
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

Chemotherapeutic coefficient for ACPCA according to seedling emergence and seedling growth test is 7.9.

Calculated physical/chemical and ecotoxicological properties of compounds by EPI Suite software are presented on Table1.

CONCLUSIONS

The received results from conducted tests clearly show that the safest product for plants is CPSDTH with chemotherapeutic coefficients 241.93 and 5193.5 respectively. The CPSH with chemotherapeutic coefficient from "Plant Vigor Test" 3 is too risky to be used as eventual fungicide because any mistake with calculation of the proper application concentration can lead to the phytotoxic damages on plants. However according to the seedlings emergence and growth, the compound is much more safe - chemotherapeutic coefficient 43.3. The ACPCA show relatively safeness for plants (60 chemotherapeutic coefficient from "Plant Vigor Test") but the trials reveal that this product is too toxic according to wheat seeds - chemotherapeutic coefficient 7.9. The data calculated by EPI Suite physical/chemical and ecotoxicological properties indicate the necessity for future investigations on environmental fate of tested compounds in order to be revealed their behavior in the environment and PECs (Predicted Environmental Concentrations) for air, water, soils and sediments.

ACKNOWLEDGEMENTS

Financial support by the Agricultural University – Plovdiv, Bulgaria (Contract 06-12) is gratefully acknowledged.

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Статията е приета на 10.12.2012 г.
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