UDC 581.2:582.22:63:576.3 **DOI:** https://doi.org/10.36495/2312-0614.2024.1.40-44

ANTAGONISTIC PROPERTIES

of preparation to themicromycetes *Fusarium oxysporum* Schltdl.

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Goal. To determine the antagonistic properties of the preparations MycoHelp, FitoHelp, Trichodermin-bio and Haupsin to the micromycetes *Fusarium oxysporum* Schltdl. **Methods.** To study the influence of biological preparations on the growth and development of *Fusarium oxysporum* micromycetes, were chosen the preparations — MycoHelp, Fitohelp, Trichodermin-bio and Haupsin. The research was conducted in the laboratory of biocontrol of agroecosystems and organic production of the Institute of Agroecology and Nature Management of the National Academy of Sciences. Determined Was determined the sensitivity of the micromycete *Fusarium oxysporum* to the studied the preparations. They were calculated rate of radial growth of the mycelium of micromycetes and the intensity of sporulation. **Results.** The micromycete *F. oxysporum* turned out to be the most sensitive to the preparations MycoHelp and Trichodermin-bio, where the colonies of the micromycete reached 8.7 and 30.5 mm. Micromycetes are less sensitive to Fitohelp and Gaupin, where the growth of the colony was 45.2 and 54.6 mm. Under the influence preparations of Trichodermin-bio, the growth rate of the *F. oxysporum* colony decreased to 0.1 mm/h, MycoHelp increased to 0.2 mm/h, Phytohelp to 0.5 mm/h, and Haupsin to 0.7 mm/h on the 4th day and decreases on the 6th day to 0.1 mm/h. On the control version, the growth rate was linear from 0.2 mm/h (on the 2nd day) to 0.7 mm/h (on the 6th day). The preparations Trichodermin-bio and Myco Help, which contain antagonistic fungi, significantly reduced the sporulation of the micromycete *F. oxysporum*, which ranged from 121.243 to 343.276 thousand units/ml. Compared to the control, which was characterized by a high intensity of sporulation, where the number of spores was above 1 million. **Conclusions.** It turns out to be the most sensitive to the preparations MycoHelp and Trichodermin-bio, where the zone of lack of growth is clearly visible, and less sensitive to the preparations Fitohelp and Gaupin. The growth rate of the colony of *F. oxysporum* under the influence of the drug Trichodermin-bio is low and reaches 0.1 mm/h. On the 2nd and 4th days, the growth rate increased to 0.2 mm/h under the influence of the drug MycoHelp, up to 0.5 mm/h under the influence of the Fitohelp preparations, and up to 0.7 mm/h under the influence of Haupsyn. This indicates that the preparations inhibit the growth of the micromycete *F. oxysporum*. The intensity of sporulation of the micromycete *F. oxysporum* significantly decreased under the influence of the preparations and ranged from 121.243 to 668.420 thousand units/ml. Compared to the control, which was characterized by a high intensity of sporulation, where the number of spores was above 1 million. The preparations Trichodermin-bio and Myco Help, which contain antagonistic fungi, reduce the sporulation of the micromycete *F. oxysporum* by 3—9 times. Research in this direction deepens knowledge of the process of interaction of micromycetes and reveals new possibilities of biological control of the number of phytopathogenic fungi in agroecosystems. This will ensure an increase in the quality of grain products and reduce the level of anthropogenic impact on the natural environment.

phytopathogenic micromycete; biological preparations; micromycete sensitivity; colony growth rate; sporulation intensity; biological control

One of the reasons for a lack of harvest of the agricultural crops in Ukraine is their damage by phytopathogenic micromycetes. Often the loss of seed yield due to diseases can reach 75% [1, 2]. And as a result, one of the most important component technologies of plant cultivation is their protection from phytopathogenic microorganisms. The intensive use of chemical plant protection agents has a negative impact on the environment and the quality of the products. The resistance of pathogens to chemicals has been constantly increasing, and the preparations lose their effectiveness over time. Fungicides of chemical origin often have a negative effect on plants and cause their growth to slow down, and sometimes this leads to the cessation of their development [3, 4].

