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Development and synthesis of compounds with fungicidal activity in suppression of fungal growth

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Abstract. The research was conducted to synthesize and study fungicidal activity of synthesized chemical compounds of various classes, triazole and imidazole, and their mode of action due to the wide spectrum of action and low application rates. The developed synthesis methods resulted in several groups of nitrogen-containing heterocyclic compounds and evaluated their fungicidal activity. Inhibitory activity of compounds to strains of *Fusarium solani* (medium resistance to fungicides) and *Sclerotinia sclerotiorum* (susceptible to most fungicides) from the collection of phytopathogenic microorganisms of Agrobiotechnological Department, RUDN University, was tested. In the synthesis of new chemical compounds with fungicidal activity, urea derivatives 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazole-5-yl)-3-(2-chlorophenyl) urea were identified and structurally confirmed. All target compounds were evaluated for their antifungal activity to inhibit mycelium growth. Preliminary screening results showed that all synthesized compounds have good fungicidal activity against *S. sclerotiorum.* The compound 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazole-5-yl)-3-(3-fluorophenyl) urea showed antifungal activity against *S. sclerotiorum*. At concentration of 100 ppm, the compound suppressed growth of *S. sclerotiorum* strain by 90.5 %. An in vitro experiment revealed that the compound 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazole-5-yl)-3-(3-fluorophenyl) urea was effective for suppressing white mold — S. *sclerotiorum*, at the dose of 100 mg/L. The significance of the research lies in the fact that production of eco-safe products in agroindustry is impossible without development of new biologically active compounds with low application rates and toxicity indicators, controlled persistence, corresponding to the world level. The results obtained can be implemented in real sector of economy engaged in production of chemical plant protection products. Synthesis and use of new fungicides are relevant in agricultural production as an element of development and intensification of existing agricultural technologies.

Keywords: triazoles, urea, plant protection products, azoles, pesticides, agriculture, chemical heterocyclic compounds, biological efficiency

Conflicts of interest. The authors declared no conflicts of interest.

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Introduction

More than 30 % of losses during crop cultivation are largely due to phytopathogenic fungi and viruses. Thus, modern agricultural production is impossible without fungicides, therefore, creation of new chemical plant protection products is relevant for modern agricultural production. Rapid growth of the world's population and the associated food shortages have led to development of highly productive agricultural industry with integrated plant protection system [1]. When affected by phytopathogenic fungi, agricultural products can suffer heavily during production, and without fungicides, they can lead to complete destruction of the crop grown. Chemicals used repeatedly in one growing season without considering mode of action become ineffective due to emergence of resistant strains of microorganisms, and therefore search for new fungicides remains invariably relevant [2, 3].

Among different classes of systemic fungicides, triazole and imidazole derivatives have low toxicity to the environment and humans. A wide spectrum of action and low application rates have led to their active use in agriculture [4, 5]. It was the global chemicalization of agriculture that made it possible to solve the problem of food shortages on a global scale [6, 7]. The mode of action of azole fungicides is to inhibit biosynthesis of ergosterol (the most important component of fungal cell membranes) at the stage of oxidative demethylation of 14a-methyl group of lanosterol (sterol-14a-demethylase (CYP51 enzyme)) [8, 9].

Along with the positive effect, use of agrochemicals in agriculture has negative consequences associated with pollution of the environment with pesticides and mineral fertilizers, deterioration of public health indicators, and global climate change [10, 11]. That is why scientists in various countries are conducting research on development of modern agricultural technologies [12, 13], the search for new chemical compounds that can reduce negative anthropogenic impact and ensure significant reduction in load on the environment associated with formation of numerous wastes [14, 15]. Effective approaches that significantly improve quality of agricultural technologies often consist of a balanced combination of modern chemical, biological and agro-technological advances at each

stage of agricultural production, including synthesis of active ingredient, studying its properties, developing formulations, conducting biological and field tests, creation of technological maps and recommendations for application [16].

