

# Efficacy of fungicides against pathogens of apple core rot from the genera *Fusarium* Link, *Alternaria* Nees and *Botrytis* (Fr.) under laboratory conditions

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**Abstract.** As a result of the studies carried out, species-specific reactions of relative sensitivity to chemical fungicides of strains of the genera *Fusarium* Link, *Alternaria* Nees and *Botrytis* (Fr.) *in vitro* were noted. Fungicide Cidely-Top, DC inhibited the growth of fungi *F. sporotrichioides*, *F. semitectum* and *A. alternata* by 95-96 %, its minimum biological effectiveness was 83 % on the *F. oxysporum* strain. Fungicide Luna Tranquility, SC showed very high antifungal activity against *F. avenacium*, *F. oxysporum* and *A. alternata* (100 %) and low antifungal activity against *F. solani* and *B. cinerea* (73-74 %), other pathogens were suppressed with average activity. The drug Tirada, SC inhibited the growth of all studied micromycetes by 98-100 %, except for *B. cinerea*, the effectiveness against which was 94 % and lower. In general, against the *B. cinerea* fungus, the effectiveness of all drugs was average or below average, which may indicate the presence of resistance in the studied strain.

## 1 Introduction

In fruit production, the apple tree ranks first, both in the North Caucasus region and in the world. A significant decrease in yield and product quality occurs, first of all, as a result of damage to fruits by mycoses. During the annual monitoring surveys of the gardens of the Krasnodar region, an increase in rotting of the core (seed cavity) of fruits caused by the pathocomplex of semi-parasitic fungi, among which the species of the genera *Fusarium* Link and *Alternaria* Nees predominate, was revealed [1–4].

Symptoms of the disease are most often found only when the fruit is cut. Infection of the apple tree even during the flowering period with the further, often asymptomatic, development of the disease makes it difficult to timely reject low-quality products. In recent years, there has been an increase in the harmfulness of the fungus *Botrytis cinerea*, which causes botrytis disease of apple fruits during storage, the yield loss from which can reach 24 % in six months. In addition, this micromycete is part of the pathocomplex of fruit core rot [5]. The growth of harmfulness of the listed fungi requires the improvement of

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protective measures to combat them. For this purpose, it is necessary to search for effective fungicides.

There is no single method for effective control of fungi of the genus *Fusarium* in different crops. Usually, the fight against pathogenic *Fusarium* species includes a set of measures - crop rotation, phytosanitary measures and the reasonable use of fungicides. Studies by V.V. Chekmarev et al. in laboratory conditions have established that the biological effectiveness of fungicides Abacus Ultra (62.5 g / l pyraclostrobin + 62.5 g / l epoxiconazole), SE and Falcon (250 g / l spiroxamine + 167 g / l tebuconazole + 43 g / l triadimenol), EC for the fungus *F. acuminatum* Ellis & Everh. (wheat) was low and amounted to 43.0-59.6 %, which may indicate the presence of resistance. The effectiveness of these agents against the fungus *F. culmorum* (Wm.G. Sm.) (wheat) was at a high level: 98.6-100 % [6-7]. A comparative assessment of the effectiveness of fungicides was given at four concentrations of the active substance (10, 100, 1000 and 10,000 parts per billion) containing pyraclostrobin (200 g / l), thiram (400 g / l), fludioxonil (25 g / l), imazalil, metalaxil and tebuconazole (50, 40 and 30 g / l) for ten strains of *Fusarium* spp. (soy, barley, wheat, tomato). The most effective in suppressing the growth of fungi was found to be a pyraclostrobin-based fungicide; the sensitivity of *F. acuminatum*, *F. graminearum* Schwabe, *F. semitectum*, *F. culmorum*, *F. sporotrichioides*, *F. equiseti* strains to fungicides was higher than that of *F. oxysporum*, *F. solani*, *F. verticillioides* Sacc. and *F. proliferatum* [8].

