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ИЗСЛЕДВАНЕ НА ТОКСИЧНОСТТА НА СПИРОХИДАНТОИНОВИ ПРОИЗВОДНИ СПРЯМО *HYPERA POSTICA* (GYLLENHAL, 1813) (COLEOPTERA: CURCULIONIDAE)
INVESTIGATION OF *SPIROHYDANTOIN* DERIVATIVES TOXICITY AGAINST *HYPERA POSTICA* (*GYLLENHAL*, 1813) (*COLEOPTERA: CURCULIONIDAE*)

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Abstract

The alfalfa weevil, *Hypera postica* (Gyll.) (*Coleoptera: Curculionidae*) is one of the most important pests on alfalfa, *Medicago sativa* L., a valuable forage crop in Bulgaria.

This article presents *in vitro* evaluation of the insecticidal activity of cyclohexanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) and 1-aminocyclo-pentanecarboxylic acid on the 3-rd instar larvae of the tested pest.

The compounds used were characterized through physicochemical parameters, IR and NMR spectral data.

It was found

that the cyclohexanespiro-5-hydantoin and the 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) manifest strong effectiveness towards the tested pest.

The mortality percentage of the larvae was calculated with the Abbott’s formula and statistically analyzed by R language for Statistical Computing, drc package for Dose-Response evaluation.

Key words: *Hypera postica* (Gyll.), insecticidal activity, spirohydantoin derivatives, drc package, R language.

INTRODUCTION

The alfalfa weevil *Hypera postica* (Gyll.) (Coleoptera: Curculionidae) is one of the most important pests of alfalfa, *Medicago sativa* L., a valuable forage crop in Bulgaria. Some authors, Popova (1968), Naidenova and Donschev (1995), Ivanova (2004) and Atanasova (2012) reported alfalfa weevil as the most destructive pest on alfalfa crop in Bulgaria.

Both adults and larvae feed on the growing tips, leaves and buds of alfalfa, which removes crop biomass and reduces harvested yield. Third and fourth instars account for most (> 90%) of the feeding (Koehler and Pimentel 1973). The most significant feeding injury occurs to the first crop of alfalfa (Liu and Fick, 1975), however, under heavy infestations, regrowth of the second crop can be affected by larval and adult feeding on stubble (Fick and Liu, 1976). Alfalfa weevil feeding injury can result in reductions in shoot length, yield, forage quality, and stand persistence (Berberet and McNew, 1986; Wilson and Quisenberry, 1986; Summers, 1998).

A lot of measures have been examined to manage alfalfa weevil populations. Tolerant cultivars are currently available, often do not provide sufficient protection from alfalfa weevil larval damage (Blodegett et al., 2000). Although biological agents have reduced weevil populations below economic injury thresholds in most regions of the world (Richardson et al., 1971), application of insecticides has been an essential component of the control programs, and it has prevented economic damage to the alfalfa crop (Karimpour, 1994).

In order to prevent resistance, effective chemical control of the pest requires new insecticides with novel modes of action. Organophosphates, carbamates, pyrthroids (Armbrust and Grysco, 1965; Pass, 1966; Stenhauer and Blickenstaff, 1967; Windbiel et al., 2005) and different compounds such as dieldrin, diazinon, aldrin, trichlorfon and lindane have been used against the pest recently (Vodjdani and Daftari, 1963). In the late 60s, the weevil developed resistance to heptachlor and dieldrin, and these agents no longer provided satisfactory control (Alder and Blickenstaff, 1964; Dorsey, 1966). The effects of some other insecticides on the pest have been studied by several researchers (Esmaili, 1970; Habibi, 1976; Karimpour and Pourmirza, 2000).

The aim of the current study is the laboratory evaluation of the insecticidal activity of some synthetic compounds: cyclohexanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), 4-(2-hydroxyethylimino)-cyclopentane-spiro-5-(2-thiohydantoin) and 1-aminocyclopentanecarboxylic acid against alfalfa weevil, *Hypera postica* (Gyll.) (Coleoptera: Curculionidae), which is the most destructive pest on alfalfa crop in Bulgaria.

