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IN VITRO SCREENING OF NEW STRAINS OF PREDACIOUS NEMATOPHAGOUS FUNGI FOR BIOCONTROL SUITABILITY WHEN PRODUCED IN LIQUID CULTURE

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Aim. To isolate, identify, and evaluate *in vitro* the predacious ability of nematophagous fungi strains, to determine their eligibility for mass production under submerged cultivation; to select the most efficient isolates of predacious nematophagous fungi as promising producers of the biopreparation to control plant-parasitic nematodes. **Methods.** Microbiological, microscopical, cultural-morphological, statistical. **Results.** The screening to determine the presence of predacious nematophagous fungi in different soils from different regions has been conducted for the first time in Ukraine. Out of 75 soil samples, isolated in Kyiv, Chernihiv, Dnipropetrovsk, Volyn and Odesa regions, 88 isolates of predacious nematophagous fungi were obtained which belong to 11 genera by their cultural-morphological characteristics: *Arthrobotrys oligospora* – 39 isolates, *A. musiformis* – 25, *A. conoides* – 11, *Drechlerella dactyloides* – 3, *A. arthrotryoides* – 2, *A. superba* – 2, *A. megalospora* – 2, *A. sphaeroides* – 1, *A. flagrans* – 1, *A. amerospora* – 1, *A. thaumasia* – 1. High nematophagous activity *in vitro* (90–100 % trapping) regarding free-living nematodes (*Rhabditis* spp). was demonstrated by 4 isolates of *A. oligospora*, 18 isolates of *A. musiformis*, 5 isolates of *A. conoides*, 3 isolates of *D. dactyloides*, and one isolate of *A. megalospora*. Among the 47 most active isolates, 4 isolates of predacious fungi formed chlamydo-spores under submerged cultivation on two types of liquid media – wort medium and corn-molasses medium: *A. musiformis*-711, *A. musiformis*-911, *A. conoides*-90, *D. dactyloides*-19. It was found that isolate *A. musiformis*-911 and *A. conoides*-90 could form conidia in the submerged culture. Further experiments in determining the character of development for two latter isolates in different types of media under submerged cultivation established that the largest number of chlamydo-spores and conidia were formed by the isolates of *A. conoides* 90 and *A. musiformis* 911 on potato dextrose medium, $7.6 \cdot 10^5$ and $6.5 \cdot 10^5$ spores/ml respectively. In the wort medium, the productivity of spore formation for the isolate of *A. conoides* 90 was 35 times lower, amounting to $2.2 \cdot 10^4$ spores/ml, and in peptone-glucose medium – 90 times lower ($8.5 \cdot 10^3$ spores/ml). The isolate of *A. musiformis* 911 in the wort medium produced 325 times fewer spores (2.0×10^3 spores/ml) than in potato-dextrose medium, while no chlamydo-spores and conidia were formed in the peptone-glucose medium. More detailed study of the ratio between isolates-producers and different sources of nutrition as the basis for the optimization of liquid media is required. **Conclusions.** According to the results of determining nematophagous activity regarding free-living nematodes, isolated from different soils in Ukraine and evaluating the character of development under submerged cultivation, two isolates of predacious fungi were selected – *Arthrobotrys conoides*-90 and *A. musiformis*-911, which are promising producers of a biological preparation eventually to be used in the biocontrol of plant-parasitic nematodes. Conidia formation of the two isolates in liquid culture under mass production conditions was observed for the first time and could also contribute to their suitability for mass production and biocontrol.

Key words: screening, *in vitro*, predacious nematophagous fungi, *Arthrobotrys*, nutrition media, submerged cultivation.

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INTRODUCTION

Plant-parasitic nematodes – obligate parasites causing considerable losses to the cultivation of agricultural

crops – are among the most pathogenic plant-related organisms. Globally losses caused by plant-parasitic nematodes, average 10–20 %, but may amount to over 50 % due to their high spreading, which caused a

global annual loss of about USD 358 billion according to the calculations for 2010–2013 (Abd-Elgawad MM, Askary TH, 2015).

Root-knot nematodes from the genus *Meloidogyne* are among the most dangerous pests of vegetables in covered soil. The accumulation of root-knot nematodes in covered soil may cause 30–90 % harvest losses of vegetables, and even a complete loss in some cases. Losses, caused by root-knot nematodes of the genus *Meloidogyne* in the European countries and the USA, were estimated as at least USD 30 billion annually (Borzykh OI et al, 2017). The infection of root-knot nematodes leads to the lost quality of vegetables and reduced resistance to many other stress factors (drought, other diseases), and can promote infections of other phytopathogens, including fungi, bacteria, and viruses (Borah B et al, 2018; Kepenekci I et al, 2018). In Ukraine, root-knot nematodes have infected up to 85 % of the greenhouse farms (Borzykh OI et al, 2017).

In Europe, chemical nematicides, are designated as highly toxic preparations and have only very restricted application. The number of registered nematicides in the world has decreased globally in the last 25 years due to concerns about their non-specific mechanisms of action, and thus about their potential toxicity and probability of harming the environment (Degenkolb T and Vilcinskis A, 2016). The application of methyl bromide for example was prohibited in 2005 (Singh A et al, 2019).

Even if all the technological regulations are followed, the application of chemical nematicides is very dangerous for human health and environment. Another drawback of chemical nematicides is their stability in the environment, promoting the development of nematode resistance which leads to the use of more toxic substances (Singh R et al, 2013).

Thus, there is a need to search for alternative controls of the number of plant-parasitic nematodes (Moosavi MR and Zare R, 2015).

Predacious nematophagous fungi from the safe group of microorganisms are among natural regulators of the number of nematodes. The fungi of this unique ecological group can change their lifestyle from saprophytic to predacious stage in the presence of nematodes, creating specialized means to catch, constrict, and consume nematodes.

The intense study of this unique group of fungi was launched after a series of publications of the American mycologist, C. Drechsler, (Drechsler C, 1933a, 1933b,

1934, 1937) on morphology and systematics of predacious fungi in the 1930s.

Since then, predacious nematophagous fungi started attracting attention of researchers as the agents of biological control of plant-parasitic nematodes.

In particular, a reason of constant interest to these fungi lies in their potential as biocontrol agents against plant- and animal-parasitizing nematodes (Askary TH, 2015).

Asexual hyphomycetes, trapping nematodes, are located within the family *Orbiliaceae* (*Orbiliales*, *Orbiliomycetes*), based on morphological and molecular studies which includes 96 species, at present related to asexual genera *Arthrobotrys* (53 species), *Dactylellina* (28 species) and *Drechslerella* (14 species) (Yu Z et al, 2014).

Numerous studies on nematophagous fungi have proven that they are present globally, even in Antarctica and in all types of climate and habitats (Zhang Y et al, 2014, Gray NF and Smith RL, 1984, Dasgupta MK and Khan MR, 2015). There are reports about their isolation from agricultural, horticultural, and forest soils, and they are especially abundant in soils, enriched with organic materials (Nordbring-Hertz B et al, 2001; Hyde K et al, 2014; Gray NF, 1985). *Arthrobotrys oligospora* Fres. 1852 is the most frequently isolated and undoubtedly most predacious fungus in the environment (Jaffee BA and Strong DR, 2005; Niu XM and Zhang KQ, 2011). No isolation studies on nematophagous fungi in different soils and regions have previously been conducted in Ukraine.

Considering active trapping of nematodes by predacious fungi, their ability to exist in soil, the possibility of cultivating large quantities in artificial media, predacious nematophagous fungi may become efficient means in the biological control of nematodes.