Nowadays, studying of the ecological features of the micromycetes of *Fusarium* spp. genus, which under the influence of environmental factors are able to change their life strategies and live as parasites on vegetative plants or as saprotrophs on the dead remains in the soil, attracts considerable attention of the scientists [5—8]. The species of fungi of the genus of *Fusarium* spp. at the end of the growing season, as well as after harvesting, are able to provide recombination of the genetic material, which allows them to constantly adapt to certain environmental conditions and acquire resistance to fungicides. This leads not only to crop losses, but also significantly worsens the sowing and nutritive quality of the grain, as well as contributes to the biological pollution of the agrocenoses [9].

Nowadays, various scientific institutions have developed integrated systems of plant protection, which include economically feasible and ecologically safe organizational, economic, agrotechnical, biological, and chemical methods [10]. Such systems became integral parts of today's biological agriculture, which is conducted with the aim of reducing the negative impact of chemical agriculture, increasing soil fertility, and maintaining the balance in the ecological system [11]. Unfortunately, in our times, using various chemical means remain a priority in the practice of protecting plants from pests and diseases. Obviously, a reliable guarantee of environmental safety is the use of biological means of protection and plant growth regulators, which, unlike the pesticides of chemical synthesis, cause qualitative and quantitative changes among biota components [12, 13]. Recently, the significance of the use of microbiological preparations in agriculture has increased all over the world, including in Ukraine. The replacement of chemical preparations of plant protection for some biological preparations is becoming more and more important. During the last decades, a significant progress in their use for biological protection of plants from pathogens caused by fungi of the genus *Fusarsum* spp. has been achieved [14].

The producers of ecologically safe plant products of organic origins do not have a sufficient selection of biological means to protect the plants from the pathogenic micromycetes. Therefore, the development of the methods that allow any kind of a rapid evaluation of a preparation capable of restraining the development and spread of the resistant microorganisms is one of the strategic scientific missions worldwide [15, 16].

The biofungicidal preparations based on fungi and antagonistic bacteria make a promising alternative for biocontrol of the number of pathogenic microbiota. *Trichoderma* species of fungi (family *Hypocreaceae,* class *Sordariomycetes*, division *Ascomycota*) have a wide spectrum of activity, due to a number of metabolites they secrete, including lytic enzymes, vitamins, growth factors, phytohormones, organic acids, antibiotics, and amino acids [17].

Besides, the *Bacillus subtilis* 26 D strain is a factor that reduces the spread of root rot by 1.8 times and contributes to an increase in the above-ground mass of plants by 55.5% [18]. The antagonistic effect of Bacillus bacteria on phytopathogenic fungi primarily takes place due to their ability to produce various antibiotics [18], and also synthesize bacillisin, mycobacillin [19; 20], polymyxin, surfactin, lichenisin, mycosubtilin, iturin, and other cyclic lipopeptides.

There are also many kinds of biological fungicides based on *Pseudomonas* bacteria [21]. The fluorescent pseudomonads are able to suppress the development of the fungi of the genus of *Fusarium*, which is the causative agent of grain ear crops [22]. It has been proven that the bacteria of *Pseudomonas aureofaciens* and *Pseudomonas putida* are characterized by a high antagonistic activity against the causative agents of septoriosis, as well as by fusariosis of the wheat ear. Despite the good prospects of using these preparations in the agroecosystems to protect plants of agricultural crops from phytopathogenic microorganisms that release antagonistic substances, their use can still be problematic. This is due to the fact that such substances can induce the resistance of phytopathogens to the mentioned compounds [23, 24].

The goal of the study. To determine the antagonistic properties of MycoHelp, FitoHelp, Trichodermin BT and Haubsin, C preparations as to the micromycete *Fusarium oxysporum* Schltdl.