MATERIALS AND METHODS

Synthetic compounds

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

The cyclohexanespiro-5-hydantoin (Fig. 1) was synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb, 1934). The cyclopentanespiro-5-(2,4-dithiohydantoin) (Fig. 2) and the 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-

thiohydantoin) (Fig. 3) were obtained in accordance with Marinov et al. (Marinov et al., 2005). The 1-aminocyclopentanecarboxylic acid (Fig. 4) was obtained according to Stoyanov and Marinov (Stoyanov and Marinov, 2012). The compounds obtained were characterized through physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical with those previously published in the literature (Enchev et al., 1999; Marinov et al., 2005; Stoyanov and Marinov, 2012).

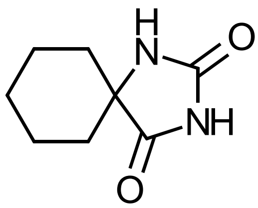

Fig. 1. *Cyclohexanespiro-5-hydantoin (CHSH)*
(Systematic name: 1,3-diazaspiro[4.5]decane-2,4-dione)

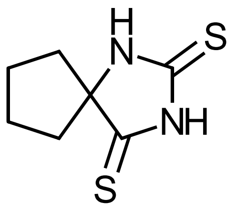

Fig. 2. *Cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH)*
(Systematic name: 1,3-diazaspiro[4.4]nonane-2,4-dithione)

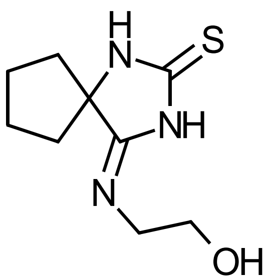

Fig. 3. *4-(2-Hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) (HEICPSTH)*
(Systematic name: 4-[(2-hydroxyethyl)imino]-1,3-diazaspiro[4.4]nonane-2-thione)

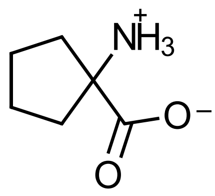

Fig. 4. *1-Aminocyclopentanecarboxylic acid (ACPCA)*

The concentrations of the compounds in water were as follows:
• cyclohexanespiro-5-hydantoin – 0.1 %;
• cyclopentanespiro-5-(2,4-dithiohydantoin) – 0.025 %;
• 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) – 0.6 %;
• 1-aminocyclopentanecarboxylic acid – 0.1 %.

Laboratory Bioassays

Laboratory experiments were conducted at 25±1 °C, 60±5 RH and photoperiod of 16:8 L:D conditions at the Laboratory of the Agricultural University of Plovdiv. A laboratory colony of *H. postica* was established in May 2014 from the 1st instars collected from an experimental field of the Agricultural University of Plovdiv. To assess the insecticidal activity of the compounds, different concentrations were prepared based on preliminary experiments.

The freshly moulted 3rd instar larvae were used in the experiments. The leaf dipping technique was used (Morse et al., 1986; Munger, 1942; Immaraju et al., 1989). Alfalfa leaves were dipped in different concentrations of tested insecticides for 30s, and left for air dryness. Each sample was transferred to a Petri-dish and provided with 15 number of 3rd instars by fine hair brush.

The control was only dipped in water. Each treatment was replicated four times. The used standard was Karate Zeon on base of lambda-Cyhalothrin – 0.00075 % according to the active substance. The mortality percentage of the larvae was recorded after 48 h, calculated with the Abbott's formula (Abbott, 1925) and statistically analyzed by R language for Statistical Computing (R Development Core Team, 2011), drc package (Ritz and Streibig, 2005) for Dose-Response evaluation.