At the same time, there is very little information in the literature on the effectiveness of chemicals used to protect fruit crops and grapes from a complex of pathogens of the genus *Fusarium*. So, the most effective chemical substances against strains of *Fusarium* fungi, which are the cause of infectious drying of the generative organs of grapes, *in vitro* experiments were: for *F. proliferatum* - thiram (chemical group of dithiocarbamates) + difenoconazole (triazoles), to a lesser extent - preparations containing methiram (dithiocarbamates) + pyraclostrobin (strobilurins); for *F. oxysporum* - difenoconazole, tetraconazole, difenoconazole + flutriafol (triazoles), as well as thiram + difenoconazole and mefentrifluconazole. From the group of strobilurins for *F. proliferatum* and *F. oxysporum*, preparations based on azoxystrobin are highly effective. For the same species, preparations of two active substances have shown high efficiency: fludioxonil (phenylpyrroles) + cyprodinil (anilinopyrimidines) [9].

In 2020, we assessed the biological effectiveness of chemical fungicides of various mechanisms of action - Chorus (750 g / kg cyprodinil), WDG; Luna Tranquility (125 g / l fluopyram + 375 g / l pyrimethanil), SC; Granuflo (800 g / kg thiram), WDG; Score (250 g / l difenoconazole), EC for species *F. avenaceum* (Fr.) Sacc., *F. solani* (Mart.) Sacc. and *F. semitectum* Berk. & Ravenel isolated from the seed cavity of infected apple fruits. Under *in vitro* conditions, a high (90-100 %) biological effectiveness of the studied drugs against *F. avenaceum* (Fr.) Sacc. Against the species *F. solani* and *F. semitectum*, the preparations Granuflo, WDG (17.3 and 17.8%) and Luna Tranquility, SC (21.9 and 24.0 %) showed insufficient effectiveness, the active substances of which belong to the chemical groups dithiocarbamates and anilinopyrimidines, respectively. The studied species lacked sensitivity to Chorus, WDG (4.1 and 6.6 %). Fungicide Score, EC inhibited the growth of *F. solani* and *F. semitectum* by 72 % [10]. Our earlier study of four fungicides of chemical origin Chorus, WDG, Luna Tranquility, SC, Granuflo, WDG, Score, CE for the species *F. sporotrichioides* and *F. semitectum*, causative agents of apple core rot, showed low biological effectiveness in two cultural characteristics. Its values, with one exception, did not exceed 50 %, and for some fungicides, in some indicators, there was no effectiveness. Species-specific reactions of relative sensitivity to chemical preparations according to different parameters *in vitro* were noted. Thus, *F. semitectum* showed a higher relative sensitivity in terms of the number of colonies, while *F. sporotrichioides* - in terms of the

development of aerial mycelium [11]. We also studied the sensitivity to fungicides *in vitro* of another causative agent of rot of the core of apple fruits - *Alternaria alternata* (Fries: Fries) Keissler. The drug Luna Tranquility, SC showed an efficiency of 95 %, fungicide Granuflo, WDG – 70 %, Chorus, WDG – 75 % [12]. In the studies of colleagues, 8-hydroxyquinoline sulfate, kresoxim-methyl (analogs of strobilurins), had the greatest efficiency in suppressing the causative agent of *A. tenuissima* Wiltshire *in vitro* complex preparations of two active ingredients: difenoconazole + flutriafol, cyprodinil + iprodione (dicarboximides). Sufficient antifungal activity was demonstrated by fluopyram, difenoconazole, fludioxonil + cyprodinil at maximum concentrations; the smallest is a drug based on a mixture of thiram + difenoconazole [13]. Long-term data of colleagues [14-15] showed that preparations Score (250 g / L of difenoconazole), EC, Maxim (25 g / L of fludioxonil), SC, Zerox (3000 mg / L of colloidal silver), WS very effectively suppressed the growth and development of *A. solani* Sorauer, *A. alternata*, *F. solani* (potato, tomato); Preparation Prestige (140 g / l imidacloprid + 150 g / l pencycuron) SC showed high efficacy against *Alternaria* (*A. solani*) (potato, tomato). It was found that silver and copper suppress *A. alternata* (tomato) and *Botrytis cinerea* Pers. (strawberries) [16]. Studies of the *in vitro* fungicidal activity of thiazolidonones 1-4 [17], 1,2,3-triazolium-5-olates and 1,1,3-trisubstituted triazines [18] showed that these compounds have a selective antifungal effect against *B. cinerea* (cucumber). For the genus *Botrytis* sp. (cobnut) the best fungicidal activity was exhibited by Klad, SC (60 g / l imazalil + 60 g / l tebuconazole; 80 g / l thiabendazole) [19].

