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SCREENING NEW *TRICHODERMA* ISOLATES FOR ANTAGONISTIC ACTIVITY AGAINST SEVERAL PHYTOPATHOGENIC FUNGI, INCLUDING *FUSARIUM* SPP.

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Aims. To obtain and characterize new isolates of *Trichoderma* antagonistic to phytopathogenic fungi, including *Fusarium* spp., and 2) to determine their suitability for mass production under different cultivation conditions. **Methods.** Microbiological, cultural-morphological, statistical. **Results.** From plants affected by phytopathogenic fungi: cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), white cabbage (*Brassica oleracea* L.), winter wheat (*Triticum aestivum* L.), spring barley (*Hordeum vulgare* L.) in the Forest-Steppe of Ukraine (Kyiv region) 11 new *Trichoderma* isolates were obtained. Preliminary, morphological determination allocated five of them to *T. viride* (isolates CK, 165, 27, 49, 35), two of them to *T. koningii* (21, 64) and four of them to *T. longibrachiatum* (161, 162, 163, 164). All isolates showed moderate to high antagonistic activity towards 8 phytopathogenic fungal species (*Fusarium oxysporum*, *Fusarium solani*, *Alternaria cucumerina*, *Colletotrichum phomoides*, *Botrytis cinerea*, *Trichothecium roseum*, *Penicillium* sp., *Cladosporium fulvum*). In a dual culture experiment they showed generally similar or often higher activity to the above-mentioned fungi than the 8 control strains used in our study, belonging to *T. viride* (5 strains), *T. koningii* (2 strains) and *T. harzianum* (1 strain), which have been maintained since long time in our laboratory. The most active new isolate CK, (presumably) *T. viride*, showed comparable high activity towards all phytopathogenic fungi as compared to our most active control strain of *T. viride*, no. 23. The latter is the basis of a biocide Trichodermin, produced by biolaboratories of Ukraine, including the Institute of Plant Protection, NAAS, Kyiv. Chlamyospore production of all isolates and strains studied in submerged culture varied from 10^6 to $3 \cdot 10^7$ spores/ml, were *T. viride* isolates and strains were on the higher end. Isolates of '*T. longibrachiatum*' did not produce chlamyospores in submerged culture. Upon superficial cultivation on barley grain, the strains and isolates of *T. viride* were also characterized by the highest production of spores ($6 \cdot 10^9$ – $9 \cdot 10^9$ spores/g) as compared to those of *T. koningii*, *T. harzianum* ($5.5 \cdot 10^9$ – $6.8 \cdot 10^9$ spores/g) and *T. longibrachiatum* ($1.3 \cdot 10^8$ – $6.8 \cdot 10^8$ spores/g). In an *in-vivo* experiment under laboratory conditions the most promising antagonistic isolate CK was used to inoculate wheat seed and tested for protection against *Fusarium* root rot (inoculum a mixture of *F. avenaceum*, *F. culmorum*, *F. gibbosum*, *F. oxysporum*, in $4 \cdot 10^4$ spores/g), where it gave an 83 % reduction in root rot as compared to the non-inoculated control. **Conclusions.** Five new isolates preliminarily (on the basis of morphological characteristics only) allocated to *T. viride* and four to *T. longibrachiatum* demonstrated *in vitro* the highest and widest antagonistic activity against the phytopathogenic fungal species *Fusarium oxysporum*, *Fusarium solani*, *Alternaria cucumerina*, *Colletotrichum phomoides*, *Botrytis cinerea*, *Trichothecium roseum*, *Penicillium* sp., *Cladosporium fulvum*, as compared to new isolates, preliminarily allocated to – *T. harzianum* and *T. koningii*. New isolate CK (allocated to *T. viride*) showed a promising and similar high antagonistic activity as compared to our *T. viride* 23 strain, which is already successfully used in the biocide Trichodermin. Since this isolate CK also produced a high number of chlamyospores in submerged culture ($3 \cdot 10^7$ spores/ml) and conidia ($8 \cdot 10^9$ spores/g) when surface cultured on barley grain respectively, it is a potential new candidate for a biocide. When this CK isolate was studied in a small laboratory pot experiment, to control *Fusarium* root rot in wheat by preventive seed inoculation, it caused an 83 % reduction in this *Fusarium* root rot. Its usefulness under field conditions and its effect on growth of plants will be investigated in future research.

Key words: mass production, submerged and surface cultivation, biological control.

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INTRODUCTION

Trichoderma fungal species are worldwide and in all climate zones an essential part of the microflora in all types of soils, both in terrestrial and marine ecosystems (Klein, Eveleigh, 1998). They occur commonly in the rhizosphere, but can also colonize (as endophytes) aboveground plant parts.

Recent investigations have shown that *Trichoderma* spp. can sense, penetrate and kill other fungi and this has led to the development of a large number of successful biopesticides with *Trichoderma* spp. as basis (Benitez T et al, 2004; Harman GE et al, 2005; Schuster, Schmoll, 2010; Kubicek CP et al, 2011; Błaszczuk et al, 2014; Sawant IS, 2014; Sood et al, 2020).

Trichoderma spp. produce a wide spectrum of (volatile) antibiotics killing a number of fungi and nematodes. Their metabolites, including enzymes are able to stimulate plant growth, to induce resistance in plants against pathogens and induce abiotic stress tolerance and are used in industrial processes (Mastouri F et al, 2010; Lorito et al, 2010; Contreras-Cornejo HA et al, 2011; Sawant IS, 2014; López-Bucio J et al, 2015; Fiorentino N et al, 2018). Some *Trichoderma* spp are noxious, because they are pathogenic to humans (Sandoval-Denis SA et al, 2014) and cultivated mushroom (Goltapeh EM, Danesh YR, 2006).

