

Evaluation of the pathogenicity of selected nematophagous fungi

MILOSLAV ZOUHAR^{1*}, ONDŘEJ DOUDA², DAVID NOVOTNÝ², JANA NOVÁKOVÁ¹
and JANA MAZÁKOVÁ¹

¹Czech University of Life Sciences, Faculty of Agrobiolgy, Food and Natural Resources, Department of Plant Protection, Kamýcká 129, 165 21 Praha 6 – Suchbátov, Czech Republic, *corresponding author, zouhar@af.czu.cz

²Research Institute of Crop Production, Division of Plant Medicine, Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic

Zouhar M., Douda O., Novotný D., Nováková J. and Mazáková J. (2010): Evaluation of the pathogenicity of selected nematophagous fungi – Czech Mycol. 61(2): 139–147.

The virulence of selected strains of six nematophagous fungi on three species of phytopathogenic nematodes was evaluated, whereby differences in pathogenicity between the investigated fungal taxa were found. *Arthrobotrys oligospora* was the most pathogenic fungus to all three tested species of nematodes.

Key words: nematophagous fungi, nematodes, pathogenicity, *Arthrobotrys*, *Dactylellina*, *Dactylella*, *Pochonia*, *Ditylenchus dipsaci*, *Globodera rostochiensis*, *Meloidogyne hapla*.

Zouhar M., Douda O., Novotný D., Nováková J. a Mazáková J. (2010): Zhodnocení patogenity vybraných druhů nematofágních hub. – Czech Mycol. 61(2): 139–147.

Byla hodnocena virulence vybraných kmenů šesti druhů nematofágních hub na tři druhy fytopatogenních háďátek. Byly zjištěny rozdíly v patogenitě mezi zkoumanými druhy hub. Druh *Arthrobotrys oligospora* byl nejvíce patogenní ze všech testovaných hub a to ke všem druhům háďátek.

INTRODUCTION

Nematophagous fungi are an interesting ecological group of fungi because they can be used to control phytoparasitic nematodes. Species of nematophagous fungi belong to many taxonomic groups. According to the type of pathogenesis in nematodes, nematophagous fungi are usually divided into endoparasites and predators (Barron 2004). So far little attention has been paid to the occurrence of nematophagous fungi in the Czech Republic (Rozsypal 1934, Ipserová 1982, Novotná 1989, Kubátová et al. 2000).

The species from the *Arthrobotrys* – *Dactylaria* – *Monacrosporium* genera complex are predators and create organs of capture (adhesive knobs or nets, con-

stricting and non-constricting rings). These fungi belong to the *Orbiliiales* order (Ascomycota) and most of them are anamorphic stages of *Orbilia* (Rubner 1996, Barron 2004, Li et al. 2005, Kirk et al. 2008).

The endoparasites produce infective conidia or zoospores that adhere and attack the nematode. E.g. *Esteya vermicola* and species of the genus *Pochonia* belong to this group (Kubátová et al. 2000, Barron 2004). Fungi of the genus *Pochonia* are close to *Verticillium* and belong to the *Hypocreales* order (Ascomycota) (Zare et al. 2001, Kirk et al. 2008).

Nematodes are very destructive organisms in agriculture and cause huge crop losses worldwide (Oka et al. 2000). The economically most important nematodes in Central Europe, including the Czech Republic, are *Ditylenchus dipsaci*, *Globodera rostochiensis* and *Meloidogyne hapla*. *D. dipsaci* (Stem and Bulb Nematode) is a significant pest of garlic and onion and is a quarantine organism in many countries of Europe. *M. hapla* (Northern Root Knot Nematode) poses especially threat to cultures of root vegetables. The quarantine species *G. rostochiensis* (Potato Cyst Nematode) causes serious losses in potato production. Phytopathogenic nematodes are difficult to control and methods to biologically control them are searched for (Brodie 1984, Janson and Lopez-Llorca 2004).

Knowledge of the pathogenicity of nematophagous fungi to nematodes is important for effective utilization of these fungi as nematode control agents. *Arthrobotrys* species, especially *A. oligospora*, have been investigated most frequently in this respect (Timper and Brodie 1993, Belder and Jansen 1994, Galper et al. 1995, Kumar and Singh 2006). The first step to recognise virulent strains is an in vitro pathogenicity test (culture of nematophagous fungi and nematode). The next step is to investigate the involved living plants (Belder and Jansen 1994, Galper et al. 1995, Kumar and Singh 2006).

