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IN VIVO INSECTICIDAL ACTIVITY OF CYCLOPENTANESPIRO-5-HYDANTOIN AND ITS TWO DERIVATIVES TOWARDS OLEANDER APHID (*APHIS NERII*) AND EFFECT ON *BUDDLEJA DAVIDII*

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***Abstract**: This paper presents in vivo insecticidal activity of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards Oleander aphid (Aphis nerii) and effect on Buddleja davidii. It was found out that the most toxic (effective) compound towards Aphis nerii is cyclopentanespiro-5-(2,4-dithiohydantoin). The safest compound towards Buddleja davidii was identified to be 1-aminocyclopentanecarboxylic acid. The dose-response modeling was conducted with R language for statistical computing and drc package.*

***Key words:** cyclopentanespiro - 5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), 1-aminocyclopentanecarboxylic acid, Oleander aphid (Aphis nerii), Buddleja davidii, drc, R language*

I. Introduction

A low toxicity as well as low sedative activity of cyclopentane-spiro-5-hydantoins is established by Oldfield and Cashin [1].

The 1-aminocyclopentane-carboxylic acid possesses antitumor activity with rodents [2-5] and is useful in the treatment of plasmocytic myeloma [6, 7].

The cyclopentanespiro-5-(2,4-dithiohydantoin) is a compound with a strong insecticidal activity against *Cladius pectinicornis*, which is an economically significant pest on roses [8].

All the compounds mentioned above have shown fungicidal activity against *Blumeria graminis* f. sp. *tritici* [9].

The Oleander aphid is a commonly found pest on oleander, butterfly weed and milkweed, appearing on buds, new shoots and foliage in the spring. Large colonies often develop during the summer and may cause damage or death to the host plant [10].

In this study we investigate the insecticidal activity of cyclopentane-spiro-5-hydantoin, cyclopentane-spiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid

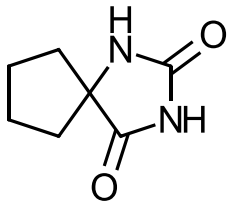
towards this pest as well as the effect of these substances on infected plant species – butterfly-bush (*Buddleja davidii*), an appreciated and widespread plant in Bulgaria.

II. Materials and methods

II.1. Synthetic compounds

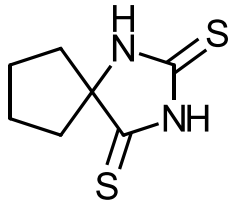
All chemicals used were purchased from Merck and Sigma-Aldrich. The cyclopentanespiro-5-hydantoin (Fig. 1, a) was synthesized *via* the Bucherer-Lieb method [11]. The cyclopentanespiro-5-(2,4-dithiohydantoin) (Fig. 1, b) was synthesized in accordance with Marinov et al. [8]. The 1-aminocyclopentanecarboxylic acid (Fig. 1, c) was obtained in accordance with Stoyanov and Marinov [12]. 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)



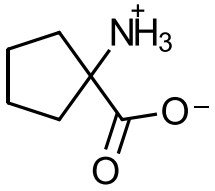
Cyclopentanespiro-5-hydantoin (CPSH)

b)



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

c)



1-Aminocyclopentane-carboxylic acid (ACPCA)

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 those previously published in the literature [12-14].

II.2. Ecotoxicological tests

A branch *in vivo* insecticidal test [15] was performed in order to establish the insecticidal activity of the tested compounds and their effect on infested plant species.

Saturated concentrations of the compounds in water were as follows:

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

Ten different concentrations were tested to determine LC_{05} (NOEL), LC_{25} (LOAEL), LC_{50} and LC_{90}. Each test variant consisted of five replications. The replications of each variant were sprayed with a solution of the tested product in tested concentration using a laboratory sprayer with a delivery rate of 1000 l ha^{-1}. Mortality was observed after 24 h and 48 h. Effectiveness was measured using the formula of Abbott [16]. Data received from the conducted tests was statistically processed with R language for statistical computing [17] and drc R language package [18]. Standard phytotoxicity tests were conducted in accordance with OECD Guide 227 - Terrestrial Plant Test: Vegetative Vigour Test [19]. The test period was 7 days. The plants were weekly observed for visual phytotoxicity manifestations (necrosis, chlorosis, whitening, deformations). Percentage Disease Indexes (PDIs) on this base with 5 grade scale were calculated [20].