In addition to high antagonistic potential, the fungi of this genus are notable for good growth rate and the possibility of cultivating on non-expensive substrates, thus, they are widely used in the elaborations of biological preparations in industrial conditions. The cultivation of *Trichoderma* fungi is done both superficially using loose substrates (Cavalcante RS et al, 2008, Motta FL et al, 2012), and by the submerged technology in liquid culture media (Jakubíková L et al, 2006; Šimkovič M et al, 2008; Al-Taweil HI et al, 2009; Kobori NN et al, 2015; Ferdous Akter M et al, 2018).

Biofungicides, based on *Trichoderma* spp. were presented worldwide in 2014 by over 250 products and comprised about 60 % of the international market of biofungicides. The most common species in most products, based on *Trichoderma*, is *T. harzianum* (83 %), followed by *T. viride*, and *T. koningii* (Woo et al, 2014). *T. harzianum* and *T. viride* are mainly used to treat soil for about 87 different crops against 70 soil pathogens and 18 foliar pathogens, predominantly fungi such as *Verticillium* spp., *Fusarium* spp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Alternaria radicina*, *Pythium* spp, and *Botrytis cinerea* (Topolovec-Pintarić, S., 2019).

Biopreparations are highly effective against root rots, wilts of vegetables, grown in covered soil (Liu JB et al, 2009; Lorito M et al, 2010; Pill et al, 2011; Yang X et al, 2011; Borrero et al, 2012; Kakvana N et al, 2013; Abo-Elyours et al, 2014; El Komy MH et al, 2015; Adhikary M et al, 2017; Bunbury-Blanchette AL, 2019; Rivera-Méndez W, 2020).

The aims of our study were 1) to obtain and characterize new isolates of *Trichoderma* antagonistic to phytopathogenic fungi, including *Fusarium* spp., and 2) to determine their suitability for mass production under different cultivation conditions.

MATERIALS AND METHODS

The studies were conducted in the Laboratory of Microbiological Methods of Plant Protection at the Institute of Plant Protection, NAAS, Kyiv in 2015–2019.

Trichoderma fungi were isolated from plants affected by phytopathogenic fungi: cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), white cabbage (*Brassica oleracea* L.), winter wheat (*Triticum aestivum* L.), spring barley (*Hordeum vulgare* L.) in the Forest-Steppe of Ukraine (Kyiv region) (Table 1). Isolates were obtained by transferring germinated conidia, which appeared on the surface of parts of plants affected by phytopathogenic fungi, to wort agar medium.

Preliminary allocation of the new isolates to a *Trichoderma* species was done on the basis of morphological features only, following the keys of Gams W and Bissett J, (2002).

The antagonistic activity of 11 new isolates and 8 collection strains of *Trichoderma* (kept for 30 years in the Laboratory of Microbiological Methods of Plant Protection at the Institute of Plant Protection, NAAS) (see Table 2) was determined by the method of blocks the dual culture assay, using different species of phytopathogenic fungi, isolated by us from diseased plant material and maintained as agar slants in our culture collection (Table 2) – *Fusarium oxysporum* (isolated from the roots of cucumbers), *Fusarium solani* (isolated from a tomato stalk), *Alternaria cucumerina* (isolated from the leaves of cucumbers), *Colletotrichum phomoides* (isolated from the fruit of the tomato), *Botrytis cinerea* (isolated from the fruit of the cucumber), *Trichothecium roseum* (isolated from soybean seeds), *Glycine max* (L) Merr), *Penicillium* sp. (isolated from soybean seeds), and *Cladosporium fulvum* (isolated from cucumber leaves).

The strains of phytopathogenic fungi (Table 2) were grown in Erlenmeyer flasks on an orbital shaker (PSU-20i, Biosan, Latvia) in liquid wort medium for 72 h at 200 rpm and 25 °C in the dark. Inoculum for flasks was obtained by rinsing with sterile water spore-mycelial mass from the surface of agar in test tubes. One ml of liquid culture of each phytopathogenic strain was added to a Petri dish and mixed with 15 ml of wort agar. *Trichoderma* isolates and strains under investigation were grown in Petri dishes using wort agar for 5 days at 25 °C. Agar blocks (5 mm diam.) were cut out with a cork borer from the margins of 7-day-old colonies and placed into the Petri dishes with the just seeded phytopathogenic fungi, 3 blocks per Petri dish, in equal distance from each other. Incubation was for 5 days at 25 °C in an incubator (TS-80, Czech Republic).

The antagonistic activity was determined in the dual culture assay on day 5 of the experiment by measuring the growth inhibition zones of test objects using the following scale: inhibition zone 0 mm, inactive; 1–14 mm poor activity; 15–25 mm moderate activity; >26 mm strong activity. The dual culture assay was done in three repeats.

The technological suitability for mass production of the new *Trichoderma* isolates was determined by submerged and surface cultivation using chlamydospore formation and biomass production as indices.

Submerged cultivation was done in 300 ml Erlenmeyer flasks with 50 ml of medium on a rotary shaker (200 rpm) at 25–26 °C for 72 h in the medium, using corn extract (side product of starch and syrup production, PrJSC ‘Dneprovsky Starch and Syrup Integrated Works’, Dniprovsk, Dnipropetrovskiy region) and

beet molasses (side product of sugar beet production, PrJCT ‘Salyvonkivskiy Sugar Plant’, Hrebinky, Kyiv region): corn extract – 1 %; molasses – 3 %; NH_4NO_3 – 0.5 %; KH_2PO_4 anhydrous – 0.5 %; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.2 %. (Tkalenko AN, Goral SV, 2013). Flasks with medium were sterilized in an autoclave at 0.1 MPa for 30 minutes. Inoculation of flasks was performed with 7-day cultures grown in test tubes. The inoculum concentration was $5 \cdot 10^6$ spores/ml.