It is not always possible to assess the effectiveness of chemical inhibition of a particular fungus species; in addition, many phytopathogens can acquire a certain level of resistance to fungicides. For this reason, it becomes necessary to study the effectiveness of drugs in relation to certain species using laboratory methods using pure cultures of fungi. The *in vitro* method allows in a fairly short time to assess the effectiveness of chemicals against pathogens [20-22].

Objective of the study: to determine the effectiveness of fungicides of chemical origin *in vitro* against some species of the genera *Fusarium*, *Alternaria* and *Botrytis*, causative agents of rot of the apple fruits core.

## 2 Materials and methods

The studies were carried out in 2020 in the laboratory of biotechnological control of phytopathogens and phytophages of the FSBSI NCF SCHVW. The objects of research were monoconidial strains of apple core rot pathogens: *Fusarium solani* (strain FR20XXV / 1.2), *Fusarium avenacium* (strain FR20XXIV / 2.2.1), *Fusarium sporotrichioides* (strain FR20XXIV / 2.4), *Fusarium oxysporum* (strain FR20XXV / 2.4.1), *Alternaria alternata* (strain FR19I / 13.2), *Botrytis cinerea* (strain SR20 / 1).

The work studied the action of fungicides Cidely-Top, DC (125 g/l difenoconazole + 15 g/l ciflufenamide) and Tirada, CE (400 g / l thiram + 30 g/l difenoconazole). The control was distilled, autoclaved water; the standard was Luna Tranquility, SC (125 g/l fluopyram + 375 g/l pyrimethanil). Solutions of fungicides were prepared from the calculation of the concentrations recommended by the manufacturer, according to the method of Chekmarev V.V. [19]. Aqueous solutions of the preparations were prepared on the day of the experiment and used fresh. In each Petri dish, 300 µL of an aqueous solution of the drug or water was applied by two methods: they were evenly distributed with a sterile glass spatula over the surface of nutrient agar and by dropping onto three paper disks (for Tirada, EC, Luna Tranquility, SC), 1 cm in diameter, 100 µL each for everyone.

The isolates of these species were inoculated with a small piece of aerial mycelium injected into the center of a Petri dish on a PGA medium (potato-glucose agar) in triplicate.

The obtained crops were placed in a thermostat for 7 days and incubated at 25 °C. All procedures related to manipulations with pure cultures were performed under sterile conditions.

The criterion for assessing the biological effectiveness (BE) of the preparations was the average growth rate (cm) of the fungal colony (aerial mycelium), which was analyzed according to the generally accepted method [20]. Using the modified paper disk method [13, 23], the antifungal activity of the preparations was determined according to the scale:

0 points - no effect; 1 point - low activity; 2 points - average activity; 3 points - high activity; 4 points - very high activity.

