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The effect of fungicides on growth of Fusarium fungi in vitro

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Abstract. Four different fungicides that recommended to control seed-borne pathogens, were tested in this study. Three fungicides contain singly pyraclostrobin (200 g/L), thiram (400 g/L), fludioxonil (25 g/L), and one fungicide contains together imazalil, metalaxyl, and tebuconazole (50, 40, and 30 g/L, respectively). Comparative assessment of the fungicides at four concentrations of active substance (10, 100, 1 000, and 10 000 ppb) on the growth of ten strains of *Fusarium* spp. was studied *in vitro* tests. To visualize the effect of fungicides on fungal growth the strains were cultivated in 50 mL liquid Czapek medium containing fungicides in the range of concentrations. The fungicide based on pyraclostrobin was the most effective in growth inhibition of *Fusarium* fungi. Broadly, the using of this fungicide is confident way to control *Fusarium* species, which are serious threat to crop production. The sensitivity of *F. acuminatum*, *F. graminearum*, *F. semitectum*, *F. culmorum*, *F. sporotrichioides*, *F. equiseti* strains to fungicides was higher in compare with the strains belonging to *F. oxysporum, F. solani, F. verticillioides,* and *F. proliferatum*.

1 Introduction

Diseases caused by *Fusarium* fungi are a common occurrence on plants, often having a significant economic impact on yield and its quality. The different *Fusarium* species causes rot of root and stem base, seedling death, wilting, stunting, leave and head blight [1]. Thus managing diseases is an essential component of production for most crops.

There is no single method to provide effective control of *Fusarium* fungi on cultivated cereals, vegetables, and others crops. Typically management of pathogenic *Fusarium* species includes crop rotation, sanitation, and judicious use of fungicides. Unfortunately, there is not sufficient number of available varieties with measurable resistance to *Fusarium* diseases. A few *Fusarium* species which infect grains produce mycotoxins that can cause severe illness or even death in humans and animals when consumed [2, 3]. In some cases, the infection with pathogenic fungi is increased greatly after seeds contact the ground.

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Therefore, the protection of plants with effective fungicides against seed-borne pathogens during its seedling and early growth stages is essential for successful plant production.

In 2019 the using of 94 fungicides based on 20 active ingredients and their combinations is allowed for seed treatment to control the *Fusarium* pathogens in Russia [4].

The aim of this study was to determine *in vitro* the sensitivity of *Fusarium* fungi to fungicides widely used for seed treatment.

2 Materials and Methods

The ten single spore-strains of different *Fusarium* species were selected to study the effect of fungicides on their growth. The taxonomic status of strains was revealed according to the sum of their macro- and micromorphological characters [5]. The species and origin of strains included in the study are shown in table 1. The strains are maintained in the collection of the Laboratory of Mycology and Phytopathology (All-Russian Institute of Plant Protection, St. Petersburg, Russia).

Table 1. Origin of *Fusarium* spp. strains included in the present work

Collection number of strains	Species	Substrate	Origin place and year
MFP 1009021	*F. acuminatum* Ellis & Everh.	soybean	Primorsky Kray, 2017
MFG 102100	*F. culmorum* (Wm.G. Sm.) Sacc.	barley	Kirov Oblast, 2017
MFP 1009031	*F. equiseti* (Corda) Sacc.	soybean	Primorsky Kray, 2017
MFP 1009041	*F. graminearum*	soybean	Primorsky Kray, 2017
MFP 1009051	*F. oxysporum* Schltdl.	soybean	Primorsky Kray, 2017
MFP 1009061	*F. proliferatum* (Matsush.) Nirenberg	soybean	Primorsky Kray, 2017
MFG 58998	*F. semitectum* Berk. & Ravenel	wheat	Krasnodar Kray, 2016
MFG 63301	*F. solani* (Mart.) Sacc.	potato	Kazakhstan, 2006
MFG 64709	*F. sporotrichioides* Sherb.	wheat	Primorsky Kray, 2007
MFG 59039	*F. verticillioides* (Sacc.) Nirenberg)	maize	Krasnodar Kray, 2016

