IN VITRO ФУНГИЦИДНА АКТИВНОСТ НА ЦИКЛОПЕНТАНСПИРО-5-ХИДАНТОИН И НЕГОВИ ПРОИЗВОДНИ СПРЯМО *BLUMERIA GRAMINIS F. SP. TRITICI*

IN VITRO FUNGICIDAL ACTIVITY OF CYCLOPENTANESPIRO-5-HYDANTOIN AND ITS DERIVATIVES TOWARDS *BLUMERIA GRAMINIS F. SP. TRITICI*

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Резюме

Спирохидантоините са биологичноактивни вещества, намиращи приложение както в хуманната медицина, така и в областта на аграрните науки. Наличните литературни данни за фунгицидно действие, проявявано от някои представители на разглежданата група съединения, определи насоката на настоящото изследване. Целта на статията е да бъде оценено въздействието на циклопентанспиро-5-хидантоин, циклопентанспиро-5-(2,4-дитиохидантоин) и 1-аминоциклопентанкарбоксилна киселина спрямо *Blumeria graminis f. sp. tritici*. Проведените *in vitro* тестове ясно показаха способността на изпитваните съединения да контролират брашнестата мана по пшеницата.

Abstract

The spirohydantoins are biologically active substances which are used in medicine as well as in the field of agricultural sciences. The available literature data on the fungicidal activity of some representatives of that group of compounds determined the direction of the study. The purpose of this paper is to assess the impact of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid on *Blumeria graminis f. sp. tritici*. The conducted *in vitro* tests clearly showed the ability of the tested products to control the wheat powdery mildew.

Ключови думи: фунгицидна активност, циклопентанспиро-5-хидантоин, циклопентанспиро-5-(2,4-дитиохидантоин), 1-аминоциклопентанкарбоксилна киселина, *Blumeria graminis*.
Key words: fungicidal activity, cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin), 1-aminocyclopentanecarboxylic acid, *Blumeria graminis*.

INTRODUCTION

The interest in the study of cyclopentanespiro-5-hydantoin and its derivatives is mainly due to the biological activity reported for these compounds. 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 confirmed for cyclopentanespiro-5-hydantoins (Oldfield and Cashin, 1965).

The dithioanalogue of the above mentioned compound – cyclopentanespiro-5-(2,4-dithiohydantoin) showed a strong insecticidal activity against *Cladius pectinicornis*, economic important pest on roses (Marinov et al., 2012).

On the other hand, 1-aminocyclopentane-carboxylic acid posses antitumor activity in rodents (Connors et al., 1958; Goldin et al., 1961; Martel and Berlinguet, 1959; Ross et al., 1961) and is useful in the treatment of plasmocytic myeloma (Benefiel et al., 1960; Krant et al., 1962). Furthermore, 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).

The available literature data for fungicidal activity of other 5-substituted hydantoins and their 2-thio analogs (Marton et al., 1993) are decisive for the purpose of this study, which is to examine the effects of cyclopentanespiro-5-hydantoin, its dithioanalogue and 1-aminocyclopentanecarboxylic acid towards *Blumeria graminis f. sp. tritici*.

Wheat is by far the most important food grain of temperate regions. Its role in human subsistence is matched by the deep significance of wheat in religion and daily life (Prance and Nesbitt, 2005). *Blumeria graminis f. sp. tritici* (wheat powdery mildew) is one of the most common and destructive diseases of cereals widely distributed in wheat growing areas throughout the world. Losses up to 45 % have been documented (Bayer CropScience Crop Compendium). Traditionally wheat powdery mildew is controlled by so called SBI fungicides (Sterol Biosynthesis Inhibitors) which represent G MoA group according to classification of fungicides by FRAC (FRAC International, 2005).