The objective of this work was to evaluate the virulence of selected strains of fungal species to the three phytopathogenic nematode species mentioned above.

MATERIALS AND METHODS

In the study, six strains of nematopathogenic fungi were used. *Arthrobotrys oligospora* Fresen. 1850 (strain CBS 115.81), *Dactylella oviparasitica* G. R. Stirling et Mankau 1978 (strain CBS 347.85), *Dactylellina candida* (Nees) Yan Li 2006 (strain CBS 546.63), *Dactylellina lysipaga* (Drechsler) M. Scholler, Hagedorn et A. Rubner 1999 (strain CBS 581.91), *Dactylellina phymatopaga* (Drechsler) Y. Li 2005 (strain CBS 450.93), and *Pochonia chlamydosporia* var. *chlamydosporia* (Goddard) Zare et W. Gams (strain CBS 113566) were obtained from the Centraalbureau voor Schimmelcultures.

Pathogenicity of the mentioned fungal species to the three species of nematodes was evaluated. The *Ditylenchus dipsaci* nematodes were extracted from infested plants of endive (*Cichorium endivia*). This population originated from Slovenia and was naturally reproduced in the Czech Republic several times. *Globodera rostochiensis* (Ro1 pathotype) was obtained from the Field Research Station of the Potato Research Institute Ltd. (Kunratice, near the town of Šluknov, North Bohemia, Czech Republic) and multiplied by the authors. The population of *Meloidogyne hapla* was acquired from a carrot field in the vicinity of the town of Lysá nad Labem (town district of Litol), Central Bohemia, Czech Republic.

The authors used original methods in the study. The upper parts (lids) of small sterile Petri dishes were inserted in large sterile Petri dishes containing sterile filter paper. The filter paper was moistened with sterile distilled water. Microscope slides with a culture of the nematophagous fungus on agar medium were placed on the smaller Petri dishes. In the experiments, rose bengal agar (dextrose – 10 g, KH_2PO_4 – 1 g, peptone from soy – 5 g, rose bengal – 0.05 g, MgSO_4 – 0.5 g, agar – 15 g, distilled water – 1000 ml, autoclaved for 15 min. at 120 °C) was used. An agar droplet was placed on a microscope slide, inoculated with the nematophagous fungus and covered with a sterile cover glass slip. The Petri dishes were wrapped in Parafilm and cultivated for 3 days at 22 °C.

After 3 days, 50 µl of the aquatic suspension containing one of the three nematode species researched was pipetted under the edge of the cover glass slip. The concentration of the nematode suspension was 1.66×10^3 specimens/ml in the case of *Meloidogyne hapla*; 2.46×10^3 /ml and 0.32×10^3 /ml in *Globodera rostochiensis* and *Ditylenchus dipsaci*, respectively. The number of inserted nematodes was determined under a stereomicroscope. In the control, nematodes were applied on the medium without nematophagous fungi. The slides were cultivated another 3 days under the same conditions, after which evaluation was performed. Ten replicates (microscopic slides) were used for each fungus and nematode species.

The total number of nematodes and specimens affected by particular nematophagous fungi was assessed under 20× and 40× magnifications, respectively. The obtained data were calculated as mortality percentage and arcsin root square transformed. A final analysis was performed by single factor ANOVA followed by Tukey's test (Statistica 8).

RESULTS AND DISCUSSION

No mortality was observed in the control variants, in which nematodes were cultivated on pure medium. This indicates that the screening method was appropriate.

Tab. 1. Comparison of the mortality of the investigated nematodes caused by nematophagous fungi in experimental variants and untreated control. P-values of Tukey's test; variants with the same number are not significantly different at $P \leq 0.05$.

