Original Contribution

Journal scientific and applied research, vol. 3, 2013
Association Scientific and Applied Research
International Journal

ISSN 1314-6289

PHYTOTOXICOLOGICAL STUDY OF SOME SPIROHYDANTOINS AND THEIR DERIVATIVES TOWARDS *PSEUDOCROSSIDIUM REVOLUTUM*

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Abstract: *This article presents a novel ecotoxicological investigation of probable deleterious effect of cyclopentanespiro-5-hydantoin, cyclohexanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards some of the most widespread moss species in the World – Pseudocrossidium revolutum.. Dose-response modeling was carried out by R language for Statistical Computing, drc package.*

Key words: *spirohydantoins, Pseudocrossidium revolutum , phytotoxicology, drc, R language*

I. Introduction

Hydantoins and their different derivatives are compounds which possess antitumor [3], anticonvulsant, antiepileptic [4], antiarrhythmic [5] and aldose reductase inhibiting properties [1, 2].

The interest in studying these substances is also due to their good ability to coordinate ions of transitional metals and to participate in obtaining complexes [6, 7] which is related to future investigations of their potential biological activity.

Pseudocrossidium revolutum (Pseudocrossidium moss) is frequently found in variety of habitats as mortar or limestone walls, limestone outcrops and quarries and other stony grounds. It is a fairly common moss species in Europe and the World [8, 9].

Moss species as common terrestrial habitats are extremely vulnerable when exposed to various toxicants [10, 11]. However, the moss has a significant role in maintaining equilibrium of ecosystems.

This paper represents a novel ecotoxicological investigation of probable deleterious effects of cyclopentanespiro-5-hydantoin, cyclohexanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards some of the most widespread moss species in the World *Pseudocrossidium revolutum*.

II. Materials and methods

II.1. Synthetic compounds

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

The cyclopentanespiro-5-hydantoin (Fig. 1, a) and cyclohexanespiro-5-hydantoin (Fig. 1, b) were synthesized *via* the Bucherer-Lieb method [12].

The cyclopentanespiro-5-(2,4-dithiohydantoin) (Fig. 1, c) was synthesized in accordance with Marinov et. al. [13].

The 1-aminocyclopentanecarboxylic acid (Fig. 1, d) was obtained in accordance with Stoyanov and Marinov [14].

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

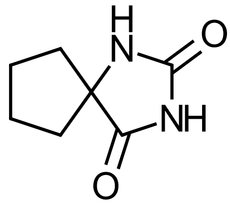
Elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106.

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.

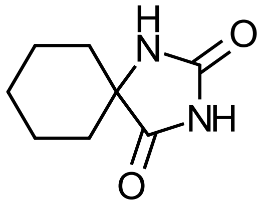
Chemical shifts were referenced to tetramethylsilane (TMS). Measurements were carried out at ambient temperature.

a)



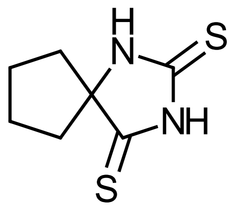
cyclopentanespiro-5-hydantoin (CPSH)

b)



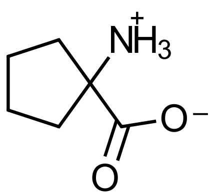
cyclohexanespiro-5-hydantoin (CHSH)

c)



cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH)

d)



1-aminocyclopentane-carboxylic acid (ACPCA)

***Fig. 1.** Structures of the compounds*

All products obtained were characterized by physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical to previously published in the literature [6, 13, 14].

II.2. Ecotoxicological tests

Naturally occurring moss colonies were taken from stony habitats in the town of Plovdiv, Bulgaria. The colonies were transferred in a standard 50 mm diameter Petri dishes with filter paper covered bottoms soaked in distilled water for 1 week, for acclimatization under laboratory conditions.

Ten different dilutions were prepared from each test compound. Filter paper disks were soaked in these solutions and were subsequently placed at the bottom of Petri dishes.

Saturated concentrations of the compounds in water were as follows:

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

A 20 mm piece of moss colony was placed in each Petri dish. The moss pieces were preliminarily weighed and soaked for 10 s in given test solution. Distilled water was used as a control variant.

After 7 days an observation of visual phytotoxic manifestations was made in regard to whitening, chlorosis and necrosis. Weight of colonies was also measured.

Based on the weight we used the formula of Abbot [15] to calculate the percent inhibition. A dose-response modeling was conducted with R language for Statistical Computing – drc package [16, 17].

III. Results and discussion

All tested compounds when in a saturated concentration in water did not have any phytotoxicological adverse effect on tested plants.

However, when soragosilicone surfactant Silwet ®L-77 – 95.5 % polyalkilene oxide modifed heptamethyltrisiloxane (Hellena Chemical Company) was added to each dilution in order to improve the wetting ability of the solutions at 0.25 % v/v concentration, all tested variants (including control variant) manifest extremely severe phytotoxicity including total necrosis of plants and a weight loss between 55-60 %.

The control variant which was set without Silwet ®L-77 did not show any signs of phytotoxicity.

Additional test with Silwet ®L-77 was conducted in ten concentrations prepared with distilled water, between 0.5 % v/v and 0.01 % v/v. All tested dilutions cause necrosis of plants except in 0.03 % and 0.01 % – Fig 2. The dose-response modeling conducted is presented on Fig. 3, Fig. 4 and Fig. 5.