The purpose of the study was to synthesize chemical compounds, to develop methods for their production, and to assess fungicidal activity in laboratory conditions.

Novelty of the research. General methods were developed for synthesis of several groups of nitrogen-containing heterocyclic compounds with assessment of their fungicidal activity. Chemical compounds were obtained that have inhibitory activity against the fungal strains *Fusarium solani* (moderate resistance to fungicides) and *Sclerotinia sclerotiorum* (susceptible to most fungicides).

Materials and methods

Fungal strains *Fusarium solani* (moderate resistance to fungicides) and *Sclerotinia sclerotiorum* (susceptible to most fungicides) from the collection of phytopathogenic microorganisms of Agrobiotechnology Department, Agrarian and Technological Institute, RUDN University, were used in the research (Table 1).

Table 1

Fungal strains

Strain	Plant	Species name	Growth on PDA medium
20MKKK 1.1	Potato	*Fusarium solani*	Slow
KTOPS1	Sunroot	*Sclerotinia sclerotiorum*	Fast

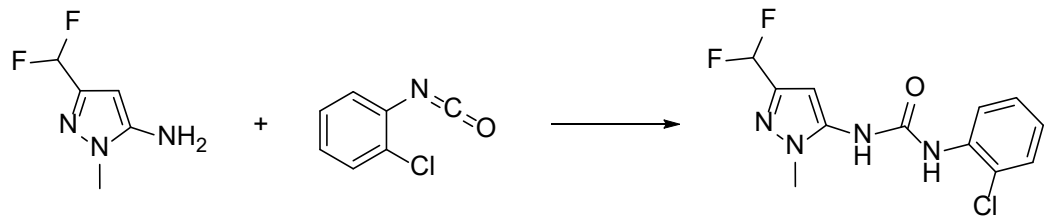
After dissolving the synthesized compound in a polar aprotic solvent dimethyl sulfoxide, a 1 % (1000 ppm) solution of the chemical was obtained. After autoclaving and solidification of PDA (Potato Dextrose Agar (composition per 1 liter of water, g: potatoes (200 g), glucose (20 g), agar (15 g), the initial solution was used to prepare mixtures of fungicides in concentrations of 10 and 100 ppm. The medium was homogenized and poured into Petri dishes with a diameter of 85 mm at the rate of approximately 20 ml per dish. An agar block with mycelium from 7-day cultures was placed in the middle of Petri dish with poisoned PDA medium. For each variant, 2 replicates were used. Control with DMSO without fungicide was grown under the same conditions. The cultures were incubated in thermostat at 22 ± 2 °C for 7 days. Effectiveness of the chemicals was assessed by measuring radial growth of colonies of each fungus. Radial growth was measured along two perpendicular axes drawn from the base of each Petri dish and intersecting at the center of the colony. The experiment had 2 replicates. As efficiency indicator, we used the percentage of growth suppression, which was calculated using the formula

$$D = \frac{D0 - Dc}{D0} 100,$$

where *D* is suppression of colony growth, %; *D*0 is colony diameter in the control; *D*c is colony diameter in the experiment.

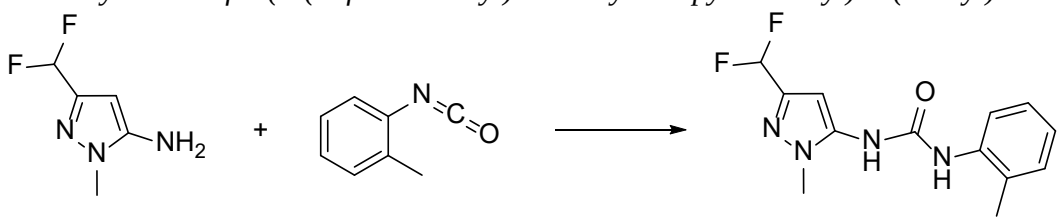
Results and discussion

1. *Synthesis of urea derivatives 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-5-yl)-3-(2-chlorophenyl) urea*