Fungus species	Nematode species		
	<i>Ditylenchus dipsaci</i>	<i>Globodera rostochiensis</i>	<i>Meloidogyne hapla</i>
<i>Dactylellina phymatopaga</i>	0.929637095991 a	0.0001308976486 d	0.000138166713 bc
<i>Arthrobotrys oligospora</i>	0.000130897649 b	0.0001308976485 c	0.000138166579 c
<i>Dactylella oviparasitica</i>	0.000165274330 c	0.0001309068053 d	0.000154478031 b
<i>Dactylellina lysipaga</i>	0.999955673983 a	0.0001308976485 b	0.000138166578 bc
<i>Dactylellina candida</i>	0.000130906649 c	0.8598005409330 a	*
<i>Pochonia chlamydosporia</i>	0.976671654438 a	0.0001309415876 d	0.000154202149 c
Control	a	a	a

* – not assessed; cultivation of the fungus failed

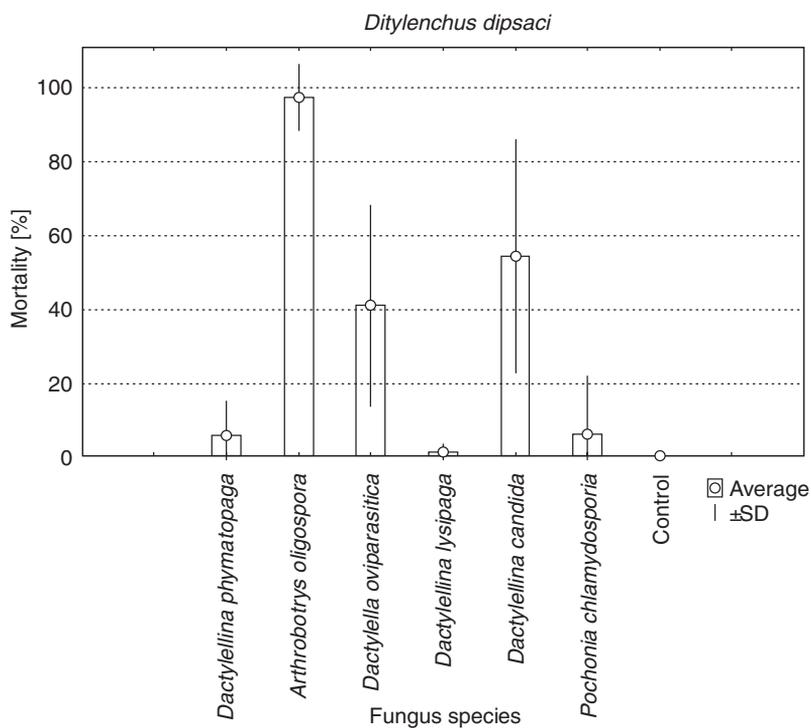


Fig. 1. Average mortality of the nematode *Ditylenchus dipsaci* caused by nematophagous fungi in experimental variants after three days of incubation.

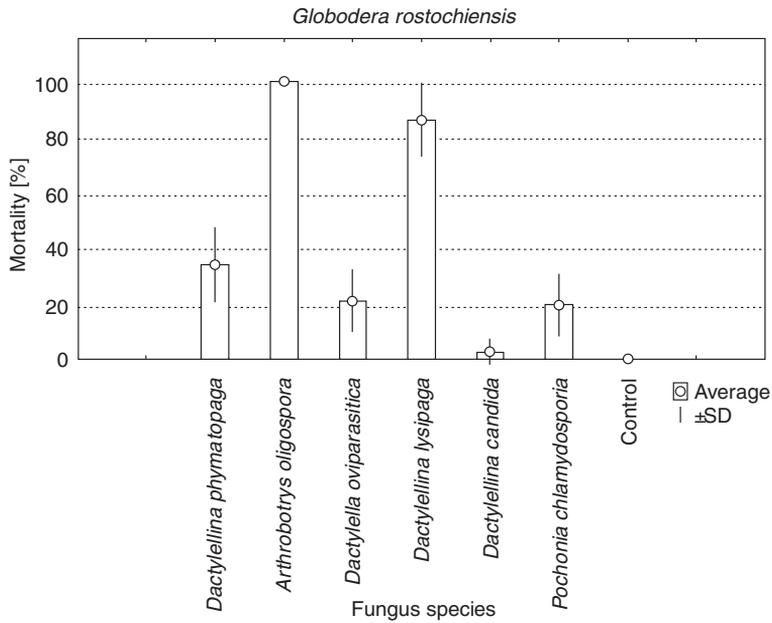


Fig. 2. Average mortality of the nematode *Globodera rostochiensis* caused by nematophagous fungi in experimental variants after three days of incubation.

