

## POTENTIAL OF OYSTER MUSHROOMS FOR THE BIOCONTROL OF SUGAR BEET NEMATODE (*HETERODERA SCHACHTII*)

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**Abstract:** The potential of oyster mushrooms to attack and kill sugar beet cyst nematode (*Heterodera schachtii*) was studied. The ability of *Pleurotus ostreatus*, *P. sajor-caju*, *P. florida*, *P. flabellatus*, *P. ostreatus* (sporeless), *P. eryngii* and *Hypsizygus ulmarius* to prey on the cyst nematode was confirmed. Nematodes were inoculated on water agar plates on which a single sparse fungal colony of one of the above mentioned fungi was grown. Nematodes were quickly immobilized after inoculation the plates with each *Pleurotus* species, with mortality of about 96%, 72%, 55%, 52%, 20%, 23% and 62%, respectively. Nematicidal activities of culture filtrates of *Pleurotus* species and *H. ulmarius* against *H. schachtii* were also studied *in vitro*. Extracts of *P. ostreatus* could paralyse 90% of nematodes whereas *P. eryngii* could paralyse only 50% of nematodes. The efficacy of old mushroom compost from cultures of *P. ostreatus* and *P. sajor-caju* in controlling cysts on sugar beet (*Beta vulgaris* ICI) were studied under greenhouse conditions. The results showed that 100 and 200 grams of mushroom compost per 3 kilogram/pot could significantly control cysts, reducing more than 85% cysts in soil. Presence of cysts on the roots and larvae inside roots were compared with the control treatment.

**Key words:** oyster mushroom, *Heterodera schachtii*, culture filtrate, paralysis, mushroom compost, *Hypsizygus ulmarius*, *Pleurotus* spp.

### INTRODUCTION

*Heterodera schachtii* Schmidt, is a major parasitic nematode of sugar beets, causing serious losses and yield reductions. It was first identified in 1859 on sugar beets near Halle, Germany (Schacht 1859). Cyst nematodes cause high levels of economic loss in a multitude of agricultural crops worldwide (Franklin 1951). *H. schachtii* can parasitize plant roots of all ages. Seedlings may be severely injured or killed resulting in poor stands. The older the plant when attacked, the less damage will occur. Young plants attacked by *H. schachtii* have elongated petioles and remain stunted until harvest.

In order to control or manage cyst nematodes, the use of nematicides is useful, but environmental side effects associated with chemical control as well as recent loss of methyl bromide as a multi-purpose soil fumigant have spurred research into alternative methods in nematode control (Hutchinson *et al.* 1999). Thorne and Barron (1984) first reported that wood-decaying fungi have the ability to capture, kill and digest nematodes. They showed that 11 species of gilled fungi (Agaricales), including *Pleurotus ostreatus* Kummer, had the ability to kill nematodes and since their report in 1984, several attempts have been made to use antagonistic fungi to control cyst nematodes. *Hirsutella rhossiliensis* Minter & Brady, infects juveniles of *H. schachtii* and reduces root penetration by them (Tedford *et al.* 1995). Researchers have identified two fungi that appear to play a significant role in sugar beet cyst nematode biocontrol in field soil in California. *Dactylella*

*oviparasitica* Stirling & Mankau, and *Fusarium oxysporum* Schlecht. were common colonizers of *H. schachtii* cysts in soil (Westphal and Becker 2001). Some of basidiomycetous fungi such as *Hyphoderma* spp. were shown to be of nematophagous fungi (Tzean and Liou 1993).

The purpose of this study was to determine the qualitative to quantitative potential of wood-decay fungi to attack and digest the sugar beet cyst nematode and to determine whether wood inhabitant fungal species could affect population dynamics of the cyst nematode in soil and diseased and dead sugar beet plants.

### MATERIALS AND METHODS

#### 1. Fungal inoculum preparation

Oyster mushrooms tested in this study were as follows. *Pleurotus ostreatus* (one sporeless isolate and one isolate with spore), *P. eryngii* Quelet Senulato., *P. sajor-caju* Singer., *P. flabellatus* (Berk. & Broome) Sacc., *P. florida* Eger. and *Hypsizygus ulmarius* (Bull.:Fr.) Redhead. Stock cultures of the oyster mushrooms were maintained on slants of malt extract agar.