EXPERIMENTAL METHODS

1.SYNTHESIS OF THE COMPOUNDS

All used chemicals were purchased from Merck and Sigma-Aldrich. The initial cyclopentanespiro-5-hydantoin (**2**, **CPSH**, Figure 1) was synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb, 1934). The cyclopentanespiro-5-(2,4-dithiohydantoin) (**3**, **CPSDTH**, Figure 1) was synthesized in accordance to Marinov et. al. (Marinov et al., 2012). The 1-aminocyclopentanecarboxylic acid (**4**, **ACPCA**, Figure 1) was obtained according to Stoyanov and Marinov (Stoyanov and Marinov, 2012). The melting points were determined with a Koffler apparatus and with a digital melting point apparatus SMP 10. The 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 (300 K).

2. FUNGICIDAL ERADICATIVE ACTIVITY TESTS

Method of leaf discs and methods adapted from those of Wong and Wilcox (Wong and Wilcox, 2000) were used for the test. Pesticide solutions with 10 different concentrations were prepared with distilled water with added standard organosilicone surfactant Silwet®L-77 manifactured by General Electric Company at concentration 0.015 % for improvement of wetting ability. The control variant was treated with solution of surfactant in distilled water, the using standard was Bayfidane ®250 EC on base of triadimenol. Fresh leaves from wheat variety "Kristi" growth stage 75, (Meier, 2001) naturally infected with the given phytopathogen were used for the purposes of test.

Leaf discs (parts) with visual powdery mildew symptoms (white powdery coating on upper leaf side) with 5 cm length were soaked in the dilutions for 5 seconds and after that placed adaxial in wetted with distilled water filter paper. Each test variant was set in five replicates. The effectiveness was measured on 24 and 48 hours after treatment by lack or presence of visual symptoms of phytopathogen (powdery mycelium) on leaves.

RESULTS AND DISCUSSION

The synthesis of the target compounds was carried out according to Figure 1. The initial cyclopentanespiro-5-hydantoin (**2, CPSH**) was synthesized *via* the Bucherer-Lieb method, based on the interaction between cyclopentanone (**1**), sodium cyanide, ammonium carbonate and ethanol (Bucherer and Lieb, 1934). The possible ways for thionation of the product obtained to cyclopentanespiro-5-(2,4-dithiohydantoin) (**3, CPSDTH**) are as follows: **Method a:** Refluxing of **2** and P_4S_{10} (mole ratio 1 : 1) in xylene for five hours (Marinov et al., 2005). **Method b:** Refluxing of **2** and 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide (Lawesson's reagent, LR) (mole ratio 1 : 2) in toluene for six hours (Marinov et al., 2005). **Method c:** Thionation of **2** with the reagent combination of P_4S_{10} and hexamethyldisiloxane (HMDO) in xylene at refluxing for an hour and a half (Marinov et al., 2012). As it is seen the method **c** is the most convenient synthetic procedure for obtaining of **3** and was used for the purpose of this paper. In addition, compound **2** was subjected to alkaline hydrolysis with barium hydroxide, resulted in hydantoin ring degradation and formation of 1-aminocyclopentane-carboxylic acid (**4, ACPCA**) (Stoyanov and Marinov, 2012). All the products obtained were characterized by physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical with the previously published in the literature (Marinov et al., 2012; Stoyanov and Marinov, 2012; Marinov et al., 2005; Enchev et al., 1999).

The compounds were tested for fungicidal activity towards *Blumeria graminis f. sp. tritici*. The Figure 2 illustrates the minimum effective concentrations of the tested products.

As it is seen the most effective product is CPSDTH which was able to completely inhibit the tested plant pathogen at 0.0000031% concentration (according to active substance). The other products were effective at following concentrations: CPSH at 0.01% and ACPCA at 0.00005%. During the test no visual phytotoxic manifestations on leaf discs were observed.