Research methodology. To study the effect of the biological preparations on the growth and development *of Fusarium oxysporum* micromycetes, the following preparations were chosen: MycoHelp, the powder (cells of bacteria *Bacillus subtilis*, *Azotobacter*, *Enterobacter*, *Enterococcus* and fungi *Trichoderma lignorum*, *Trichoderma viride*, the total number of viable cells is not less than 1.0 × 10^9 КУО/г), FitoHelp, suspension (*Bacillus subtilis* bacteria cells, titr 1.0 × 10^9 — 1.0 × 10^{10} КУО/см^3), Trichodermin BT, powder (*Trichoderma lignorum* fungi spores, the strain(M-40, spore titer 1—10 млрд/см^3) and Haubsin, C (composite *Pseudomonas chlororaphis subsp. aureofacien*, İMB B-7097 and *Pseudomonas chlororaphis subsp. aureofaciens*, İMB B-7096, the title is no less 4 × 10^9 КУО/см^3) which are included in the «List of pesticides and agrochemicals allowed for use in Ukraine for 2023» [25].

The research was conducted in the laboratory of biocontrol of the agroecosystems and organic production of the Institute of Agroecology and Nature Management of the National Academy of Sciences.

The antifungal activity of the preparations as to the phytopathogenic micromycetes was determined using the «disc» method in order to find out the sensitivity of phytopathogenic fungi to the preparations of biological origins [26]. The growth of the colony was recorded at regular intervals during the study period from the second to the sixth days, and the rate of the radial growth of the mycelium of micromycetes was calculated using the following formula:

$$r = \frac{r1 - r0}{r1 - r0}$$

where *r* — the radial growth rate of colonies; *r*0 — the radius of colonies at time *t*0; *r*1 — the radius of the colonies at time *t*1.

The number of the infectious structures was determined in the Goryaev-Tom chamber [26; 27]. The number of the spores in 1 ml of suspension was calculated using the formula below:

$$M = \frac{(a \times 1000)}{(h \times S)},$$

where, *M* — the number of cells in 1 ml of suspension; *a* — the average number of spores per square; *h* — chamber depth in mm; *S* — the area of the grid square in mm^2.

The difference between the control and the experimental indicators was considered reliable when the probability of the difference was P <0.05.

Research results and discussion. The sensitivity of the micromycete *Fusarium oxysporum* to the following preparations: MycoHelp, FitoHelp, Trichodermin BT and Haubsin, C, was determined (see Fig. 1).

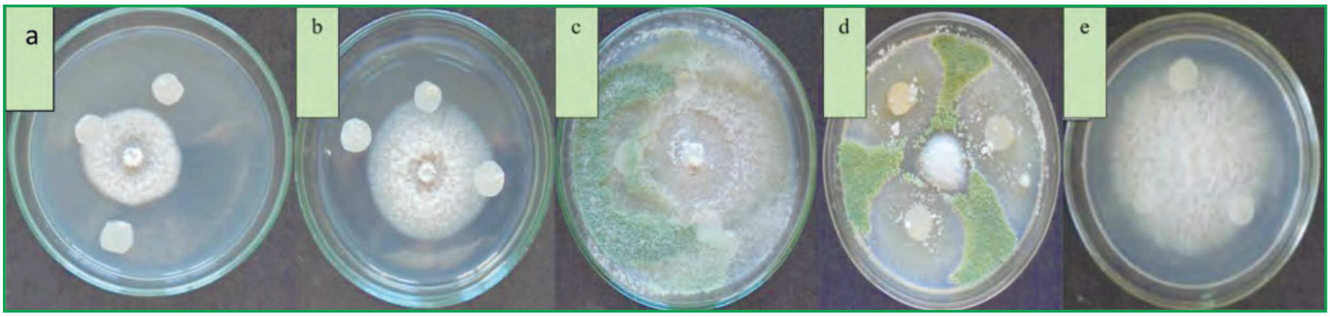

Fig. 1. Sensitivity of the micromycete F. oxysporum to the studied drugs: a — Fitohelp; b —Haupsin; c — Trichodermin BT; d — MycoHelp; e — control (water)

According to the results of the study presented in the table, it was determined that *F. oxysporum* micromycete was the most sensitive to the preparations MycoHelp and Trichodermin BT where the zone of the lack of growth was significantly visible. The studied micromycete colonies stopped growing on the 6^{th} day and reached 8.7 and 30.5 mm, compared to the control elements, where the diameter of the colony on the 6^{th} day reached 68.3 mm.