#### 2. Nematode inoculum preparation

Sugar beet cyst nematodes were collected from a field in Khorasan province (Iran). Newly formed females and cysts were washed off from the collected sugar beet roots with a vigorous water stream through an 850  $\mu$  aperture sieve onto a 250  $\mu$  aperture sieve and extracted by centrif-

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ugation in 76% (w/v) sucrose solution. Eggs were released from the cysts by breaking the cysts in a 40-ml glass tissue grinder (Barker 1985). The eggs were separated from debris by centrifugation in a 35% (w/v) sucrose solution for 5 min at 1500 rpm, transferred to an antibiotic solution (streptomycin sulfate 6000 ppm), and maintained at 4°C for two days before being used.

### 3. In vitro studies

#### 3.1. Effect of mycelium

The anti-nematode ability of bio-control organisms was tested by transferring a 4 mm disc of each fungus culture to the centre of water agar plates and incubated for 5 days at 24±1°C. By this time a thin weft of sparse hyphal growth had radiated from the disc through the adjacent agar. Twenty active nematodes were hand picked from the nematode culture and placed on water agar plates in the vicinity of fungi hyphal growth. Periodic observations on interaction were made under a stereo-microscope (Nikon).

#### 3.2. Effect of fungal culture filtrate

To study the effect of culture filtrate on nematodes, the cultures of fungi were prepared in malt extract broth, incubated for 14 days at 24±1°C, sterilized using filter papers as well as milipore filters. Several concentrations (5×, 25×, 50× and 100×) from culture filtrate were prepared. Two ml of culture filtrate was poured into counting plates. Twenty active hand picked nematodes were added to the culture filtrate. Nematode activity was observed under a stereo-microscope at suitable intervals.

#### 3.3. Sugar beet seeds preparation

The sugar beet seed Cv. ICI were collected and sterilized with 50% NaOCl solution.

### 4. In vivo studies (greenhouse experiments)

Inoculation substrate was prepared by boiling of wheat grains in water for 15 min till they were soft. Water excess was drained off and the substrates were spread on the surface of a clean blotting paper and air dried for 15 min to remove the excess water. Then Ca<sup>2+</sup> was added and the substrates put into milk glasses.

The glasses were autoclaved for 60 min at 121°C and cooled. Straws were chopped into small pieces (1±2 cm), soaked in water overnight, then boiled in water for 60 min. Water excess was drained off and allowed to cool up to 25°C and sterilized. Wet straw (~85% moisture) was mixed with 20% grain spawn of each fungal species. The spawned substrates were then put into 30 cm×42 cm polyethene bags.

The bages were tightly closed and pin holes were made on the surfaces. The bags were subsequently kept in a spawn running room at 25±1°C under dark conditions till primordia were formed. After primordial formation, large holes were made in the polythene bags to allow the normal development of fruiting bodies. The bags were then kept at 22±1°C with a 12 h photoperiod and 85–90% of relative humidity. Mushrooms were manually harvested in clusters from the substrates three days after primordial initiation.

Spent oyster mushroom composts were used in this trials. The amount of each two composts (100 and 200 g) of *P. ostreatus* and *P. sajor-caju* were mixed with a fine soil (same mixture of pit, perlite and clay). The sterilized seeds were cultivated. After 2–3 weeks, the plants were inoculated with 2000 second-stage juvenils of *H. schachtii* per plant. Sterile wet straws (without fungus) were used as controls. Plants were maintained in greenhouse at 24±26°C. Plants were extracted from pots after 70 days, fresh and dry weight index and population of cysts and juveniles were evaluated. Each treatment had four replications.

### 5. Statistical analysis

Analysis of data was carried out by MSTATC and SAS. Data were subjected to the analysis of variance, and means were compared and grouped according to the Duncan's multiple range test.