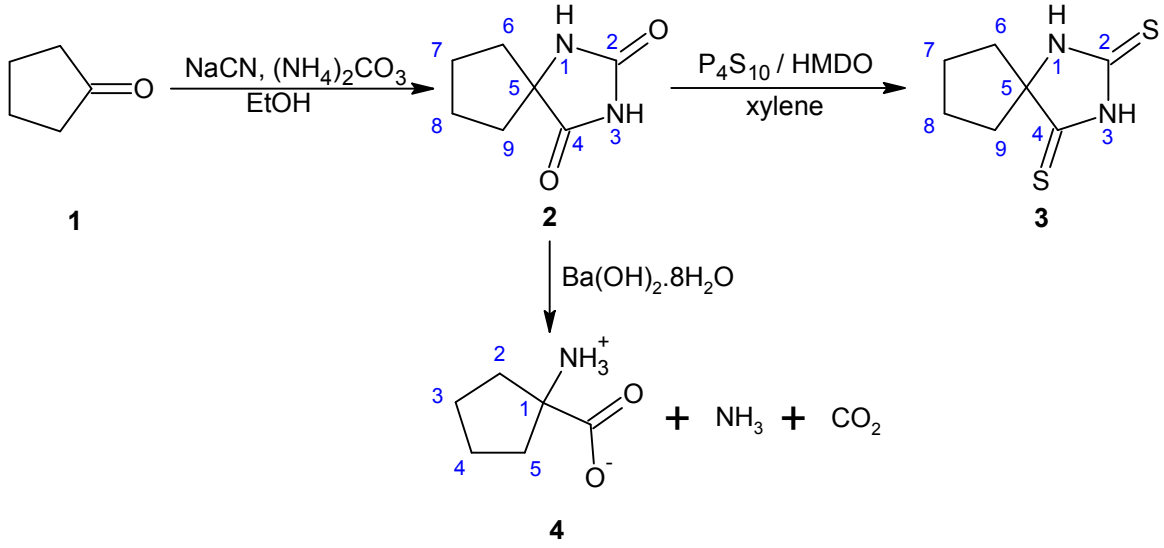

Fig. 1. *Synthetic routes to cyclopentanespiro-5-hydantoin (**2, CPSH**), cyclopentanespiro-5-(2,4-dithiohydantoin) (**3, CPSDTH**) and 1-aminocyclopentanecarboxylic acid (**4, ACPCA**)*

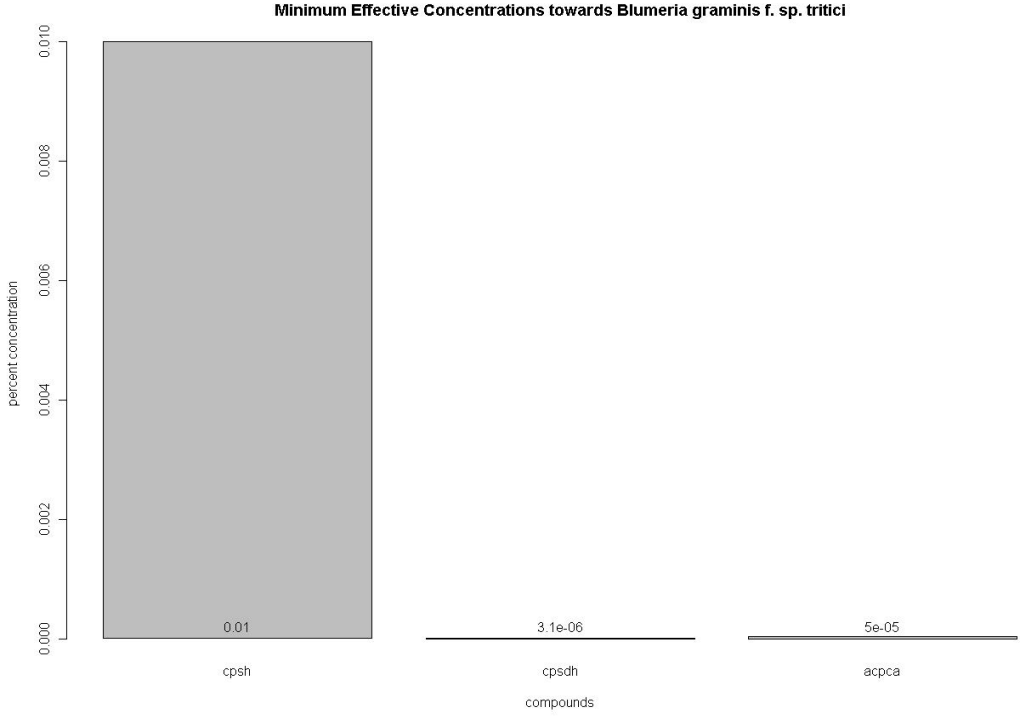

Fig. 2. *Effectiveness of the compounds*

The pictures below show the eradicative effect of the compounds to the control variant:

