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Antagonistic effect of the promising fungal producer strain of microbiopreparation T-1 *Trichoderma* sp. on oil flax Fusarium blight

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ABSTRACT

Fusarium blight is the most harmful disease of oil flax, which affects crops in all cultivation regions during the growing season. Considering the value of flaxseed oil, which is also used for medical purposes, it is advisable to use biological preparations to obtain environmentally safe products, which allow not only to ensure the protection of plants from diseases but also to improve the ecological situation. In recent years (2018-2020), at the biomethod laboratory of V.S. Pustovoit All-Russian Research Institute of Oil Crops has been carried out the search of the promising producer strains for developing microbiopreparations against the pathogen of oil flax Fusarium blight with a multifunctional type of action and a prolonged shelf life. These studies involve the study of the antagonistic mechanisms of promising producer strains of microbiopreparations on the disease pathogen. The article presents the effect of the promising antagonist strain T-1 *Trichoderma* sp. on the pathogen of oil flax Fusarium blight *Fusarium oxysporum* Schlecht. emend. Shyd. et Hans. var. *orthoceras* (App. et Wr.) Bilai, identified as a result of a gradual screening. High competition of the antagonist strain for the feeding area, its hyperparasitic and antibiotic activity was established during the co-cultivation of the antagonist and the pathogen by the method of double cultures. During the study of the mechanism of the antagonistic action of the promising producer strain on the Fusarium blight pathogen using a light microscope, it was found that even before coalescence with the antagonist colony on the second day of coincubation, the shrinking of certain areas in the pathogen mycelium was noted, which proves the antibiotic activity of the fungal antagonist T-1 *Trichoderma* sp. On the 4th day of co-cultivation, after coalescence of the pathogen and antagonist colonies, aggregation of the pathogen mycelium into the cords and the effect of the antagonist mycelium coiling around the pathogen hyphae were observed . On the 6th day, the effect of the antagonist mycelium coiling around the pathogen hyphae increased. By the 8th-10th days of interaction, there was observed degradation and lysis of the aerial and substrate mycelium of the pathogen of oil flax Fusarium blight. Over the entire observation period, there was noted the normal development of the oil flax Fusarium blight pathogen *Fusarium oxysporum* var. *orthoceras* in the control.

Key words: Oil flax, Fusarium blight, T-1 *Trichoderma* sp., Antagonist strain.
Article type: Research Article.

INTRODUCTION

Fusarium blight is the most harmful disease of oil flax, which affects the crops in all cultivation regions during the growing season. Considering the value of flaxseed oil, which is also used for medical purposes, it is advisable to use biological preparations to obtain environmentally safe products. Moreover, in recent years, environmentally safe techniques more and more often included during developing of the systems of protective measures (Jaber *et al.* 2020; Pavlyushin 2020). At the biomethod laboratory of the V.S. Pustovoit All-Russian Research Institute of Oil Crops, one of the few in Russia, for many years research has been carried out on the development of microbiological means of protecting oil crops from diseases. In recent years (2018-2020), there has been carried

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out the search of the promising producer strains for developing microbiopreparations against the pathogen of oil flax Fusarium blight with a multifunctional type of action and a prolonged shelf life. There are many studies about fungi around the world (Moradi *et al.* 2016; Karimi *et al.* 2017). One of the promising producer strains of microbiopreparation identified as a result of a gradual screening for the pathogens of oil flax Fusarium blight *Fusarium oxysporum* Schlecht. emend. Shyd. et Hans. var. *orthoceras* (App. et Wr.) Bilai and *Fusarium poae* (Peck) Wollenw., Lewis is the fungal strain T-1 *Trichoderma* sp. (Maslienko *et al.* 2019). It is known that the species of fungi from the genus *Trichoderma* are spread in all types of soils and are considered potential antagonists against many pathogenic soil microorganisms capable of minimizing the use of chemical fungicides (Zhang *et al.* 2013). The antagonistic activity of different species of fungi of the genus *Trichoderma* against the fungi of the genus *Fusarium* has been established (Gwa & Nwankiti 2017; Yassin *et al.* 2021). The development of effective microbiological plant protection products involves the study of the mechanisms of antagonistic action of the promising producer strains of the microbiopreparations on pathogens. The purpose of this work is studying the mechanism of antagonistic action of the promising fungal producer strain of the microbiopreparation T-1 *Trichoderma* sp. against the most spread pathogen of oil flax Fusarium blight *Fusarium oxysporum* var. *orthoceras*.

