Pakistan J. Zool., vol. 49(1), pp 35-43, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.1.35.43

Potential of Some Nematophagous Fungi against *Meloidogyne hapla* Infection in Czech Republic

Manzoor Hussain* , Miloslav Zouhar and Pavel Ryšánek
Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic

ABSTRACT

Five fungal species *Arthrobotrys oligospora*, *Dactylella oviparasitica*, *Clonostachys rosea*, *Stropharia rugosoannulata* and *Lecanicillium muscarium* isolated from root and soil samples collected in Prague, Czech Republic, were cultured on agar media and tested against *Meloidogyne hapla* both *in-vitro* and *in-vivo*. All fungi proved to be efficient in reducing final population of northern root knot nematode *M. hapla* and giving vigour to the plants. In laboratory experiment, *L. muscarium* was the most effective against nematode eggs (95.6%) and second stage juveniles (J2) (95.8%) infection. In greenhouse experiment, similar trend was found. *L. muscarium* proved to be more effective against *M. hapla* whereas *S. rugosoannulata* and *C. rosea* showed better results among other tested fungi in experiments. Moreover, plant growth parameters were also improved due to antagonistic relationship of fungi and nematodes in soil.

Article Information
Received 2 June 2016
Revised 29 June 2016
Accepted 16 July 2016
Available online 01 October 2016

Authors' Contributions
MH designed study, collected data and wrote manuscript. MZ and PR supervised the study and proofread manuscript.

Key words
***Meloidogyne incognita, Lecanicillium muscarium, Arthrobotrys oligospora, Dactylella oviparasitica*, nematicides**

INTRODUCTION

Nematophagous fungi have been extensively studied to control soil-borne plant parasitic nematodes since use of nematicides, soil sterilants and fumigants brings about several ecological and toxicology threats (Jatala, 1986; Santos *et al*., 1992). Biological suppressive soil has mitigating influence on soil borne pathogens and provoked plant vigor and yield despite of virulence of pathogen, conducive environment and susceptible hosts (Cook and Baker, 1983; Hornby, 1990; Becker *et al*., 2013; Renčo, 2013). Nematophagous fungi have developed exclusive abilities to parasitize on nematodes in a various way. They are constituted to different taxonomic groups with complex types of prey devices (adhesive knobs or nets, constricted and non-constricted rings) (Barron, 1977; Bird and Herd, 1995; Zouhar *et al*., 2013). Organisms constitute complex type of networks below soil line with several complex interspecific relationships. However, antagonistic relationship of nematodes and fungi is of more concerned these days (Janson and Lopez-Llorca, 2004). The fungi may harm plant parasitic nematodes in different ways by producing different types of complex trapping devices and specialized organs (Gray, 1987; Jafee, 2004; Zouhar *et al*., 2013). Plant parasitic nematodes pose a serious danger to several crops worldwide but endoparasitic nematodes (*Meloidogyne* spp., *Heterodera* spp., or *Globodera* spp.) are the most alarming pathogens which reside inside the roots for maximum of their life span (Hussey and Grundler, 1998; Renčo *et al*., 2012). Due to their feeding behavior and life cycle, it is challenging to control them with nematicides and microbial antagonists while they get established into host tissues (Stirling, 1991; Renčo *et al*., 2011). Plan parasitic nematodes despoil all economically important crops though vegetables are more prone to them, (Hussain *et al*., 2016); causing estimated overall losses of 5%- 43% (Sasser and Freckman, 1987). Overall plant parasitic nematodes cause damages to agriculture and forests amounting to 78 billion US dollars per year worldwide (Barker, 1998; Zhang *et al*., 2008). *Meloidogyne hapla* has been a conspicuous vegetable pest in the Czech Republic over the past few years (Nováková and Zouhar, 2009). Losses occur due to prevalence of *M. hapla* may reach 50-90% of the total crop (Nováková and Zouhar, 2009). More specifically, production losses have been reported for carrots as well as parsley grown in the sandy soils of the Elbe lowland in the Czech Republic (Douda *et al*., 2010).

Although scientists are struggling to control soil borne pathogens especially plant parasitic nematodes by introducing different biocontrol agents such as fungi, bacteria and predatory insects but main problem is insufficient knowledge on ecology. Biocontrol agents could be more advantageous including germination, proliferation, sensitivity to population variation, and control efficiency if they apply in the same ecosystem where isolated.

The objectives of our study were: 1) to evaluate the potential of the fungi against conspicuous pathogen,

* Corresponding author: hussain@af.czu.cz
0030-9923/2017/0001-0035 $ 9.00/0

M. hapla in laboratory and greenhouse conditions; 2) to determine whether the parasitism was restricted only to eggs or juveniles of nematodes.

MATERIALS AND METHODS

Nematode culture

Nematode galled roots of tomato plants from our greenhouse were collected and eggs masses were isolated and a single egg mass was used to establish a nematode population. Eggs were extracted from 45-days old galled tomato seedlings roots by using 0.05% NaOCl. Extracted eggs were gently washed with tap water to remove NaOCl (Hussey and Barker, 1973). *Meloidgyne hapla* specie was identified based on morphological and morphometrical characteristics (Eisenback, 1985). Extracted eggs were placed at room temperature of 25˚C to hatch J2. Eggs and J2 were counted and exposed to test fungi.

