Common location approach in experimental studies of phytotoxic and antagonistic properties of soil micromycetes

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Paper received 28.01.17; Accepted for publication 05.02.17.

Abstract. The phytotoxic and antagonistic properties of *Trichoderma* and *Fusarium* fungi strains isolated jointly in various agrocenoses were studied experimentally. The maximum antagonism towards *Fusariums* was produced by *Trichoderma* strains, extracted from wheat isogenic lines. The strains of *Trichoderma* showing the remarkable effect against fusariums also appeared to be the most phytotoxic. In tests on phytotoxicity the seeds and seedlings of wheat or mustard often were more susceptible responders to exometabolites in fungi-conditioned, cell free culture medium than water-cress, which is the classic object for such assays.

Keywords: *antagonistic properties, phytotoxicity, Trichoderma, Fusarium.*

Introduction. Getting high crops and preserving the necessary quality of farming plant cultures on the background of high specialization and concentration of agricultural production is a priority in many countries across the world, including Ukraine [1, 2]. In modern paradigm the use of biological preparations based on active strains of microorganisms with antagonistic and fungicide properties is considered as the most perspective and promising alternative for chemical methods of plant protection from phytopatogens [2, 3]. It is also well known that the interactions between soil microorganisms belonging to different systematic and physiological groups can vary considerably – from mutually beneficial coexistence to active competitive conflicts. One of the forms of the substrate competition is the antagonism, which means that one active strain produces the anti-microbial substances (antibiotics, bactericines, lysis enzymes, etc.), while the other members of microbiocenosis react on that within the limits of their physiology. For example, in a number of studies the authors showed that the soil micromycetes of *Fusarium* Link genus are the facultative parasites for many agricultural plants [4, 5]. At the other hand, the species of *Trichoderma* Pers. are the natural antagonists of *Fusariums*, and this fact is used in practice [2, 3]. Usually the assessment of antagonistic properties of manufactured biopreparations is carried out experimentally, by co-culturing only the antagonist and the responding organism on the solid substratum [6, 7]. However, the phytotoxic effects produced by antagonistic strains have been studied insufficiently. The most frequent responding object in such experiments is the watercress seeds, while the reactions of plants, which should be treated with the preparations *per se*, are not examined, that renders such results suspect.

Study objectives. Considering listed above, the aim of present work was to evaluate the antagonistic and phytotoxic activities of several species of soil micomycetes of *Fusarium* and *Trichoderma* genera, which were extracted from soils under various agricultural plants.

Materials and Methods. For present research the matched sets of soil fungi species were selected in the collection of microorganisms maintained at the Plant Physiology and Biochemistry of V.N. Karazin National University, Kharkiv, Ukraine. All micromycetes were extracted from soils of various agrocenoses located in Kharkiv region, North-Eastern Ukraine. Strains *Fusarium heterosporum* TrA3 and *Trichoderma* TrA763 were isolated from the rhizosphere of the winter wheat *Triticum aestivum* L., isogenic line *Vrn–A1a*. Species *F. heterosporum* TrB21 and *Tr.* TrB were found in the rhizosphere of wheat, isogenic line *Vrn–B1a*, and *F. heterosporum* TrD32 and *Trichoderma* TrD – from the rhizosphere of wheat, isogenic line *Vrn–D1a*. From the rhizosphere of white mustard, *Sinapis alba* L., the micromycetes *F. heterosporum* S25, *F. oxysporum* S1, *Tr. koningii* S83 and *Tr. aureoviride* S407 were isolated. The strain of *Tr. aureoviride* Tr405 was found in the rhizosphere of summer wheat. Strains *F. heterosporum* P23, *Tr. koningii* P59 and *Tr. aureoviride* P578 were extracted from the top soil layer of the fallow land.

For the experiments the microorganisms were cultured on solid substratum (mash-agar) or in bulk culture using diluted non-hop beer mash [8]. The antagonistic activity of all *Trichoderma* strains against all *Fusarium* strains were measured in the pairwise comparison in conditions of their direct co-culturing [9, 10]. If an obvious, but not very significant growth delay was observed for one of the species after 2-3 days of co-culturing, the effect was estimated as antagonistic relationships (marked as +). If one of the paired strains showed some overlap with its counterpart in the borderline zone between them, and the former oppressed the growth of the latter, then the moderate antagonism was recorded (++). The occurrence of the remarkable, clearly seen zone of the delayed, oppressed growth for one of the strains was considered as the marker of a strong antagonism (+++).

