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THE USE OF *Trichoderma* SPECIES AGAINST THE MAIN MYCOTIC PATHOGENS OF WALNUT

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Summary. Walnut plantations suffer from the impact caused by a number of pathogenic mycotic microorganisms. The most common pathogen fungus is *Alternaria alternata* and *Fusarium poae*. In the framework of the research, we aimed to determine the antagonistic activity of the strain *Trichoderma harzianum* CNMN - FD - 16 against the identified pathogens. Thus, on the 10th day the inhibition of the pathogen *A. alternata* by *T. harzianum* is 74%. On the same day, the inhibition of *F. poae* by *T. harzianum* is 73%. *Trichoderma harzianum* suppressed the colonies of *A. alternata* of 83% - this means 4 points, and the suppression of the colonies of *F. poae* was 69% - registering 3 points.

Keywords. *Trichoderma harzianum CNMN – FD – 16, Alternaria spp., Fusarium spp., pathogenic microorganisms, antifungal activity, dual culture.*

Introduction. Walnut (*Juglans regia* L.) is highly valued by the entire human community. The worldwide production of walnuts in between the years of 2021-2022 is estimated at 2 million tons, this demonstrating the importance of the crop [7].

Worldwide in 2022, the main walnut producer leaders were: People's Republic of China (2,521,504 t.), United States of America (592,390 t.), together they produce more than 60% of the world's nuts. They are followed by Iran (321,074 t.), Turkey (225,000 t.), Mexico (171,368 t.) Ukraine (125,850 t.). [1].

In the Republic of Moldova, walnut culture is given more special attention. Enumerating the benefits that the walnut has for humanity, they are: medicinal, food, industrial and last but not least, cultural. More than that, walnut has a personalized Law No. 658-XIV of 29.10.1999, which regulates the activities

related to the cultivation of walnuts, the production, collection, processing and marketing of its fruits and derived products, regardless of the type of ownership, the legal form of the economic entity and the location of the plantations [3].

With the increase in the attractiveness of the nut crop, it received the status of an industrial crop, the area sown in the Republic of Moldova increased 10 times - from 3,7 thousand hectares to 36,8 thousand hectares in 2021. The fruitful areas in the same year were 22,5 thousand hectares. Gross harvest increased from 6,0 thousand tons to 19,1 thousand tons, but walnut production per 1 ha for 2015-2021 in farms of all ownership types was only 0,62-0,96 t/ha, the correlations are shown in table 1.

Growing walnuts, like most nut crops, is a complex technological process. This crop makes the main requirements for soil and climate: it prefers rich, deep, well-drained soils, and does not tolerate waterlogging. The most favorable areas for growing are regions where the temperature does not fall below -25ºC. Photophilous, moisture-loving, sensitive to drought, requires a good supply of water in spring. It suffers from frosts, strong winds, it does not tolerate transplantation well. According to literary data, only 7% of the world's land area is suitable for growing walnuts [9]. Like any agricultural crop, walnut is a subject to attacks by a number of harmful microorganisms. Among the most harmful mycotic microorganisms, there are species of *Alternaria sp.* and *Fusarium sp.*

The fact that outlines the harmfulness of *Alternaria alternata* on walnut culture, is manifested by a decrease in yield due to the decrease in the photosynthetic surface of the leaves. Many species of *Alternaria sp.* causes leaf spots and thus affects the photosynthetic apparatus of the plant, which, with a strong infection, leads to significant yield losses, especially in weakened plants of sensitive varieties. As a human health hazard, conidia of small-spored *Alternaria* species are one of the most abundant indoor and outdoor airborne allergens [8].

Table 1. Indicators of the development of the walnut crop in Republic of Moldova by years [5]

Nr.	Indicators	Years							
		2000	2005	2010	2015	2018	2019	2020	2021
1.	Cultivated areas, thousands of ha.	3,7	3,7	9,4	25,2	30,8	35,2	34,7	36,8
2.	Fruit-bearing areas, thousands ha.	3,3	3,2	4,1	15,0	18,9	21,4	20,9	22,5

3.	Gross harvest, thousands t.	6,0	13,4	11,6	11,0	20,2	19,7	14,6	19,1
4.	Productivity in farms of all categories, t/ha	1,82	4,20	2,79	0,7	0,96	0,83	0,62	0,75
5.	Productivity in private farms, t/ha	2,40	6,0	3,78	1,71	1,97	1,41	1,26	1,39

Another pathogen identified in walnut culture is the microorganism *Fusarium poae*. *Fusarium* wilt caused especially by *F. poae* affects the stems and causes significant damage, as it allows the accumulation of mycotoxins in agricultural crops, the walnut crop entering the same category of crops subject to attack by mycotic pathogens [6].

In September 2019, twig canker symptoms were observed on English walnut in several orchards, with approximately 15% to 40% disease incidence in southern Xinjiang region, People's Republic of China. The branch lesions were long oval, concave, and black to brown. Leaves of the affected branches turned yellow and the branches eventually died. According to the morphological characteristics, the seven isolates were identified as a member of the *Fusarium* species [4].