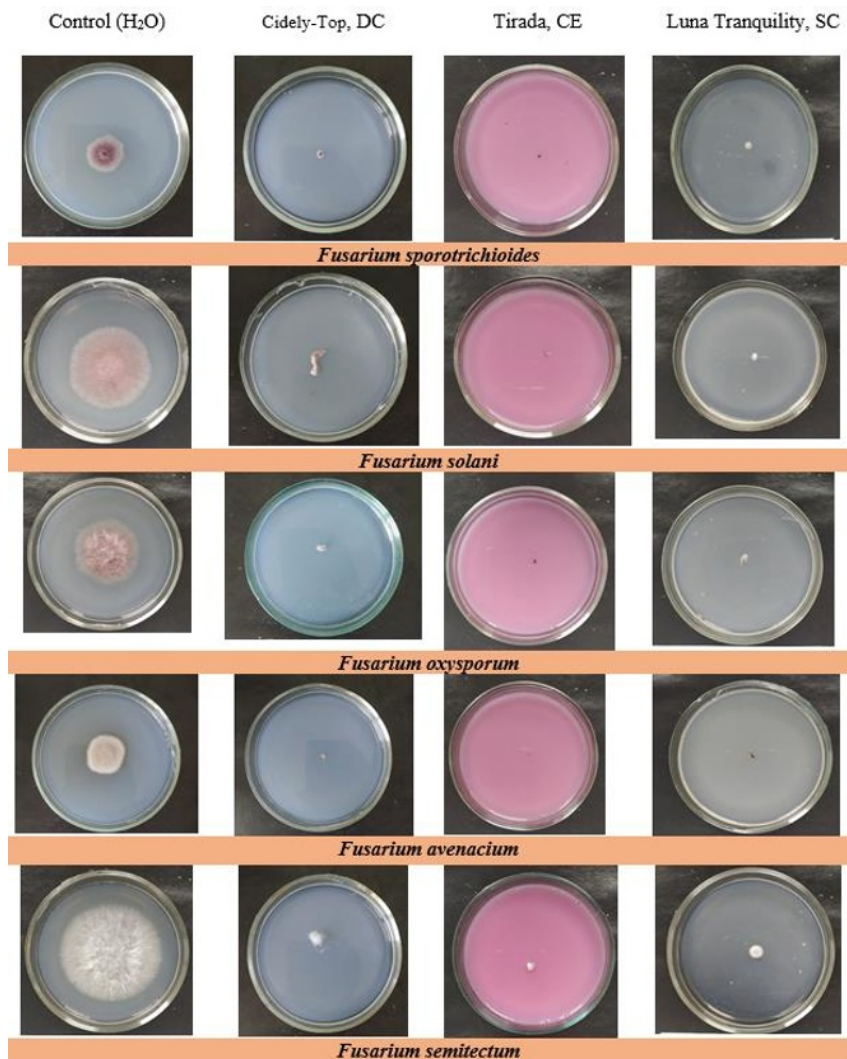
This method makes it possible to assess the effect of the concentration gradient of the active substance on the growth of the fungal mycelium.

### 3 Results and discussion

The results obtained by various methods on the effectiveness of drugs against causative agents of rot of the core of apple fruits gave comparable data. So, with a uniform application of the drug to the agar surface, it was found that all the studied fungicides inhibited the growth of aerial mycelium of species of the genus *Fusarium*, but to varying degrees (Table 1, Figure 1). On the 7th day of fungi growth, the diameter of the culture ranged from 2.5 cm to 4.8 cm. The highest sensitivity to all tested preparations was shown by *F. sporotrichioides* and *F. avenacium*, the lowest - by *F. solani* and *F. semitectum*.

**Table 1.** Influence of fungicides on the growth of fungi of the genus *Fusarium in vitro*, 7th day after inoculation

Experience Option	Culture diameter, cm	Biological efficiency, %
<i>Fusarium sporotrichioides</i>		
Control (H <sub>2</sub> O)	2.6	-
Cidely-Top, DC	0.1	96.1
Tirada, CE	0	100
Luna Tranquility, SC	0.2	92.3
LSD* <sub>05</sub> 1,0		
<i>Fusarium solani</i>		
Control (H <sub>2</sub> O)	3.9	-
Cidely-Top, DC	0.3	92.3
Tirada, CE	0	100
Luna Tranquility, SC	1.0	74.3
LSD <sub>05</sub> 1,2		
<i>Fusarium oxysporum</i>		
Control (H <sub>2</sub> O)	3.0	-
Cidely-Top, DC	0.5	83.3
Tirada, CE	0	100
Luna Tranquility, SC	0	100
LSD <sub>05</sub> 1,1		
<i>Fusarium avenacium</i>		
Control (H <sub>2</sub> O)	2.5	-
Cidely-Top, DC	0.3	88.0
Tirada, CE	0	100
Luna Tranquility, SC	0	100
LSD <sub>05</sub> 1,1		
<i>Fusarium semitectum</i>		
Control (H <sub>2</sub> O)	4.8	-
Cidely-Top, DC	0.2	95.8
Tirada, CE	0.06	98.7
Luna Tranquility, SC	0.5	89.5
LSD <sub>05</sub> 1,3		
*least significant difference at p≤0.05		