RESULTS AND DISCUSSION

The conducted trials reveal the strong insecticidal action of two of the tested compounds: CHSH and HEICPSTH. The Dose-Response Curves are presented in the following graphics:

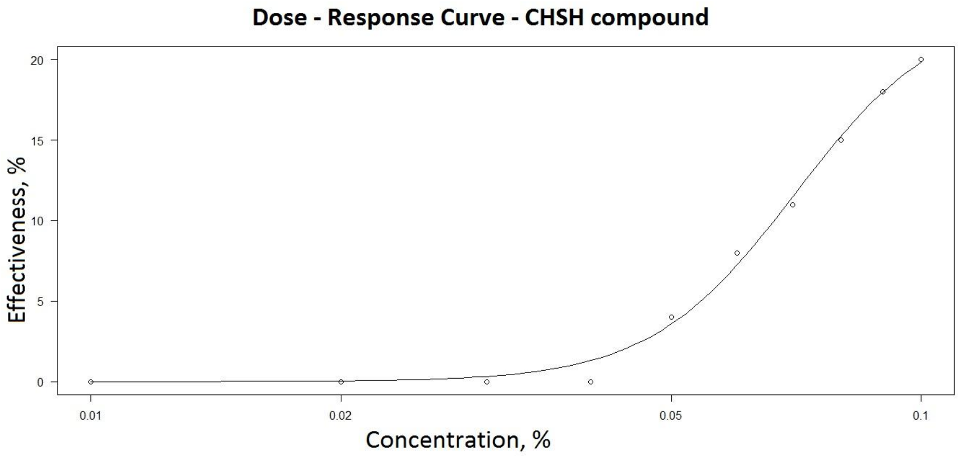

Fig. 5. *Dose-response curve of cyclohexanespiro-5-hydantoin (CHSH)*

The toxicological data are:

- NOAEC (No Observed Adverse Effect Concentration) = 0.03 %;
- LD_{50} = 0.07 %;
- LD_{90} = 0.1 %;
- AIC of the Model = 24.00.

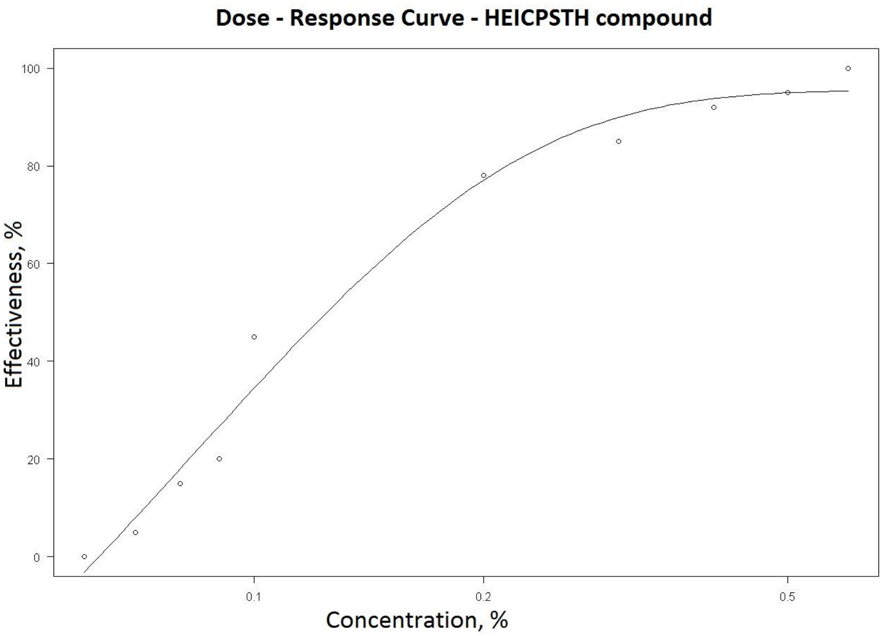

Fig. 6. *Dose-response curve of 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) (HEICPSTH)*

The toxicological data are:

- NOAEC (No Observed Adverse Effect Concentration) = 0.04 %;
- LD_{50} = 0.15 %;
- LD_{90} = 0.4 %;
- AIC of the Model = 73.84.

CONCLUSIONS

The received results show that according to the NOAEC there is almost no difference between CHSH and HEICPSTH. However according to the LD_{50} and LD_{90} the CHSH manifests insecticidal action at significantly lower concentrations. The used standard Karate Zeon was able completely to kill all tested individuals at registered concentration.

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