For these reasons, the importance of combating pathogens is imposed as a necessity. The importance of the control is accentuated by the use of biorational and harmless methods to the entire biodiversity.

Materials and methods. The microbiological cultures investigated were: *Trichoderma harzianum* CNMN – FD – 16, *Alternaria alternata* and *Fusarium poae*. *Trichoderma harzianum* CNMN – FD – 16 was taken from the collection of the Laboratory of Phytopathology and Biotechnology of USM and is registered in the National Collection of Nonpathogenic Microorganisms (CNMN) of the Republic of Moldova.

The pathogens *Alternaria alternata* and *Fusarium poae* were isolated from the experimental walnut orchard of the Botanical Garden (Institute) "Alexandru Ciubotaru".

The research was carried out in laboratory (Institute of Genetics, Physiology and Plant Protection of Moldova State University, in the Laboratory of Phytopathology and Biotechnology). At the first stage, the antagonistic

activity of mycelial culture of *Trichoderma sp.* against the pathogens *A. alternata* and *F. poae* was studied by the method of dual culture. The microorganisms were cultivated at optimum temperature for these fungi of 25-26^{0}C. Colony radius were measured daily by the linear method (mm). On the 5th and 10th days, the index of inhibition (%) of the pathogen by *Trichoderma harzianum* CNMN – FD – 16 towards pathogens was calculated and the type of interaction between fungi in dual culture. The degree of growth of *Trichoderma sp.* on the *Alternaria* and *Fusarium* colonies was assessed in points: 0 points - no growth, 1 point - antagonist occupies 25% of the pathogen colony area, 2 points - antagonist occupies 25-50% of the pathogen colony, 3 points - antagonist occupies 51-75% of the colony area pathogen, 4 points - the antagonist occupies 76-100% of the area of the pathogen colony [10].

Formula for determining the percentage of inhibition of the growth of mycotic pathogens towards to the antagonist (*Trichoderma sp.*), on the 10th day, were:

$$I = \frac{M - m}{M} * 100\%;$$

As:

I – is the inhibition index, %,

M- average growth of the phytopathogen in the control;

m- it is the growth of the phytopathogen in the experiment [2].

Results and discussions. Initially, before proceeding, the researched pathogenic mycotic microorganisms were studied under a microscope to determine their purity and their integrity.

1 2





Figure: **1** Pathogenic microorganism *Alternaria alternata* visualized under the photonic microscope Bel Fotonics, ob. 10 x 40
2 Pathogenic microorganism *Fusarium poae* visualised under photonic microscope Bel Fotonics, ob.

Tab.2. The data of the antagonistic activity of *Trichoderma harzianum* CNMN – FD – 16 in relation to the pathogenic microorganisms *Alternaria alternata* and *Fusarium poae* on the 5th and 10th day

Nr.	Species name, strain	Colony radius, mm		Inhibition %		Degree of suppression of pathogens by *Trichoderma harzianum* CNMN – FD – 16 on the 10th day, %	Degree of suppression of pathogens by *Trichoderma harzianum* CNMN – FD – 16 on the 10th day, points
		5th day	10th day	5th day	10th day		
1.	***Alternaria alternata***	**6 ± 0,5**	**10 ± 0,4**	**0**	**0**	**83**	**4**
	***Trichoderma harzianum* CNMN – FD – 16**	**75 ± 0,5**	**85 ± 0,5**	**58,3**	**74**		
2.	***Fusarium poae***	**22 ± 0,5**	**25 ± 0,2**	**0**	**0**	**69**	**3**
	***Trichoderma harzianum* CNMN – FD – 16**	**62 ± 0,3**	**75 ± 0,4**	**65**	**73**		

On the 10th day the inhibition of the pathogen *A. alternata* by *T. harzianum* is 74% and there is no inhibition of *T. harzianum* by the pathogen. On the same day, the inhibition of *F. poae* by *T. harzianum* is 73%. *Trichoderma harzianum* CNMN – FD – 16 suppressed the colonies of *A. alternata* of 83% - this means 4 points, and the suppression of the colonies of *Fusarium poae* was 69% - registering 3 points.

Conclusions:

1. The strain *Trichoderma harzianum* CNMN - FD - 16 has antagonistic influence on the group of mycotic pathogens isolated from the walnut crop during the research (*Alternaria alternata* and *Fusarium poae*);
2. The microorganism *Trichoderma harzianum* CNMN - FD - 16 on the 10th day shows antagonistic activity by inhibiting: *Alternaria alternata* - by 83% registering 4 suppression points and *Fusarium poae* - by 69% with 3 suppression points.
3. The strain *Trichoderma harzianum* CNMN - FD - 16 can be recommended as a potential means of combating alternariosis and fusariosis wilt of the walnut culture.

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