**Fig. 1.** The effect of drugs on the growth of fungi of the genus *Fusarium* *in vitro*, 7th day after inoculation

The maximum biological effectiveness of the fungicide Cidely-Top, DC was revealed for the fungi *F. sporotrichioides* (the most common species) and *F. semitectum*, the minimum - for *F. oxysporum* and *F. avenacium*. The last two types of the drug Luna Tranquility, SC suppressed by 100 %, and showed minimal efficacy against the species *F. solani*. The drug Tirada, EC suppressed the growth of all studied micromycetes by 98-100 %, showing the best result.

Analysis of the biological effectiveness of fungicides against *A. alternata* *in vitro* revealed its high sensitivity to all drugs.

The *B. cinerea* strain turned out to be the fastest growing culture in this experiment and had the lowest sensitivity to all studied drugs (Table 2, Figure 2).

**Table 2.** Influence of fungicides on the growth of fungi of the genus *Alternaria* and *Botrytis* *in vitro*, 7th day after inoculation

Experience Option	Culture diameter, cm	Biological efficiency, %
<i>Alternaria alternata</i>		
Control (H <sub>2</sub> O)	3.0	-
Cidely-Top, DC	0.1	96.6
Tirada, CE	0	100
Luna Tranquility, SC	0	100
LSD <sub>05</sub> 0,9		
<i>Botrytis cinerea</i>		
Control (H <sub>2</sub> O)	7.6	-
Cidely-Top, DC	0.5	93.4
Tirada, CE	0.4	94.7
Luna Tranquility, SC	2.1	72.3
LSD <sub>05</sub> 1,3		

Thus, having studied the biological effectiveness of three fungicides *in vitro* against pathogens of rot of the apple fruits core, it was found that drugs, where one of the active substances belongs to triazoles, suppressed the growth of pathogens of the genus *Fusarium* by 98-100 %, which coincides with the data of other researchers [13]. The efficacy of a preparation containing an active substance from the chemical group of anilinopyrimidines was found to be selective for the different species of this genus assessed. Thus, the effectiveness of Luna Tranquility, SC for strains *F. solani* and *F. semitectum* was lower. Earlier, we obtained similar results for *F. solani* [10].

The effectiveness of the preparations Tirada, EC and Luna Tranquility, SC in suppressing *A. alternata* was 100 %. For the preparation Luna Tranquility, SC, this result coincides with our previously obtained data [11]. At the same time, according to colleagues, the drug with the active substances thiram and difenoconazole showed lower values of effectiveness in the control of *A. tenuissima*, the causative agent of grape alternaria, in comparison with other studied drugs [13]. Differences in the results can be associated with both the characteristics of the strains and the emergence of resistance during long-term treatments. This work will be continued by us in the direction of expanding the number of studied strains, spectra of chemical groups of fungicides, as well as for other species of the genus *Alternaria*, causative agents of apple rot.

In relation to the *B. cinerea* fungus, the effectiveness of all drugs was average or below average, which indicates a low sensitivity of the studied strain. The chemical compounds were studied by us had a selective antifungal effect against this pathogen; the data obtained coincided with the results of colleagues [17-18]. For *B. cinerea*, the search for highly effective fungicides will continue, as well as the selection of the necessary concentrations *in vitro*.

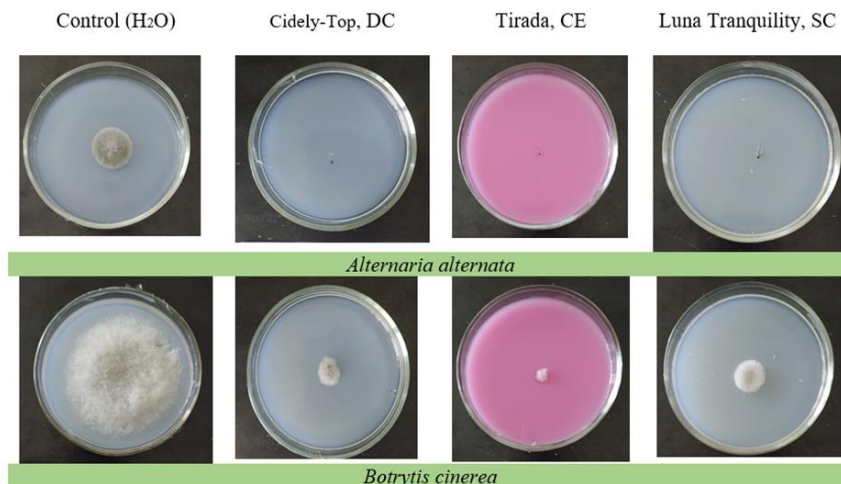
In the analysis by the disk diffusion method, two preparations were evaluated, one of which contained a fungicide from the group of triazoles (Tirada, CE), and the other - aniline pyrimidines (Luna Tranquility, SC).

