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IN VITRO SCREENING FOR INSECTICIDAL ACTIVITY OF CYCLOPENTANESPIRO-5-HYDANTOIN AND ITS FOUR DERIVATIVES AGAINST POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA* ZELLER (LEPIDOPTERA: GELECHIIDAE)

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Abstract

This article presents in vitro evaluation of the insecticidal activity of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin), cyclopentanespiro-5-(2-thiohydantoin) and 1-aminocyclopentanecarboxylic acid on the first instar larvae of the potato tuber moth Phthorimaea operculella Zeller (Lepidoptera: Gelechiidae). It was found out that the 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) manifest a strong effectiveness towards the tested pest.

Key words: *Phthorimaea operculella Zeller, insecticidal activity, cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin), cyclopentanespiro-5-(2-thiohydantoin), 1-aminocyclopentanecarboxylic acid, drc, R language*

1. INTRODUCTION

The potato tuber moth *Phthorimaea operculella* Zeller is one of the major economic pests of potato in tropical and sub-tropical regions. It is among the most destructive potato insect pest under both field and storage conditions (Haines 1977; Raman and Palacios 1982). The larvae feed on leaves and stems but they are most harmful to potato tubers (Varela and Bernays 1988). The heaviest infestations take place when potatoes are kept in stores, where the adult moth lays its eggs on the tubers (Raman et al. 1987). The potato tuber moth originates from Central and South America, and in the early 20th century invaded in Europe. In Bulgaria the pest is established for the first time in 1950 on potatoes in the area of Petrich (Stanev and Kaytazov 1962). Since 2008 there has been an expansion in the distribution of the species in the country, spreading in the regions of Kyustendil, Pernik, Samokov, Ichtiman, Pazardjik, Plovdiv, Smolyan, Burgas, Blagoevgrad, Kardzhali, Balchik and Dobrich (Vaneva-Gancheva and Grigorova 2010; Subchev et al. 2013).

The major control of the potato tuber moth is the chemical one. The control over this pest is very difficult due to its high reproductive capacity, polyvoltinity and adaptability to seasonal changes in the weather conditions. There are some reports about efficacy of insecticides from different groups against *Phthorimaea operculella* (Foot 1974; Foot 1975; Kroschel and Koch 1996; Kay 2006; Clough et al. 2010; Vaneva-Gancheva and Dimitrov 2013).

The available literature data till now for 1-aminocyclopentanecarboxylic acid describe antitumor activity in rodents (Connors et al. 1958; Goldin et al. 1961; Martel and Berlinguet 1959; Ross et al., 1961) and its usage in the treatment of plasmocytic myeloma (Benefiel et al. 1960; Krant et al. 1962). Moreover, carboxy-labeled ^{11}C-1-aminocyclopentanecarboxylic acid is a potential tumor-localizing agent for detecting cancer in humans by nuclear medicine scanning techniques (Hayes et al. 1976).

There can be mentioned that Oldfield and Cashin synthesized and tested for toxicity, anticonvulsant and analgesic activity in mice a number of different cycloalkanespiro-5-hydantoins. A low toxicity and low sedative activity was established for cyclopentanespiro-5-hydantoins (Oldfield and Cashin, 1965).

In previous works of ours we have investigated the insecticidal activity of cyclopentanespiro-5-hydantoin and its derivatives as follows: 1) *In vitro* insecticidal activity of cyclopentanespiro-5-(2,4-dithiohydantoin) against *Cladius pectinicornis* (Marinov et al. 2012), 2) *In vivo* insecticidal activity of cyclopentanespiro-5-hydantoin and its two derivatives towards Mealy plum aphid (*Hyalopterus pruni*) (Ganchev et al. 2013) and 3) *In vivo*

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insecticidal activity of cyclopentanespiro-5-hydantoin and its two derivatives towards Oleander aphid (*Aphis nerii*) (Marinov et al. 2013).

The aim of the current study is *in vitro* evaluation of the insecticidal activity of some synthetic compounds:

cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2, 4-dithiohydantoin), 4-(2-hydroxyethylimino) –

cyclopentanespiro-5-(2-thiohydantoin), cyclopentanespiro-5-(2-thiohydantoin) and 1-

aminocyclopentanecarboxylic acid against potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae).

2. MATERIALS AND METHODS

2.1. Synthetic compounds

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

The cyclopentanespiro-5-hydantoin (Figure 1) was synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb 1934). The cyclopentanespiro-5-(2,4-dithiohydantoin) (Figure 2), the 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) (Figure 3) and the cyclopentanespiro-5-(2-thiohydantoin) (Figure 4) were obtained in accordance with Marinov et. al (Marinov et al. 2005). The 1-aminocyclopentanecarboxylic acid (Figure 5) 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).