For the studying of the micromycetes' phytotoxicity their sterilized exometabolites were obtained; for that the conditioned culture medium, collected after 5-7 days of the single strain fungi culturing, was filtered through the Millipore™ membrane syringe filters with pore diameter 0,2 μm. This conditioned, cell-free medium was used for the biotest against water-cress seeds or the seeds of that agricultural plant, which rhizosphere the particular fungi strain was initially extracted from. The impact of exometabilites was assessed on the seeds germination and on the length of the seedling roots measured on the 7^{th} day of growth [8]. The micromycetes culture was considered as toxic, if its medium caused at least 30 % decrease in seeds germination rate or seedling root growth in compare with the relevant matched control (fresh medium) [2].

Considering that the micromycetes can grow not only on leafs but also in plant phylosphere (leaf surface), the additional assay was performed the isolated leaf plates for data completeness [8]. In this experiment the white mustard and wheat sprouts of equal age were used; the plants were grown in the factorostatic chamber at constant temperature of about +22-24°C and illumination lasted 16 h per day. The equal-size fragments of the leaf plates were isolated and treated with the cell-free medium collected from fungi cultures; in the matched control series the sterile, non-hop beer mash was used instead of conditioned medium. The observations were conducted during three days; the appearance of necrotic spots, burns, changes of the color on the leaf plates were recorded [8]. Each of experimental series was carried out three times, and data collected in these repeats were combined and averaged for the analysis in present work.

Results and discussion.

The analysis of data available in the literature showed that currently there are about 50 bio-preparations in the world, based on *Trichoderma* micromycetes [7], and the majority preparations are aimed at the oppression of the pathogens' growth and dissemination. However, there are gaps in our knowledge on whether these properties are present in all strains of this species. The strains of micromycetes tested in present work were extracted from the rhizosphere of the same agricultural plants, and noteworthy the wheat didn't carry the signs of fusariosis-type damage. That can be an evidence of rather high, natural fungistatic background, which was maintained, among other factors, by *Trichoderma* fungi. In our study the antagonistic properties of the selected *Trichoderma* strains towards *Fusarium* were the highest in strains found in the rhizospere of wheat isogenic lines (Table 1). This finding can be partially explained by the fact of the high concentration of phytopathogens, including *Fusariums*, present in the rhizophere of the wheat in natural conditions [3]. Thus these strains of *Trichoderma*, competing with *Fusariums*, are permanently ready to synthesize a lot of compounds that decrease the growth of phytopathogens. Therefore these strains also appeared to be more active in the experiment. However, the strongest antagonistic impact against all tested *Fusarium* strains was observed from *Tr. koningii* P59, which was extracted from the top soil layer of the fallow land. The lowest rate of antagonistic activity was detected for the strain *Tr. koningii* S83 that was found in the white mustard rhizosphere, and also from the strain *Tr. aureoviride* P578, extracted from the soil of the fallow land.

Table 1. The assessment of the antagonistic activity of *Trichoderma* micromycetes towards various strains of *Fusarium*

Micromycetes	*Fusarium heterosporum* TrA3	*F. heterosporum* TrB21	*F. heterosporum* TrD32	*F. heterosporum* S25	*F. heterosporum* P23	*F. oxysporum* S1
Trichoderma sp. TrA21	++	++	++	++	++	++
Trichoderma sp. TrB13	+++	+++	++	++	++	++
Trichoderma sp. TrD6	++	++	+++	+	++	+
Tr. koningii P59	+++	+++	+++	++	+++	+++
Tr. koningii S83	+	+	+	++	++	+
Tr. aureoviride P578	+	+	+	++	+	+
Tr. aureoviride S407	++	++	+++	++	++	++
Tr. aureoviride Tr405	++	+++	++	++	+	+

Fungi strains' phytotoxicity was evaluated using a widely accepted methodology that included measurements of responses of water-cress seeds, but also white mustard and wheat seeds were added as responding objects. The results of these experiments are presented in Table 2.