The micromycete *F. oxysporum* turned out to be less sensitive to the preparations like FitoHelp and Haubsin, C, and for it the growth of the colony on the 6th day reached 45.2 and 54.6 mm. The mentioned preparations were characterized by a low antagonistic property comparing to the data for the micromycete *F. oxysporum*.

The growth rate of the micromycete *F. oxysporum* under the influence of various preparations was also analyzed (see Table 1).

Under the influence of Trichodermin BT preparation, the colony growth diameter increased up to 8.7 mm on the 6th day, and the *F. oxysporum* colony growth rate was low and amounted to 0.1 mm/h. Under the influence of MycoHelp preparation, the diameter of the colony on the 6th day grew to 30.5 mm, and the growth rate on the 2nd and 4th days increased up to 0.2 mm/h, but on the 6th day it decreased. At the same time, under the effect of FitoHelp and Haubsin, C preparations, the growth of the colony was higher and reached 45.2 and 54.6 mm on the 6th day respectively, and the growth rate of the micromycete changed on the 4th day, increasing under the effect of FitoHelp preparation up to 0.5 mm/h, and under the influence of Haubsin, C preparation it raised up to 0.7 mm/h, on the 6^{th} day mycelial growth stopped, and then the growth rate decreased down to 0.1 mm/h. This indicates that the preparations inhibit the growth of the micromycete of *F. oxysporum*. On the control variety, the growth rate was linear and ranged from 0.2 mm/h (on the 2^{nd} day) to 0.7 mm/h (on the 6^{th} day).

The effect of the studied preparations on the intensity of sporulation of the micromycete *F. oxysporum* (see Fig. 2) was determined.

Trichodermin BT and MycoHelp preparations, which contain antagonistic fungi, significantly reduced the sporulation of the micromycete *F. oxysporum*, which ranged from 121.243 to 343.276 thousand units/ml. Compared to the control variant, which was characterized by a high intensity of sporulation, its number of spores totaled the number above 1 million. FitoHelp and Haubsin, C preparations had a lesser effect on the intensity of sporulation of the fungus, at that the number of the spores was lower than the one for the control variant, and it ranged from 549.350 to 668.420 thousand pcs./ml. This shows that the studied preparations have different effects on the reproductive capacity of the micromycete *F. oxysporum*. These studies will allow to select a fungicide with a greater antifungal effect against the micromycete *F. oxysporum*. This will

The effect of the biopreparations on the growth of the micromycete F. oxysporum colonies

Preparations	Colony diameter, mm			Mycelial growth rate, mm/h		
	2 days	4 days	6 days	2 days	4 days	6 days
MycoHelp	8.7±0.02	26.3±0.04	30.5±0.06	0.2±0.004	0.2±0.006	0.1±0.002
Trichodermin BT	6.3±0.02	8.0±0.04	8.7±0.04	0.1±0.002	0.1±0.004	0.1±0.002
Haubsin, C	6.7±0.001	38.0±0.06	54.6±0.01	0.2±0.004	0.7±0.03	0.1±0.002
FitoHelp	10.3±0.02	30.3±0.06	45.2±0.01	0.2±0.004	0.5±0.01	0.1±0.002
Control (water)	11.8±0.02	51.2±0.01	68.3±0.2	0.2±±0.006	0.4±0.004	0.7±0.002
Note: P — 0.05						