Surface cultivation was done in 1 L Erlenmeyer flasks with barley seeds. The flasks were filled with substrate to 1/3 volume, moistened with water (70–80 % by weight) and sterilized in an autoclave at 0.1 MPa for 30 minutes. Inoculation of flasks was performed with 7-day cultures grown in test tubes. The inoculum concentration was $1 \cdot 10^6$ spores/g. (Bondarenko et al, 1985) Cultivation was performed for 10 days till massive formation of air-distributed conidia at 25–26 °C occurred.

To determine the biomass of *Trichoderma* fungi, 10 ml of liquid culture was filtered through a paper filter (pore size 4–7 μm), washed with distilled water and dried in an drying oven at 110 °C for 1 hour. The biomass was determined by weighing using a precision scale (Axis, Poland). The productivity of fungal spore formation was determined via direct calculation of spores in a Goryaev’s chamber (haemocytometer with 90 nL capacity instead of the usual 100 nL) with a microscope (Zeiss Primo Star, $\times 400$).

The calculation of optimal inoculum doses of a liquid biopreparation based on isolate *T. viride* CK (3.5×10^7 chlamydospores/ml) was done using the pretreatment of winter wheat seeds of Myronivska 808

Table 1. Culture number and sources of isolation of new *Trichoderma* isolates from Kyiv region

Provisional species allocation and collection number of isolate	Source of isolate
<i>T. viride</i> CK	cucumber roots affected by root rot
<i>T. viride</i> 165	stem of a tomato plant affected by Fusarium wilt
<i>T. viride</i> 27	tomato fruit affected by Alternaria
<i>T. viride</i> 49	leaves of white cabbage, affected by phytopathogens
<i>T. viride</i> 35	cucumber roots affected by root rot
<i>T. koningii</i> 21	roots of winter wheat affected by root rot
<i>T. koningii</i> 64	tomato affected by root rot
<i>T. longibrachiatum</i> 161	roots of winter wheat affected by root rot
<i>T. longibrachiatum</i> 162	roots of spring barley affected by root rot
<i>T. longibrachiatum</i> 163	roots of spring barley affected by root rot
<i>T. longibrachiatum</i> 164	roots of winter wheat affected by root rot

variety (as it is nationally widely cultivated) in three concentrations (0.04, 0.2, 1 %). For this experiment, performed under laboratory conditions, artificial inoculated sterilized potting soil was used. The experiment was performed in plastic containers the size 50×40×10 cm. The soil was inoculated with a mixture of four *Fusarium* spp. (*F. avenaceum*, *F. culmorum*, *F. gibbosum*, *F. oxysporum*, cultivated in wort medium for 72 h on an orbital shaker, 200 rpm) in a final concentration of $4 \cdot 10^4$ spores/g soil. Winter wheat seeds were soaked in suspensions of liquid culture of *T. viride* CK for 2 hours and sown 100 seeds per container. The containers were kept at room temperature and natural light. The experiment was done in three repeats.

Results of studies. During our studies (2015–2019) 11 *Trichoderma* isolates were obtained (Table 1) and on the basis of the morphologically characteristics we made

a presumptive species identification: *T. viride*, isolates CK, 165, 27, 49, 35; *T. koningii*, isolates 21, 64; *T. longibrachiatum*, isolates 161, 162, 163, 164.

It was determined that the 11 new *Trichoderma* isolates differed in their antagonistic activity against the eight phytopathogenic fungi tested (Table 2). In general, the isolates, morphologically allocated to *T. viride* and *T. longibrachiatum* showed a higher activity and broader spectrum of action than the isolates allocated to *T. harzianum* and *T. koningii*.

Seven out of ten investigated isolates and strains of *T. viride* (23, CK, Tp-1, 75, 27, 49, 35) demonstrated high antagonistic activity regarding all fungal spp. tested, both in producing antibiotic substances and in hyperparasitic activity with the build-up of phytopathogen colonies and abundant spore formation (Table 2). Isolate *T. viride* CK showed the highest activity apart from the control strain for this species, *T. viride* 23 (Fig. 1).

Table 2. The antagonistic activity of 11 newly obtained isolates of *Trichoderma* and control strains for the species *T. viride*, *T. koningii* and *T. harzianum*