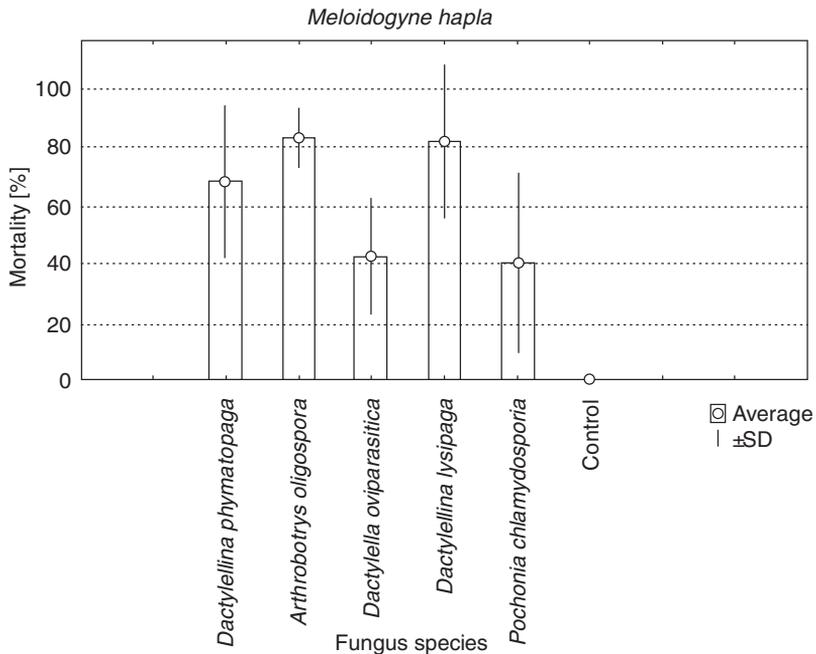


Fig. 3. Average mortality of the nematode *Meloidogyne hapla* caused by nematophagous fungi in experimental variants after three days of incubation.

Within the *Ditylenchus dipsaci* variants, statistically significant differences were discovered in the cases of *Arthrobotrys oligospora*, *Dactylella oviparasitica* and *Arthrobotrys candida*. *A. oligospora* was the most effective in *D. dipsaci* trapping (average mortality 97 %) while *A. candida* and *D. oviparasitica* were much less effective (average mortality 54 % and 40 %) (Fig. 1, Tab. 1).

Globodera rostochiensis was suppressed with *Dactylellina phymatopaga*, *Arthrobotrys oligospora*, *Dactylella oviparasitica*, *Dactylellina lysipaga* and *Pochonia chlamydosporia* species. *A. oligospora* was the most effective, because no living nematodes (average mortality 100 %) were found in this variant. High mortality was recorded in *G. rostochiensis* in the experiment with *D. lysipaga* (average mortality 86 %). The mortality caused by *D. phymatopaga*, *D. oviparasitica* and *P. chlamydosporia* was significantly lower (34, 21, and 20 %, respectively) (Fig. 2, Tab. 1).

In the case of the nematode *Meloidogyne hapla*, all tested fungi were effective. *A. oligospora* was again the most efficient; the average mortality was close to 80 %. However, a nearly identical result was found in the experiment with *D. lysipaga* (average mortality 79 %). The mortality caused by *D. phymatopaga* (66 %), *D. oviparasitica* (41 %) and *P. chlamydosporia* (39 %) was significantly lower (Fig. 3, Tab. 1). *D. candida* was not tested since the cultivation failed.

The pathogenicity of nematophagous fungi under in vitro conditions was investigated on a limited number of nematodes species. Using this approach, phytopathogenic (Timper and Brodie 1993, Belder and Jansen 1994, Galper et al. 1995, Jacobs 1997, Kumar and Singh 2006), free living (Galper et al. 1995; Gomes et al. 1999, 2001) and gastrointestinal parasitic (Gomes et al. 1999, 2001) nematodes were investigated. The pathogenicity of nematophagous fungi on *Meloidogyne hapla* and *Globodera rostochiensis* under in vitro conditions had been only investigated by Belder and Jansen (1994). In her study of fungi associated with cysts of *G. rostochiensis*, Novotná (1989) did not record any nematophagous fungi and therefore did not investigate the pathogenicity of nematophagous fungi on this nematode species. Before our study, nobody had studied the pathogenicity of nematophagous fungi on *Ditylenchus dipsaci* under in vitro conditions.