MATERIAL AND METHODS

The antagonistic activity of the antagonist strain to the Fusarium blight pathogen was determined by the method of double cultures. Pure cultures of the antagonist T-1 *Trichoderma* sp. and the disease pathogen *Fusarium oxysporum* var. *orthoceras* were grown separately for 5 days on potato sucrose agar (PSA; Bilay 1977). The blocks with the mycelium of the antagonist and pathogen (7 mm in size), were cut out with a sterile drill and placed in a Petri dish at 6 cm. Co-cultivation of the antagonist and the pathogen was also carried out on PSA, for ten days at the temperature of 25 ºC. The control was cultures of separately sown antagonist and pathogen. The counting was carried out on the 10th day of cultivation. The growth of the pathogen and antagonist in cm^2 and % of the Petri dish area (competition for the feeding area) and the nature of their interaction were noted: the presence or absence of growth inhibition zones (sterile zone, mm); the antagonist growth per pathogen colony (hyperparasitic zone, cm^2; Maslienko 2005). The mechanism of antagonistic action of a promising producer strain on the pathogen of oil flax Fusarium blight was determined according to an authentic procedure using a light microscope "Motic BA 300." Double cultures were plated with agar blocks on a PSA medium poured in a thin layer in Petri dishes and on glass slides. The slides were placed in humidity chambers and observed daily for the development of the pathogen and antagonist within 10 days (Maslienko 2005).

RESULTS AND DISCUSSION

During the co-cultivation of the antagonist and the pathogen by the method of double cultures, there was determined the antagonistic activity of the fungal strain T-1 *Trichoderma* sp. on the pathogen of oil flax Fusarium blight *Fusarium oxysporum* var. *orthoceras* (Fig. 1).

1 2

Fig. 1. Antagonistic activity of the fungal strain T-1 *Trichoderma* sp. on the pathogen of oil flax Fusarium blight *Fusarium oxysporum* var. *orthoceras* after 10 days of the cultivation on a PSA medium (orig.):
1 - Double culture of the antagonist with the pathogen; а - antagonist; б - pathogen;
2 - Control - the Fusarium blight pathogen.

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10 days after co-cultivation on a PSA medium, there was determined high competition of the antagonist strain for the feeding area in relation to *Fusarium oxysporum* var. *orthoceras*. The area of the nutrient medium growth with the antagonist was 70.4%, with the pathogen - 29.6%, while the pathogen occupied 100% of the medium surface in the control. In addition, the hyperparasitic activity of the antagonist to the Fusarium blight pathogen has been established. The size of the antagonist growth zone per pathogen (hyperparasitic zone) was 24.0 cm^2. The antibiotic activity of the antagonist strain was noted, the sterile zone between the colonies was 3.0 mm. Our data are consistent with the results of research by the teams of scientists from Israel, Austria, Spain, China, and Pakistan that the antagonistic activity of *Trichoderma* fungi against various plant pathogens occurs through different mechanisms of action, including competition for nutrients and space, antibiosis and mycoparasitism (Druzhinina *et al*. 2011; Ghazanfar *et al.* 2018). During the study of the antagonistic action mechanism of the promising producer strain on the pathogen of oil flax Fusarium blight using a light microscope, it was found that the fungal antagonist T-1 *Trichoderma* sp. showed a positive taxis against the pathogen colony. Even before coalescence with the antagonist colony, the shrinking of certain areas of the mycelium was noted in the pathogen mycelium on the second day of coincubation, which proves the antibiotic activity of the fungal antagonist (Fig. 2).