Species and number of isolate/strain	Diameters of growth inhibition zones of phytopathogenic fungi, mm; n = 3							
	<i>Fusarium oxysporum</i>	<i>Fusarium solani</i>	<i>Alternaria cucumerina</i>	<i>Colletotrichum phomoides</i>	<i>Botrytis cinerea</i>	<i>Trichothecium roseum</i>	<i>Penicillium sp.</i>	<i>Cladosporium fulvum</i>
<i>T. viride</i> 23	36 ± 3.0	40 ± 0	> 50	> 50	> 50	> 50	> 50	> 50
<i>T. viride</i> CK (new)	40 ± 7.54	30 ± 3.2	> 50	48 ± 1.7	> 50	> 50	38 ± 1.7	> 50
<i>T. viride</i> 165 (new)	16 ± 8.6	15 ± 2.6	50 ± 9.6	> 50	50 ± 8.0	5 ± 0.9	31 ± 6.8	0
<i>T. viride</i> Tp1	> 50	25	49 ± 5	> 50	> 50	> 50	22 ± 4	> 50
<i>T. viride</i> 75	> 50	25	> 50	> 50	> 50	> 50	35 ± 4	> 50
<i>T. viride</i> 27 (new)	> 50	15	> 50	> 50	> 50	> 50	31 ± 3	> 50
<i>T. viride</i> 49 (new)	> 50	20	49 ± 2	> 50	> 50	> 50	15 ± 2	> 50
<i>T. viride</i> 35 (new)	40 ± 5	25	> 50	> 50	> 50	> 50	23 ± 1	> 50
<i>T. viride</i> TM	0 ± 0	0	12 ± 4	26	25	25 ± 5	0 ± 0	20
<i>T. viride</i> T3	10 ± 2	0	25 ± 6	20	25	43 ± 4	21 ± 4	30
<i>T. koningii</i> M10	0 ± 0	0	6 ± 6	21	15	22 ± 3	5 ± 1	15
<i>T. koningii</i> 21 (new)	15 ± 3	10	15 ± 3	33	18	24 ± 3	0 ± 0	10
<i>T. koningii</i> 64 (new)	20 ± 2	10	18 ± 4	27	24	22 ± 1	8 ± 1	10
<i>T. harzianum</i> 8900	45 ± 5	25	35 ± 7	42	45	40 ± 10	30 ± 2	40
<i>T. harzianum</i> 8995	58 ± 5	20	40 ± 7	48	43	> 50	20 ± 4	38
<i>T. longibrachiatum</i> 161 (new)	17 ± 5.0	5 ± 0.7	> 50	> 50	> 50	> 50	20 ± 3.2	> 50
<i>T. longibrachiatum</i> 162 (new)	17 ± 5.0	5 ± 0.3	> 50	> 50	> 50	> 50	25 ± 2.9	> 50
<i>T. longibrachiatum</i> 163 (new)	24 ± 1.9	0	50 ± 0	> 50	> 50	30 ± 0	13 ± 3.3	> 50
<i>T. longibrachiatum</i> 164 (new)	19 ± 1.9	0	> 50	> 50	> 50	40 ± 0	16 ± 0.9	> 50

Note. Data presented as mean values ± standard error.

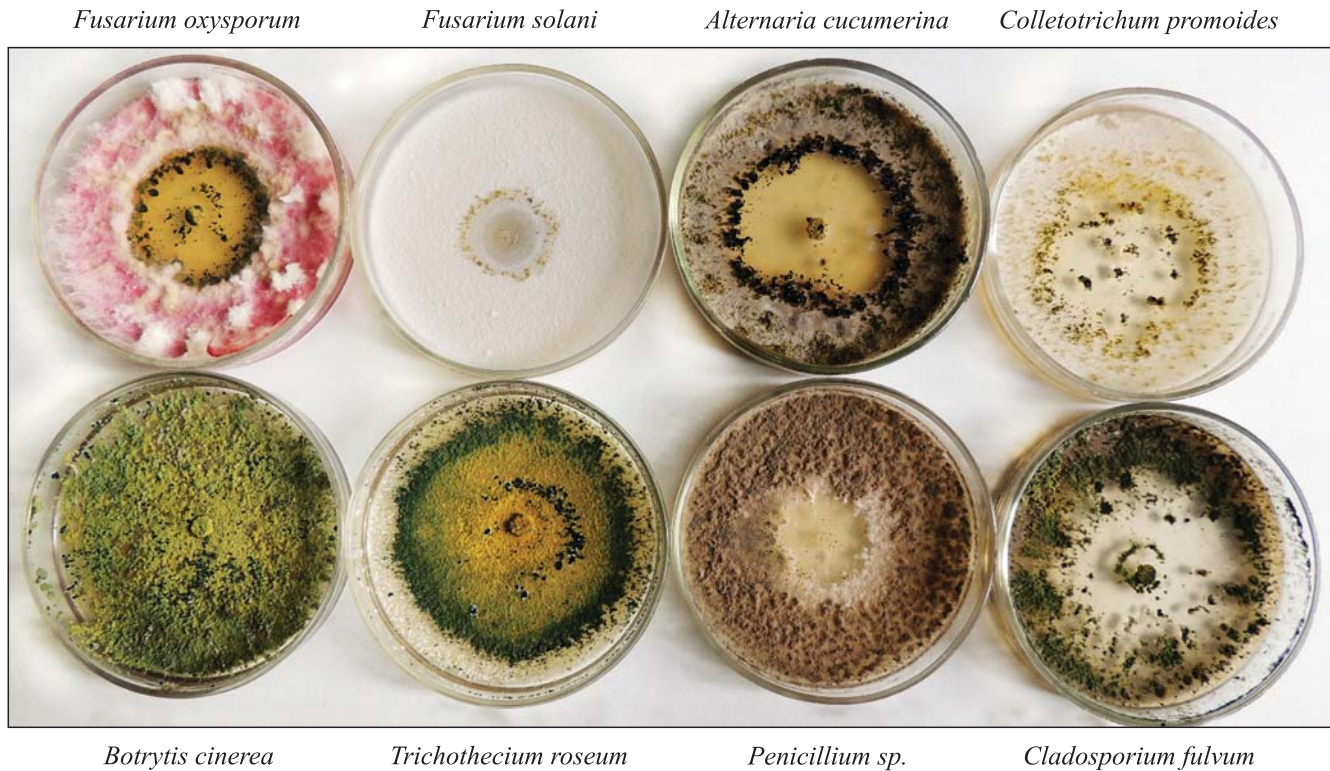


Fig. 1. Growth inhibition of different phytopathogenic fungi by a new isolate of presumptive *T. viride* (*T. viride* CK) on wort agar, 7 days after co-incubation at 25 °C in the dark

Isolate *T. viride* 165 demonstrated much lower antagonistic activity regarding two *Fusarium* species (2–2.5 times smaller diameters of the growth inhibition zones of phytopathogens compared with isolate *T. viride* CK and strain *T. viride* 23), and this isolate did not inhibit the growth of *Trichothecium roseum* and *Cladosporium fulvum*.