Four commercially available fungicides, containing following active substances: # 1 (50 g/L of imazalil, 40 g/L of metalaxyl, 30 g/L tebuconazole), # 2 (200 g/L of pyraclostrobin), # 3 (25 g/L of fludioxonil) and # 4 (400 g/L of thiram) were chosen for the study. The effect of each fungicide was evaluated by the accumulation of biomass of *Fusarium* fungi during submerged cultivation in 50 mL of liquid Czapek medium (CZ) with fungicide in a 750-mL Erlenmeyer flask in compare with the cultivation in the same medium without fungicide. The fungicides were diluted in sterile water to obtain a concentration of the active substance of 10 g/L, in the case of a three-component fungicide # 1 – 10 g/L of imazalil. Then ten-fold dilutions of the obtained solutions from 1 g/L to 1 mg/L were prepared. In each flask 50 µL of the fungicide solution was added so that the final concentration of active substance reached to 10, 100, 1 000, and 10 000 ppb.

Each flask was inoculated with 50 µL suspension (2×10^4 CFU/mL) obtained from the fungal colony growing on potato-sucrose agar. In control, only inoculum was added to the medium without fungicides.

The flasks were incubated in INNOVA 44 (Eppendorf, Germany) for 6 days at 24 °C with constant shaking at 100 rpm. To determine fungal growth the culture supernatant was separated by vacuum filtration using pump Millipore XF5423050 (Alsace, France). The biomass was dried at 50 °C for a day and then weighed. The experiment was carried out in triplicate. Using probit analysis (Probit 2.0.0.6 program), the concentration of each fungicide leading to a 50% decrease in the fungal biomass (IC50) was calculated.

3 Results

The dry weight of biomass after cultivation in liquid CZ in the control was 358±35 mg for *F. acuminatum, F. culmorum* – 178±24 mg*, F. equiseti* – 277±21 mg*, F. graminearum* – 283±7 mg*, F. oxysporum* – 365±18 mg*, F. proliferatum* – 336±27 mg*, F. semitectum* – 256±6 mg*, F. solani* – 313±23 mg*, F. sporotrichioides* – 243±11 mg, and *F. verticillioides* – 245±19 mg. The addition of the fungicides to the CZ led to significant effect, contributing to a decrease in the weight of fungal biomass (Fig. 1). It depends on the *Fusarium* species ($p < 0.0001$), as well as on fungicide ($p = 0.02$).

