In vitro screening for fungicidal activity of cyclopentanespiro-5-hydantoin and its two derivatives towards *Alternaria solani* and effect on tomato plant varieties

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In vitro screening for fungicidal activity of cyclopentanespiro-5-hydantoin and its two derivatives towards Alternaria solani and effect on tomato plant varieties: *This paper presents in vitro trials (radial growth assays) with cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards Alternaria solani and investigation of their phytotoxic action towards tomato plant varieties. It was found that the tested compounds have no effectiveness towards the cited phytopathogen. No phytotoxic action of the compounds was observed towards tomato plants of three different varieties.*

Key words: *Cyclopentanespiro-5-hydantoin, Cyclopentanespiro-5-(2,4-dithiohydantoin),1-Aminocyclo pentanecarboxylic acid, Alternaria solani, Tomato plant varieties.*

INTRODUCTION

Alternaria solani is a phytophatogen which causes tomato leaf spots and as such it is a commonly found fungal pest in Bulgaria. The pathogen can significantly damage crops, thus causing economic losses to production [1].

The biological activity [2] and coordination properties [3-5] of cyclopentanespiro-5-hydantoin and its derivatives are well documented. In previous works of ours we have investigated the fungicidal activity of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclo-pentanecarboxylic acid towards *Blumeria graminis* f. sp. *tritici* [6], as well as the insecticidal activity of cyclopentanespiro-5-(2,4-dithiohydantoin) towards *Cladius pectinicornis* [7].

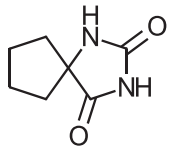
In the current research we have conducted *in vitro* trials (radial growth assays) with cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards *Alternaria solani*. An investigation of their phytotoxic action towards tomato plant varieties has also been performed.

EXPERIMENTAL

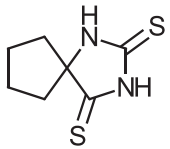
Synthetic compounds

The cyclopentanespiro-5-hydantoin (Fig. 1, a) was synthesized *via* the Bucherer-Lieb method [8]. The cyclopentanespiro-5-(2,4-dithiohydantoin) (Fig. 1, b) was synthesized in accordance with Marinov et al. [7]. The 1-aminocyclopentanecarboxylic acid (Fig. 1, c) was obtained in accordance with Stoyanov and Marinov [9].

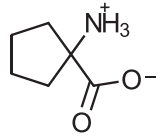
The products obtained were characterized through physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical with those previously published in the literature [3, 9, 10].



Cyclopentanespiro-5-hydantoin (CPSH)
a)



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



1-Aminocyclopentane-carboxylic acid (ACPCA)
c)

Fig. 1

***In vitro* tests**

Radial growth assays *in vitro* trials were conducted according to methods of Thornberry [11]. 1 ml of the tested solution was added to preliminarily sterilized Petri dishes (1 ml sterile distilled water for control), followed by addition of 9 ml PDA (potato dextrose agar). After mixing the solution with PDA through vigorous shaking, inoculation was conducted to 10 mm PDA disks with developed mycelium of the tested pathogen (two disks per Perti dish). The inoculated Petri dishes were incubated in thermostat under 22-25°C. Each compound was tested at 10 different concentrations in 5 replications – degrees of freedom = 36.

Observations were conducted on 3, 7, 10, and 14 days after inoculation with ruler, measuring the mycelium zone around inoculated disks. On the basis of these results, Area Under Disease Progressive Curve (AUDPC) [12] was calculated by R language agricolae package [13].

Effectiveness was calculated based on the values of AUDPC with Abbott's formula [14]. One-way ANOVA analysis was conducted to determine statistically proven differences between control and tested solutions through R Program Language for Statistical Computing [15].

***In vivo* tests**

Standard phytotoxicity tests were conducted in accordance with OECD Guide 227 - Terrestrial Plant Test: Vegetative Vigour Test [16]. The test period was 7 days. The plants were weekly observed for visual phytotoxicity manifestations (necrosis, chlorosis, whitening, deformations). On this basis, Percentage Disease Indexes (PDIs) were calculated in a 5-grade scale [17]. Based on the PDIs, LC_{05} (NOEL), LC_{25} (LOAEL), LC_{50} were determined, through the use of R language and drc package. The chemotherapeutic indexes were calculated as a ratio between LC_{50} obtained from phytotoxicity test and LC_{90} obtained from fungicidal test.

RESULTS AND DISCUSSION

The conducted radial growth assays with a saturated concentration of the compounds in water (1 % m/v for CPSH; 0.025 % m/v for SPSDTH and 0.1 % m/v for ACPCA) showed that the products are ineffective towards the tested phytopathogen.

Fig. 2 and Fig. 3 depict the ANOVA analysis conducted with R language for statistical computing.

```
> summary(aov(values~ind, data=test))
            Df Sum Sq Mean Sq F value Pr(>F)
ind          4   8269  2067.2   3.947  0.022 *
Residuals   15   7857   523.8
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Fig. 2

It is obvious from Fig. 3 that there are significant differences only between control variant and used standard mankozeb at 0.16 % (trade name Dithane M 45 – 0.2 %). P > 0.05 between tested products and control variant means there is no effectiveness towards this pathogen.

Conducted *in vivo* phytotoxicity test shows that in the saturated concentration of the compounds in water, they do not have any deleterious effects on tomato plants of three different varieties (Miljana, Ideal and Buffalo Heart).

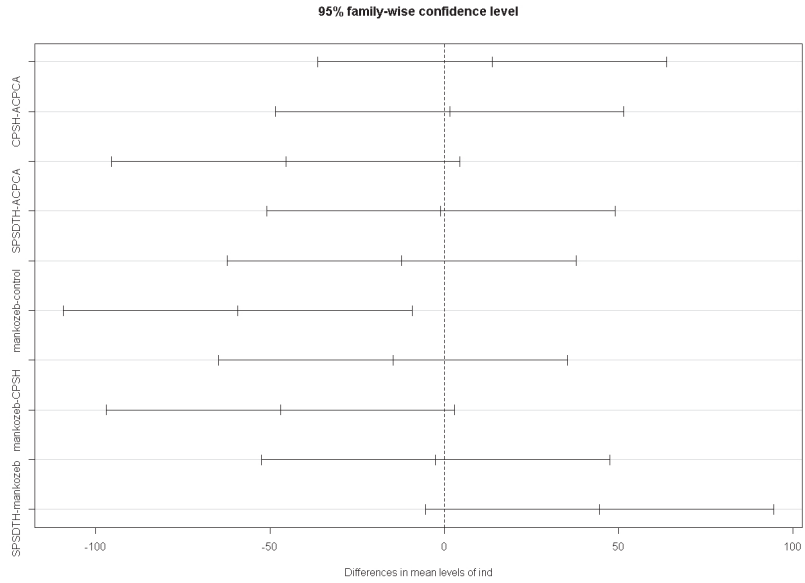


Fig. 3

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This paper has been reviewed