Some of our laboratory strains showed rather disappointing results concerning antagonism of some phytopathogenic fungi: high activity of the *T. viride* T-3 strain was found only against *Trichothecium roseum*, as for *Alternaria cucumerina*, *Colletotrichum phomoides*, *Botrytis cinerea*, *Penicillium sp.*, *Cladosporium fulvum*, it had moderate activity against *F. oxysporum* and no activity against *Fusarium solani*. *T. viride* TM demonstrated poor antagonistic activity against *Alternaria cucumerina*, *Colletotrichum phomoides*, *Botrytis cinerea*, *Cladosporium fulvum*, and did not inhibit the growth of *F. oxysporum*, *F. solani*, *Penicillium sp.*

Both laboratory strains of *T. harzianum* demonstrated high antagonistic activity, inhibiting the growth of all the investigated species of phytopathogenic fungi.

The isolates of *T. longibrachiatum* showed also a good antagonistic activity, except against both *Fusarium* spp. tested.

The isolates and control strains of *T. koningii* were characterized by low to moderate antagonistic activity only against all fungal spp. tested.

Under submerged cultivation conditions in liquid corn extract/molasse medium, all 19 investigated isolates and strains produced almost a similar amount of biomass over a period of 72 hours cultivation at 25 °C, namely 10.2–14.7 g/l (Table 3).

All studied strains and isolates of *T. viride* (Fig.2), *T. harzianum* and *T. koningii* produced chlamydo-spores in the submerged culture.

Spore formation productivity of the new isolate *T. viride* CK was on the level of the biocide Trichodermin control strain *T. viride* 23, namely $3 \cdot 10^7$ chlamydo-spores/ml after 72 hours growth in corn extract/molasse medium at 25 °C. Other isolates and strains of *T. viride* produced a smaller amount of chlamydo-spores, $1.0 \cdot 10^7$ – $1.5 \cdot 10^7$ /ml. The productivity of *T. harzianum* strains was $8 \cdot 10^6$ chlamydo-spores/ml. A considerably smaller number of chlamydo-spores was produced by the *T. koningii* control strain and the two new isolates – $0.7 \cdot 10^6$ – $1.4 \cdot 10^6$ chlamydo-spores/ml.

The new isolates of *T. longibrachiatum* did not produce chlamydo-spores in the submerged culture (Fig. 3).

Under surface cultivation on barley seeds, *T. viride* isolates and strains were characterized by higher productivity compared to strains of the other three species (*T. koningii*, *T. harzianum*, *T. longibrachiatum* of aerial spores (conidia) – $6.2 \cdot 10^9$ – $9.2 \cdot 10^9$ spores/g, and only two strains of this species (TM and T-3) produced a smaller number of conidia, viz. $3.8 \cdot 10^9$ – $4.3 \cdot 10^9$ spores/g. The productivity of *T. koningii* and *T. harzianum* control strains was on the level of $5.5 \cdot 10^9$ – $6.8 \cdot 10^9$ spores/g, and the productivity of conidia formation in the four new *T. longibrachiatum* isolates was the lowest, namely $1.3 \cdot 10^8$ – $6.8 \cdot 10^8$ spores/g. *T. longibrachiatum* isolates were notable for later formation of spores (by 7–10 days) as compared to *T. viride* strains and isolates.

The treatment of seeds with a preparation with *T. viride* isolate CK in a 1 % concentration decreased the infection of plants with root rots by 83 % as compared to the control. After twenty-fold decrease in the preparation concentration the biological efficacy decreased twice (40 %), see Table 4.

DISCUSSION

Mass production of biological control preparations requires the availability of initial strains with high antagonistic activity and specific, suitable growth characteristics: high productivity, synchronic growth (biomass) and spore formation. Due to observed loss of their initial antagonistic traits, physiological features, change in morphological characteristics, once active strains may show lower or zero activity after long-term storage and subculturing on artificial media. Therefore, existing industrial strains of microorganisms require periodic check-up by the main indices – sporulation rate, technological specificities, activity rate.

This study was aimed at estimating the antagonistic activity and suitability for cultivation of 19 *Trichoderma* spp. isolates and strains – 11 new isolates and 8 collection strains, the latter have been maintained by us for a long time and used to produce biopreparation Trichodermin.

Trichoderma spp. strains produce different types of propagula – mycelium fragments, conidia (air-

Table 3. Biomass and spore production of 11 newly obtained *Trichoderma* isolates and control strains of *T. viride*, *T. koningii* and *T. harzianum* and isolates of *T. longibrachiatum* under submerged and surface cultivation conditions