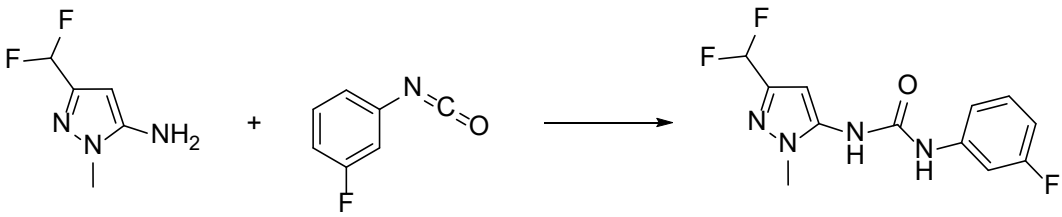
2-chlorophenyl isocyanate (0.148 g, 0.96 mmol) was added to the solution of aminopyrazole (0.129 g, 0.88 mmol) in dichloromethane (5 ml) and boiled for 6 hours. After reaction, the product was purified by column chromatography on SiO_2 on automatic chromatograph in mixture of EtOAc/Hexane, which gave 0.176 g (66 %) of urea.^{1}H NMR spectrum (400 MHz, DMSO): δ 9.52 (s, 1H), 8.65 (s, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 7 .9 Hz, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.06 (dd, *J* = 11.3, 4.5 Hz, 1H), 6.89 (t, J_F = 54.6 Hz, 1H), 6.48 (s, 1H), 3.77 (s, 3H). NMR^{19}F (283 MHz, DMSO).

2. *Synthesis of 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-5-yl)-3-(o-tolyl) urea*



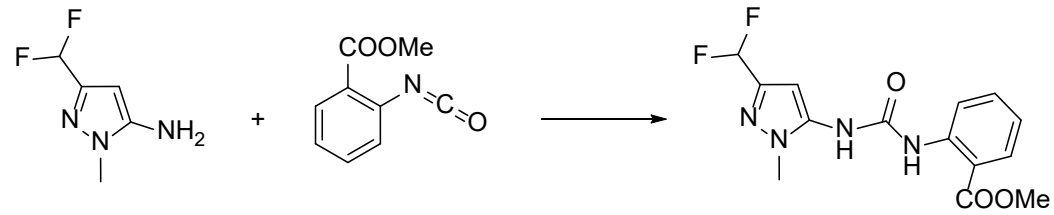
2-tolylisocyanate (0.128 g, 0.96 mmol) was added to the solution of aminopyrazole (0.129 g, 0.88 mmol) in dichloromethane (5 ml) and boiled for 18 hours. After reaction, the product was purified by column chromatography on SiO_2 (Agilent cartridge) on automatic chromatograph in mixture of EtOAc/Hexane, which gave 0.198 g (80 %) of urea.^{1}H NMR spectrum (400 MHz, DMSO): δ 9.11 (s, 1H), 8.24 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.29–7 .09 (m, 2H), 6.99 (d, *J* = 9.0 Hz, 1H), 6.86 (t, J_F = 46.4 Hz, 1H), 6.46 (s, 1H), 3.75 (s, 3H), 2.26 (s, 3H). NMR^{19}F (376 MHz, DMSO).

3. *Synthesis of 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-5-yl)-3-(3-fluorophenyl) urea*



3-fluorophenyl isocyanate (0.131 g, 0.96 mmol) was added to the solution of aminopyrazole (0.129 g, 0.88 mmol) in dichloromethane (5 ml) and boiled for 4 hours. After reaction, the product was purified by column chromatography on SiO_2 (Agilent cartridge) on automatic chromatograph in mixture of EtOAc/Hexane, which gave 0.195 g (78 %) urea.^{1}H NMR spectrum (400 MHz, $CDCl_3$): δ 9.23 (s, 1H), 8.86 (s, 1H), 7.49 (d, J = 11.7 Hz, 1H), 7.41–7.25 (m, 1H), 7.16 (d, J = 8.0 Hz, 1H), 6.88 (t, J_F = 54.8 Hz, 1H), 6.82 (t, J = 8.2 Hz, 1H), 6.45 (s, 1H), 3.72 (s, 3H).^{19}F (376 MHz, DMSO).