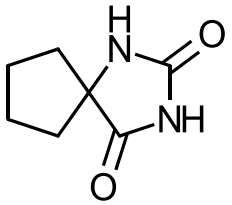


Figure 1. Cyclopentanespiro-5-hydantoin (CPSH)

(Systematic name: 1,3-diazaspiro[4.4]nonane-2,4-dione)

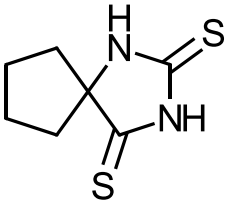


Figure 2. Cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH)

(Systematic name: 1,3-diazaspiro[4.4]nonane-2,4-dithione)

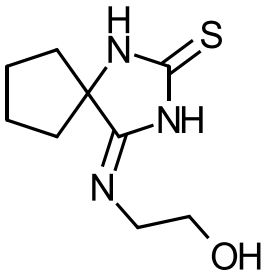


Figure 3. 4-(2-Hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) (HEICPSTH)
(Systematic name: 4-[(2-hydroxyethyl)imino]-1,3-diazaspiro[4.4]nonane-2-thione)

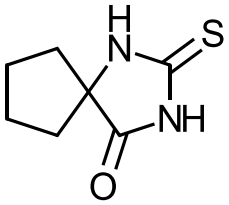


Figure 4. Cyclopentanespiro-5-(2-thiohydantoin) (CPSTH)
(Systematic name: 2-thioxo-1,3-diazaspiro[4.4]nonan-4-one)

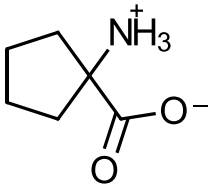


Figure 5. 1-Aminocyclopentanecarboxylic acid (ACPCA)

Saturated concentrations of the compounds in water were as follows:

• cyclopentanespiro-5-hydantoin – 1 %;

• cyclopentanespiro-5-(2,4-dithiohydantoin) – 0.025 %.

• 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) – 0.6 %;

• cyclopentanespiro-5-(2-thiohydantoin) – 0.06 %.

• 1-aminocyclopentanecarboxylic acid – 0.1 %.

2.2. Phthorimaea operculella

The laboratory population of the potato tuber moth was established by infested potato tubers, obtained from stores. The tubers were kept together with uninfested potato tubers in an insect-cage. Following emergence of moths, fresh tubers were provided for oviposition. The potato tuber moth was grow at a temperature of 26±1°C, 60±5% relative humidity and photoperiod of 16: 8 h (L : D) by the methodology of Rafiee-Dastjerdi et al. (Rafiee-Dastjerdi et al. 2013) at the Laboratory of Agricultural University, Plovdiv.

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Two adult pairs (♂ & ♀) were kept in plastic oviposition containers about 10 cm in diameter and 20 cm in height covered with fine gauze material fitted tightly over the opening with an elastic band. A filter paper as the substrate for better egg laying were used. It is placed flat on the upper outside of the fine gauze material with glass Petri-dish as weight on top which ensure contact of the filter paper with gauze material. Moths lay their eggs on the underside through the gauze material onto the paper. The filter paper is removed and replaced every morning. With this method, eggs can be removed without opening the container (Maharjan and Jung 2011). A piece of filter paper with one day old eggs were kept in Petri-dishes till hatching.

2.3. Bioassay

Laboratory bioassay was carried out to evaluate the insecticidal activity of some synthetic compounds against first instar larvae of the potato tuber moth *Phthorimaea operculella* (Zeller) using dipping method (Park et al. 2002).

Potato slices (1 cm thickness) were dipped in the tested products for 20 seconds and left for air dryness. Each slice was transferred to a Petri-dish and provided with 20 number of first instar larvae (1-2 days after being hatched) by fine hair brush. Each treatment was replicated four times. Mortality percentages were recorded daily and corrected according to 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.

3. RESULTS AND DISCUSSION

The initial cyclopentanespiro-5-hydantoin (Figure 1) was obtained *via* the Bucherer-Lieb method, based on the interaction between cyclopentanone, sodium cyanide, ammonium carbonate and ethanol (Bucherer and Lieb 1934). This product was subjected to a thionation with P_4S_{10} in a medium of xylene. As a result of this interaction, the cyclopentanespiro-5-(2,4-dithiohydantoin) (Figure 2) was synthesized (Marinov et al. 2005). The latter was treated with 2-aminoethanol to the 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) (Figure 3). Then, this compound was refluxed in 20 % HCl and the cyclopentanespiro-5-(2-thiohydantoin) (Figure 4) was obtained (Marinov et al. 2005). The cyclopentanespiro-5-hydantoin (Figure 1) was subjected to alkaline hydrolysis with barium hydroxide, resulted in hydantoin ring degradation and formation of 1-aminocyclopentanecarboxylic acid (Figure 5) (Stoyanov and Marinov 2012).