Species and number of the strain	Submerged cultivation		Surface cultivation, aerial spore production $\times 10^9$ /g
	biomass, g/l	chlamyospores production $\times 10^6$ /ml	
<i>T. viride</i> 23	14.7 \pm 0.7	32.4 \pm 3.7	7.8 \pm 0.7
<i>T. viride</i> CK (new)	13.7 \pm 0.5	31.6 \pm 3.2	7.9 \pm 0.5
<i>T. viride</i> 165 (new)	11.6 \pm 0.7	15.3 \pm 0.33	6.2 \pm 0.5
<i>T. viride</i> Tp1	12.1 \pm 0.9	9.5 \pm 0.45	8.4 \pm 0.5
<i>T. viride</i> 75	12.7 \pm 0.7	9.7 \pm 0.70	7.2 \pm 0.4
<i>T. viride</i> 27 (new)	10.9 \pm 0.1	10.4 \pm 0.11	8.1 \pm 0.7
<i>T. viride</i> 49 (new)	12.2 \pm 0.5	9.7 \pm 0.70	7.9 \pm 0.3
<i>T. viride</i> 35 (new)	13.0 \pm 0.2	11.5 \pm 1.0	9.2 \pm 1.0
<i>T. viride</i> TM	11.7 \pm 0.8	10.2 \pm 0.6	4.3 \pm 0.3
<i>T. viride</i> T 3	10.5 \pm 0.6	9.9 \pm 1.23	3.8 \pm 0.7
<i>T. koningii</i> M10	11.1 \pm 0.4	0.7 \pm 0.12	5.4 \pm 0.8
<i>T. koningii</i> 21 (new)	11.3 \pm 0.7	1.4 \pm 0.33	6.8 \pm 1.1
<i>T. koningii</i> – 64 (new)	11.5 \pm 0.7	1.1 \pm 0.05	6.2 \pm 1.0
<i>T. harzianum</i> 8900	10.2 \pm 1.1	7.8 \pm 0.62	5.5 \pm 0.4
<i>T. harzianum</i> 8995	12.1 \pm 0.7	8.0 \pm 0.31	5.9 \pm 0.7
<i>T. longibrachiatum</i> -161 (new)	11.8 \pm 0.3	not produced	0.13 \pm 0.02
<i>T. longibrachiatum</i> -162 (new)	14.5 \pm 0.2	not produced	0.22 \pm 0.01
<i>T. longibrachiatum</i> -163 (new)	13.9 \pm 0.7	not produced	0.68 \pm 0.04
<i>T. longibrachiatum</i> -164 (new)	14.2 \pm 0.3	not produced	0.17 \pm 0.01

Note. Data presented as mean values \pm standard error.

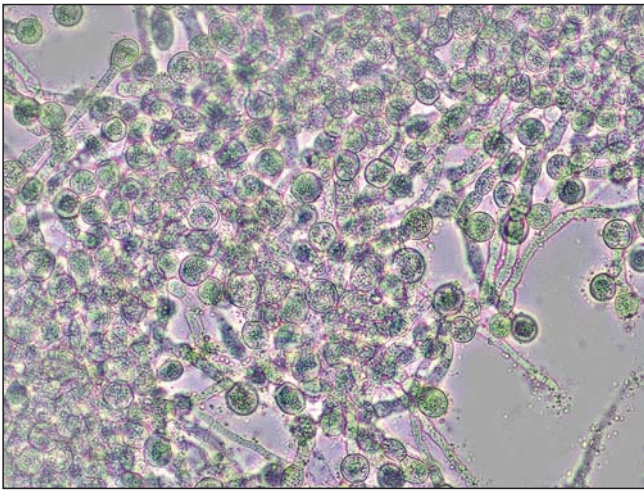


Fig. 2. The growth of *T. viride* CK in the submerged culture (after 72 hours growth in corn extract/molasse medium at 25 °C) producing chlamydo-spores

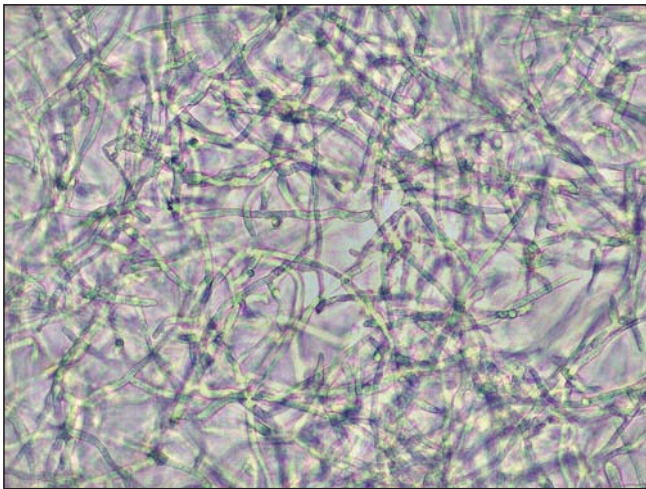


Fig. 3. The growth of *T. longibrachiatum* in the submerged culture (after 72 hours growth in corn extract/molasse medium at 25 °C)

Table 4. Determination of the effective dose of a Trichodermin biological preparation, based on new *T. viride* isolate CK, for pre-sowing treatment of winter wheat seeds

Content of preparation in a working suspension, % of a $3 \cdot 10^7$ spores/ml concentrate in liquid culture	The decrease in the number of plants, infected with root rot, % (as compared to the control) on day 12, n = 3
1.00	82.9 ± 4.73
0.20	57.2 ± 5.34
0.04	40.0 ± 3.91

Data presented as mean values \pm standard error

distributed or submerged), chlamydo-spores (submerged) with different biological efficacy or storage stability (Lewis JA, Papavizas GS, 1984). Most bio-preparations of *Trichoderma spp.* are produced using air-distributed conidia, formed on moistened grain of cereals via solid-phase fermentation (Motta FL et al, 2014; Woo et al, 2014; Cruz-Quiroz RD et al, 2017; Hamrouni R et al, 2019).

Submerged cultivation has several advantages, including shorter period of cultivation and the possibility of automation of many operations. Since not all the fungi are capable of spore formation in the submerged culture, the selection of the corresponding strain is of utmost significance (Jakubiková L. et al, 2006).

Submerged conidia, produced by some strains of *Trichoderma spp.*, have thinner cell wall compared to air-distributed conidia and lower survival rate while stored (Watanabe S et al, 2006). Chlamydo-spores are suggested as the most promising propagules to produce preparations using the submerged technology, as they are capable of surviving in soil for a long time, having faster germination in soil and lower sensitivity to soil fungistasis than conidia. Chlamydo-spore-based formulations have other beneficial features, including resistance to drying and low temperatures, insensitivity to soil antibiotics, and extended preservation time, which in turn simplifies processing, storage, and transport of the biological control agent (Beagle-Ristaino JE, Papavizas GC, 1985; Li et al, 2016).