Based on PDIs, LC_{05} (NOEL), LC_{25} (LOAEL), LC_{50} were determined using R language and drc package. Chemotherapeutic indexes were calculated as a ratio between LC_{50} obtained from phytotoxicity test and LC_{90} from insecticidal test.

III. Results and Discussion

Dose-response curves from the conducted insecticidal tests are depicted in Figures 2 to 4.

Toxicological indicators of CPSH are established as follows:

1. NOAEL (No Observed Adverse Effect Level) LC_{05} = 0.16 %;
2. LOAEL (Lowest Observed Adverse Effect Level) LC_{25} = 0.28 %;
3. LC_{50} = 0.38 %;
4. LC_{90} = 0.74 %.

Toxicological indicators of CPSDTH are established as follows:

1. NOAEL (No Observed Adverse Effect Level) LC_{05} = 0.0034 %;
2. LOAEL (Lowest Observed Adverse Effect Level) LC_{25} = 0.0038 %;
3. LC_{50} = 0.006 %;
4. LC_{90} = 0.014 %.

Toxicological indicators of ACPCA are established as follows:

1. NOAEL (No Observed Adverse Effect Level) LC_{05} = 0.012 %;
2. LOAEL (Lowest Observed Adverse Effect Level) LC_{25} = 0.029 %;
3. LC_{50} = 0.045 %;
4. LC_{50} = 0.045 %.

It is clearly evident, from the results presented, that the most toxic (effective) compound towards *Aphis nerii* is CPSDTH (LC_{90} = 0.014 %) followed by ACPCA (LC_{90} = 0.045 %). CPSH is less toxic (LC_{90} = 0.74 %). However, even at this concentration – 0.74 %, it means that this tested compound is as effective as many of the commercially available modern products for plant protection.