Fig. 2. Mycelium of the Fusarium blight pathogen *Fusarium oxysporum* var. *orthoceras* before coalescence with the colony T-1 *Trichoderma* sp. on the second day of coincubation on PSA (orig.):
a – The shrinking of an aerial mycelium of pathogen.

After coalescence of the pathogen mycelium with the antagonist, the effect of mycelium modification of *Fusarium oxysporum* var. *orthoceras* increased (Fig. 3). On the fourth day of co-cultivation, after coalescence of the pathogen and antagonist colonies, aggregation of the pathogen mycelium into the cords was noted (Fig. 3.1 а). In addition, there were noted isolated cases of the antagonist mycelium coiling around the pathogen hyphae (Fig. 3.1 в). On the sixth day, the effect of the antagonist mycelium coiling around the pathogen hyphae increased (Fig. 3.2 г). By the eighth day, the destruction of aerial (Fig. 3.3 д) and substrate mycelium (Fig. 3.3 е) cords was observed. By the tenth day of incubation, the degradation and lysis of the pathogen mycelium increased (Fig. 3.4 з). Lysis of Fusarium mycelium can be explained by the ability of fungi of the genus *Trichoderma* to produce the enzymes that destroy the cell wall, including β- (1,6) -glucanases, chitinases, and proteases (Sood *et al.* 2020). Thus, even before coalescence with the antagonist colony, on the second day of coincubation, the shrinking of certain areas in the pathogen mycelium was noted, which proves the antibiotic activity of the fungal antagonist. Subsequently, there was observed the aggregation of the mycelium of Fusarium blight pathogen into the cords and the hyperparasitic action of the antagonist, which was expressed in the effect of the antagonist mycelium coiling around the pathogen hyphae. By the eighth-tenth days of interaction, degradation and lysis of the aerial and substrate mycelium of the pathogen occurred. Over the entire observation period, there was noted the normal development of the oil flax Fusarium blight pathogen *Fusarium oxysporum* var. *orthoceras* in the control (Fig. 4).