Fungus culture

Lecanicillium muscarium was isolated from egg masses of root knot nematodes collected from twenty vegetable fields of tomato, eggplants, and carrot whereas other fungi *(Arthrobotrys oligospora*, *Dactylella oviparasitica*, *Clonostachys rosea)* were isolated directly form the soil collected from localities of Semice (N 50.16265 E 14.89643) and Litol (N 50.18404 E 14.83742) in Central Bohemia, Czech Republic by soil dilution method. *Stropharia rugusoannulata* isolate was obtained from commercial production of mushroom seed company, the Mycelium Wolf. Uprooted roots with combined infection of nematode and fungi were observed. The black colored egg masses were associated with fungi. On uprooting the plants, roots with shared infection of nematode and fungi were observed. The egg masses were associated with fungi with black color. These egg masses were inoculated axenically on petri plates containing potato dextrose agar (PDA) amended with streptomycin at 1g/L after surface sterilization with 0.5% NaOCl for 2 min (Singh and Mathur, 2014). Petri plates were incubated at 25°C±2°C for ten days. Fungal colonies were isolated and purified by repetitive sub culturing, and later identified and confirmed by using molecular technique polymerase chain reaction (PCR) in laboratory.

All tested fungi were grown on potato dextrose broth (PDB) and kept under room temperature on orbital shaker. After two weeks, fungal mycelia were harvested and culture filtrates were prepared in distilled water and standardized. Each isolate was replicated five times and experiment was repeated once to authenticate our findings.

Effect of fungi on egg parasitism

Each fungus isolate was inoculated to the center of Petri dish containing PDA medium amended with antibiotic streptomycin at 1g/L. After inoculation, plates were incubated at 25˚C for one week, and each plate was spread uniformly with 100 *M. hapla* eggs. Five replicates for each fungus were used whereas eggs without fungi were kept as control for comparison. Evaluation of fungus was noticed after 24, 48, and 72 h and percent egg parasitism was measured by staining with cotton blue and counted under Stereo-binocular microscope. Eggs either infected by direct penetration of hyphae or disintegration of their contents were considered as infected (Khan *et al*., 2006; Singh and Mathur, 2010). Eggs with live J2 and hatched J2 from eggs were counted as viable.

Effect of fungi on nematode activity and mortality

Five mL of each fungal culture filtrate and 1 mL of nematode suspension containing 100 J2 were placed in sterile Petri plates. Nematode activity was noticed after 24, 48, and 72 h. Fungus efficacy was noticed according to percentage of paralyzed nematodes. Rigid, elongated, and bent nematodes were considered as immobilized if they do not react after probed with fine needle (Cayrol and Pijarowski, 1989; Singh and Mathur, 2010). Revival test was run by centrifugation for 3 min at 1000 rpm, and incubated in sterile water for one day. If nematodes were found inactive after one day, they were considered as dead. Five replications were used in this study.

Greenhouse studies

This experiment was carried out in greenhouse of Czech University of Life Sciences, Prague, Czech Republic. The experiment contained two parts i.e.; one was designed for *M. hapla* eggs while the other was constructed for second stage juveniles (J2). To investigate the effectiveness of fungi against nematodes, susceptible variety of carrot “Darina” was used. Based on initial inoculum, the experiment was categorized into two. In one experiment freshly extracted nematode eggs (500 eggs/pot); while in the other, one day old second stage juveniles (500 J2/pot) were used. For both experiments, one carrot seedling aged of two weeks was used and one week later inoculated simultaneously with fungi and nematodes eggs and J2 respectively.

Mycelia from all of five fungi were harvested from PDB media, weighed and standard solution (w/v) was prepared in distilled water. 20 ml of the 30% (w/v) solution of each fungus was pipetted on top of soil in each pot. In the control, nematodes were applied without nematophagous fungi. The pots were placed in completely randomized design (CRD) with five replications on a bench in a greenhouse. The pots were irrigated after two-day intervals throughout the period of study. The daily temperature ranged between 25° and 28°C.

Data collection

After 75 days, plants were carefully removed from the pots and their roots were cut off from the shoots. The roots were gently washed under tap water and blotted dry. Fresh root shoot weights and lengths were recorded. The numbers of galls were counted under Stereomicroscope at magnification 40X. For determination of total nematode population, the eggs were obtained from the roots by using 0.5% NaOCl solution passing through a sieve with pore size of 74 and 25µm (Hussey and Barker, 1973; Hussain *et al*., 2016). The juveniles were extracted from the soil of each individual plant from their respective pots (Whitehead and Hemming, 1965). The total number of eggs and nematodes in soil were also noted and constituted the total nematode population. The potential of nematophagous fungi was evaluated in terms of nematode reproduction factor (Rf); where Rf is the final nematode population at harvest (Pf) divided by the initial nematode population (Pi) at inoculation. The reproduction was calculated by dividing the final population by 500 in both experiments.

Statistical analysis

All experiments were repeated once. All pool data from two experiments were subjected to analysis of variance (ANOVA). The means were compared by the Duncan Multiple Range Test (DMRT) at P=0.05 by using software Statistics 8.1.