However, there is comparatively little success in elaborating commercially acceptable formulations of nematophagous fungi. In most cases, fungi are massively produced on solid media, such as cereal grain or chaff, and the colonized substrate is applied to the soil (Moosavi MR and Askary TH, 2015; Cayrol JC, 1988; Kumar D et al, 2005). There are much fewer studies, substantiating the obtaining of preparations under submerged technology in liquid media (Gardner K et al, 2000; Lee et al, 2004; Teplyakova TV, 2019).

The elaboration of new bioproducts based on fungi depends on the availability of proper isolates. Therefore, a maximal possible number of isolates should

be studied during screening programs. Sometimes it takes the study of hundreds or thousands of isolates to determine a few with the relevant combination of characteristics for the biocontrol. Only a small share of candidate isolates (under 1 %) may be producers of biocontrol agents (Moosavi R and Askary TH, 2015).

The aim of the study was as follows: to isolate, identify, and evaluate *in vitro* the predacious ability of nematophagous fungi strains, to select the most efficient isolates of predacious nematophagous fungi as promising producers of the biopreparation to control plant-parasitic nematodes.

MATERIALS AND METHODS OF STUDIES

Soil samples were selected from different biotopes (agricultural land, greenhouses, uncultivated soil from the banks of water bodies, forests, city parks, building surrounding grounds, roadside ditches, etc.) predominantly in Kyiv (Forest-Steppe zone) region, as well as in Chernihiv, Volyn (Polissia zone), Odesa, Dnipropetrovsk (Steppe zone) regions. In total 75 soil samples (150–300 g each) were taken from a depth of 5–15 cm.

Free-living nematodes (*Rhabditis* spp.), used as test-objects, were extracted from partially rotten apples, lying on the ground, by the flotation method. Nematodes were identified using specialized taxonomic keys (Tarjan A, 1977), and grown at room temperature on damp oat-flakes in Petri dishes. To support the population, every 10–15 days the nematodes, which moved from the substrate to the lids of Petri dishes, were washed with water and transferred into the fresh medium (Heintz CE, 1978).

The soil sprinkling technique was used to find predacious nematophagous fungi (Drechsler C, 1941a; Duddington CL, 1955; Eren J and Pramer D, 1965; Gray NF, 1984). Soil (and plant residues, if present in the samples), were dispersed along the surface of sterile water agar (agar – 2 %, water – 1000 ml), leaving clean areas of agar to facilitate the direct study, into 7–10 Petri dishes per soil sample (1 g per one dish on average) with immediate subsequent addition of several drops of the suspension with free-living nematodes (100–200) to stimulate the development of predacious fungi. The dishes were incubated at 20–25 °C. The time of the appearance of nematophagous fungi varied considerably, thus, regular daily examination of the dishes with solid medium was done for 10–15 days. The presence of nematophagous fungi was determined with further identification during direct observation, finding fungi by searching for nematodes, trapped by fungi, and

the availability of remarkable conidiophores directly above the surface of agar, using the stereomicroscope MBI-9 at a magnification of 20x–40x. When the conidiophores of predacious fungi appeared, the latter were isolated into pure culture using micromanipulations with conidia with a thin sterile needle.

The identification was conducted using the material, infected with nematodes, as the features of these fungi were more constant in the presence of nematodes than in a pure culture (Cooke RC and Godfrey BES, 1964).

The fungi were identified by morphological and morphometric characteristics based on specialized taxonomic keys (Yu et al, 2014).

The determination of micromorphological properties of the extracted isolates, their identification and microphotography were conducted using Zeiss Primo Star microscope at 400× magnification and Axio Vision software.

The cultural-morphological and biological properties were studied in the following media: wort agar (beer wort – 25 %, agar – 2 %), potato dextrose agar (PDA), agar with the extract of cornmeal (CMA, (20 g of cornmeal in 1000 ml of water was heated at 70 °C for 1 h, then filtered through cotton wool and agar was added (Duddington CL, 1955a).

The experiments on determining nematophagous activity were conducted in Petri dishes with the diameter of 4 cm. Agar blocks with the diameter of 5 mm from the colonies of 7-day-old fungi media on PDA, cut out with a forstner drill bit, were placed in the center of the dish on the surface of water agar, and a few drops of the suspension with free-living nematodes (about 300 specimen) were added. The dishes with nematodes, not inoculated with fungi, served as control. The experiments were done in three repeats. The mortality of nematodes was determined on Day 5 and calculated using Abbott's formula:

$$\% \text{ mortality} = \frac{X_{\text{control}} - X_{\text{treatment}}}{X_{\text{control}}} \times 100,$$

where X_{control} – the average number of live nematodes in the control group, $X_{\text{treatment}}$ – the average number of live nematodes in the variants of the inoculation with fungi.

The isolates, which trapped over 90 % nematodes *in vitro*, were estimated as highly active, 50–89 % – as having medium activity, under 50 % – poorly active.

The submerged cultivation of the extracted isolates was done in Erlenmeyer flasks of 300 ml with 50 ml

of the medium on the orbital shaker PSU-20i (Biosan, Latvia) at 200 rpm and the temperature of 25–26 °C for 72 h. The following media were used for submerged cultivation: wort (25 % beer wort), corn-molasses (corn extract – 1 %, molasses – 4 %, KH_2PO_4 – 0.5%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.05 %), peptone-glucose (peptone – 2 %, glucose – 2 %, KH_2PO_4 – 0.5 %, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.05 %). The flasks with media were sterilized in an autoclave at 0.1 MPa for 30 min and inoculated with suspensions of spore-mycelium mass, washed with 5 ml of sterile water from the tubes with 7-day-old media of fungi on PDA, 1 ml suspension per flask. The experiments were done in three repeats.

To determine the biomass of fungi under submerged cultivation, 10 ml of liquid medium were filtered through previously dried and weighed paper filters (“white stripe”, pore diameter of 8–12 μm), washed with distilled water, dried at +110 °C for 1 h and weighed using the assay balance.

The productivity of spore formation was done by counting spores in the hemocytometer (Gorjaev’s chamber).

The data on nematode-trapping activity of isolates and productivity in submerged cultivation were subjected to dispersion analysis (One-way ANOVA).

RESULTS OF INVESTIGATIONS

Some 88 isolates of predacious nematophagous fungi, related to 11 genera, were extracted out of 75 selected soil samples: *Arthrobotrys oligospora* – 39 isolates, *A. musiformis* – 25, *A. conoides* – 11, *Drechlerella dactyloides* – 3, *A. artrobotryoides* – 2, *A. superba* – 2, *A. megalospora* – 2, *A. sphaeroides* – 1, *A. flagrans* – 1, *A. amerospora* – 1, *A. thaumasia* – 1. Predacious fungi were found in all but 3 soil samples.

In terms of the mechanism of trapping nematodes, there was prevalence of species, forming three-dimensional adhesive networks and only isolates of *D. dactyloides* formed single trapping constricting rings.

The results of the search demonstrate wide spread occurrence in Ukrainian soils of this ecological group of fungi. Predacious fungi were found in most samples of different types of soil, including the ones, poor in organic substances (sandy and sandy loam soils). However, their number in soil was insignificant. In the different soil samples, there were 0.02–5 colony-forming units (CFU) in 1 g or 1 CFU in 0.5–20 g of soil.

Nematophagous fungi were observed in all types of habitats, but no correlation was found between the species of fungi and habitats.

The main aim of the studies was to select the most efficient isolates, which would be promising producers of the biopreparation eventually to be used in the biocontrol of plant-parasitic nematodes. The evaluation criteria for such isolates were nematophagous activity regarding test-objects, character and rate of growth of agar media and in submerged culture, intensity and type of spore-formation (chlamydospores and conidia), formation of spontaneous trapping devices (without the presence of nematodes).