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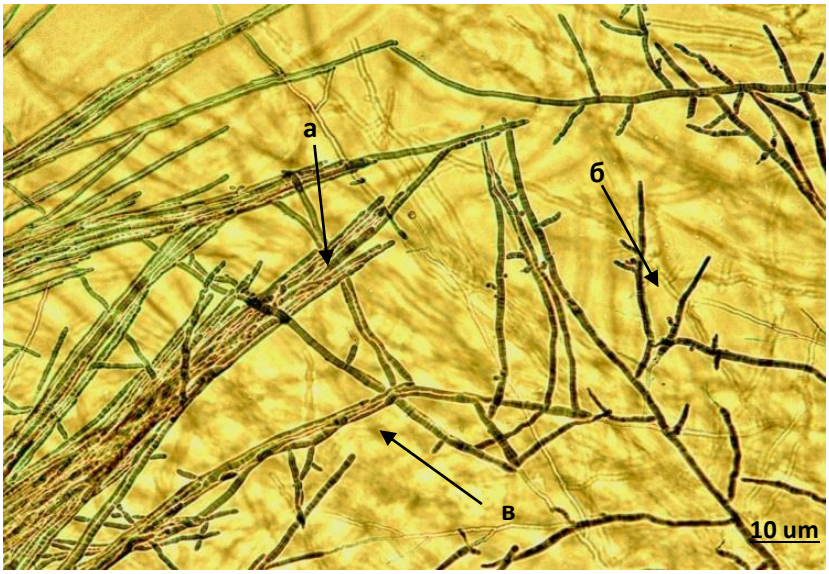
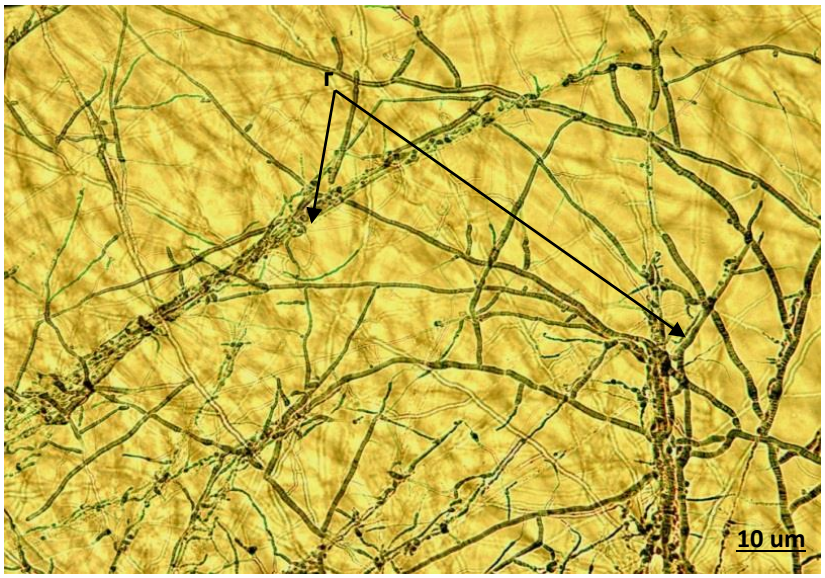
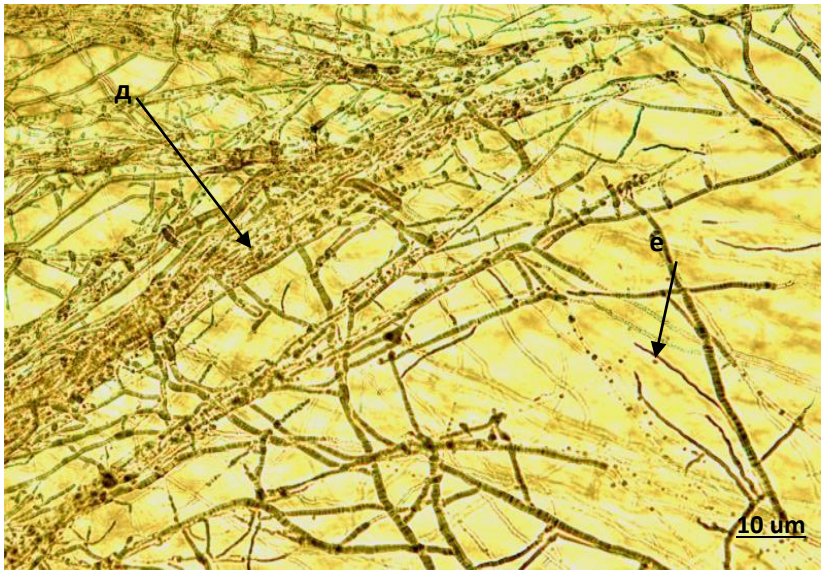
1. 

2. 

3. 

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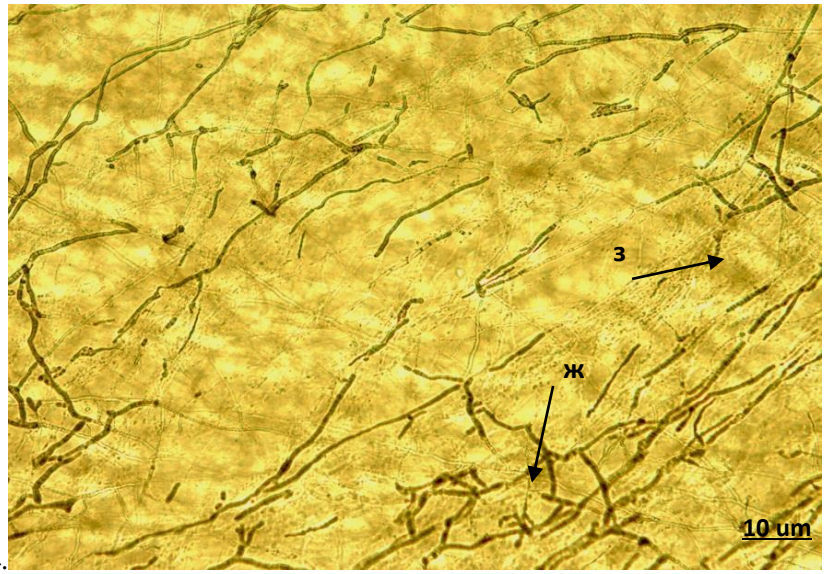
4. 