The conducted test revealed the lack of any insecticidal activity of the cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH) and 1-aminocyclopentanecarboxylic acid (ACPCA) evident by potato tuber moth at their saturated concentrations in water. No mortality was registered in the variants.

However, the 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) (HEICPSTH) manifests a strong effectiveness expressed on Dose-Response Curve depicted on Figure 6.

As is shown – typical S-shaped curve described the toxicity of the component towards *Phthorimaea operculella*. The toxicological indexed of HEICPSTH are as follows:

- NOAEC (No Observed Adverse Effect Concentration) = 0.02 %;
- LOAEC (Lowest Observed Adverse Effect Concentration) = 0.04 %;
- LC_{50} = 0.07 %;
- LC_{90} = 0.2 %.

The other products – cyclopentanespiro-5-hydantoin (CPSH) and cyclopentanespiro-5-(2-thiohydantoin) CPSTH also manifest insecticidal activity but at very low levels (Figures 7 and 8):

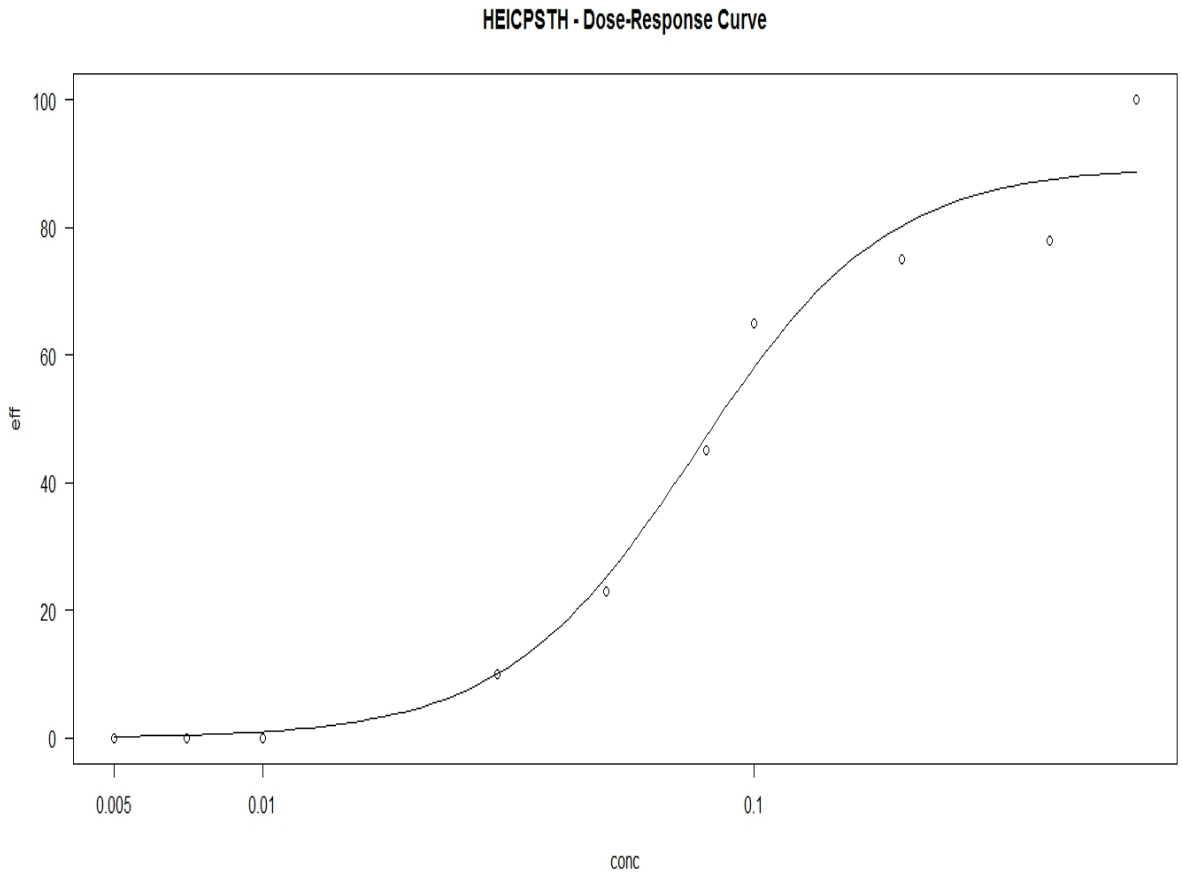


Figure 6. Dose-response curve of 4-(2-hydroxyethylimino)-cyclopentanespiro-5-(2-thiohydantoin) (HEICPSTH)