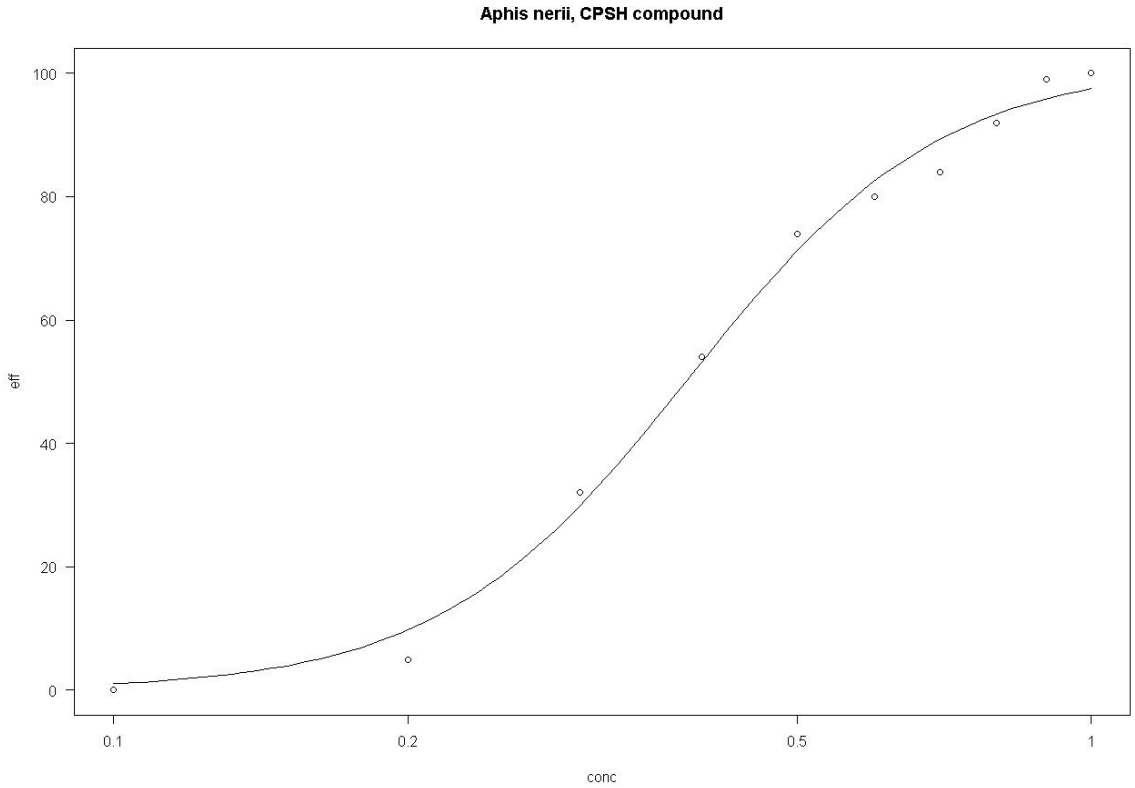


Fig. 2. *Aphis nerii, CPSH compound dose-response curve*

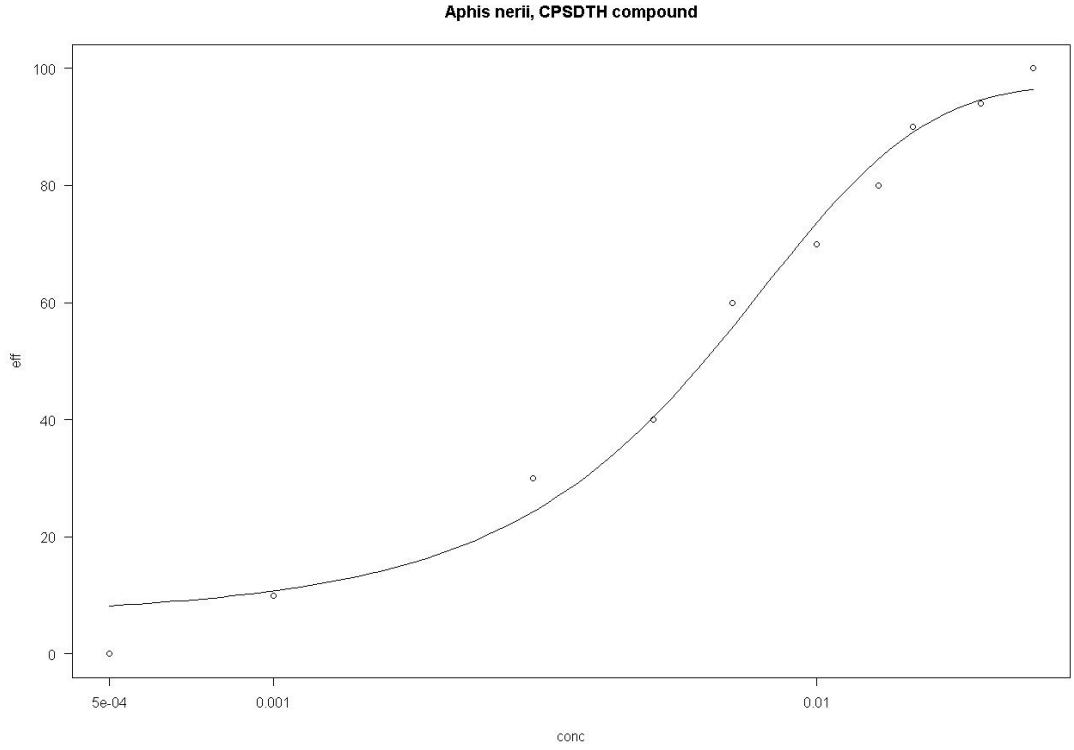


Fig. 3. *Aphis nerii, CPSDTH compound dose-response curve*

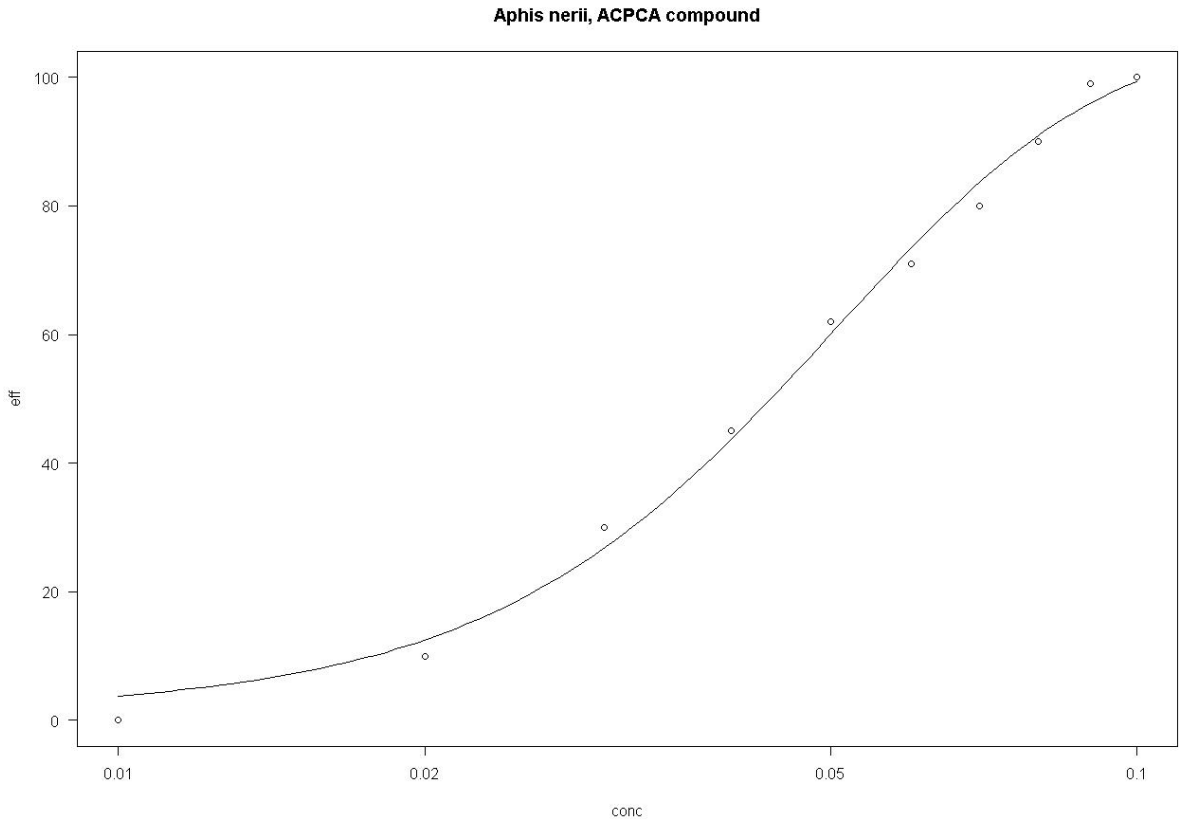


Fig. 4. *Aphis nerii, ACPCA compound dose-respond curve*

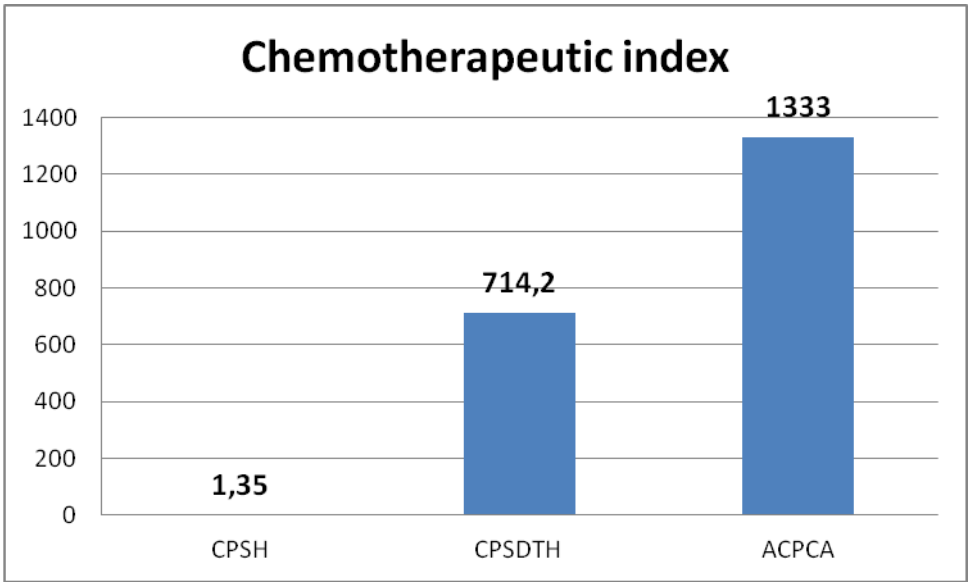


Fig. 5. *Chemotherapeutic indexe*

Figure 5 depicts phytotoxicity test conducted with a plant - *Buddleja davidii*, infested with *Aphis nerii*.

It is clear from phytotoxicity tests conducted that the ACPCA compound is the safest to a plant treated with a combination of strong

insecticidal activity to *Aphis nerii* (LC_{50} = 0.045 %). The most toxic compound to the tested aphid specie CPSDTH (LC_{50} = 0.014 %) also has a very high Chemotherapeutic index – 714.2.

Although the CPSH possesses the lowest insecticidal effectiveness (LC_{50} = 0.74 %), it has a very low Chemotherapeutic index – 1.35, which means that this compound is dangerous as a plant protection product. It can be concluded from these results that the ACPCA and CPSDTH are promising novel compounds which can be further developed as insecticidal plant protection products.

Acknowledgements

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