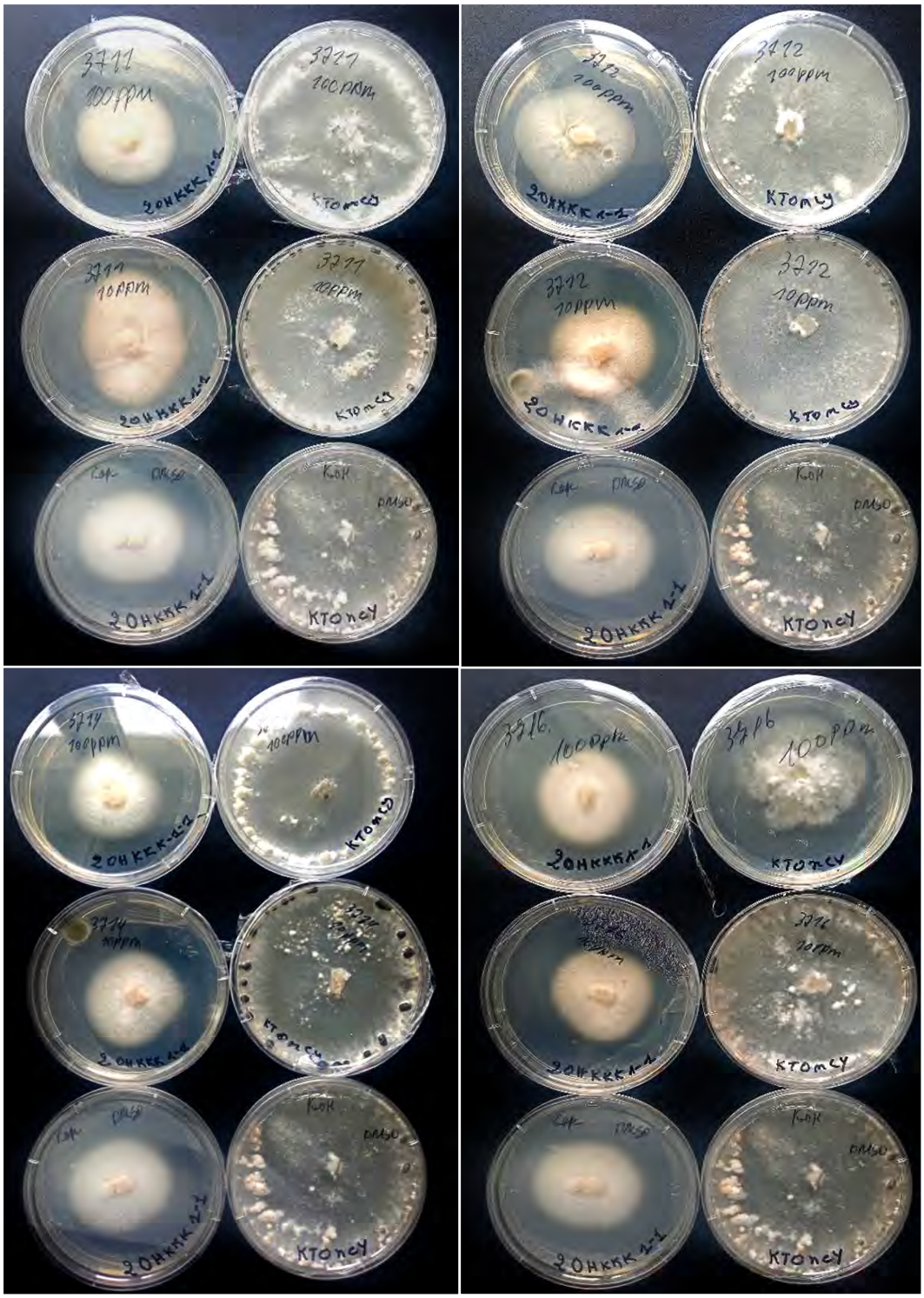
4. *Synthesis of 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-5-yl)-3-(2-methoxyphenyl) urea*



2-(carboxymethyl) phenyl isocyanate (0.170 g, 0.96 mmol) was added to the solution of aminopyrazole (0.129 g, 0.88 mmol) in dichloromethane (5 ml) and boiled for 4 hours. After reaction, after evaporation and washing with ether and hexane mixture, the crude product was obtained; 0.035 g (12 %) of urea was used without purification.