Using the paper disk method, it was also found that the preparations Tirada, CE and Luna Tranquility, SC had antifungal activity against the studied pathogens of apple core rot (Figures 3, 4, 5). Both drugs showed very high antifungal activity (4 points) against *F. oxysporum* (LSD<sub>05</sub> 0.9) and medium (2 points) against *B. cinerea* (LSD<sub>05</sub> 1.2).

The fungicide Luna Tranquility, SC showed very high antifungal activity against the species *F. avenacium* (LSD<sub>05</sub> 0.9) and *A. alternata* (LSD<sub>05</sub> 0.7), and low for *F. solani* (1 point) (LSD<sub>05</sub> 1.0, the difference is insignificant); the drug suppressed other pathogens with medium activity (2 points). Fungicide Tirada, CE, on the contrary, for the pathogens *F.*

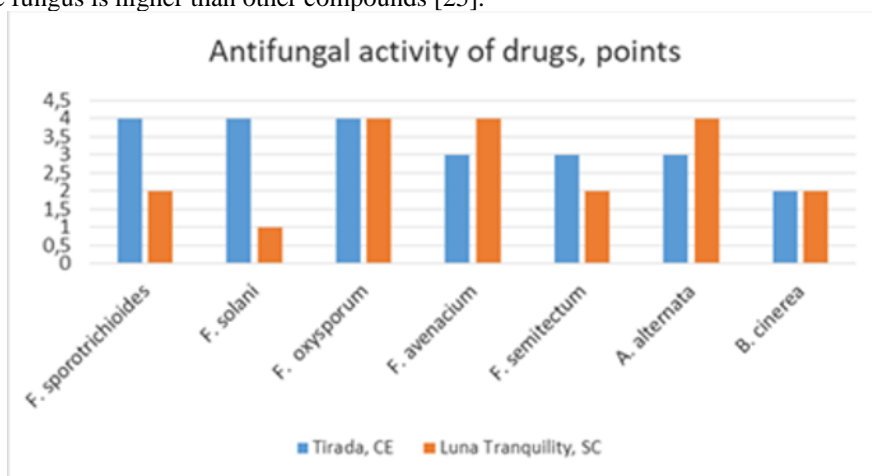


solani and *F. sporotrichioides* had the maximum antifungal activity (4 points) and for *F. semitectum* it was high (3 points).