## RESULTS

### 1. In vitro studies

#### 1.1. The effect of mycelium

Results showed that there were significant nematocidal differences between fungal species at various times (Table 1). Nematodes were inactivated/immobilized within 15–30 minutes of their exposure to oyster mushrooms culture.

Table 1. Analysis of variance in mortality [%] of *H. schachtii* subjected to oyster mushrooms hyphae in water agar after four, eight and 24 hours

Source	df	ss	Ms
Species	7	230.97	32.99*
Time	2	167.36	83.68*
Time×species	14	34.14	2.43*
Error	46	12.55	0.27*
total	69	445.041	

CV% = 12.33%

df – degree of freedom

ss – sum of squares

ns – mean of squares

\*significant = at ( $\alpha = 0.05$ )

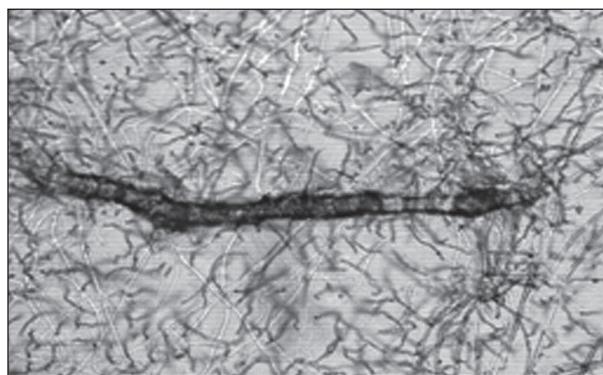


Fig. 1. Disintegration of nematode *Heterodera schachtii* body after penetration by *P. sajor-caju* hyphae

However, these nematodes were not killed but moved feebly if placed in a drop of water. Subsequently, these nematodes were observed under a microscope after 4, 8 and 24 hours.

It was indicated that fungal hyphae had grown towards the nematode and eventually penetrated it through one of the body orifices. It was further observed that nematode body had been filled with the hyphae and body contents were digested within 2–3 days after inoculation (Fig. 1).

Table 2. Grouping mortality per cent of *H. schachtii* subjected to fungus hyphae using Duncan's multiple range test

Fungal species	Time [hrs]	Mortality [%]
<i>P. ostreatus</i>	24	96 (7.8) ab*
	8	35 (6) c
	4	8 (3) ghi
<i>P. sajor-caju</i>	24	72 (8.5) a
	8	33 (5.8) cd
	4	17 (4.2) fg
<i>P. florida</i>	24	55 (7.4) b
	8	27 (5.2) cde
	4	15 (4) gh
<i>P. flabellatus</i>	24	52 (7.2) b
	8	30(5.5) cd
	4	10(3.2) hi
<i>Hypsizogus ulmarius</i>	24	62(8) ab
	8	30 (5.5) cd
	4	3(1.9) j
<i>P. ostreatus</i> (sporeless)	24	20 (4.5) efg
	8	9 (3) i
	4	0 (0.7) j
<i>P. eryngii</i>	24	23 (4,8) def
	8	10 (3.2) hi
	4	0 (0.7) j
Control	24	0 (0.7) j
	8	0 (0.7) j
	4	0 (0.7) j

\*mortality [%] followed by different letter are significantly different from each other

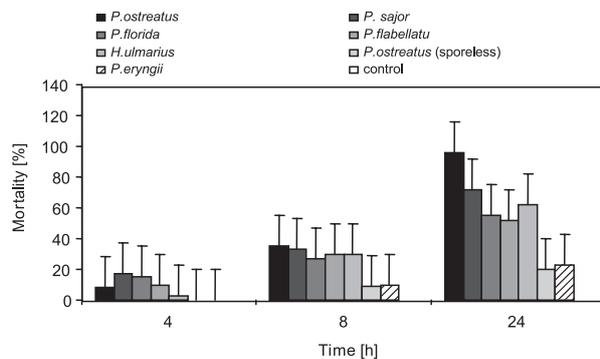


Fig. 2. Mortality per cent of *H. schachtii* juveniles after 4, 8 and 24 hours

However, maximum nematocidal activity was observed in *P. ostreatus* that accounted for the mortality rate of 96% within 24 hours, while *P. ostreatus* (sporeless) led to the nematicidal rate of 20% after 24 hours. Mortality per cent was also increased with passage of time (Table 2, Fig. 2)

### 1.2. Effect of culture filtrate

Results showed that there were significant differences in the paralytic activities among several species and several concentrations at different times intervals (Tables 3, 4, 5).