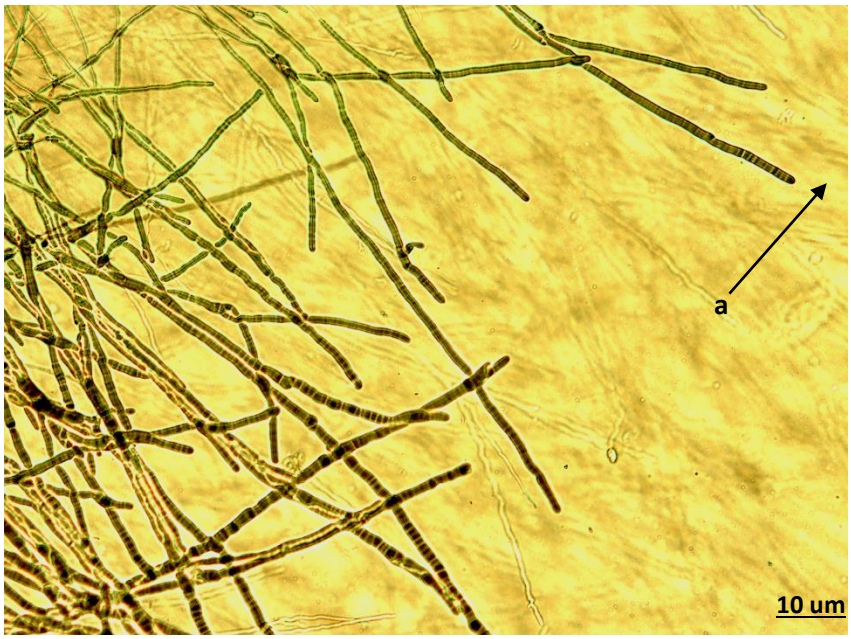
Fig. 3. Mycelium of the Fusarium blight pathogen *Fusarium oxysporum* var. *orthoceras* after the coalescence with the antagonist colony of the T-1 *Trichoderma* sp. (orig.) on the fourth (1), sixth (2), eighth (3) and tenth (4) days of incubation on PSA (orig.):

1 - Cords from the pathogen mycelium (а), the antagonist mycelium (б), the beginning of the coiling of pathogen hyphae with the antagonist mycelium (в);

2 - Intensive coiling of pathogen hyphae with the antagonist mycelium (г);

3 - Destruction of aerial (д) and substrate mycelium cords of the pathogen (е);

4 - Mycelium of the antagonist (ж), destroyed mycelium of the pathogen (з).

1. 

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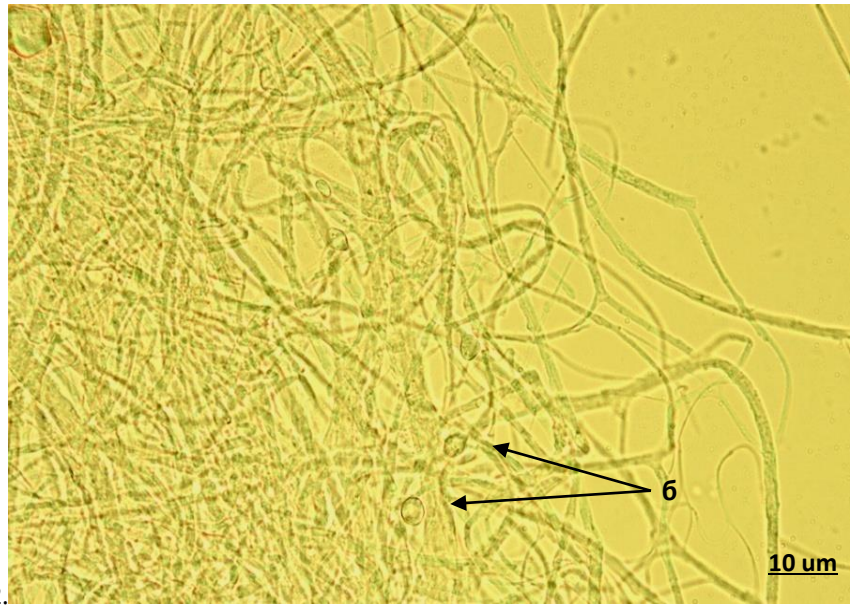