RESULTS

In vitro study

Lecanicillium muscarium had more parasitic activity in terms of *M. hapla* egg infection after 72h followed by *S. rugosoannulata* (89%). Whereas other species also showed better results in egg infection. There were a few eggs able to hatch while exposed to fungi *L. muscarium* and *D. oviparasitica* (Table I). It was seen that *L. muscarium* and *D. oviparasitica* invaded eggs in early stages of their development and parasitized them with emerging juveniles. In case of *S. rugosoannulata* J2 were trapped by acanthocytes which can be seen in Figure 1. During early stage of infection, fungal hyphae completely occupied the embryo within egg and consumed all contents of eggs leaving them empty. None of fungi produce their resting spores such as chlamydospores etc in nematode eggs, although conidia were seen occasionally in Petri plates. Eggs containing second stage juveniles were escaped first but later they were preyed by fungi as well. In case of inactivity and mortality of nematode juveniles, all fungi behaved excellent but the percentage of egg and J2 parasitism was higher in case of *L. muscarium* and *S. rugosoannulata.* After revival test, a few juveniles were able to stay alive which is more obvious in Table I.

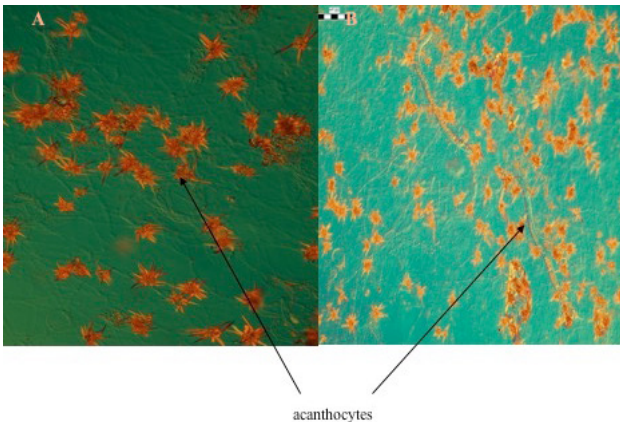

Fig. 1. Production of specialized structure of large spike cells acanthocytes produced by fungi, *S. rugosoannulata* (A), and its parasitic action on nematode juveniles (B)

Table I.- Parasitic efficiencies of fungi on eggs and juveniles (J2) of root knot nematodes, *Meloidogyne hapla*

Fungi/ Treatments	Egg infection (%)			Inactive nematode at various interval (%)			Active neatodes (%)*
	24h	48h	72h	24h	48h	72h	
A. oligospora,	24.6± 3.36c	54.4± 5.37c	88.6± 2.07b	53.8± 7.40 a	72.2± 5.81 b	84.8± 3.70 c	6.8± 1.48 b
D. oviparasitica	28.4± 3.51bc	63± 2.74ab	88.2± 1.30b	55± 3.81 a	74.6± 2.70 b	85.8± 3.63 bc	7.8± 0.84 ab
S. rugosoannulata	32.6± 3.51b	58.4± 5.13bc	89.4± 2.88ab	53.6± 5.50 a	72.6± 3.05 b	89.4± 2.70 b	7± 1.58 b
C. rosea	30.8± 4.82b	64.6± 1.52a	86.4± 10.29b	52.4± 4.67 a	70.2± 4.21 b	86± 2.92 bc	9.6± 2.41 a
L. muscarium	42± 2.24a	66.2± 2.59a	95.6± 2.41a	55± 3.39 a	83.2± 3.56 a	95.8± 2.39 a	2.4± 1.67 c
Control (PDB)	00	00	00	00	00	00	00
Control (Water)	00	00	00	00	00	00	00

* After revival in water; Data are mean of ten replications. Means in each column with different letters differ significantly according to Duncan's Multiple Range Test at P = 0.05.

Table II.- Influence of nematophagous fungi to *Meloidogyne hapla* reproduction and plant growth parameters of carrot in greenhouse, 75-days after inoculation with an initial population density (Pi) of 500 eggs and 500 J2 per plant