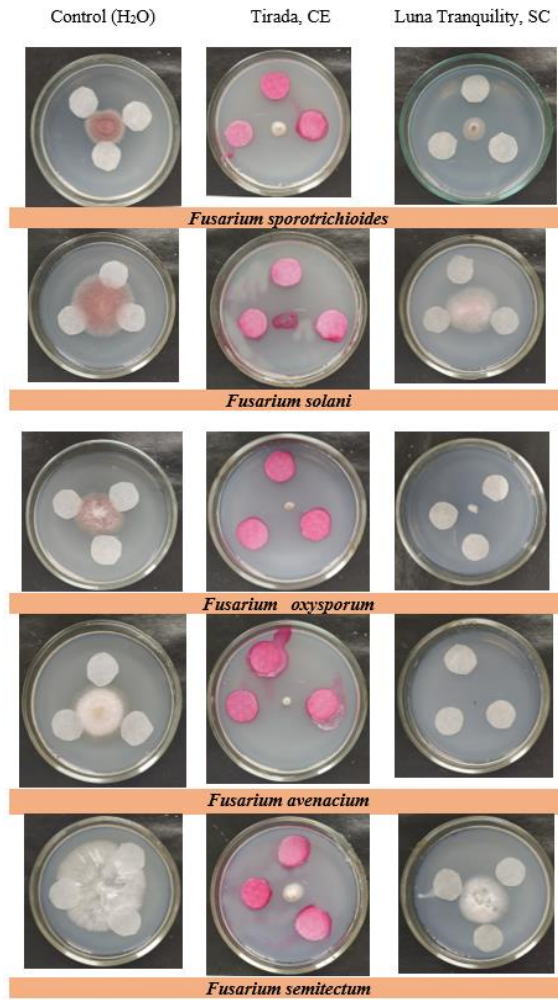


**Fig. 2.** The effect of drugs on the growth of fungi of the genus *Alternaria* and *Botrytis* *in vitro*, 7th day after inoculation

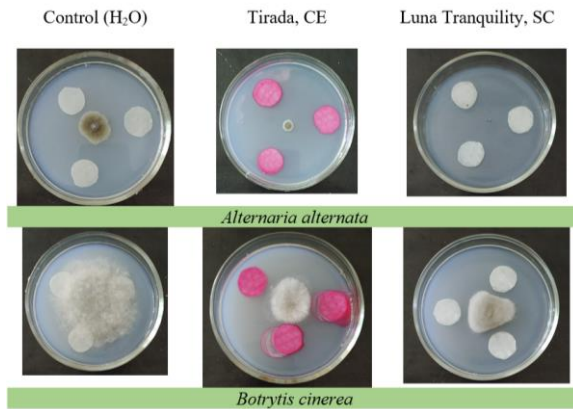
Thus, the disk diffusion method, as well as the method of uniform application of fungicides to the agar surface, showed a high efficiency of the preparation containing a compound from the triazole group as one of the active substances in relation to a larger number of pathogens. In this case, the drug Tirada, CE contains the second component, difenoconazole. This active ingredient is one of the most effective in the class of triazoles; proven to be effective against many fungi. For example, for the causative agent of apple scab, the fungicide remains highly effective after many years of use, and only isolated cases of a shift in sensitivity to it have been revealed [24]. It is noted that the resistance of triazoles is quantitative or dose-dependent, and the activity of difenoconazole against the same fungus is higher than other compounds [25].



**Fig. 3.** Antifungal activity of the fungicides Tirada, CE and Luna Tranquility, SC against fungi of the genera *Fusarium*, *Alternaria* and *Botrytis*, causative agents of rot of the core of apple fruits, *in vitro*, 7 days after inoculation, paper disc method



**Fig. 4.** The effect of drugs on the growth of fungi of the genus *Fusarium* *in vitro*, 7th day after inoculation, paper disc method



**Fig. 5.** The effect of drugs on the growth of fungi of the genus *Alternaria* and *Botrytis* *in vitro*, 7th day after inoculation, paper disk method



## 4 Conclusion

As a result of the studies, species-specific reactions were noted regarding the sensitivity to chemical preparations of strains of the genera *Fusarium*, *Alternaria* and *Botrytis* under *in vitro* conditions. Fungicide Cidely-Top, DC inhibited the growth of fungi *F. sporotrichioides*, *F. semitectum* and *A. alternata* by 95-96 %, its minimal inhibition was 83 % on the *F. oxysporum* strain. Fungicide Luna Tranquility, SC showed very high antifungal activity against the species *F. avenacium*, *F. oxysporum*, and *A. alternata* (100 %) and low against *F. solani* and *B. cinerea* (BE 73-74 %), suppressed other pathogens with an average activity. The drug Tirada, CE inhibited the growth of all studied micromycetes by 98-100 %, except for *B. cinerea*, the effectiveness against which was 94 % and lower. In general, against the fungus *B. cinerea*, the effectiveness of all drugs was average or below average, which may indicate a low sensitivity of this pathogen to the studied two-component fungicides.

A higher efficiency against a greater number of species of causative agents of rot of the apple fruits core was noted in preparations having a compound from the group of triazoles as one of the active substances.

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