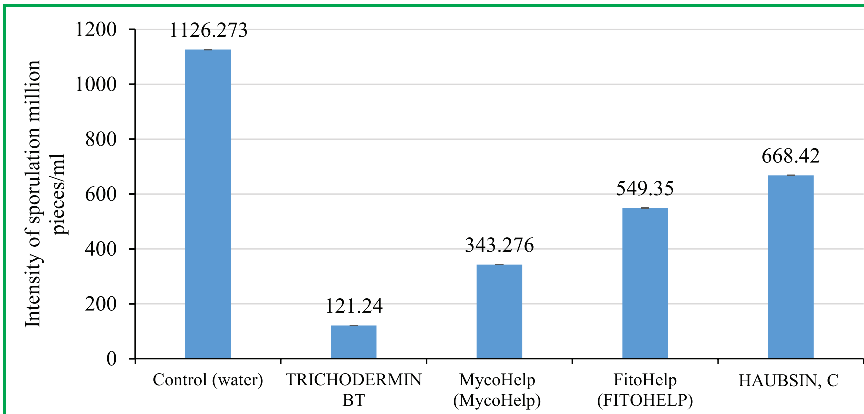

Fig. 2. Intensity of sporulation of the micromycete F. oxysporum under the influence of the studied preparations

ensure an increase in the quality of agricultural products and reduce the level of anthropogenic impact on the natural environment.

Therefore, the studied preparations, namely MycoHelp, FitoHelp, Trichodermin BT and Haubsin, C significantly influence the growth and development of the colonies of *F. oxysporum* micromycetes. The preparations named Trichodermin BT and MycoHelp showed themselves as the most effective, and were characterized by high antagonistic properties to the studied micromycete. A further research in this direction can deepen the knowledge of the process of interaction of the micromycetes and reveal new possibilities of biological control of the number of phytopathogenic fungi in the agroecosystems. This will help to provide an increase in the quality of grain products and reduce the level of anthropogenic impact on the natural environment.

CONCLUSIONS

The sensitivity of the micromycete of *F. oxysporum* to the studied preparations varied significantly. It turned out to be the most sensitive to the preparations named Trichodermin BT and MycoHelp, whereas the zone of a lack of the growth was clearly visible, and the mentioned mycromycete was less sensitive to Fitohelp and Haubsin, C preparations.

The growth rate of the colony of *F. oxysporum* under the influence of the preparation Trichodermin BT was determined as low and it reached 0.1 mm/h. Under the influence of MycoHelp preparation, the growth rate of the crops on the 2nd and the 4th day increased up to 0.2 mm/h, whereas under the influence of the preparation named FitoHelp it increased on the 4th day up to 0.5 mm/h, and also under the influence of Haubsin, C preparation, the growth rate of the studied crops increased up to 0.7 mm/hour, and then on the 6th day it went down to 0.1 mm/h. This indicated that the preparations inhibit the growth of the micromycete *F. oxysporum*.

The intensity of the sporulation of *F. oxysporum* micromycete significantly decreased under the influence of the mentioned preparations and ranged from 121.243 to 668.420 thousand units/ml. Compared to the control variation, the process was characterized by a high intensity of the sporulation, and in these terms the number of spores was found to be above 1 million ones. The preparations of Trichodermin BT and MycoHelp, which contain antagonistic fungi, reduce the sporulation of the micromycete *F. oxysporum* by 3—9 times.

Financing. The research was carried out within the framework of the task 24.01.02.05.F. «Development of scientific and methodological bases for the regulation of the number of micromycetes in agrocenoses of agricultural crops in the Central Forest Steppe of Ukraine».

Conflict of interest. The authors declare no conflict of interest.

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Антагоністична властивість препаратів до мікроміцету *Fusarium oxysporum* Schltdl.