Fungi/Treatments	Galls/ root system*	Egg masses/ root system*	Egg production/ root system	J2 production/ 100cc of soil	Reproduction factor ** (Pf/Pi)	Fresh shoot wt. (g)	Fresh root wt. (g)	Fresh root length (cm)	Fresh shoot length (cm)
After inoculation of Pi of 500 eggs/plant									
A. oligospora,	11.00± 0.11b	5.00±0.39b	138.00± 1.3b	215.00± 0.45b	2.42± 0.023b	13.00± 0.98c	16.86± 1.2c	9.14± 1.2b	36.29± 3.5d
D. oviparasitica	9.00± 0.14b	7.00±0.48b	122.00± 1.2b	212.00± 0.42b	2.36± 0.33b	14.00±.92bc	28.86± 2.3b	9.29± 1.23b	37.29± 3.7cd
S. rugosoannulata	8.00± 0.32b	5.00±0.36b	111.00± 1.09b	175.00± 0.31b	1.97± 0.01b	15.00± 0.87b	14.86± 1.4cd	10.00± 0.99ab	39.43± 4.1c
C. rosea	7.00± 0.22b	4.00±0.01 b	118.00±1.02 b	199.00± 0.38b	2.23± 0.03b	13.14± 1.0c	13.14± 0.98d	10.00± 1.3ab	44.14± 4.3b
L. muscarium	3.00±0.03 c	2.00± 0.02c	99± 0.99b	102.00± 0.36b	1.22± 0.04b	17.86± 2.1a	56.00± 3.4a	11.00± 0.97a	50.00± 5.2a
Control	40.0± 1.9a	27.00± 1.1a	2671.0± 2.1a	1518.0± 0.35a	20.52± 1.2a	10.43± 1.2d	8.57± 1.2e	4.43± 0.77c	26.14± 2.1e
After the inoculation of Pi of 500 J2 /plant*									
A. oligospora,	9.00± 0.23b	5.00± 0.34bc	241.00± 2.1b	818.00± 1.34b	8.66±0.98 b	12.75± 1.2b	17.20± 1.2c	8.10± 0.87b	35.20± 1.23c
D. oviparasitica	10.00± 0.34b	7.00± 0.31b	222.00± 2.3b	713.00± 1.44b	7.57± 0.99b	13.21± 0.99b	29.75± 2.1b	8.00± 0.68b	35.23± 1.34c
S. rugosoannulata	8.00± 0.07b	6.00± 0.41c	211.00± 1.99b	612.00± 1.41b	6.54± 0.93b	13.00± 0.98b	16.15± 1.4c	8.30± 0.67b	37.00± 1.20c
C. rosea	9.00± 0.03b	5.00± 0.23bc	208.00± 2.3b	519.00± 1.43b	5.60± 0.77b	13.19± 0.93b	13.24± 1.25d	9.50± 0.34ab	43.41± 1.24b
L. muscarium	7.00±0.02 b	5.00± 0.13c	163.00± 1.98b	319.00± 1.23b	3.51± 0.67c	17.25± 0.88a	55.25± 1.68a	9.80± 0.37a	49.23± 1.51a
Control	38.00±1.42a	28.00± 1.2a	3953± 2.3a	1099± 2.4a	18.89± 0.99a	11.20± 1.2c	9.20±.89 e	5.10±0.32 c	25.21± 1.6e

Data are mean of ten replications; Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P = 0.05$.

*Gall and egg mass indices: 0-5 scale; where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses, and 5 = > 100 galls or egg masses per root system (Quesenberry *et al.,* 1989).

**RF, Reproduction factor whereas Pf is final nematode population density divided by initial nematode population density.

Fig. 2. Improvement of plant growth parameters and reduction of root galls and egg masses in carrots after treatments with nematophagous fungi; (A) *A. oligospora*, (B) *L. muscarium*, (C) *D. oviparasitica*, (D) *C. rosea*, (E) *S. rugosoannulata*, as compared to control (F) *M. hapla* eggs

Fig. 3. Improvement of roots in carrot plants after treatment of fungus, *L. muscarium* (A) as compared to control (B), *M. hapla* (J2)

Greenhouse study

In greenhouse experiments, all tested fungi significantly suppressed *M. hapla* reproduction by reducing number of galls and egg masses on roots and J2 in soil in comparison to control ($P < 0.05$). The highest reduction of final nematode population in soil treated with *L. muscarium* have been found followed by *S. rugosoannulata* and *D. oviparasitica* (Table II). Of the media tested, *L. muscarium* gave the best results of biomass production for most fungal species. It is also found that *L. muscarium* colonized the gelatinous matrix and preyed upon eggs more than J2 of nematodes. The behavior of other fungi in both studies of *M. hapla* egg and J2 infection was also similar but less pronounced to *L. muscarium* (Table II). In comparison to others, *A. oligospora* was found less reactive whereas *C. rosea* and *S. rugosoannulata* showed better results in parasitism of eggs and J2 as well (Table II). In general, plant development was more in case of fungal treatment

as compared to their respective controls. The growth of shoots and roots were improved in all of treated plants (Fig. 2). In comparison, *L. muscarium* proved to be the best nematophagous fungi against *M. hapla* followed by C. *rosea* and *S. rugosoannulata*. The greater root weight and longer shoot length in case of carrot resulted better developed roots systems rather than an increased weight of galls on roots. In treatments receiving nematodes, roots were distorted and bushy with galls. The treated roots were in proper shaped with higher contents of food as compared to control (Figs. 2 and 3). In case of *L. muscarium* root shoot weights and lengths were improved in both forms of inoculums; eggs and J2, which is more prominent in Table II. Furthermore, the higher percentage of root shoot lengths and weights with low reproduction factor declared that *L. muscarium* was more potent than other fungi in experiments (Table II). A very few nematodes were recovered from the roots of the plants and their respective pots, treated with fungal species. Roots were observed healthy and sturdy with fabulous shape, and were eatable.

DISCUSSION

All of fungal species produced excellent results in parasitism of eggs and juveniles. In our study, five fungi including *A. oligospora*, *D. oviparasitica*, *C. rosea*, *S. rugosoannulata*, and *L. muscarium* were isolated from vegetable fields in association with egg mass of *M. hapla*, where *A. oligospora* Fresen, is a nematode destroying predacious fungi (Shepherd, 1961) which develop a characteristic organs of three dimensional networks sticky hyphal anastomosing loops to capture prey (Nansen *et al*., 1986). Moreover, the fungus has also been reported to control animal parasitic nematodes (Descazeaux, 1939; Roubaud and Deschiens, 1941; Soprunov, 1958; Pandey, 1973; Gronvold *et al*., 1984; Gruner *et al*., 1985).