A remarkable specificity of *D. dactyloides* isolates is the formation of a great number of spontaneous constricting rings both on agar media and under submerged cultivation. This ability was preserved after multiple subculturing. As for the remaining defined species of predacious fungi, the formation of spontaneous traps was observed only for several isolates of *A. musiformis* (in 7 out of 25 extracted ones) immediately after the extraction into a pure culture and in a much smaller quantity as compared with *D. dactyloides*, and this ability was gradually lost after 1–2 times of subculturing.

Contrary to species with adhesive properties, *D. dactyloides* isolates were characterized by slow growth both on agar media and in the submerged culture. For instance, the diameter of *D. dactyloides* colonies on wort agar at 25 °C was 2–2.5 cm on Day 12, 4–4.5 cm on Day 20, 7–9 cm on Day 30, whereas the colonies of fast-growing species with adhesive network were spreading along the whole Petri dish on Day 8–10.

The results of evaluating the nematophagous activity of the extracted isolates of predacious fungi are presented in Table 1.

Out of 39 investigated isolates of *A. oligospora*, high activity was shown for 4 (the mortality of nematodes – 95–100 %), 24 demonstrated medium activity (the mortality of nematodes – 52–88 %), 11 had poor activity (the mortality of nematodes – 15–45 %).

High nematophagous activity was found for *A. musiformis* isolates (Fig. 1). 18 out of 25 isolates demonstrated high nematophagous activity (90–100 %), medium activity was registered for 7 isolates (the mortality of nematodes – 49–89 %).

Out of 11 *A. conoides* isolates, 5 had high activity (the mortality of nematodes – 91–100 %), 5 – medium activity (58–82 %), and one had poor activity (the mortality of nematodes – 20 %).

High activity was determined for all three isolates from the genus *D. dactyloides* – 97–100 %.

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Table 1. The nematophagous activity of the extracted isolates of predacious nematophagous fungi isolated from different Ukrainian soils

Species and number of isolates	The reduction in the number of nematodes as compared with the control, %, on Day 5	Species and number of isolates	The reduction in the number of nematodes as compared with the control, %, on Day 5
<i>A. oligospora-1</i>	26 ± 3.0	<i>A. musiformis-251</i>	95 ± 2.5
<i>A. oligospora-4</i>	42 ± 4.4	<i>A. musiformis-261</i>	89 ± 3.5
<i>A. oligospora-512</i>	25 ± 5.0	<i>A. musiformis-293</i>	91 ± 0.3
<i>A. oligospora-6</i>	37 ± 6.6	<i>A. musiformis-34</i>	94 ± 2.9
<i>A. oligospora-7151</i>	69 ± 6.0	<i>A. musiformis-351</i>	97 ± 0.6
<i>A. oligospora-9</i>	70 ± 10.0	<i>A. musiformis-231</i>	97 ± 1.7
<i>A. oligospora-10</i>	44 ± 6.6	<i>A. musiformis-391</i>	100 ± 0.0
<i>A. oligospora-12</i>	62 ± 3.9	<i>A. musiformis-431</i>	99 ± 0.6
<i>A. oligospora-132</i>	38 ± 3.3	<i>A. musiformis-62</i>	75 ± 5.0
<i>A. oligospora-21</i>	45 ± 5.0	<i>A. musiformis-64</i>	95 ± 1.5
<i>A. oligospora-252</i>	83 ± 6.6	<i>A. musiformis-65</i>	100 ± 0.0
<i>A. oligospora-262</i>	70 ± 5.0	<i>A. musiformis-68</i>	60 ± 10.0
<i>A. oligospora-27</i>	81 ± 6.6	<i>A. musiformis-69</i>	95 ± 2.5
<i>A. oligospora-292</i>	70 ± 5.0	<i>A. musiformis-711</i>	99 ± 0.6
<i>A. oligospora-352</i>	75 ± 5.0	<i>A. musiformis-731</i>	99 ± 0.6
<i>A. oligospora-392</i>	99 ± 0.6	<i>A. musiformis-741</i>	98 ± 1.0
<i>A. oligospora-40</i>	95 ± 1.2	<i>A. musiformis-802</i>	100 ± 0.0
<i>A. oligospora-432</i>	70 ± 5.0	<i>A. musiformis-831</i>	100 ± 0.0
<i>A. oligospora-61</i>	45 ± 5.0	<i>A. musiformis-861</i>	95 ± 1.0
<i>A. oligospora-63</i>	45 ± 3.3	<i>A. musiformis-911</i>	100 ± 0.0
<i>A. oligospora-66</i>	15 ± 5.0	<i>A. conoides-82</i>	58 ± 6.6
<i>A. oligospora-67</i>	75 ± 6.6	<i>A. conoides-232</i>	69 ± 3.8
<i>A. oligospora-70</i>	60 ± 10.0	<i>A. conoides-24</i>	70 ± 5.0
<i>A. oligospora-71</i>	35 ± 5.0	<i>A. conoides-372</i>	98 ± 1.0
<i>A. oligospora-700</i>	53 ± 3.3	<i>A. conoides-41</i>	20 ± 5.0
<i>A. oligospora-712</i>	88 ± 3.3	<i>A. conoides-722</i>	73 ± 6.6
<i>A. oligospora-721</i>	60 ± 6.6	<i>A. conoides-733</i>	82 ± 6.6
<i>A. oligospora-732</i>	80 ± 6.6	<i>A. conoides-78</i>	97 ± 1.0
<i>A. oligospora-742</i>	81 ± 3.3	<i>A. conoides-841</i>	100 ± 0.0
<i>A. oligospora-743</i>	80 ± 3.5	<i>A. conoides-90</i>	100 ± 0.0
<i>A. oligospora-752</i>	80 ± 2.9	<i>A. conoides-913</i>	95 ± 2.5
<i>A. oligospora-79</i>	73 ± 6.6	<i>A. artrobotryoides-21</i>	70 ± 5.0
<i>A. oligospora-801</i>	100 ± 0.0	<i>A. artrobotryoides-83</i>	38 ± 6.6
<i>A. oligospora-81</i>	65 ± 5.0	<i>A. superba-18</i>	55 ± 5.0
<i>A. oligospora-842</i>	99 ± 0.6	<i>A. superba-19</i>	73 ± 6.6
<i>A. oligospora-843</i>	88 ± 2.9	<i>A. megalospora-27</i>	20 ± 3.5
<i>A. oligospora-912</i>	85 ± 3.3	<i>A. megalospora-42</i>	90 ± 2.5
<i>A. oligospora-92</i>	77 ± 6.6	<i>A. sphaeroides-213</i>	15 ± 2.9
<i>A. oligospora-93</i>	75 ± 5.0	<i>A. flagrans-371</i>	15 ± 2.9
<i>A. musiformis-52</i>	65 ± 5.0	<i>A. amerospora 751</i>	10 ± 2.9
<i>A. musiformis-61</i>	50 ± 3.3	<i>A. thaumasia-89</i>	60 ± 4.3
<i>A. musiformis-7</i>	65 ± 3.3	<i>D. dactyloides-201</i>	100 ± 0.0
<i>A. musiformis-81</i>	55 ± 3.5	<i>D. dactyloides-30</i>	97 ± 1.5
<i>A. musiformis-131</i>	95 ± 1.5	<i>D. dactyloides-19</i>	100 ± 0.0

Note. The data are presented as mean values ± standard deviation.



Fig. 1. A *Rhabditis* nematode in the adhesive hyphae of *Arthrobotrys musiformis-911* on water agar). Bar: 100 μm

Out of two isolates of *A. megalospora*, one was highly active, causing the mortality of nematodes at the level of 90 % and one had poor activity (20 %).