Only a few nematophagous fungal species have been studied for pathogenicity in vitro. *A. oligospora* is the most frequently investigated taxon (Timper and Brodie 1993, Belder and Jansen 1994, Galper et al. 1995, Gomes et al. 2001). *P. chlamydosporia* is frequently studied as well, but as a pathogen of nematode eggs only (Braga et al. 2008, Singh and Mathur 2010). *D. candida*, *Monacrosporium* spp., *Nematoctonus* sp. and various *Arthrobotrys* species have been examined much less frequently (Timper and Brodie 1993, Galper et al. 1995, Jacobs 1997, Gomes et al. 1999, 2001, Kumar and Singh 2006). Nobody had investigated the pathogenicity of *D. oviparasitica*, *D. lysipaga* and *D. phymatopaga* before our study.

In the present study, *A. oligospora* was the most pathogenic to all nematode species tested. It caused mortalities of 97 %, 100 % and 80 % in *D. dipsaci*, *G. rostochiensis* and *M. hapla*, respectively. This fungal species thus appears to be very pathogenic to various nematode species. Similar results were obtained by Timper and Brodie (1993), Belder and Jansen (1994), and Galper et al. (1995). Belder and Jansen (1994), who observed high pathogenicity of *A. oligospora* to *M. hapla*, *M. incognita*, and *Pratylenchus penetrans*, found a lower pathogenicity to *G. rostochiensis* (mortality approx. 20 %) than according to our results (mortality 100 %). Various strains were investigated in these studies and the virulence of each strain to *G. rostochiensis* turned out to be different.

Many strains of pathogenic fungi which are kept under in vivo conditions for many years show lower virulence than those newly obtained from natural substrates. The virulence observed in the present study could have been influenced by this factor, because the used strains were isolated at different times, the first one in 1963. Age could be the reason of the lower virulence of the *D. candida* strain, which was the oldest one. The second oldest was the *A. oligospora* strain, which was the most pathogenic species and this strain could have been more virulent if it had been obtained more recently. Age influences the virulence of the strains, but the pathogenicity of each species is determined by way the nematodes are trapped, and cannot be influenced by time.

Timper and Brodie (1993), Belder and Jansen (1994), Galper et al. (1995), Kumar and Singh (2006), and Gomes et al. (1999, 2001) used a slightly different way of evaluating the pathogenicity of nematophagous fungi in vitro, namely on agar medium in small Petri dishes. In the present study, the pathogenicity was studied on agar medium on a microscopic slide. We found the same results as were obtained by Timper and Brodie (1993), Belder and Jansen (1994), and Galper et al. (1995), who used a slightly different method.

D. candida seems to be less pathogenic to nematodes when compared with *A. oligospora* (Belder and Jansen 1994, Galper et al. 1995) and *A. superba* (Jacobs 1997). Our results are similar. In the present study, this species caused a mortality of more than 50 % in *Ditylenchus dipsaci*, but no statistically significant mortality in *Globodera rostochiensis* was recorded (the mortality of *M. hapla* was not evaluated.)

A. oligospora, which was the most pathogenic fungus, will be used for our in vivo experiments in an application with nematophagous fungi suppressing the three phytopathogenic nematodes.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Agriculture of the Czech Republic, project no. QH81163, and the Ministry of Education, Youth and Sports of the

Czech Republic, project no. MSM6046070901. The authors are grateful to Mr. Jan Urban for helping with processing the samples and to Dr. Gregor Urek and Mrs. Marie Brožová for supplying the nematodes.