Dactylella oviparasitica (Stirling and Mankau, 1978) was first isolated from *Meloidogyne* egg masses collected from peach orchards in the San Joaquin Valley, California. This fungus proved to be more aggressive against *Meloidogyne* eggs rather than J2 and any other nematodes spp. *D. oviparasitica* is believed to produce appressoria as well as chitinase enzyme (Stirling and Mankau, 1979) which might be responsible for successful penetration into eggs, eggmasses and J2. *Clonostachys rosea* is a facultative saprophyte (Schroers *et al*., 1999) and potential bioagent which is extensively being used against plant pathogenic fungi i.e., *Botrytis cinerea* (Morandi *et al*., 2003), and plant parasitic nematodes (Zhang *et al*., 2008). Fungus is capable to produce large number of conidia in a very short time which stick to nematodes and proliferate into body cavity after germination. The fungus is believed to produce hydrolytic enzymes such as proteases, collagenase, and chitinase which may be involved in nematode cuticle penetration and host cell degradation (Tunlid *et al*., 1994; Åhman *et al*., 2002; Yang *et al*., 2007). *S. rugosoannulata* is related to nematophagous fungi found in woods, grasslands and composts (Luo *et al*., 2006) which produce specialized structure of large spike cell called acanthocytes (Farr, 1980), which mechanically destroy nematodes eggs as well as J2. Lastly, *L. muscarium* is an entomopathogenic fungi which produce mucilaginous matrix enables to attach with host surface. Fungal conidia produce fine hyphae on germination of conidia and hydrolytic enzymes including chitinases and gluconases which help fungi to penetrate nematode eggs and J2 mechanically or by enzymatic actions (Yang *et al*., 2005). Observation on the proliferation of these fungi in *M. hapla* eggs and J2 showed that appressoria were formed by most of fungi especially in case of *D. oviparasitica*. Appressoria is an important device of fungi to get attachment and penetration in host (Emmett and Parberry, 1975). In our understanding, plant parasitic fungi most commonly penetrate their hosts mechanically but enzymatic penetration or combination of both could be happened in some fungi (Emmett and Parberry, 1975). Most of entomopathogenic fungi such as *L. muscarium* produce hydrolytic enzymes which enable them to penetrate cuticle of nematode eggs and J2 (Gabriel, 1968; Latge, 1974; Leopold and Samsinokova, 1970; Shinya *et al*., 2008). Mechanical penetration was also observed in most of fungi tested, but since chitin is the main constituent of *Meloidogyne* egg shells (Bird, 1976), production of chitinase in culture indicates that enzymatic penetration was also occurred. Behaviour of *A. oligospora* was irregular because it had better results in mortality of J2 after 24h and 48 h but after 72h it was not quite significant which might be its growth factor in culture. Growth of *A. oligospora* was slowest in culture media due to which it did not have prominent parasitic effects on nematode eggs and J2. But it is believed that *A. oligospora* produce three dimensional hyphae trap as well as toxins which paralyze nematodes very effectively (Bird and Herd, 1995; Nansen *et al*., 1986). Mat of mycelium was observed around eggs and J2 in case of *C. rosea* and *S. rugosoannulata* which was clear evidence of their successful penetration and parasitism. Both are referred to nematophagous fungi producing mechanical traps (Barron, 1977; Zouhar *et al*., 2013) as well as hydrolytic enzyme which enable them to dissolve cuticle and produce successful infection (Zhang *et al*., 2008). These fungi were assessed against *M. hapla* in greenhouse. All fungi had enormous effects on plant growth as well as nematode reproduction including gall and egg masses indices and egg and J2 production in roots and soil. *L. muscarium* proved to be more effective in reducing the infection level of nematodes as compared to control plants. Agility of this fungus in parasitism was more in nematode eggs rather than J2 which could be its multiple way of action. It is believed that *L. muscarium* produce very fine hyphae to rupture the cuticle of nematode eggs mechanically; also produce chitinases which facilitate in

penetration of egg shells and J2. Furthermore, this fungus was also documented to stimulate induced resistance in plants (Hirano *et al*., 2008). Presence of number of galls on roots and J2 in soil of control plants validated the studies done by Larkin *et al*. (1993), which stated that maximum population of nematodes was found in fumigated soil rather than non-fumigated. Efficiency of *L. muscarium* may also be correlated to temperature which produces high level of conidia and enzymes, in wide range of temperature (5-30°C) with an optimum at 25C (Fenice *et al*., 1996; 1997). Below the soil line, several biological interactions are happening which are necessary to maintain ecosystem. These nematophagous fungi produce different types of mechanical traps for nematodes which may restrict the free movement of nematodes in soil (Jensen *et al*., 2004). In this way, nematodes could have limited chance to approach root zones to get penetrated into host plants. Nematophagous fungi also produce different types of enzymes for example chitinases, proteases, amylases etc to pin down nematode population. Overall all fungi behaved excellent in our greenhouse studies but *L. muscarium, S. rugosoannulata* and *C. rosea* produced best results in reducing nematode reproduction factor (Pf/Pi) and escalation of plant growth. Root systems of treated plants were colonized with fungi when observed under microscope which was clear picture of protecting shield against plant parasitic nematode (Koike *et al*., 2001).

CONCLUSIONS

All of fungi were highly effective at managing root knot nematode population up to maximum extent. Among fungi, *L. muscarium* had greater potential to reduce infestation level in soil up to greater extent which happened due to their multiple parasitic actions; production of enzymes, fine hyphae and induced resistance at wide range of temperature. It was further documented that the efficient antagonistic relationship between fungi and nematodes had positive effects on plant growth compared to controls.