The ability of the investigated *Trichoderma* strains to produce chlamydo-spores in the submerged culture (spores of vegetative reproduction), which are the forms in the cycle of fungus development, most resistant to the impact of unfavorable environmental factors, ensuring their survival in soil, is the prerequisite for the elaboration of the submerged technology of liquid preparation production.

Therefore, following the results of our initial laboratory assessment, the new *T. viride* CK isolate was selected for further studies, as it is the most promising candidate for a microbiological preparation, judging by its antagonistic activity and technological traits under submerged cultivation. Growth characteristics of *T. viride* isolate CK after incubation of 5 days on wort agar at 25 °C are shown in Fig. 4.

The suitability of *Trichoderma spp.* isolates and strains for mass cultivation was determined by the indices of conidia formation during superficial cultivation on grain and the capability of producing chlamydo-

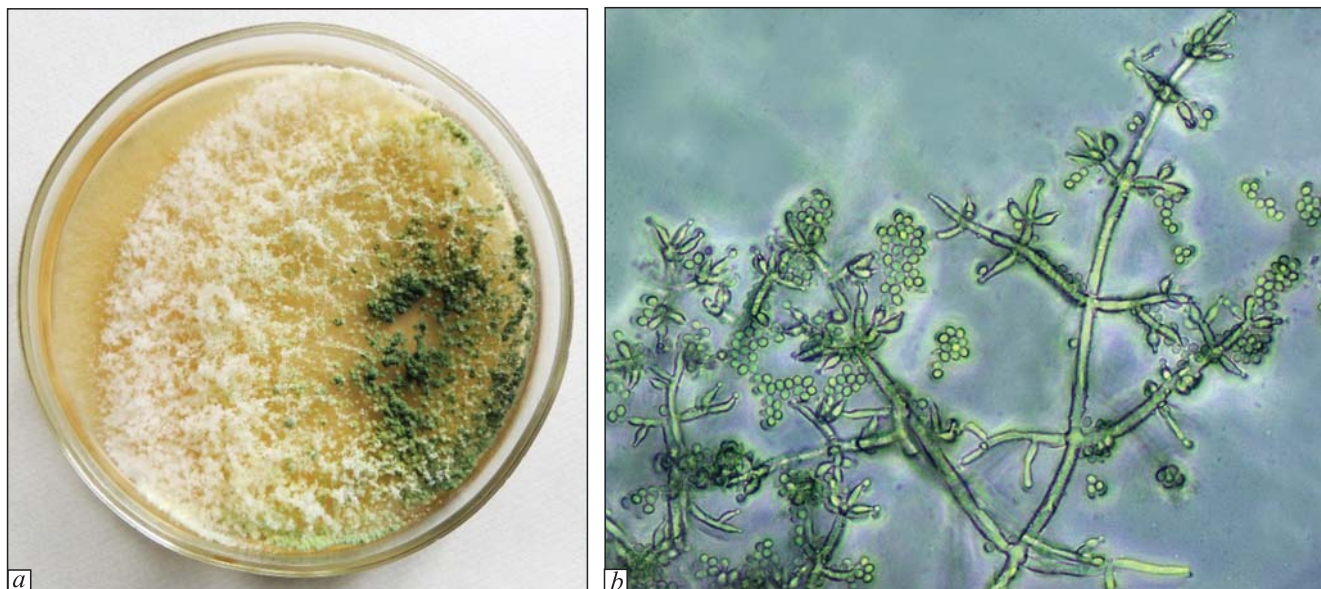


Fig. 4. *T. viride* CK strain: *a* – growth after cultivation of 5 days at 25 °C on wort agar; *b* – conidiophores and conidia of the fungus

spores in the submerged culture. Submerged cultivation was conducted using a culture medium, based on cheap organic sources of nutrients – corn extract and molasses, which can completely meet the demands of fungi for necessary nutrients and are economically reasonable, being by-products of food industry. Optimization of the culture medium, selection of the components and determination of their optimal content for the cultivation of the new *T. viride* isolate CK will be a further direction of our research.

T. viride isolate CK, a promising highly antagonistic isolate *in vitro*, also showed promising antagonistic activity towards *Fusarium* spp. (reduced the number of plants affected by root rot by more than 80 %) when tested in a small laboratory based experiment where its effect on the reduction of root rot in wheat, variety Myronivska 808, was investigated.

In our earlier studies, we tested a preparation based on the *T. viride* CK isolate in the field using cucumber. Pre-treatment of cucumber seeds hybrid Courage F1 at a rate of 2 ml/kg provided complete protection against plant loss during the seedling phase as a result of root rot caused by *Fusarium* spp. in the greenhouse (Balvas-Hremiakova K, 2019).

We also found that in addition to the protective effect, the *T. viride* CK isolate also demonstrated its growth-stimulating activity, increasing the germination by 20–25 %, furthermore metabolites, synthesized this isolate, stimulated growth activity of meristem cells – the length of the root and stem of cu-

cumber plants was 25–30 % larger during the seedling phase as compared to the control (Balvas-Hremiakova K and Goral S, 2019).

CONCLUSIONS

Five new isolates preliminarily (on the basis of morphological characteristics only) allocated to *T. viride* and four allocated to *T. longibrachiatum*, demonstrated *in vitro* the highest and widest antagonistic activity against the phytopathogenic fungal species *Fusarium oxysporum*, *F. solani*, *Alternaria cucumerina*, *Colletotrichum phomoides*, *Botrytis cinerea*, *Trichothecium roseum*, *Penicillium* sp., *Cladosporium fulvum*, as compared to new isolates, preliminarily allocated to *T. harzianum* and *T. koningii*. New isolate CK (allocated to *T. viride*) showed a promising and similar high antagonistic activity as compared to our *T. viride* 23 strain, which is already successfully used in the biocide Trichodermin. Since this isolate CK also produced a high number of chlamydospores in submerged culture ($1 \cdot 10^7$ – $3 \cdot 10^7$ spores/ml) and conidia ($6 \cdot 10^9$ – $9 \cdot 10^9$ spores/g) respectively when surface cultured on barley grain, it is a potential new candidate for a biocide. When this CK isolate was studied in a small laboratory pot experiment, to control *Fusarium* root rot in wheat, by preventive seed inoculation, it caused an 83 % reduction in this *Fusarium* root rot. Its usefulness under field conditions and its effect on growth of wheat plants will be investigated in future research.