Table 3. Analysis of variance of *H. schachtii* paralysis [%] subjected to oyster mushrooms culture filtrate after four hours

Source	df	ss	Ms
Species	6	30.069	5.011*
Time	4	219.368	54.842*
Time×species	24	40.556	1.690*
Error	68	50.620	0.744*
Total	102	340.613	

CV% = 23.62%, \*significant at ( $\alpha=0.05$ )

Table 4. Analysis of variance of *H. schachtii* paralysis [%] of subjected to oyster mushrooms culture filtrate after eight hours

Source	df	ss	Ms
Species	6	28.392	4.732*
Time	4	414.056	103.514*
Time×species	24	14.954	0.623*
Error	68	22.459	0.330*
Total	102	479.861	

CV% = 12.02%, \*significant at ( $\alpha=0.05$ )

Table 5. Analysis of variance of *H. schachtii* paralysis [%] of subjected to oyster mushrooms culture filtrate after 24 hours

Source	df	ss	Ms
Species	6	30.045	5.008*
Time	4	453.376	113.344*
Time×species	24	19.152	0.798*
Error	68	19.465	0.286*
Total	102	522.038	

CV% = 8.41%, \*significant at ( $\alpha = 0.05$ )

On the transfer of active nematodes to different oyster mushroom culture filtrates, the nematodes became sluggish. There were only 33 per cent of active nematodes after four hour exposure to *P. ostreatus* culture filtrate (Table 6). The population of active nematodes reduced to 45 per cent after eight hour exposure to *P. sajor-caju* and *P. florida* (Table 7). Also after 24 hour exposure to *P. ostreatus* 90% of nematodes were inactivated (Table 8). Inactivated nematodes, however, remained alive for 24 hours and resumed activity when transferred to tap water, but were killed after 48 hours exposure.

On the other hand, nematodes remained active in tap water up to 48 hours when the last observation was made. It appears that *Pleurotus* spp. secretes some toxic metabolite(s) which considerably inactivate nematodes.

Table 6. Grouping paralysis per cent of *H. schachtii* subjected to culture filtrate after four hours

Treatment	Fungal species						
	<i>P. ostreatus</i>	<i>P. sajor-caju</i>	<i>P. florida</i>	<i>P. ostreatus</i> (sporeless)	<i>H. ulmarius</i>	<i>P. flabellatus</i>	<i>P. eryngii</i>
0	0.707 g	0.707 g	0.707 g	0.707 g	0.707 g	0.707 g	0.707 g
0.05	3.254 cde	0.707 g	0.707 g	0.707 g	0.707 g	1.552 fg	1.552 fg
0.25	4.099 bcd	0.707 g	2.396 ef	3.240 cde	0.707 g	3.669 bcde	2.396 ef
0.50	4.528 abc	4.430 abcd	4.099 bcd	4.099 bcd	2.825 def	3.669 bcde	3.240 cde
1	5.803 a	5.042 ab	5.191 ab	4.528 abc	4.099 bcd	3.669 bcde	3.240 cde

Means followed by different letter are significantly different ( $p > 0.01$ ) according to Duncan's multiple range test

Table 7. Grouping paralysis per cent of *H. schachtii* subjected to culture filtrate after eight hours

Treatment	Fungal species						
	<i>P. ostreatus</i>	<i>P. sajor-caju</i>	<i>P. florida</i>	<i>P. ostreatus</i> (sporeless)	<i>H. ulmarius</i>	<i>P. flabellatus</i>	<i>P. eryngii</i>
0	1.253 h	1.253 h	1.253 h	1.253 h	1.253 h	1.253 h	1.253 h
0.05	5.191 de	5.191 de	3.240 g	4.099 fg	3.240 g	4.430 ef	3.240 g
0.25	6.491 abc	6.364 abc	4.528 ef	5.526 cde	5.033 def	5.510 ced	4.529 ef
0.50	6.739 ab	7.221 a	5.523 cde	7.101ab	5.510 cde	6.618 ab	6.618 ab
1	6.739 ab	7.445 a	5.523 cde	7.445 a	5.948 bcd	6.739 ab	6.739 ab

Means followed by different letter are significantly different ( $p > 0.01$ ) according to Duncan's multiple range test

Periliminary studies on the characterization of the toxic metabolite revealed that it is a heat stable and dialyzable low molecular weight compound.