ACKNOWLEDGEMENT

We are thankful to Czech University of Life Sciences Prague for providing funds to conduct part of this research under project number PROJ201500056.

Conflict of interest statement

The authors have declared no conflict of interest

REFERENCES

Åhman, J., Johanson, T., Olsson, M., Punt, P.J., van den Hondel, C.A.M.J.J. and Tunlid, A.S., 2002. Improving the pathogenicity of a nematode-trapping fungus by genetic engineering of a subtilisin with nematotoxic activity. *Appl. environ. Microbiol.*, **689**: 3408-3415. http://dx.doi.org/10.1128/AEM.68.7.3408-3415.2002

Barker, K.R., 1998. Introduction and synopsis of advancements in nematology. In: *Plant and nematode interactions* (eds. K.R. Barker, G.A. Pederson and G.L. Windham), Am. Soc. of Agronomy, Inc., Crop Sci. Am. Inc., & Soil Sci. Soc. Am. Inc, Madison, WI. http://dx.doi.org/10.2134/agronmonogr36.c1

Barron, G.L., 1977. *The nematode destroying fungi*. Canadian Biological Publications, Guelph, Ont. pp. 140.

Becker, J.S., Borneman, J. and Becker, J.O., 2013. *Dactylella oviparasitica* parasitism of the sugar beet cyst nematode observed in trixenic culture plates. *Biol. Contr*., **64**: 51-56. http://dx.doi.org/10.1016/j.biocontrol.2012.10.007

Bird, A., 1976. The development and organization of skeletal structures in nematodes. In: *The organization of nematodes* (ed. N.A. Croll). Academic Press, New York. pp. 107-137

Bird, A.F. and Bird, J., 1991. *The structure of nematodes*. Academic Press, San Diego.

Bird, J. and Herd, R.P., 1995. *In vitro* assessment of two species of nematophagous fungi (*Arthrobotrys oligospora and Arthrobotrys flagrans*) to control the development of infective cyathostome larvae from naturally infected horses. *Vet. Parasitol*., **56**:181-187. http://dx.doi.org/10.1016/0304-4017(94)00663-W

Butt, T.M., 2002. Use of entomogenous fungi for the control of insect pests. In: *The Mycota XI: Agricultural applications* (ed. F. Kempken). Springer-Verlag Berlin, pp. 111-34. http://dx.doi.org/10.1007/978-3-662-03059-2_7

Cayrol, J.C., Djian, C. and Pijarowski, L., 1989. Study of the Nematocidal properties of the culture filtrate of the nematophagus fungus *Paecilomyces lilacinus*, *Rev. Nematol.,* **12**: 331-336.

Cook, R.J. and Baker, K.F., 1983. *The nature and practice of biological control of plant pathogens*. APS, St. Paul.

Descazeaux, J., 1939. Action des champignons *Hyphomycetes* predateur sur les larves de certain nematodes parasites des ruminants. *Bull. Soc. Pathol. Exot*., **32**: 457-459.

Douda, O., Zouhar, M., Mazáková, J., Nováková, E. and Pavela, R., 2010. Using plant essences as alternative mean for northern root-knot nematode (*Meloidogyne hapla*) management. *J. Pestic. Sci*., **83**: 217-221. http://dx.doi.org/10.1007/s10340-010-0287-4

Eisenback, J.D., 1985. Detailed morphology and anatomy of second stage juvenile males, and females of the genus *Meloidogyne* (root knot nematode).

In: *An advanced treatise on Meloidogyne*, Vol. 1. *Biology and control* (ed. J.N. Sasser and C.C. Carter). North Carolina State University Graphics, Raleigh.

Emmett, R.W. and Parberry, D.G., 1975. Appressoria. *Annu. Rev. Phytopathol*., **13**: 147-167. http://dx.doi.org/10.1146/annurev.py.13.090175.001051

Farr, D.F., 1980. The acanthocyte, a unique cell type in *Stropharia* (Agaricales). *Mycotaxon*, **11**: 241-249.

Fenice, M., Seibmann, L., Giambattista, R., Petruccioli, M. and Federici, F., 1996. Production of extracellular chitinolytic activities by a strain of the antarctic entomogenous fungus *Verticillium* cfr. *Lecanii*. In: *Chitin enzymology*. Volume 2 (ed. R.A.A. Muzzarelli), Grottammare, Atec Edizioni Italy, pp. 285-292

Fenice, M., Seibmann, L., Zucconi, L. and Onofri, S. 1997. Production of extracellular enzymes by antarctic fungal strain. *Polar Biol*., **17**: 275-280. http://dx.doi.org/10.1007/s003000050132

Gabriel, B.P., 1968. Enzymatic activities of some entomophthorous fungi. *J. Inverteb. Pathol*., **11**: 70-81. http://dx.doi.org/10.1016/0022-2011(68)90055-4

Gray, N.F., 1987. Nematophagous fungi with particular reference to their ecology. *Biol. Rev.,* **62:** 245-304. http://dx.doi.org/10.1111/j.1469-185X.1987.tb00665.x

Gronvold, J., 1984. *The influence of earthworms on the transmission of infective larvae of* Bovinetri chostrongyles. Ph.D. Diss., Royal Veterinary and Agricultural University, Copenhagen.