The isolate of the genus *A. thaumasia* demonstrated medium nematophagous activity (60 %). The isolates of the genera *A. sphaeroides*, *A. flagrans*, *A. amerospora* had poor activity (the mortality of nematodes – 10–15 %).

The suitability of 47 most active isolates of 5 genera of predacious fungi to submerged cultivation conditions using two nutrient media was studied (Table 2).

The isolates belonging to species with reticulate trapping networks, grew well in the submerged culture. All the isolates of *A. musiformis* formed more than 10.1–14.8 g/l of biomass in both media. Out of 18 tested isolates of *A. oligospora*, 12 formed the amount of biomass over 10 g/l.

Out of 6 isolates of *A. conoides*, the most productive isolate was the one of *A. conoides-90* (13.1–13.5 g/l).

In the submerged culture, the isolates of *D. dactyloides* were also considerably inferior to the isolates with reticulate networks by their indices of growth intensity and biomass accumulation. For instance, after 96 h of submerged cultivation, the biomass yield on wort and corn-molasses media was 3.5–4.1 g/l. All three isolates of this genus formed spontaneous constricting rings both in the submerged culture and on agar media.

Only the isolates of *A. musiformis-711*, *A. musiformis-911*, *A. conoides-90*, *D. dactyloides-19* were characterized by the ability to produce chlamydo spores in the submerged culture. In addition to chlamydo spores, the isolates of *A. conoides-90* and *A. musiformis-911* also formed submerged conidia, and *A. musiformis-911* formed single spontaneous traps as well (Fig. 2). The isolates of *D. dactyloides* formed a great number of spontaneous constricting rings in the submerged culture, as well as on agar media. The remaining isolates

from different species formed only mycelium in the submerged culture.

The ability of the investigated isolates to form conidia and 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 their application as producers of the biological preparation against plant-parasitic nematodes.

The isolates of *A. conoides 90* and *A. musiformis 911* were characterized with intense spore-formation on agar media and the formation of chlamydo spores in aging cultures. The submerged conidia of *A. conoides 90* measured 12–28 (20.7) \times 8–17 (11.7) μm and were formed on short conidiophores (Fig. 3).

In the next stage of the research, the character of development was determined for *A. conoides 90* and *A. musiformis 911* on different types of liquid nutrient media – wort, potato dextrose, peptone-glucose media (Table 3).

The largest number of chlamydo spores and conidia was formed by the isolates of *A. conoides 90* and *A. musiformis 911* on potato dextrose medium – $7.6 \cdot 10^5$ and $6.5 \cdot 10^5$ spores/ml, respectively. In the wort medium, the productivity of spore formation for the isolate of *A. conoides 90* was 35 times lower, amounting to $2.2 \cdot 10^4$ spores/ml, and in peptone-glucose medium – 90 times lower ($8.5 \cdot 10^3$ spores/ml). The isolate of *A. musiformis 911* in the wort culture produced 325 times fewer spores ($2.0 \cdot 10^3$ spores/ml) than in potato-dextrose medium, while no chlamydo spores and conidia were formed in the peptone-glucose medium.

The results of the studies demonstrate the need for more detailed investigation on the ratio between isolates-producers and different nutrition sources as the basis for the optimization of liquid media. Further studies will also include the determination of the biocon-

Table 2. The technological properties of the isolates of predacious nematophagous fungi under submerged cultivation (in 72 h)

Species and number of the isolate	Biomass yield, g/l, in media		The character of fungus development in the submerged culture
	wort	corn-molasses	
<i>A. oligospora-252</i>	10.1 ± 0.7	10.8 ± 0.5	mycelium
<i>A. oligospora-27</i>	9.8 ± 0.7	9.9 ± 1.1	mycelium
<i>A. oligospora-392</i>	12.2 ± 0.3	12.4 ± 0.3	mycelium
<i>A. oligospora-40</i>	10.1 ± 0.5	9.8 ± 0.6	mycelium
<i>A. oligospora-63</i>	12.2 ± 0.8	12.4 ± 0.5	mycelium
<i>A. oligospora-70</i>	8.0 ± 1.0	8.2 ± 0.5	mycelium
<i>A. oligospora-712</i>	6.7 ± 0.6	6.5 ± 0.3	mycelium
<i>A. oligospora-721</i>	8.1 ± 0.2	9.5 ± 0.5	mycelium
<i>A. oligospora-732</i>	13.1 ± 0.6	12.8 ± 0.3	mycelium
<i>A. oligospora-742</i>	7.4 ± 0.3	8.1 ± 0.7	mycelium
<i>A. oligospora-743</i>	11.2 ± 0.7	12.4 ± 0.2	mycelium
<i>A. oligospora-752</i>	9.8 ± 0.3	10.1 ± 0.3	mycelium
<i>A. oligospora-79</i>	8.5 ± 0.5	9.1 ± 0.6	mycelium
<i>A. oligospora-801</i>	11.4 ± 1.2	12.0 ± 0.2	mycelium
<i>A. oligospora-842</i>	10.7 ± 0.7	10.4 ± 0.4	mycelium
<i>A. oligospora-843</i>	12.6 ± 0.8	13.1 ± 0.5	mycelium
<i>A. oligospora-912</i>	12.0 ± 1.0	12.6 ± 0.6	mycelium
<i>A. oligospora-93</i>	11.1 ± 1.2	11.5 ± 0.8	mycelium
<i>A. musiformis-131</i>	12.5 ± 0.6	13.5 ± 0.2	mycelium
<i>A. musiformis-251</i>	11.2 ± 0.3	11.5 ± 0.7	mycelium
<i>A. musiformis-261</i>	12.5 ± 0.6	12.0 ± 1.0	mycelium
<i>A. musiformis-293</i>	10.4 ± 0.8	11.2 ± 0.4	mycelium
<i>A. musiformis-34</i>	11.8 ± 0.3	12.1 ± 0.7	mycelium
<i>A. musiformis-351</i>	10.1 ± 0.6	10.9 ± 0.3	mycelium
<i>A. musiformis-231</i>	12.3 ± 0.3	13.0 ± 0.3	mycelium
<i>A. musiformis-391</i>	13.1 ± 0.3	13.4 ± 0.1	mycelium
<i>A. musiformis-431</i>	10.5 ± 0.7	10.8 ± 0.5	mycelium
<i>A. musiformis-64</i>	13.8 ± 0.5	12.8 ± 0.4	mycelium
<i>A. musiformis-65</i>	14.5 ± 0.1	13.2 ± 0.3	mycelium
<i>A. musiformis-69</i>	13.0 ± 0.6	13.6 ± 0.2	mycelium
<i>A. musiformis-711</i>	11.1 ± 0.3	11.5 ± 0.6	mycelium, chlamydospores
<i>A. musiformis-731</i>	13.8 ± 0.3	13.2 ± 0.3	mycelium
<i>A. musiformis-741</i>	13.7 ± 0.2	13.0 ± 0.1	mycelium
<i>A. musiformis-802</i>	11.4 ± 0.5	11.7 ± 0.6	mycelium
<i>A. musiformis-831</i>	12.2 ± 0.6	12.8 ± 0.4	mycelium
<i>A. musiformis-861</i>	11.2 ± 0.6	11.6 ± 0.8	mycelium
<i>A. musiformis-911</i>	12.4 ± 0.5	13.1 ± 0.2	mycelium, chlamydospores, conidia, traps
<i>A. conoides-372</i>	9.4 ± 0.3	10.1 ± 0.3	mycelium
<i>A. conoides-733</i>	10.0 ± 0.6	11.2 ± 0.7	mycelium
<i>A. conoides-78</i>	8.7 ± 0.3	8.8 ± 0.3	mycelium
<i>A. conoides-841</i>	10.5 ± 0.6	11.2 ± 0.6	mycelium
<i>A. conoides-90</i>	13.1 ± 0.3	13.5 ± 0.2	mycelium, conidia, chlamydospores
<i>A. conoides-913</i>	9.8 ± 0.2	10.4 ± 0.5	mycelium
<i>A. megalospora-42</i>	10.1 ± 0.6	10.5 ± 0.5	mycelium
<i>D. dactyloides-201</i>	3.5 ± 0.3	3.8 ± 0.2	mycelium, constricting rings
<i>D. dactyloides-30</i>	3.6 ± 0.2	3.9 ± 0.3	mycelium, constricting rings
<i>D. dactyloides-19</i>	3.9 ± 0.3	4.1 ± 0.3	mycelium, chlamydospores, constricting rings

Note. The data are presented as mean values ± standard deviation.