Мета. Визначити антагоністичні властивості препаратів МікоХелп, п. (клітини бактерій *Bacillus subtilis*, *Azotobacter*, *Enterobacter*, *Enterococcus* та гриби *Trichoderma lignorum*, *Trichoderma viride*, загальна кількість життєздатних клітин не менше 1,0 × 10⁹ КУО/г), ФітоХелп, суспензія (клітини бактерій *Bacillus subtilis*, титр 1,0 × 10⁹ — 1,0 × 10¹⁰ КУО/см³), Триходермін БТ, п. (спори гриба *Trichoderma lignorum*, штам М-40, титр спор 1—10 млрд/см³) і Гаубсин, С (суміш *Pseudomonas chlororaphis subsp. aureofacien*, IMB B-7097 та *Pseudomonas chlororaphis subsp. aureofaciens*, IMB B-7096, титр не менше 4 × 10⁹ КУО/см³) до мікроміцету *Fusarium oxysporum* Schltdl. **Методи.** Для дослідження впливу біологічних препаратів на ріст і розвиток мікроміцетів *Fusarium oxysporum* було обрано препарати — МікоХелп, ФітоХелп, Триходермін БТ та Гаубсин, С. Дослідження проводили у лабораторії біоконтролю агроекосистем та органічного виробництва Інституту агроекологіїі природокористування НААН. Визначено чутливість мікроміцету *Fusarium oxysporum* до досліджуваних препаратів. Обраховували швидкість радіального росту міцелію мікроміцетів та інтенсивність споруляції. **Результати.** Найчутливішим виявився мікроміцет *F. oxysporum* до препаратів МікоХелп та Триходермін БТ, де колонії мікроміцету досягли 8,7 та 30,5 мм. Менш чутливий мікроміцет до препаратів ФітоХелп та Гаубсин, С, де ріст колонії становив 45,2 та 54,6 мм. За дії препаратів Триходермін БТ швидкість росту колонії *F. oxysporum* була низькою і становила 0,1 мм/год, МікоХелп — на 4-ту добу зростає до 0,2 мм/год, ФітоХелп — до 0,5 мм/год, Гаубсин, С — до 0,7 мм/год та знижується на 6-ту добу до 0,1 мм/год. На контрольному варіанті швидкість росту була лінійною від 0,2 мм/год (на 2-гу добу) до 0,7 мм/год (на 6-ту добу). Препарати Триходермін БТ та МікоХелп, які в своєму складі містять гриби антагоністи, істотно знижували споруляцію мікроміцету *F. oxysporum*, що варіювала від 121,243 до 343,276 тис. шт./мл. Контроль характеризувався високою інтенсивністю споруляції — кількість спор була понад 1 млн. **Висновки.** Найчутливіший мікроміцет *Fusarium oxysporum* Schltdl. до препаратів МікоХелп та Триходермін БТ, де суттєво видно зону відсутності росту, менш чутливий — до препаратів ФітоХелп і Гаубсин, С. Швидкість росту колонії *F. oxysporum* за дії препарату Триходермін БТ низька і сягає 0,1 мм/год. За дії препарату МікоХелп швидкість росту на 2-гу та 4-ту добу зростала до 0,2 мм/год, за дії препаратів ФітоХелп та Гаубсин, С відповідно до 0,5 та 0,7 мм/год. Це свідчить про те, що препарати пригнічують ріст мікроміцету *F. oxysporum*. Інтенсивність споруляції мікроміцету *F. oxysporum* істотно знижувалася за впливу препаратів і варіювала від 121,243 до 668,420 тис. шт./мл, порівняно з контролем, який характеризувався високою інтенсивністю споруляції — кількість спор становила понад 1 млн. Препарати Триходермін БТ та МікоХелп, які в своєму складі містять гриби антагоністи, у 3—9 разів знижують споруляцію мікроміцету *F. oxysporum*. Дослідження в цьому напрямі поглиблюють знання процесу взаємодії мікроміцетів і розкривають нові можливості біологічного контролю чисельності фітопатогенних грибів в агроекосистемах. Це забезпечить підвищення якості зернової продукції та знизить рівень антропогенного впливу на навколишнє природне середовище.

фітопатогенний мікроміцет; біологічні препарати; чутливість мікроміцету; швидкість росту колонії; інтенсивність споруляції; біологічний контроль

Received by the editors: *05.12.2023*
Accepted for publication: *24.02.2024*
Printed and published online: *March 2024*