REFERENCES

- BARRON G. L. (2004): Fungal parasites and predators of rotifers, nematodes and other invertebrates. – In: Mueller G. M., Bills G. F. and Foster M. S. (eds.), Biodiversity of fungi, inventory and monitoring methods, p. 435–450, Amsterdam.
- BELDER E. DEN and JANSEN E. (1994): Capture of plant-parasitic nematodes by an adhesive hyphae forming isolate of *Arthrobotrys oligospora* and some other nematode-trapping. – *Nematologica* 40: 423–437.
- BRAGA F. R., ARAUJO J. V., CAMPOS A. K., SILVA A. R., ARAUJO J. M., CARVALHO R. O., CORREA D. N. and PEREIRA C. A. J. (2008): In vitro evaluation of the effect of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Schistosoma mansoni* eggs. – *World J. Microbiol. Biotechnology* 24(11): 2713–2716.
- BRODIE B. B. (1984): Nematode parasites of potato. – In: Nikle W. R. (ed.), Plant and insects nematodes, p. 167–212, New York.
- GALPER S., EDEN L. M., STIRLING G. R. and SMITH L. J. (1995): Simple screening methods for assessing the predacious activity of nematode-trapping fungi. – *Nematologica* 41: 130–140.
- GOMES A. P. S., ARAÚJO J. V. and RIBEIRO R. C. F. (1999): Differential in vitro pathogenicity of predatory fungi of the genus *Monacrosporium* for phytonematodes, free-living nematodes and parasitic nematodes of cattle. – *Braz. J. Med. Biol. Res.* 32: 79–83.
- GOMES A. P. S., VASCONCELLOS R. S., RAMOS M. L., GUIMARAES M. P., YATSUDA A. P. and VIEIRA-BRESSAN M. C. R. (2001): In vitro interaction of Brazilian strains of the nematode-trapping fungi *Arthrobotrys* spp. on *Panagrellus* sp. and *Cooperia punctata*. – *Mem. Inst. Oswaldo Cruz* 96(6): 861–864.
- IPSEROVÁ E. (1982): Nematofágní houby a jejich výzkum u háďátka řepného [Nematophagous fungi and their research on beet nematode]. – 38 p., ms. [Postgradual Thesis: Library of Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, Praha, Czech Republic] (in Czech).
- JACOBS P. (1997): Untersuchungen über die Wirkung nematophager Pilze auf *Meloidogyne* sp. in vitro und auf deren Befall an *Lycopersicon esculentum*. – *Z. Pflanzenkrankh. Pflanzensch.* – *J. Pl. Dis. Prot.* 104(2): 153–165.
- JANSON H.-B. and LOPEZ-LLORCA L. V. (2004): Control of nematodes by fungi – In: Arora D. K. (ed.), Fungal biotechnology in agricultural, food and environmental applications, p. 205–215, New York and Basel.
- KIRK P. M., CANNON P. F., MINTER D. W. and STALPERS J. A., eds. (2008): Ainsworth & Bisby's dictionary of the fungi. 10th edition. – 771 p. Wallingford.
- KUBÁTOVÁ A., NOVOTNÝ D., PRAŠIL K. and MRÁČEK Z. (2000): Nematophagous hyphomycete *Esteya vermicola* found in the Czech Republic. – *Czech Mycol.* 52: 227–235.
- KUMAR D. and SINGH K. P. (2006): Assessment of predacity of *Arthrobotrys dactyloides* for biological control of root knot disease of tomato. – *J. Phytopathology* 154: 1–5.
- LI Y., HYDE K. D., JEEWON R., CAI L., VIJAYKRISHNA D. and ZHANG K. (2005): Phylogenetics and evolution of nematode-trapping fungi (*Orbiliiales*) estimated from nuclear and protein coding genes. – *Mycologia* 97: 1034–1046.
- NOVOTNÁ J. (1989): Mikroskopické houby na cystách háďátka bramborového *Globodera rostochiensis* Wollenw. [Microscopic fungi on cysts of potato nematode *Globodera rostochiensis* Wollenw.]. – *Česká Mykol.* 43: 96–107 (in Czech).

- OKA Y., KOLTAI H., BAR-EYAL M., MOR M., SHARON E., CHET I. and SPIEGEL Y. (2000): New strategies for the control of plant-parasitic nematodes. – *Pest Management Science* 56: 983–988.
- ROZSYPAL J. (1934): Houby na háďátku řepném *Heterodera Schachtii* Schmidt v moravských půdách [Fungi on beet nematode *Heterodera schachtii* Schmidt in Moravian soils]. – *Věstn. Čs. Akad. Zeměd.* 10: 413–422 (in Czech).
- RUBNER A. (1996): Revision of predacious hyphomycetes in the *Dactylella* – *Monacrosporium* complex. – *Stud. Mycol.* 39: 1–134.
- SINGH S. and MATHUR N. (2010): In vitro studies of antagonistic fungi against the root-knot nematode, *Meloidogyne incognita*. – *Biocontrol Sci. Techn.* 20(3): 275–282.
- TIMPER P. and BRODIE B. B. (1993): Infection of *Pratylenchus penetrans* by nematode-pathogenic fungi. – *J. Nematol.* 25(2): 297–302.
- ZARE R., GAMS W. and EVANS H. C. (2001): A revision of *Verticillium* sect. *Prostrata*. V. The genus *Pochonia*, with notes on *Rotiferophthora*. – *Nova Hedwigia* 73(1–2): 51–86.