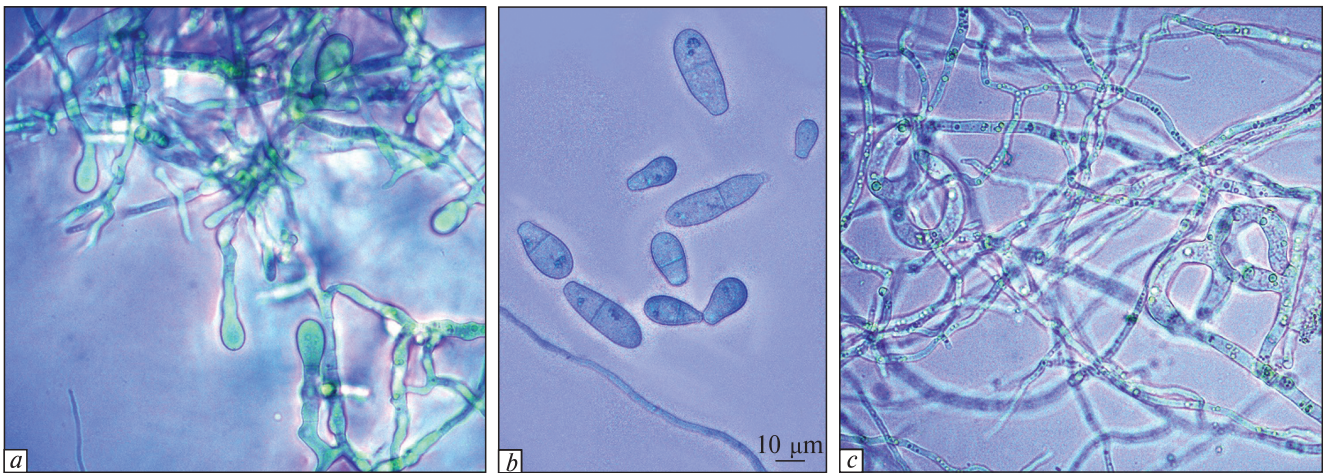


Fig. 2. The formation of submerged conidia and traps by *A. musiformis* 911 strain in the submerged culture (corn-molasses media). *a* – conidia formation (24 hours); *b* – conidia (72 hours); *c* – traps (72 hours). Bar: 10 µm

tol effect on plant parasitic nematodes of the two most promising strains.

DISCUSSION

We conducted the first screening of predacious nematophagous fungi in different soils and regions of Ukraine.

We did not intend to study the flora of predacious nematophagous fungi in detail, as the main aim was to conduct the screening of the most efficient isolates which would be promising biocontrol agents for plant-parasitic nematodes. The evaluation criteria were as follows: the nematophagous activity *in vitro*, the character and rate of growth on agar media and in the submerged culture, the intensity and type of spore-formation.

Our studies demonstrated that predacious nematophagous fungi are widespread in soils of different ecologic origin – both agricultural and uncultivated land. The most frequently isolated species was *A. oligospora* (44 %).

No predacious fungi were found only in 3 soil samples. About 37 % soil samples contained more than one species of predacious fungi, 2 species were found in 24 soil samples, and 4 samples contained 3 species each which demonstrates that different species of predacious fungi may take similar ecologic niches.

In addition to the mentioned species, 4 soil samples had predacious fungi which did not form traps, but nematodes were caught by adhesion directly to mycelium. Fungi with adhesive hyphae are likely to belong to the genus *Stylopage* (Drechsler C, 1941, b). However, it was found impossible to isolate these species into a

pure culture and identify them due to the absence of spore formation therein.

Five samples were also positive for the development of predacious fungi *Arthrobotrys* spp., but due to the presence of mites – microbophages which got to the dishes from soil we did not succeed in isolating these fungi into a pure culture.

The studies on nematophagous activity *in vitro* using free-living *Rhabditis* spp. as test object demonstrated that this activity was considerably different for different fungal isolates belonging to species with reticulate adhesive networks and varied from 10 to 100 %.

The highest activity in trapping nematodes was noted for *D. dactyloides* with constricting rings. All three isolates of this species were active nematophagous at a level of 97–100 % and formed many trapping rings in a pure culture on different agar media (wort agar, PDA, CMA) and in the submerged culture spontaneously, without the presence of nematodes. The formation of spontaneous rings in *D. dactyloides* was also observed by Cooke RC, 1963; Lawton JR, 1957; Gray NF, 1985.

However, the isolates of *D. dactyloides* grew rather slowly on agar media and in the submerged culture. A low rate of the growth of the species with constricting rings as compared with *Arthrobotrys* spp., which have adhesive networks, was also noted by other researchers (Duddington CL, 1955(b); Cooke RC, 1963; Kumar D et al, 2005; Lee GJ et al, 2008; Swe A et al, 2009). It was observed that slowly growing species may form their organs of trapping without any stimulation, whereas the ones, demonstrating faster saprotrophic growth, mainly *Arthrobotrys* spp., require external stimuli to cause the formation of a trap (Cooke RC, 1963; 1964,