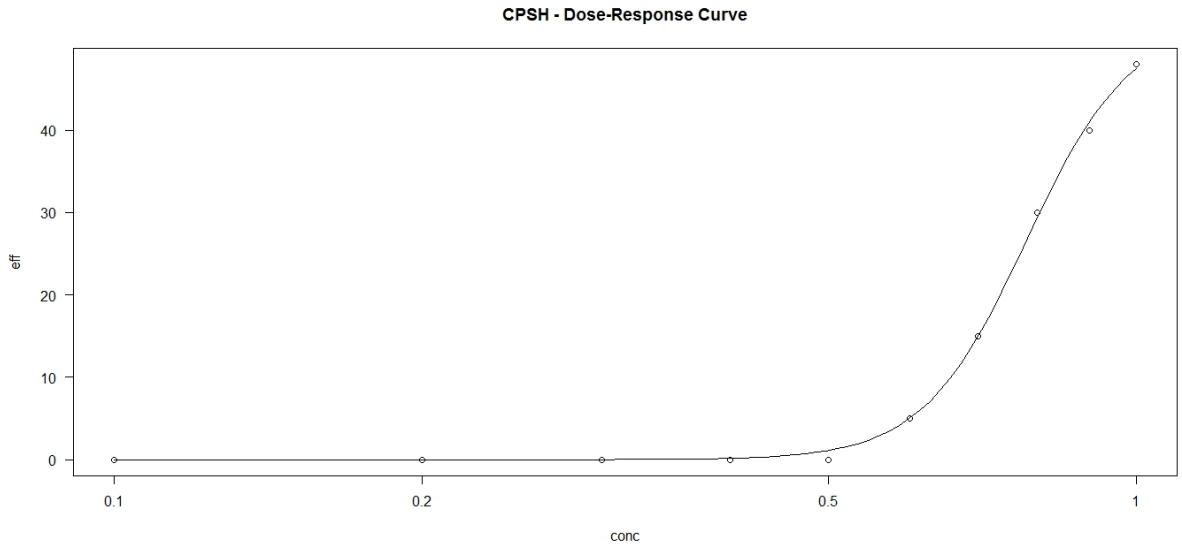


Figure 7. Dose-response curve of cyclopentanespiro-5-hydantoin (CPSH)

- NOAEC (No Observed Adverse Effect Concentration) = 0.55 %;
- LOAEC (Lowest Observed Adverse Effect Concentration) = 0.68 %.

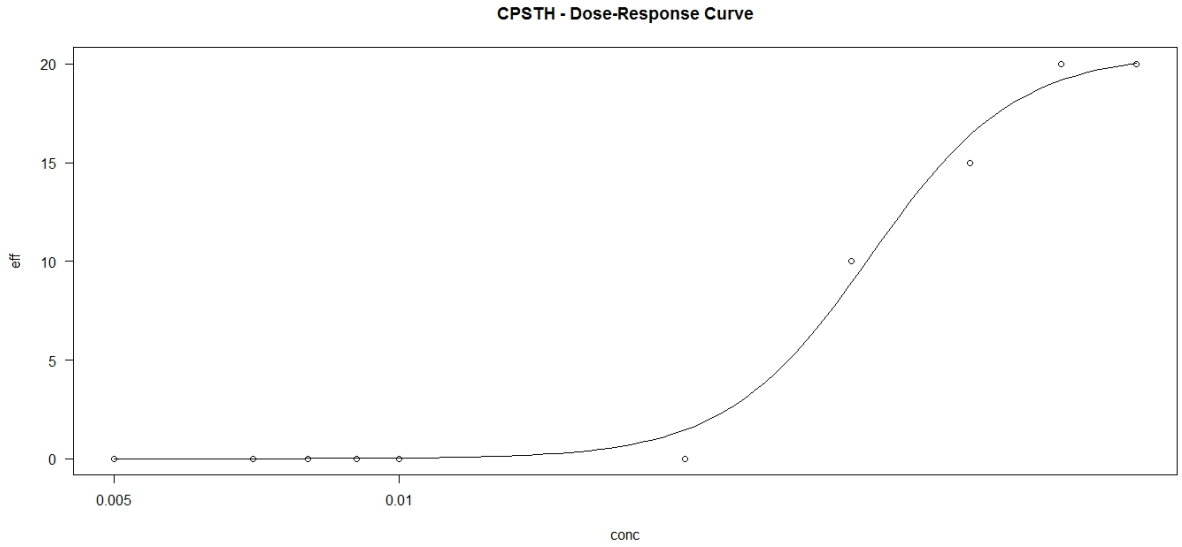


Figure 8. Dose-response curve of cyclopentanespiro-5-(2-thiohydantoin) (CPSTH)

- NOAEC (No Observed Adverse Effect Concentration) = 0.018 %.

As depicted on the presented Dose-Response Curves, CPSH and CPSTH express extremely low effectiveness versus the tested pest in contrast to HEICPSTH which was able to terminate the pest at 0.2 % LC_{90} concentration.

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