Gruner, L., Peloille, M., Sauve, C. and Cortet, J., 1985. Survie et conservation de Factivite predatrice vis-a-vis de nematodes trichostrongylides apres ingestion par des Ovins de trois hyphomycetes predateurs. C. *R. Acad. Sci. Paris*, **300**: 525-528.

Hirano, E., Koike, M., Aiuchi, D. and Tani, M., 2008. Pre-inoculation of cucumber roots with *Verticillium lecanii* (*Lecanicillium muscarium*) induces resistance to powdery mildew. *Res. Bull. Obihiro Univ*., **29:** 82-94.

Hornby, D., 1990. *Biological control of soil-borne plant pathogens.* CAB International, Wallingford.

Hussain, M., Kamran, M., Singh, K., Zouhar, M., Rysanek, P. and Anwar, S.A., 2016. Response of selected okra cultivars to *Meloidogyne incognita*. *Crop Protect*., **82**: 1-6. http://dx.doi.org/10.1016/j.cropro.2015.12.024

Hussey, R.S. and Barker, K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Pl. Dis. Rep*., **57**: 1025-1028.

Hussey, R.S. and Grundler, F.M.W., 1998. Nematode parasitism in plants. In: *The physiology and biochemistry of free-living and plant parasitic nematodes* (ed. R.N. Perry and D.J. Wright). CAB International, Wallingford, pp. 213-243.

Irshad, U., Mukhtar, T., Ashfaq, M., Kayani, M.Z., Kayani, S.B., Hanif, M. and Aslam, S., 2012. Pathogenicity of citrus nematode (*Tylenchulus semipenetrans*) on *Citrus jambhiri*. *J. Anim. Pl. Sci*., **22**: 1014-1018.

Jaffee, B.A., 2004. Wood, nematodes, and the nematode-trapping fungus *Arthrobotrys oligospora*. *Soil. Biol. Biochem.,* **36**: 1171-1178. http://dx.doi.org/10.1016/j.soilbio.2004.03.003

Janson, H.B. and Lopez-Llorca, L.V., 2004. *Control of nematodes by fungi. In: Fungal biotechnology in agricultural, food and environmental applications* (ed. D.K. Arora). New York and Basel, pp. 205-215.

Jatala, P., 1986. Biological control of plant parasitic nematodes. *Annu. Rev. Phytopathol.*, **24**: 453-489.

Jensen, B., Knudsen-Inge, M.B., Madsen, M. and Jensen, D.F., 2004. Biopriming of infected carrot seed with an antagonist, *Clonostachys rosea*, selected for control of seed borne *Alternaria* spp. *Biol. Contr*., **94**: 551-560.

Khan, A., Williams, K.L. and Nevalainen, H.K., 2006. Control of plant parasitic nematodes by *Paecilomyces lilacinus* and *Monacrocporium lysipagum* in pot trials. *Biol. Contr.*, **51**: 463-658.

Koike, N., Hyakumachi, M., Kageyama, K., Tsuyumu, S. and Doke, N., 2001. Induction of systemic resistance in cucumber against several diseases by plant growth promoting fungi lignification and superoxide generation. *Eur. J. Pl. Pathol*., **107**: 523-533. http://dx.doi.org/10.1023/A:1011203826805

Latgé, P., 1974. Activitds protfiolytique et chitinolytique de Cordyceps militaris. *Entomophaga*, **19**: 41-53. http://dx.doi.org/10.1007/BF02371507

Leopold, J. and Samsinokova, A., 1970. Quantitative estimation of chitinase and other enzymes in the fungus *Beauveria bassiana*. *J. Inverteb. Pathol.,* **15**: 34-42. http://dx.doi.org/10.1016/0022-2011(70)90095-9

Larkin, R.P., Hopkins, D.L. and Martin, F.N., 1993. Ecology of *Fusarium oxysporum* sp. niveum in soils suppressive and conducive to Fusarium wilt of watermelon. *Phytopathology*, **83**: 1105-1116.

Luo, H., Li, X., Li, G., Pan, Y. and Zhang, G. 2006. Acanthocytes of *Stropharia rugosoannulata* function as a nematode-attacking device. *Appl. environ. Microbiol.,* **72**: 2982 -2987. http://dx.doi.org/10.1128/AEM.72.4.2982-2987.2006

Morandi, M.A.B., Maffia, L.A., Mizubuti, E.S.G., Alfenas, A.C. and Barbosa, J.G., 2003. Suppression of *Botrytis cinerea* sporulation by *Clonostachys rosea* on rose debris: a valuable component in *Botrytis* blight management in commercial greenhouses. *Biol. Contr*., **26**: 311-317. http://dx.doi.org/10.1016/S1049-9644(02)00134-2

Nansen, P., Gronvold, J., Henriksen-Sv, A.A. and

Wolstrup, J., 1986. Predacious activity of the nematode-destroying fungus, *Arthrobotrys oligospora*, on pre-parasitic larvae of *Cooperia oncophora* and on soil nematodes. *Proc. helminthol. Soc. Wash*., **53**: 237-243.

Nováková´, E. and Zouhar, M., 2009. *Meloidogyne hapla*–škůdce, kterého možná neznáte. *Zahradnictví*, **7**:15-17 (in Czech).