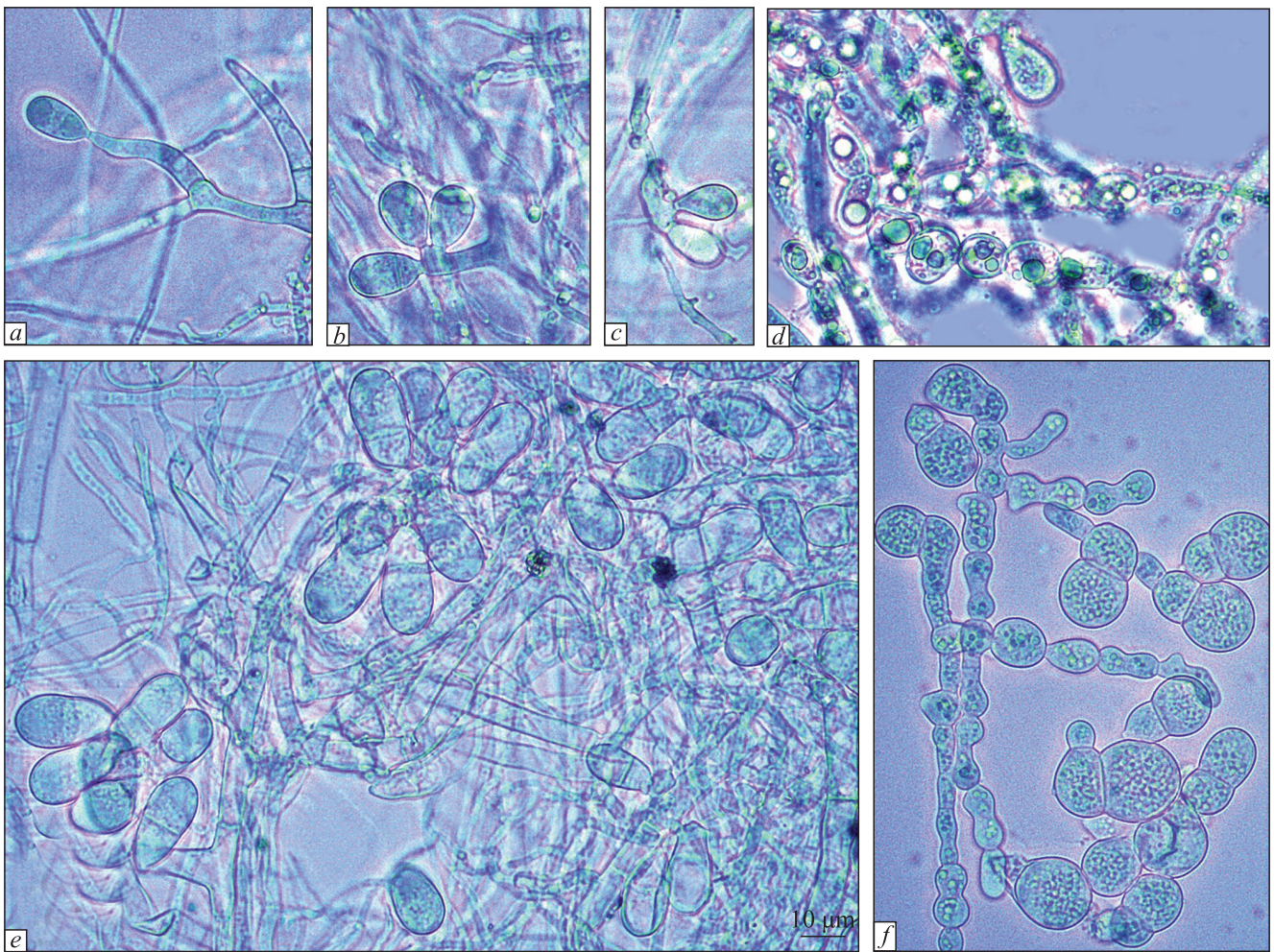


Fig. 3. *A. conoides 90*: chlamydospores and conidia in the submerged culture. *a, b, c* – conidia formation (24 hours); *e* – conidia (72 hours); *d* – chlamydospores (72 hours) on corn-molasses medium; *f* – submerged conidia germinated at once, forming the chains of chlamydospores (glutamic acid was used as the only source of nitrogen, 72 hours). Bar: 10 µm

1968). The species with constricting rings are less competitive in soil as compared with the network-forming species (Cooke RC, 1963; Kumar D et al, 2015).

The suitability of 47 most active isolates of 5 species of predacious fungi was evaluated for the conditions of submerged cultivation on two media – wort and corn-molasses. Submerged cultivation has many advantages over surface cultivation, including a shorter cultivation period and the automation possibility for many operations. As not all the fungi can form spores in the submerged culture, the selection of a relevant strain is extremely important. Under submerged cultivation on both media, only 4 isolates – *A. musiformis*-711, *A. musiformis*-911, *A. conoides*-90, *D. dactyloides*-19 – formed chlamydospores. In addition, the isolates of *A. musiformis*-911 and *A. conoides*-90 can form conidia in the submerged culture. The formation conidia by *Arthrobotrys* spp. fungi under submerged

cultivation was, as far as could be determined, not previously reported.

Our study demonstrated again the important role of chlamydospores in the cycle of the development of predacious fungi, as they are the most promising propagules for the production of biological preparations under submerged technology due to their ability to survive in soil for a long time, faster germination in soil and weaker sensitivity to soil fungistasis, stability to drying and low temperatures and continuous conservation, which, in its turn, facilitates processing, storing, and transporting (TePLYakova, 2019; Castillo-Saldarriaga C et al, 2020; Federica SM et al, 2013; Li et al, 2016).

Thus, according to the results of our primary laboratory evaluation, the strains *A. musiformis*-911 and *A. conoides*-90 were selected for further studies, as they are most promising producers of the microbiological

Table 3. The development of *A. conoides* 90 and *A. musiformis* 91-1 isolates on different types of media in submerged culture

Medium	Biomass, g/l	Productivity of conidia and chlamydo spores/ml
<i>A. conoides</i> 90		
Wort	10.2 ± 0.3	2.2 · 10 ⁴
Potato dextrose	10.8 ± 0.2	7.6 · 10 ⁵
Peptone-glucose	8.7 ± 0.2	8.5 · 10 ³
<i>A. musiformis</i> 91-1		
Wort	10.1 ± 0.6	2.0 · 10 ³
Potato dextrose	7.8 ± 0.1	6.5 · 10 ⁵
Peptone-glucose	8.7 ± 0.3	0

Note. The data are presented as mean values ± standard deviation

preparation due to their nematophagous activity and technological traits under submerged cultivation. Although the isolates of *D. dactyloides* manifested high nematophagous activity, we consider them potential biocontrol agents of little promise due to their low growth rate.

Our studies demonstrated also that the intensity of the formation for chlamydo spores and conidia of these two isolates depends considerably on the type of the nutrient medium. One further experiment of investigating the impact of sources of nitrogen and carbon nutrition on the growth of *A. conoides*-90 in the submerged culture (the data are not presented) demonstrated that depending on different sources of nitrogen and carbon, the isolate could produce predominantly either conidia or chlamydo spores. Moreover, when glutamic acid was used as the only source of nitrogen, submerged conidia germinated at once, forming the chains of chlamydo spores, and chlamydo spores were formed directly out of conidia (Fig. 3, f).

The results of the studies demonstrate the need for more detailed investigation on the ratio between isolates-producers and different nutrition sources as the basis for the optimization of liquid media. Moreover, further studies should evaluate the activity of the selected isolates regarding plant-parasitic nematodes.

CONCLUSIONS

Out of 75 soil samples, isolated in Kyiv, Chernihiv, Odesa, Dnipropetrovsk, Volyn, Odesa regions, 88 iso-

lates of predacious nematophagous fungi were obtained which belong to 11 genera: *Arthrobotrys oligospora* – 39 isolates, *A. musiformis* – 25, *A. conoides* – 11, *Drechlerella dactyloides* – 3, *A. artrobotryoides* – 2, *A. superba* – 2, *A. megalospora* – 2, *A. sphaeroides* – 1, *A. flagrans* – 1, *A. amerospora* – 1, *A. thaumasia* – 1.

According to the results of determining nematophagous activity regarding free-living nematodes and evaluating the character of development under submerged cultivation, two isolates of predacious fungi were selected – *A. conoides*-90, *A. musiformis*-911, which are promising producers of a biological preparation eventually to be used in the control of plant-parasitic nematodes.

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

Conflict of interest. Authors declare no conflict of interest.