After the confirmation of nematocidal nature of *Pleurotus* under *in vitro* conditions, further studies were made to decipher the possibility of its use under various conditions.

## 2. In vivo studies

*In vivo* studies indicated that the application of completely spawn run compost could reduce some indices of the disease caused by *H. schachtii*. This application could reduce population of *Heterodera* in roots in pot cultures which is one of the important indices in disease diagnosis (Fig. 3).

The number of cysts per gram of soil was significantly lower in all soils treated with spent oyster mushroom composts than that in the non-treated, suppressive control, despite that plants in all pots had received the same nematode inoculum (Fig. 4). The average number of eggs per cysts in treated with fungi was 1000 eggs while the average number of eggs per cyst in the non-treated, was 21750 (Fig. 5). Also, the number of cyst on roots was lower in all plants treated with spent oyster mushroom composts (Fig. 6). The number of eggs per cyst was significantly higher in the treatments with 100g of spent oyster mushroom composts than treatments with 200 g. In all treatments, plants weight were not significantly different among each other and control (Table 9).

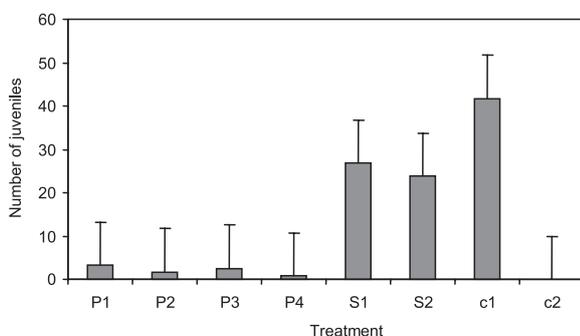


Fig. 3. Analysis of mean number of juveniles in roots. P1=100g *P. ostreatus*+nematode; P2=200g *P. ostreatus*+nematode; P3=100g *P. sajor-caju*+nematode; P4=200g *P. sajor-caju*+nematode; S1=100g wet straws+nematode; S2=200g wet straws+nematode; C1=only nematode and C2=nematode+nematicide

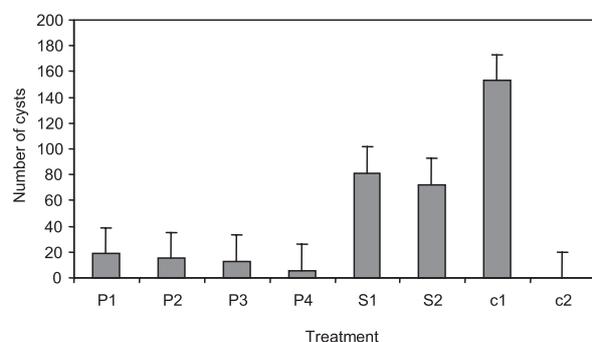


Fig. 4. Analysis of mean number of cysts in the soil. P1=100 gr *P. ostreatus*+nematode; P2=200g *P. ostreatus*+nematode; P3=100g *P. sajor-caju*+nematode; P4=200g *P. sajor-caju*+nematode; S1=100g wet straws+nematode; S2=200g wet straws+nematode; C1=only nematode and C2=nematode+nematicide

Table 8. Grouping paralysis per cent of *H. schachtii* subjected to culture filtrate after 24 hours