Pandey, V.S., 1973. Predatory activity of nematode trapping fungi against the larvae of *Trichostrongylus axei* and *Ostertagia ostertagi:* a possible method of biological control. *J. Helminthol*., **47:** 35-48. http://dx.doi.org/10.1017/S0022149X00023725

Quesenberry, K.H., Baltensperger, D.D., Dunn, R.A., Wilcox, C.J. and Hardy, S.R., 1989. Selection for tolerance to root-knot nematodes in red clover. *Crop Sci*., **29**: 62-65. http://dx.doi.org/10.2135/cropsci1989.0011183X002900010014x

Renčo, M., 2013. Organic amendments of soil as useful tools of plant parasitic nematodes control Review Article. *Helminthologia*, **50**: 3-14. http://dx.doi.org/10.2478/s11687-013-0101-y

Renčo, M., Sasanelli, N. and Kováčik, P., 2011. The effect of soil compost treatments on potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida*. *Helminthologia*, **48**: 184-194. http://dx.doi.org/10.2478/s11687-011-0027-1

Renčo, M., Sasanelli, N., Papajová, I. and Maistrello, L., 2012. The nematicidal effect of chestnut tannin solutions on the potato cyst nematode *Globodera rostochiensis* (Woll.) Behrens. *Helminthologia*, **2**: 108-114.

Roubaud, E. and Deschiens, R., 1941. Essais relatifs a la prophylaxie de 1'anguillulose du mouton par 1'usage des hyphomycetes predateur de sol. C. *R. Seanc. Biol., Paris*. **135**: 687-690.

Santos, M.A., Ferraz, S. and Muchovej, J.J., 1992. Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* race 3. *Nematropica*, **22**: 183-192.

Sasser, J.N. and Freckman, D.W., 1987. A world perspective on Nematology: The role of society. In: *Vistas on nematology* (eds. J.A. Veech and D.W. Dickson). SON Inc., Hyattsville, MD. pp. 7-14.

Schroers, H.J., Samuels, G.J., Seifert, K.A. and Gams, W., 1999. Classification of the mycoparasite *Gliocladium roseum* in *Clonostachys* as *C. rosea*, its relationship to *Bionectria ochroleuca*, and notes on other *Gliocladium*-like fungi. *Mycologia*, **91**: 365-385. http://dx.doi.org/10.2307/3761383

Shepherd, 1961. Nematode-trapping fungi in Danish agricultural soils. *Horticultura*, **15**: 94-96.

Shinya, R., Aiuchi, D., Kushida, A., Tani, M., Kuramochi, K. and Koike, M., 2008. Effects of fungal culture filtrates of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains on *Heterodera glycine* eggs and juveniles. *J. Inverteb. Pathol*., **97**: 291-297. http://dx.doi.org/10.1016/j.jip.2007.11.005

Singh, S. and Mathur, N., 2010. *In vitro* studies of antagonistic fungi against the root knot nematode, *Meloidogyne incognita*. *Biocontr. Sci. Technol*., **20**: 275-282. http://dx.doi.org/10.1080/09583150903484318

Soprunov, F.F., 1958. *Predacious hyphomycetes and their application in the control of pathogenic nematodes. Academy of Sciences of the Turkmen SSR, Ashkhabad* (English translation 1966 by Israel Program for Scientific Trans- lations, Jerusalem). pp. 1-292.

Stirling, G.R. and Mankau, R., 1978. *Dactylella oviparasitica*, a new fungal parasite of *Meloidogyne* eggs. *Mycologia*, **70**: 774-783.

Stirling, G.R. and Mankau, R., 1979. Mode of parasitism of *Meloidogyne* and other nematode eggs by *Dactylella oviparasitica. J. Nematol*., **11**: 282-288. http://dx.doi.org/10.2307/3759357

Stirling, G.R., 1991. *Biological control of plant parasitic nematodes*. CAB International, Wallingford.

Tunlid, A., Rosen, S., Ek, B. and Rask, L., 1994. Purification and characterization of an extracellular serine protease from the nematode- trapping fungus *Arthrobotrys oligospora*. *Microbiol*ogy, **140**: 1687-1695.

Whitehead, A.G. and Hemming, J.R., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annls. Appl. Biol*., **55**:25-38.

Yang, J., Huang, X., Tian, B., Sun, H., Duan, J., Wu, W. and Zhang, K., 2005. Characterization of an extracellular serine protease gene from the nematophagous fungus *Lecanicillium psalliotae. Biotechnol. Lett*., **27**: 1329-1334.

Yang, J.K., Tian, B.Y., Liang, L.M. and Zhang, K.Q., 2007. Extracellular enzymes and the pathogenesis of nematophagous fungi. *Appl. Microbiol. Biotechnol*., **75**: 21-31.

Zhang, L., Yang, J., Niu, Q., Zhao, X., Ye, F., Liang, L. and Zhang, K.Q., 2008. Investigation on the infection mechanism of the fungus *Clonostachys rosea* against nematodes using the green fluorescent protein. *Appl. Microbiol. Biotechnol.*, **78**: 983-990.

Zouhar, M., Douda, O., Nováková, J., Doudová, E., Mazáková, J., Wenzlová, J., Ryšánek, P. and Renčo, M., 2013. First report about the trapping activity of *Stropharia rugosoannulata* acanthocytes for Northern Root Knot Nematode. *Helminthologia*, **2:** 127-131.