It can be seen that all tested micromycetes showed some impact on both seeds germination and the length of the seedling roots in responder plants, but the extent of this influence varied between species and strains. The oppressive action produced by *Fusariums* was more apparent. The conditioned medium after culturing *F. heterosporum* S25 decreased significantly the germination of water-cress and wheat seeds; the mustard seeds appeared to be less susceptible towards the exometabolites of that fungi strain. The strains of *F. heterosporum*, extracted from the rhizosphere of the isogenic wheat lines, were more effective in causing a decrease in the germination of water-cress and wheat seeds, and also influenced the length of seedling root length. The data analysis also showed that the seeds and sprouts of the wheat were more sensitive towards the exometabolites of *Fusarium* species; meanwhile the water-cress, being a classic responder for such tests, appeared to be less responsive. The influence of the *Fusarium*-conditioned medium on the mustard was found to be less effective, except the significant impact of these exometabolites on the seedling root length. It can be explained by the fact that the mustard seeds contain the substances or compounds, which have phytoncide properties and thus decrease the phytopathogens growth [11]; during the germination of the seeds the amount and concentration of these substances declines, and the seedlings become more vulnerable to the damage caused by fungi exometabolites.

The experiment with testing of the phytotoxicity of conditioned medium from various cultured fungi strains against isolated leaf plates didn't provide striking results with clear tendencies (data not shown). Clearly detectable damage to wheat and mustard leafs was noticed for the medium conditioned by *Fusarium* species, extracted from the rhizosphere of wheat isogenic lines *Vrn–B1a* and *Vrn–D1a*. Other *Fusarium* strains and all *Trichoderma* species didn't produced significant injuries in the leaf plates.

Conclusions. The study showed that different strains of the same *Trichoderma* fungi species, originating from different locations, had different antagonistic activity towards *Fusarium* species and strains. The most active strains were extracted from the rhizosphere of isogenic wheat lines. The biotests for the phytotoxicity were performed for both genera of micromycetes against water-cress seeds but also against wheat and mustard seeds. This testing showed that *Trichoderma* strains, which were active antagonists towards *Fusariums*, also produced a significant phytotoxicity against responding plants. The alteration of the seedlings root length appeared to be a useful parameter in such experiments, because it was able to reveal the toxic action of conditioned medium in series, where no effect on the seeds germination was detected. Noteworthy, in some cases the phytotoxic outcome was negligible in water-cress, but very strong in the agricultural plants used as responders. In total, the data obtained in present study, contribute to our knowledge about the antagonistic relationships between different participants of soil fungi communities and as well between micromycetes and plants. The results can be used in practice for identification of fungi strains, which potentially can be the producers of biopreparations for agricultural plant protection.

Table 2. The influence of the medium, conditioned by cultured micromycetes, on the seeds germination and the length of the seedling roots of the responding plants (% of the control)

Micromycetes	Responding plants					
	Lepidum sativum L.		*Sinapis alba* L.		*Triticum aestivum* L.	
	Seeds germination	Roots length	Seeds germination	Roots length	Seeds germination	Roots length
Fusarium heterosporum TrA3	75	80	98	77	55	67
F.heterosporum TrB21	78	81	97	79	56	70
F.heterosporum TrD32	70	83	97	81	55	70
F. heterosporum S25	50	98	87	80	60	75
F. heterosporum P23	81	71	97	81	61	70
F. oxysporum S1	80	94	89	88	65	69
Trichoderma sp. TrA21	99	98	99	98	80	80
Trichoderma sp. TrB13	80	100	98	99	82	82
Trichoderma sp. TrD6	98	102	97	96	89	87
Tr. koningii P59	88	71	97	97	88	86
Tr. koningii S83	95	72	96	99	82	87
Tr.aureoviride P578	99	107	98	98	85	88
Tr.aureoviride S407	97	103	98	98	86	85
Tr.aureoviride Tr405	65	87	71	97	88	80

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Оценка фитотоксических и антагонистических свойств почвенных микромицетов с учетом совместного местообитания

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Аннотация. В эксперименте изучены фитотоксические и антагонистические свойства изолятов почвенных микромицетов родов *Trichoderma* и *Fusarium*, выделенных из разных агроценозов. Максимальный антагонистизм в отношении фузариев проявляли изоляты триходерм, выделенные из ризосферы изогенных линий пшеницы. Изоляты *Trichoderma*, показавшие наиболее отчеливый эффект на фузариях, также обладали повышенной фитотоксичностью. В тестах на фитотоксичность семена и проростки горчицы и пшеницы зачастую оказывались более чувствительными к влиянию экзометаболитов грибов, чем семена кресс-салата, считающегося классическим тест-объектом в таких исследованиях.

Ключевые слова:*антагонистические свойства, фитотоксичность, Trichoderma, Fusarium*.