Treatment	Fungal species						
	<i>P. ostreatus</i>	<i>P. sajor-caju</i>	<i>P. ostreatus</i> (sporeless)	<i>P. florida</i>	<i>P. flabellatus</i>	<i>H. ulmarius</i>	<i>P. eryngii</i>
0	2.942 j	2.942 j	2.942 j	2.942 j	2.942 j	2.942 j	2.942 j
0.05	6.331 e	6.331 e	5.523 efgh	4.528 i	5.033 fghi	4.762 hi	4.855 ghi
0.25	8.396 bcd	8.190 bcd	5.510 efgh	6.229 e	5.791 efg	6.364 e	5.948 ef
0.50	9.513 a	8.588 abc	8.487 bcd	8.490 bcd	7.550 d	8.396 bcd	7.554 d
1	9.513 a	8.878 ab	8.686 abc	8.686 abc	7.875 bcd	8.396 bcd	7.760 cd

Means followed by different letter are significantly different ( $p > 0.01$ ) according to Duncan's multiple range test

Table 9. Grouping mean measurement indices, interaction between *H. schachtii* and oyster mushrooms

Composts amounts [g]		nematode	Cysts on roots	Cysts in soil	Eggs in cysts	Juveniles in roots
<i>P. ostreatus</i>	100	no nematode	0 e	0 d	0 f	0 e
		with nematode	5.75 c	18.75 c	2500 d	3.25 d
	200	no nematode	0 e	0 d	0 f	0 e
		with nematode	2 d	15.25 c	1000 ef	1.75 de
<i>P. sajor-caju</i>	100	no nematode	0 e	0 d	0 f	0 e
		with nematode	2 d	13 c	3125 d	2.5 de
	200	no nematode	0 e	0 d	0 f	0 e
		with nematode	3 d	5.75 cd	1725 de	0.75 de
straw	100	no nematode	0 e	0 d	0 f	0 e
		with nematode	11 b	81.5 b	12000 b	26.75 b
	200	no nematode	0 e	0 d	0 f	0 e
		with nematode	11.75 b	72.5 b	8500 c	23.75 c
Control	no nematode	0 e	0 d	0 f	0 e	
	with nematode	19 a	153.25 a	21750 a	41.75 a	

Means followed by different letter are significantly different ( $p > 0.01$ ) according to Duncan's multiple range test

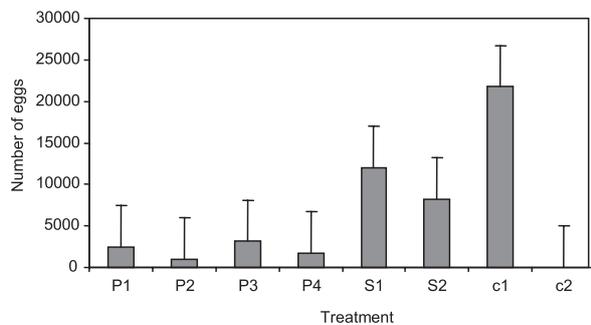


Fig. 5. Analysis of mean number of eggs in the cysts. P1=100g *P. ostreatus*+nematode; P2=200g *P. ostreatus*+nematode; P3=100g *P. sajor-caju*+nematode; P4=200g *P. sajor-caju*+nematode; S1=100g wet straws+nematode; S2=200g wet straws+nematode; C1=only nematode and C2=nematode+nematicide

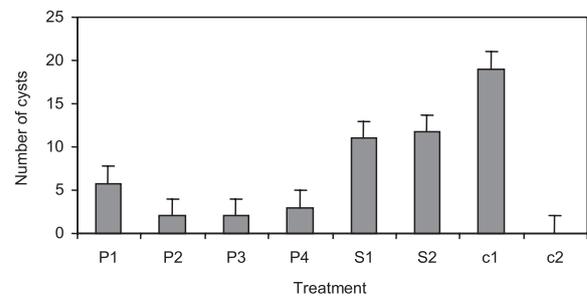


Fig. 6. Analysis of mean number of cysts on roots. P1=100g *P. ostreatus*+nematode; P2=200g *P. ostreatus*+nematode; P3=100g *P. sajor-caju*+nematode; P4=200g *P. sajor-caju*+nematode; S1=100g wet straws+nematode; S2=200g wet straws+nematode; C1=only nematode and C2=nematode+nematicide

## DISCUSSION

Experimental results showed the effects of *Pleurotus* spp. and *H. ulmarius* on *Heterodera* population densities under both *in vitro* and *in vivo* conditions. The study confirmed the ability of *Pleurotus* to capture, kill and digest the cyst nematode. The mode of infecting nematodes by all strains of *Pleurotus* species was consistent with that of *P. ostreatus* described by Thorne *et al.* (2000), Hibbet and Thorne (1994), Sharma (1994) and Thorne and Barron (1984).