Adherence to ethical principles. All experiments described in this paper were non animal based.

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Скринінг нових ізолятів *Trichoderma* на антагоністичну активність щодо кількох фітопатогенних грибів, у тому числі *Fusarium spp.*

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Мета. Отримати та охарактеризувати нові ізоляти *Trichoderma* з антагоністичними властивостями відносно фітопатогенних грибів – збудників хвороб пшениці, у тому числі *Fusarium spp.* та визначити їх придатність для масового виробництва в різних умовах вирощування. **Методи.** Мікробіологічні, культурально-морфологічні, статистичні. **Результати.** З рослин, уражених фітопатогенними грибами: огірок (*Cucumis sativus* L.), помідор (*Solanum lycopersicum* L.), білокачанна капуста (*Brassica oleracea* L.), озима пшениця (*Triticum aestivum* L.), ярий ячмінь (*Hordeum vulgare* L.) у Лісостепу України (Київська область), виділено 11 нових ізолятів *Trichoderma*. За культурально-морфологічними характеристиками п'ять з них були визначені як *T. viride* (ізоляти СК, 165, 27, 49, 35), два – *T. koningii* (21, 64), чотири – *T. longibrachiatum* (161, 162, 163, 164). Усі ізоляти виявляли помірну та високу антагоністичну активність щодо 8 видів фітопатогенних грибів (*Fusarium oxysporum*, *Fusarium solani*, *Alternaria cucumerina*, *Colletotrichum phomoides*, *Botrytis cinerea*, *Trichothecium roseum*, *Penicillium sp.*, *Cladosporium fulvum*). У експерименті з подвійною культурою вони виявили загалом подібну або в деяких випадках вищу активність до вищезазначених грибів, ніж 8 контрольних штамів, використаних у нашому дослідженні, що належать до *T. viride* (5 штамів), *T. koningii* (2 штамів) та *T. harzianum* (1 штам), які тривалий час підтримуються в мікробіологічній лабораторії Інституту захисту рослин НААН. Найактивніший новий ізолят СК, визначений як *T. viride*, проявив високу активність відносно всіх фітопатогенних грибів порівняно з високоактивним контрольним штамом *T. viride* 23, який є продуцентом біопрепарату Триходермін, що виробляється біолабораторіями України, в тому числі Інституту захисту рослин НААН (Київ). Продуктивність утворення хламідоспор всіх ізолятів та штамів, що вивчалась при глибинному культивуванні, коливалась від 10^6 до $3 \cdot 10^7$ спор/мл, при цьому ізоляти

та штам *T. viride* мали найвищу продуктивність спорування. Ізоляти '*T. longibrachiatum*' не утворювали хламідоспор у глибинній культурі. При поверхневому вирощуванні на зерні ячменю штамів та ізоляти *T. viride* також характеризувалися найвищою продуктивністю спорування ($6 \cdot 10^9$ – $9 \cdot 10^9$ спор/г) порівняно з *T. koningii*, *T. harzianum* ($5,5 \cdot 10^9$ – $6,8 \cdot 10^9$ спор/г) та *T. longibrachiatum* ($1,3 \cdot 10^8$ – $6,8 \cdot 10^8$ спор/г). Вексперименті *in vivo* в лабораторних умовах найбільш перспективний антагоністичний ізолят СК був використаний для передпосівної обробки насіння пшениці на штучному інфекційному фоні, створеному внесенням в ґрунт збудників кореневих гнилей (суміш *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium gibbosum*, *F. oxysporum*, $4 \cdot 10^4$ спор/г), знизивши ураження рослин кореневими гнилями на 83 % порівняно з контролем. **Висновки.** П'ять нових ізолятів, попередньо (лише на основі морфологічних характеристик) ідентифіковані як *T. viride*, і чотири – *T. longibrachiatum*, проявили *in vitro* найвищу антагоністичну активність відносно фітопатогенних грибів *Fusarium oxysporum*, *Fusarium solani*, *Alternaria cucumerina*, *Colletotrichum phomoides*, *Botrytis cinerea*, *Trichothecium roseum*, *Penicillium sp.*, *Cladosporium fulvum*, порівняно з новими ізолятами, попередньо визначеними як *T. harzianum* і *T. koningii*. Новий ізолят СК (визначений як *T. viride*) показав високу антагоністичну активність, що була на рівні штаму *T. viride* 23, який є продуцентом біопрепарату Триходермін. Ізолят СК продукував велику кількість хламідоспор у глибинній культурі ($3 \cdot 10^7$ спор/мл) та конідій при культивуванні на зерні ячменю ($8 \cdot 10^9$ спор/г), і є перспективним в якості продуцента біопрепарату. Передпосівна обробка насіння пшениці рідкою культурою ізоляту СК на штучному інфекційному фоні в лабораторних умовах знижувала ураження рослин фузаріозними кореневими гнилями на 83 %. Ефективність його застосування у польових умовах та вплив на ріст рослин будуть досліджені в майбутніх дослідженнях.

Ключові слова: масове виробництво, глибинне та поверхнєве вирощування, біологічний захист.

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