To evaluate fungicidal properties by in vitro method, we used strains of *Fusarium solani* (moderate resistance to fungicides) and *Sclerotinia sclerotiorum* (susceptible to most fungicides) (Fig.).

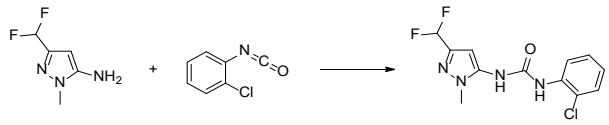
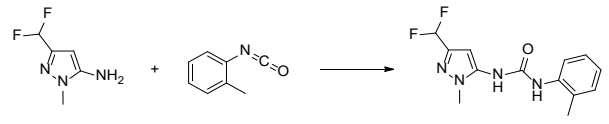
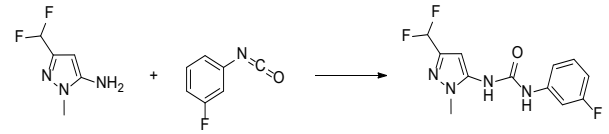
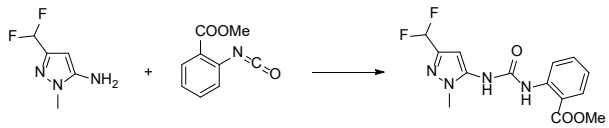
Table 2 shows that the compounds 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-5-yl)-3-(2-methoxyphenyl) urea have higher fungicidal activity compared to widely used triazole (Control).



Effect of synthesized compounds on fungal growth
Source: made by authors

Table 2

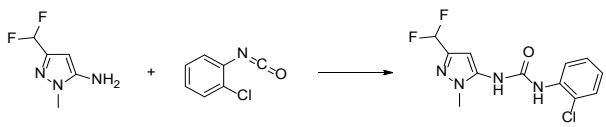
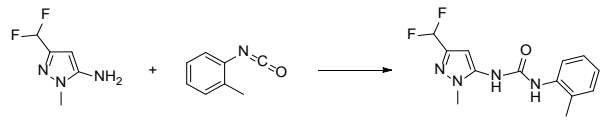
Characteristics of synthesized compounds

Compound	Weight, mg	Molecular weight	Base solution (1000 ppm)
	43	280	43 mg + 4300 µl DMSO
	40	301	40 mg + 4000 µl DMSO
	42	284	42 mg + 4200 µl DMSO
	35	340	35 mg + 3500 µl DMSO

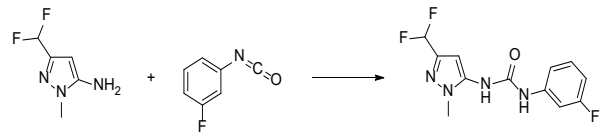
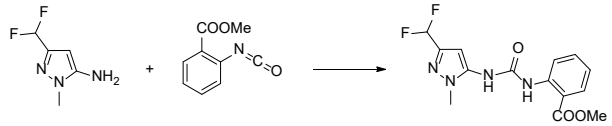
The result of the synthesis is chemical compound 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-5-yl)-3-(3-fluorophenyl) urea, which has fungicidal activity (Table 3).