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Скринінг *in vitro* нових штамів хижих нематофагових грибів на придатність до біоконтролю при виробництві в рідкій культурі

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Мета. Виділити, ідентифікувати та оцінити *in vitro* хижацьку здатність штамів нематофагових грибів, визначити їхню придатність для масового виробництва в умовах глибинного культивування; відібрати найбільш ефективні ізоляти хижих нематофагових грибів, перспективні в якості продуцентів біопрепарату для боротьби з фітопаразитичними нематодами. **Методи.** Мікробіологічні, мікроскопічні, культурально-морфологічні, статистичні. **Результати.** Вперше в Україні проведено скринінг у природних умовах хижих нематофагових грибів. З 75 проб ґрунту, відібраних в Київській, Чернігівській, Одеській, Дніпропетровській, Волинській, Одеській областях виділено 88 ізолятів хижих нематофагових грибів, які за культурально-морфологічними характеристиками відносяться до 11 родів: *Arthrobotrys*

oligospora – 39 ізолятів, *A. musiformis* – 25, *A. conoides* – 11, *Drechslereella dactyloides* – 3, *A. artrobotryoides* – 2, *A. superba* – 2, *A. megalospora* – 2, *A. sphaeroides* – 1, *A. flagrans* – 1, *A. amerospora* – 1, *A. thaumasia* – 1. Високу нематофагову активність *in vitro* відносно вільноживучих нематод *Rhabditis* sp. (90–100 % вловлювання) проявили 4 ізоляти *A. oligospora*, 18 ізолятів *A. musiformis*, 5 ізолятів *A. conoides*, 3 ізоляти *D. dactyloides*, один ізолят *A. megalospora*. З 47 найбільш активних ізолятів 4 ізоляти хижих грибів в умовах глибинного культивування на двох типах рідких середовищ – суловому і кукурудзяно-мелясному утворювали хламідоспори: – *A. musiformis*-711, *A. musiformis*-911, *A. conoides*-90, *D. dactyloides*-19. Встановлено, що ізоляти *A. musiformis*-911 і *A. conoides*-90 здатні утворювати в глибинній культурі конідії. В подальших дослідях з визначення характеру розвитку двох останніх ізолятів у різних типах середовищ при глибинному культивуванні встановлено, що найбільшу кількість хламідоспор і конідій ізоляти *A. conoides* 90 та *A. musiformis* 911 утворювали на картопляно-глюкозному середовищі – відповідно $7,6 \cdot 10^5$ та $6,5 \cdot 10^5$ спор/мл. У суловому середовищі продуктивність спорування ізоляту *A. conoides* 90 була в 35 разів нижчою і становила $2,2 \cdot 10^4$ спор/мл, а в пептоно-глюкозному – в 90 разів нижчою ($8,5 \cdot 10^3$ спор/мл). Ізолят *A. musiformis* 911 в суловому середовищі продукував в 325 разів менше спор ($2,0 \times 10^3$ спор/мл), ніж у картопляно-глюкозному середовищі, а в пептоно-глюкозному середовищі хламідоспори та конідії не утворювались. Необхідне більш детальне вивчення відношення ізолятів-продуцентів до різних джерел, живлення як базової основи до оптимізації рідких середовищ. **Висновки.** За результатами визначення нематофагової активності відносно вільноживучих нематод та оцінки характеру розвитку в умовах глибинного культивування відібрано два ізоляти хижих грибів – *A. conoides*-90, *A. musiformis*-911, перспективні в якості продуцента біологічного препарату для боротьби з фітопаразитичними нематодами. Утворення конідій двох ізолятів у рідкій культурі в умовах масового виробництва спостерігалось вперше, а також могло сприяти їх придатності для масового виробництва та біоконтролю.

Ключові слова: скринінг, *in vitro*, хижі нематофагові гриби, *Arthrobotrys*, живильні середовища, глибинне культивування.

REFERENCES

- Abd-Elgawad MM, Askary TH. (2015) Impact of phytonematodes on agriculture economy. In Biocontrol agents of phytonematodes (pp. 3–49). (Askary TH; Martinelli PRP. eds.) Wallingford, UK CAB International. doi: 10.1079/9781780643755.0003.
- Askary TH. (2015) Nematophagous fungi as biocontrol agents of phytonematodes. In Biocontrol Agents of Phytonematodes. (Askary TH; Martinelli PRP. eds.) Wallingford, CAB. International. 81–125. doi: 10.1079/9781780643755.0081.
- Borah B, Ahmed R, Hussain M, Phukon P, Wann SB, Sarmah DK, Bhau BS. (2018) Suppression of root-knot disease in *Pogostemon cablin* caused by *Meloidogyne incognita* in a rhizobacteria mediated activation of phenylpropanoid pathway. *Biological Control*. **119**:43–50. <https://doi.org/10.1016/j.biocontrol.2018.01.003>.
- Borzykh OI, Sigariova DD, Pylypenko LA, Kovtun AM. (2017) The most dangerous nematodes of plants and the systems of protective measures. Kyiv. NVP Interservis LLC. 140 p. (in Ukrainian)
- Castillo-Saldarriaga C, Céspedes-Gutiérrez E, Cubides-Cárdenas JA, Gómez-Álvarez M, Márquez-Lara D. (2020) Biphasic fermentation strategy: an alternative to produce chlamydospores of nematophagous fungus *Duddingtonia flagrans*. *Biocontrol Sci Technol*. **30**(10):1100–1115. <https://doi.org/10.1080/09583157.2020.1793908>.
- Cayrol JC. (1988) Lutte biologique contre les *Meloidogyne* au moyen du champignon prédateur *Arthrobotrys irregularis*. *EPPO Bulletin*, **18**(1):73–75. <https://doi.org/10.1111/j.1365-2338.1988.tb00350.x>. (in French).
- Cooke RC. (1963) Ecological characteristics of nematode-trapping Hyphomycetes: I. Preliminary studies. *Ann. Appl. Biol.* **52**(3):431–437. <https://doi.org/10.1111/j.1744-7348.1963.tb03767.x>.
- Cooke RC. (1964) Ecological characteristics of nematode-trapping Hyphomycetes: II. Germination of conidia in soil. *Ann. Appl. Biol.* **54**(3):375–379. <https://doi.org/10.1111/j.1744-7348.1964.tb01201.x>.
- Cooke R. (1968) Relationships between nematode-destroying fungi and soil-borne phyto-nematodes. *Phytopathology*. **58**(7):909–913.
- Cooke RC, Godfrey BES. (1964) A key to the nematode-destroying fungi. *Transactions of the British mycological society*. **47**(1):61–74. [https://doi.org/10.1016/S0007-1536\(64\)80081-4](https://doi.org/10.1016/S0007-1536(64)80081-4).
- Dasgupta MK, Khan MR. (2015) Nematophagous fungi: ecology, diversity and geographical distribution. In Biocontrol agents of phytonematodes. (Askary TH; Martinelli PRP. eds.) Wallingford, UK: CABI Publishing, 126–162. doi: 10.1079/9781780643755.0126.
- Degenkolb T, Vilcinskas A. (2016) Metabolites from nematophagous fungi and nematicidal natural products from fungi as an alternative for biological control. Part I: metabolites from nematophagous ascomycetes. *Appl. Microbiol. Biotechnol.* **100**(9):3799–3812. doi: 10.1007/s00253-015-7233-6.
- Drechler C. (1933) Morphological diversity among fungi that prey on nematodes. *J. Wash. Acad. Sci.* **23**:138–141. <https://www.jstor.org/stable/24530416>.
- Drechler C. (1933) Morphological features of some fungi that capture and kill nematodes. *J. Wash. Acad. Sci.* **23**:267–270. <https://www.jstor.org/stable/24530516>.
- Drechler C. (1934) Organs of capture in some fungi preying on nematodes. *Mycologia*. **26**:134–144. doi: 10.2307/3754035.