Fig. 2. *Control variant, Silwet ®L-77 – 0.1 %, Silwet ®L-77 – 0.01 %*

```
> summary(drm(per_inhib~conc, data=silwet, fct=LL.3()))

Model fitted: Log-logistic (ED50 as parameter) with lower limit at 0 (3 parms)

Parameter estimates:

                Estimate Std. Error    t-value p-value
b:(Intercept) -7.0474483  1.3833907 -5.0943296  0.0014
d:(Intercept) 55.0072093  1.9615933 28.0421077  0.0000
e:(Intercept)  0.0836933  0.0020153 41.5293536  0.0000

Residual standard error:

 3.383857 (7 degrees of freedom)
> |
```

Fig. 3. *Dose-Response Modeling – drc package, R language*

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> ED(drm(per_inhib~conc, data=silwet, fct=LL.3()), c(5, 25, 50), interval="delta")

Estimated effective doses
(Delta method-based confidence interval(s))

     Estimate Std. Error     Lower  Upper
1:5  0.0551114  0.0045545 0.0443417 0.0659
1:25 0.0716126  0.0026200 0.0654173 0.0778
1:50 0.0836933  0.0020153 0.0789279 0.0885
> |
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Fig. 4. *EDx estimation - drc package, R language*

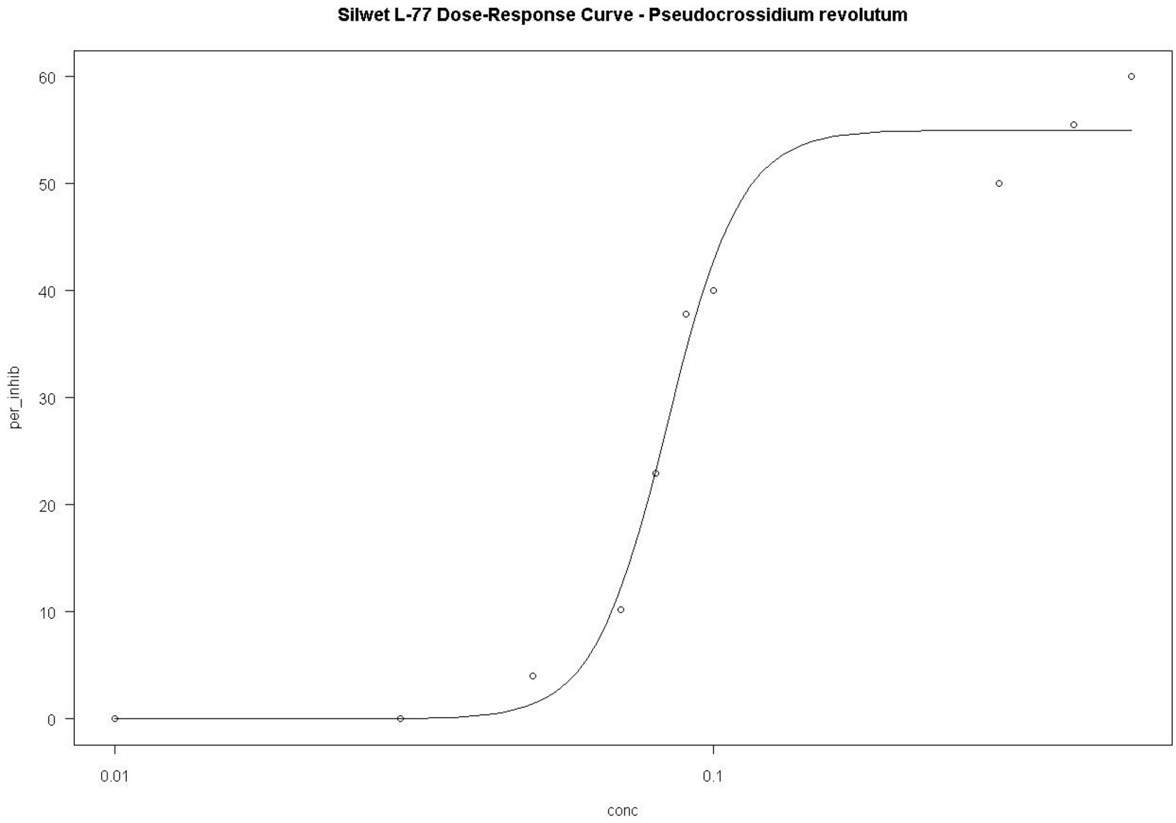

Fig. 5. *Dose-Response Curve - drc package, R language*

IV. Conclutions

Trials conducted reveal the compounds' safety with regard to populations of *Pseudocrossidium revolutum* moss. They do not cause any phytotoxic manifestations in the saturated concentration in water. On the other hand, tests showed extremely severe phytotoxic action of one of the most popular agricultural adjuvants to mosses.

Although, recommended rate of Silwet ®L-77 is 0.25 % v/v concentration in water, the trials reveal that NOAEC (LD_{05}) is 0.055 % v/v (LD_{50} = 0.08 % v/v) which is far below this value. This means that application and use of organosilicone sufactant must be conducted with a due care to the environment, especially when farm lands are close to nature parks and reserves.

Acknowledgements

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

We are grateful also to Mr. G. Marinov, Sofia for stimulating discussions.

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