Table 3

Fungicidal activity of synthesized chemical compounds

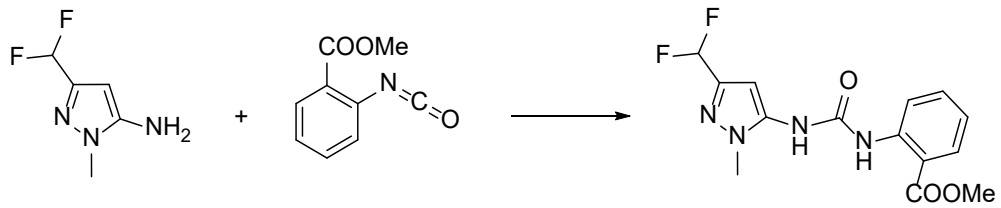
Compound	Variant	Reduced growth of mycelium, % compared to control	
		Fusarium solani	*S. sclerotiorum*
	Control	49.5	65
	10 ppm	60	75
	100 ppm	79.5	85
	Control	49.5	75
	10 ppm	59.5	73
	100 ppm	79.5	77.2

Ending of table 3

Compound	Variant	Reduced growth of mycelium, % compared to control	
		Fusarium solani	*S. sclerotiorum*
	Control	49.5	65
	10 ppm	49.5	70
	100 ppm	49.5	72
	Control	49.5	75
	10 ppm	54.3	85
	100 ppm	65.5	90.5

Conclusion

Structures with fungicidal activity were identified, containing the synthesis of 1-(3-(Difluoromethyl)-1-methyl-1H-pyrazol-5-yl)-3-(2-methoxyphenyl) urea, at the concentration of 100 mg/L in the solvent Dimethyl sulfoxide.



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Разработка и синтез соединений с фунгицидной активностью в подавлении роста грибов

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Аннотация. Проведено исследование с целью синтеза и изучения фунгицидной активности синтезированных химических соединений различных классов, в частности триазола и имидазола, ввиду широкого спектра действия и малых норм расхода. Разработанными методами синтеза получены несколько групп азотсодержащих гетероциклических соединений и проведена оценка их фунгицидной активности. Проверена ингибирующая активность соединений к штаммам грибов *Fusarium solani* (штамм, обладающий средней устойчивостью к фунгицидам) и *Sclerotinia sclerotiorum* (штамм, восприимчивый к большинству фунгицидов) из коллекции культур фитопатогенных микроорганизмов агробиотехнологического департамента РУДН. При синтезе новых химических соединений, обладающих фунгицидной активностью, установлены и структурно подтверждены производные мочевины 1-(3-(Дифторметил)-1-метил-1*H*-пиразол-5-ил)-3-(2-хлорфенил) мочевина. Все целевые соединения оценивались на их противогрибную активность по ингибированию роста мицелия. Предварительные результаты скрининга показали, что все синтезированные соединения обладают хорошей фунгицидной активностью в отношении S. sclerotiorum. Соединение 1-(3-(Дифторметил)-1-метил-1H-пиразол-5-ил)-3-(3-фторфенил) мочевина проявляла противогрибную активность в отношении *S. sclerotiorum*. В концентрации 100 ppm соединение подавляло рост штамма *S. sclerotiorum* на 90,5 %. Эксперимент in vitro выявил, что соединение 1-(3-(Дифторметил)-1-метил-1H-пиразол-5-ил)-3-(3-фторфенил) мочевина было эффективным для подавления склеротиниозной гнили — *S. Sclerotiorum* — в дозе 100 мг/л. Значимость исследований состоит в том, что производство экологически безопасной продукции в агропромышленном комплексе невозможно без разработки новых биологически активных соединений, обладающих низкими нормами расходов и показателями токсичности, контролируемой персистентностью, соответствующими мировому уровню. Полученные результаты можно внедрить в реальный сектор экономики, занимающийся производством химических средств защиты растений. Синтез и использование новых фунгицидов актуальны в сельскохозяйственном производстве как элемент развития и интенсификации существующих аграрных технологий.

Ключевые слова: триазолы, мочевина, средства защиты растений, азолы, пестициды, земледелие, химические гетероциклические соединения, биологическая эффективность

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