- Drechsler C. (1937) Some hyphomycetes that prey on free-living terricolous nematodes. *Mycologia*. 29(4):447–552. <https://doi.org/10.2307/3754331>.
- Drechsler C. (1941, a) Predacious fungi. *Biol. Rev. Camb. Philosoph. Soc.* 16, 265–290. <https://doi.org/10.1111/j.1469-185X.1941.tb01104.x>.
- Drechsler C. (1941, b) Four phycomycetes destructive to nematodes and rhizopods. *Mycologia*. 33(3):248–269. doi: 10.2307/3754760.
- Duddington CL. (1955a) Notes on the technique of handling predacious fungi. *Transact. Brit. Mycol. Soc.* 38(2):97–103. [https://doi.org/10.1016/S0007-1536\(55\)80021-6](https://doi.org/10.1016/S0007-1536(55)80021-6).
- Duddington CL. (1955b) Fungi that attack microscopic animals. *Bot. Rev.* 21(7):377–439. <https://doi.org/10.1007/BF02872434>.
- Eren J, Pramer D. (1965) The most probable number of nematode-trapping fungi in soil. *Soil Sci.* 99(4):285. doi: 10.1097/00010694-196504000-0001.
- Federica SM, Alberto FL, Emilia IL, Carina MF, Alfredo SC. (2013) Optimization of production of chlamydospores of the nematode-trapping fungus *Duddingtonia flagrans* in solid culture media. *Parasitol. Res.* 112(3):1047–1051. <https://doi.org/10.1007/s00436-012-3231-0>.
- Gardner K, Wiebe MG, Gillespie AT, Trinci AP. (2000) Production of chlamydospores of the nematode-trapping *Duddingtonia flagrans* in shake flask culture. *Mycol. Res.* 104(2):205–209. <https://doi.org/10.1017/S0953756299001124>.
- Gray NF, Smith RL. (1984) The distribution of nematophagous fungi in the maritime Antarctic. *Mycopathol.* 85 (1):81–92. <https://doi.org/10.1007/BF00436707>.
- Gray NF. (1985) Ecology of nematophagous fungi: effect of soil moisture, organic matter, pH and nematode density on distribution. *Soil Biol. Biochem.* 17(4):499–507. [https://doi.org/10.1016/0038-0717\(85\)90017-3](https://doi.org/10.1016/0038-0717(85)90017-3).
- Gray NF. (1984) Ecology of nematophagous fungi: Methods of collection, isolation, and maintenance of predatory and endoparasitic fungi. *Mycopathologia* 86:143–153. <https://doi.org/10.1007/BF00441123>.
- Hyde K, Swe A, Zhang KQ. (2014) Nematode-Trapping Fungi. In: Zhang KQ., Hyde K. (eds) *Nematode-Trapping Fungi*. Fungal Diversity Research Series, vol 23. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-8730-7_1.
- Heintz CE. (1978) Assessing the predacity of nematode-trapping fungi *in vitro*. *Mycologia*. 70(5):1086–1100. <https://doi.org/10.2307/3759140>.
- Jaffee BA, Strong DR. (2005) Strong bottom-up and weak top-down effects in soil: nematode-parasitized insects and nematode-trapping fungi. *Soil Biol. Biochem.* 37:1011–1021. <https://doi.org/10.1016/j.soilbio.2004.05.026>.
- Kepenekci I, Hazir S, Oksal E, Lewis EE. (2018) Application methods of *Steinernema feltiae*, *Xenorhabdus bovienii* and *Purpureocillium lilacinum* to control root-knot nematodes in greenhouse tomato systems. *Crop Protect.* 108:31–38. <https://doi.org/10.1016/j.cropro.2018.02.009>.
- Kumar D, Singh KP, Jaiswai RK. (2005). Screening of different media and substrates for cultural variability and mass culture of *Arthrobotrys dactyloides* Drechsler. *Mycobiology*. 33(4):215–222. doi: 10.4489/MYCO.2005.33.4.215.
- Kumar D, Maurya N, Kumar P, Singh H, Addy SK. (2015) Assessment of germination and carnivorous activities of a nematode-trapping fungus *Arthrobotrys dactyloides* in fungistatic and fungicidal soil environment. *Biol. Cont-rol.* 82:76–85. doi: 10.1016/j.biocontrol.2014.12.014.
- Lawton JR. (1957) The formation of constricting rings in nematode-catching Hyphomycetes grown in pure culture. *J. Exp. Bot.* 8(1):50–54. <https://doi.org/10.1093/jxb/8.1.50>.
- Lee GJ, Koo CD, Sung JH. (2008) Characteristics of pine-wood nematode trapping by nematophagous *Arthrobotrys* spp. *Korean J. Mycol.* 36(2):153–162. <https://www.koreascience.or.kr/article/JAKO200805441020294.pdf> (in Korean).
- Lee JK, Kim DG, Lee SB. (2004) Nutritional requirements and mass production of nematode-trapping fungus, *Arthrobotrys oligospora*. *J. Asia-Pacific Entomol.* 7(3):325–329. [https://doi.org/10.1016/S1226-8615\(08\)60234-4](https://doi.org/10.1016/S1226-8615(08)60234-4).
- Moosavi MR, Askary TH. (2015). Nematophagous fungi-commercialization. In *Biocontrol agents of phytonematodes*. (Askary TH; Martinelli PRP. eds.) Wallingford, UK: CABI Publishing, 187–202. doi: 10.1079/9781780643755.0187.
- Moosavi MR, Zare R. (2015) Factors affecting commercial success of biocontrol agents of phytonematodes, 423–445. *Biocontrol Agents of Phytonematodes* (Eds: TH Askary and PRP Martinelli), CABI Publishing, Wallingford. doi: 10.1079/9781780643755.0423.
- Niu XM, and Zhang KQ. (2011). *Arthrobotrys oligospora*: a model organism for understanding the interaction between fungi and nematodes. *Mycology*. 2(2):59–78. <https://doi.org/10.1080/21501203.2011.562559>.
- Nordbring-Hertz B, Jansson H-B, Tunlid A. (2001) Nematophagous fungi. In *Encyclopedia of life sciences*, London: Macmillan. <https://doi.org/10.1002/9780470015902.a0000374.pub3>.
- Sasser JN. (1989) Plant-parasitic nematodes: the farmer's hidden enemy. *Plant-parasitic nematodes: the farmer's hidden enemy*. <https://www.cabdirect.org/cabdirect/abstract/19902302327>.
- Satchuthanathavale V, Cooke RC. (1967) Carbohydrate nutrition of some nematode-trapping fungi. *Nature*. 214(5085):321–322. <https://doi.org/10.1038/214321a0>.
- Singh A, Sharma P, Kumari A, Kumar R, Pathak DV. (2019) Management of root-knot nematode in different crops using microorganisms. In: Varma A., Tripathi S., Prasad

- R. (eds) Plant Biotic Interactions. Springer, Cham. https://doi.org/10.1007/978-3-030-26657-8_6.
- Singh R, Trivedi D, Srivastava A. (2013) Role of nematode-trapping fungi for crop improvement under adverse conditions. In: Tuteja N., Gill S. (eds) Crop Improvement Under Adverse Conditions. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-4633-0_12.
- Swe A, Jeewon R, Pointing SB, Hyde KD. (2009) Diversity and abundance of nematode-trapping fungi from decaying litter in terrestrial, freshwater and mangrove habitats. *Biodiver. Conser.* 18(6):1695–1714. <https://doi.org/10.1007/s10531-008-9553-7>.
- Tarjan A, Esser R, Chang S. (1977) An illustrated key to nematodes found in fresh water. J. (Water Pollution Control Federation). 49(11):2318–2337. <http://www.jstor.org/stable/25039452>.
- Tepliyakova TV (2019) Scientific basis for the effective use of predatory fungi-hyphomycetes in the fight against parasitic nematodes of plants. *Horticulture and viticulture.* 1:43–56. <https://www.cabdirect.org/cabdirect/abstract/20203394161> (in Russian).
- Yu Z, Mo M, Zhang Y, Zhang KQ. (2014) Taxonomy of nematode-trapping fungi from *Orbiliaceae*, Ascomycota. In: Zhang KQ., Hyde K. (eds) Nematode-Trapping Fungi. Fungal Diversity Research Series, vol 23. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-8730-7_3.
- Zhang Y, Zhang KQ, Hyde K. (2014) The ecology of nematophagous fungi in natural environments. In: Zhang KQ., Hyde K. (eds) Nematode-Trapping Fungi. Fungal Diversity Research Series, vol 23. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-8730-7_4.