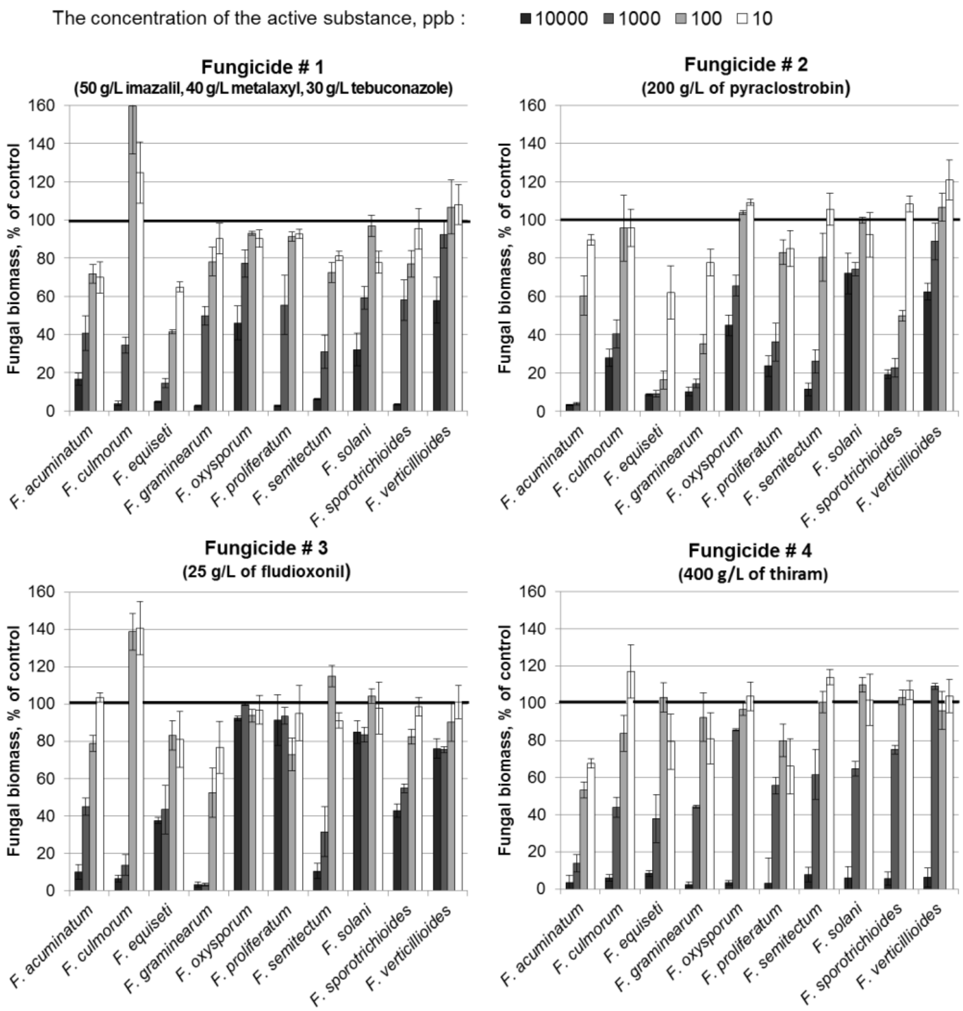

Fig. 1. The effect of fungicides in various concentrations on the accumulation of biomass of *Fusarium* spp. after cultivation in liquid Czapek medium (24 °C, 6 days)

The significant variation of sensitivity of *Fusarium* fungi to the different fungicides was observed. The high concentrations of the active substance of all fungicides (1 000 and 10 000 ppb) reduced the growth of all fungal strains. The exception was fungicide # 3 which did not affect the growth of *F. oxysporum*, *F. proliferatum*, *F. solani* and

F. verticillioides strains. The low concentrations of the active substance of some fungicides stimulated the accumulation of fungal biomass. For example, the addition in CZ medium 100 ppb of the fungicides # 1 and # 3 led to an increase of *F. culmorum* growth in compare with control (p= 0.04 and p= 0.02, respectively).

4 Discussion

Studies *in vitro* have been conducted on the effect of some fungicides on growth of *Fusarium* fungi as a seed-treatment to improve seedling health. The results of probit analysis (Table 2) showed that on average the fungicide # 1 (50 g/L of imazalil, 40 g/L of metalaxyl, 30 g/L tebuconazole) the most effectively limited the growth of all ten strains belong to different *Fusarium* species.

Table 2. The concentration of fungicides that leads to half-maximum inhibition (IC50) of the biomass of *Fusarium* strains after submerged cultivation

***Fusarium* fungi**	**Concentration of active substance (IC50), ppb**			
	# 1	**# 2**	**# 3**	**# 4**
F. acuminatum	532	117	660	61
F. culmorum	815	1445	575	672
F. equiseti	245	13	3523	544
F. graminearum	485	58	66	774
F. oxysporum	784	7810	>10000	4905
F. proliferatum	779	684	>10000	1037
F. semitectum	357	547	861	1491
F. solani	2799	>10000	>10000	1528
F. sporotrichioides	662	98	869	1992
F. verticillioides	14541	19498	>10000	6302

A similar reaction of the strains *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. verticillioides* to the addition of fungicides was revealed. The fungicide # 3 (25 g/L of fludioxonil) did not inhibit growth of these fungi. Generally these strains showed low sensitivity to the active substances (IC50 from 684 to >10000 ppb) compared with other fungi included in the study.

Overall, *F. acuminatum*, *F. equiseti*, *F. semitectum,* and *F. sporotrichioides* strains showed an average level of sensitivity to the fungicides (IC50 varied between 13 and 3523 ppb). The fungicide # 2 (200 g/L of pyraclostrobin) was most effective for control of these strains.

The strains of closely related species *F. culmorum* and *F. graminearum* turned out to be the most sensitive to fungicides (IC50 varied between 58 and 1445 ppb), among which fungicide # 3 (25 g/L of fludioxonil) was the most effective. These species are most aggressive to cereals and dangerous due to the production of mycotoxins. Perhaps the high sensitivity of these species to analyzed fungicides is the result of screening the substances for antifungal activity against these pathogens.

Knowledge of the sensitivity of different fungal species to particular active substances is extremely important when plan protection systems and choose the most effective fungicides. Accurate diagnosis of the etiology of crop diseases, as well as the correct identification of *Fusarium* pathogen and determination of its area, are necessary to predict the effect of used fungicides. The seed treatment with fungicides should be a mandatory technique for obtaining a high quality yield.

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