Figure 3 shows the control variant plant parts pictures, with significant presence (infestation) of tested phytophatogen. In the contrast to the control, in the all other tested variants figures show complete eradication of phytophatogen mycelium caused by examined compounds.

CONCLUSIONS

The conducted *in vitro* test clearly showed the possibility of tested products to control wheat powdery mildew. This indicates the good base for future *in vivo* field tests against this phytopathogen and eventual potential such compounds to be developed as synthetic fungicides.

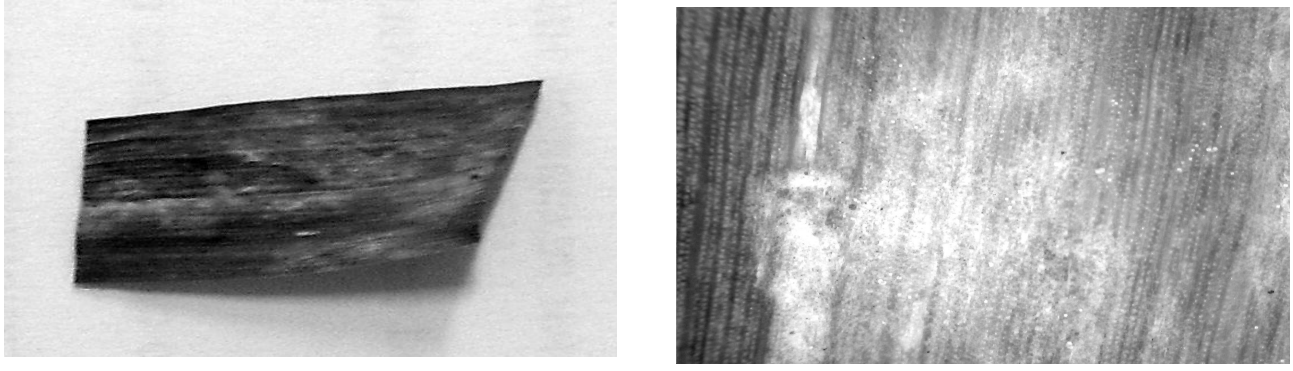

Fig. 3. *Control variant*

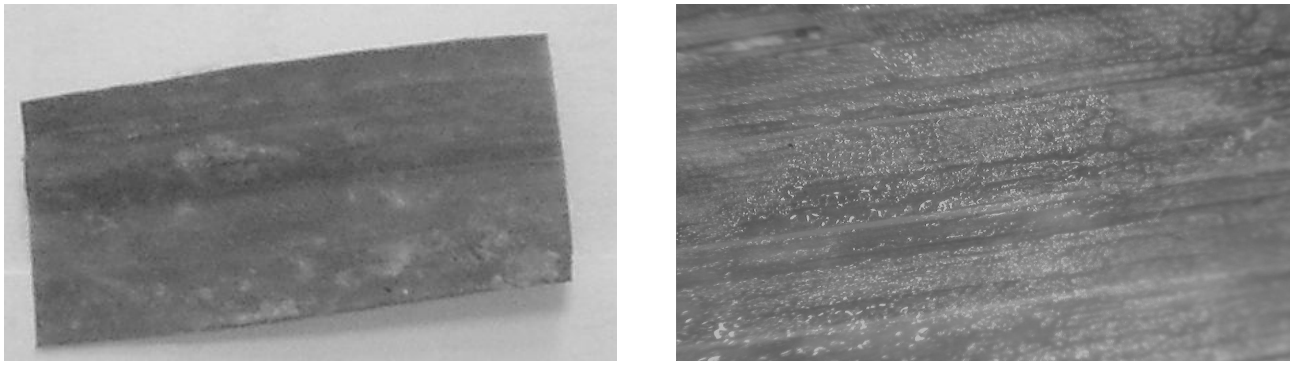

Fig. 4. *CPSH treated variant*

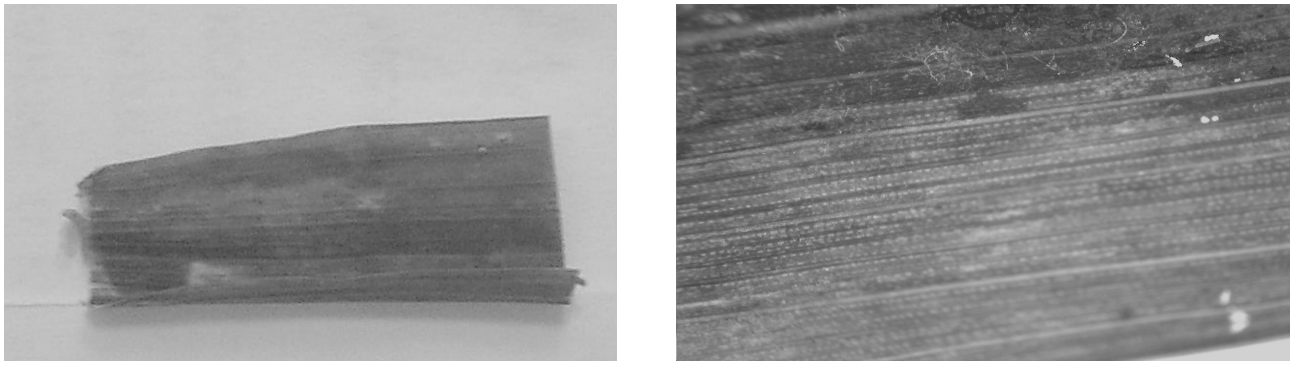

Fig. 5. *CPSDTH treated variant*

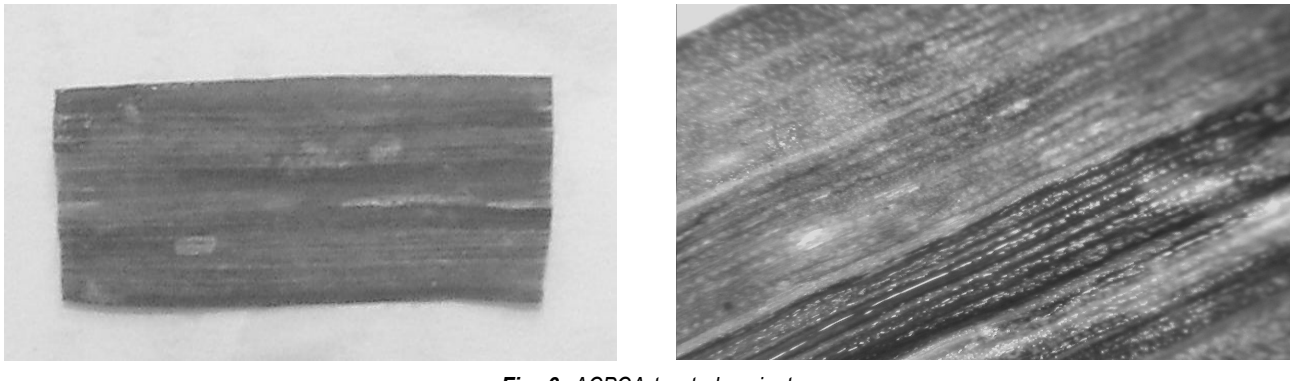

Fig. 6. *ACPCA treated variant*

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ACKNOWLEDGEMENTS

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

Статията е приета на 10.12.2012 г.
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