Barron and Thorne (1987) indicated that the ability to infect nematodes was because of a toxin released as droplets by secretory hyphal cells. Tiny secreted droplets were commonly observed on the hyphae of all tested strains of *Pleurotus* species on water agar (Barron and Thorne 1987; Chitwood 2004).

The mentioned results were confirmed in 2006 (Heydari *et al.* 2006; Palizi *et al.* 2006; Palizi *et al.* 2007). Also Barron and Thorne (1987) indicated the oriented/directed growth of the hyphae which then entered with a great precision the head of nematode as directed hyphae. Oriented hyphae were commonly observed on dead nematodes attacked by the *Pleurotus* species. *Pleurotus* spp. and *H. ulmarius* killed the cyst nematode after only a short period of exposure to their hyphae. Nematodes were immobilized as soon as they approached the fungal colony. Paralysed nematodes were characterized by their immobilized, and straightened bodies.

The hyphae of these fungi colonized paralysed nematodes after prolonged exposure of the nematodes to the fungal colonies. Hutchison *et al.* (1996) reported the interaction between the lawn inhabiting agaric *Conocybe lacteal* and fungal feeding nematode *Aphelenchoides* sp. in which the hyphae killed nematodes with an antifeedant. Results from the multiplication experiments were in accordance with the nematophagous ability of basidiomycetous fungi (Chitwood 2004). The sugar beet nematode could not reproduce in the cultures of these species of oyster mushrooms.

Finally, our experiments revealed that *Pleurotus* spp. and *H. ulmarius* could reduce *H. schachtii* population densities under both *in vitro* and *in vivo* conditions and it could therefore be regarded as one of the best potential biocontrol agents in the country.

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## POLISH SUMMARY

### POTENCJAŁ GRZYBÓW OSTRYGOWYCH I *HYPsizYGUS ULMARIUS* DO BIOLOGICZNEGO ZWALCZANIA MATWIKI BURAKOWEGO (*HETERODERA SCHACHTII*)

Badano potencjał grzybów ostrygowych do atakowania i zabijania mątwika burakowego (*Heterodera schachtii*). Potwierdzono zdolność pasożytowania *Pleurotus ostreatus*, *P. sajor-caju*, *P. florida*, *P. flabellatus*, *P. ostreatus* (niezależnie), *P. eryngii* i *Hypsizygus ulmarius* na mątwiku burakowym. Płytki z agarem wodnym, na którym rosły rzadkie kolonie tych grzybów zaszczepiono mątwikiem burakowym. Po inokulacji nicienie były szybko unieruchomione na płytkach z każdym gatunkiem *Pleurotus*, a śmiertelność nicieni wynosiła odpowiednio 96%, 72%, 55%, 52%, 20%, 23% and 62%. Badano także aktywność

nicieniobójczą *in vitro* filtratów kultur gatunków *Pleurotus* i *H. ulmarius* przeciwko mątwikowi burakowemu. Wyciągi z *P. ostreatus* sparaliżowały 90% nicieni, podczas gdy *P. erynii* sparaliżował tylko 50% nicieni. Efektywność starego kompostu z kultur *P. ostreatus* i *P. sasor-caju* w zwalczaniu cyst mątwika buraka (*Beta vulgaris* ICI)

badano w warunkach szklarniowych. Wyniki wykazały, że 100 i 200 gramów kompostu z grzybów na 3-kilogramowy wazon mogły istotnie zwalczyć cysty, zmniejszając ich ilość w ziemi o ponad 85%. Ilość cyst i larw w korzeniach porównano z kombinacją kontrolną.