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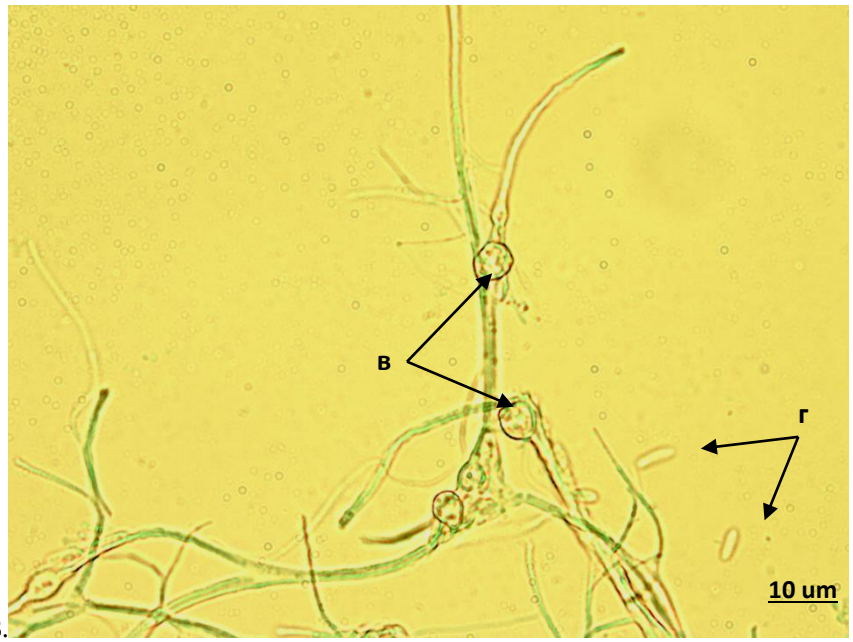

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Fig. 4. Mycelium of the Fusarium blight pathogen *Fusarium oxysporum* var. *orthoceras* on the fourth (1), eighth (2) and tenth (3) days of incubation on PSA (control) (orig.):
1 - Aerial mycelium of the pathogen (а);
2 - The beginning of the formation of chlamydospores in the substrate mycelium (б);
3 - Chlamydospores (в) and conidia (г) in the substrate mycelium.

On the eighth day of Fusarium blight pathogen cultivation on PSA, there was noted the beginning of the formation of chlamydospores in the substrate mycelium (Fig. 4.2 б). On the tenth day, there was noted mass formation of chlamydospores and conidia of the pathogen in the substrate mycelium (4.3 в, г).

CONCLUSION

The antagonistic activity of the fungal strain T-1 *Trichoderma* sp. on the pathogen of oil flax Fusarium blight *Fusarium oxysporum* var. *orthoceras* was established during the co-cultivation of the antagonist and the pathogen by the method of double cultures: high competition of the antagonist strain for the feeding area, its hyperparasitic and antibiotic activity. During the study of the mechanism of the antagonistic action of the promising producer strain on the Fusarium blight pathogen using a light microscope, it was found that even before coalescence with

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the antagonist colony on the second day of coincubation, the shrinking of certain areas in the pathogen mycelium was noted, which proves the antibiotic activity of the fungal antagonist T-1 *Trichoderma* sp. After the coalescence of the pathogen and antagonist colonies, aggregation of the pathogen mycelium into the cords and a hyperparasitic action was observed, which was expressed in the effect of the antagonist mycelium coiling around the pathogen hyphae.

By the eighth-tenth days of interaction, degradation and lysis of the aerial and substrate mycelium of the pathogen occurred. Over the entire observation period, there was noted the normal development of the oil flax Fusarium blight pathogen *Fusarium oxysporum* var. *orthoceras* in the control.

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