e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 67 (2019), No 3
https://doi.org/10.3409/fb_67-3.11

The Ovistatic Effect of Saprotrophic Soil Fungi on *Ascaris suum* Eggs

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Accepted August 06, 2019 *Published online August 22, 2019* *Issue online September 30, 2019*

Original article

KOŁODZIEJCZYK L., MAZURKIEWICZ-ZAPAŁOWICZ K., TWARUŻEK M., GRAJEWSKI J., ŁOPUSIEWICZ Ł., RYBIŃSKA A., DZIKA, E., PILARCZYK B. 2019. The ovistatic effect of saprotrophic soil fungi on *Ascaris suum* eggs. Folia Biologica (Kraków) **67**: 109-118.

The aim of the study was to evaluate the potential use of selected species of soil fungi (*Fusarium oxysporum*, *F. sulphureum*, *F. verticillioides*, and *Penicillium expansum*) for the bioregulation of the dispersive stages of a parasitic nematode – the large roundworm of pig (*Ascaris suum*). Experimental cultures containing *A. suum* eggs with soil fungi and control cultures without fungi were incubated at 26°C for 28 days. Microscopic observations of the developmental stages of the *A. suum* eggs (zygote, 2-8 blastomeres, morula/blastula, gastrula, and larva) were performed at 7, 14, 21, and 28 days. The API-ZYM test was used to semi-quantitatively determine the activity of 19 hydrolytic fungal enzymes. The cytotoxicity of the fungi was determined with a tetrazole salt MTT assay. Microscopic observations of *A. suum* eggs incubated in the presence of fungi up to day 28 did not show any signs of destruction to egg shells and/or penetration of the fungi into the eggs. The ovistatic effect of all tested fungi (*F. sulphureum*, *P. expansum*, *F. verticillioides*, and *F. oxysporum*; $p<0.05$) was seen only on the 7th day of incubation, whereas on the 14th day, only *F. verticillioides* and *F. oxysporum* showed an inhibitory effect on the embryogenesis of *A. suum*, and by the 28th day, only *P. expansum*. The API-ZYM test showed differences in the hydrolytic activity of the tested strains, while the MTT assay showed the high cytotoxicity of *F. sulphureum*, the moderate cytotoxicity of *F. verticillioides* and *P. expansum*, and the low cytotoxicity of *F. oxysporum*. Among the fungal strains studied, *F. sulphureum* showed the highest ovistatic effect, which may be related to its enzymatic activity and cytotoxicity.

Key words: *Ascaris suum*, ovistatic effect, saprotrophic soil fungi.

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Microbes, including fungi, constitute the main component of soil biomass, and the amount of carbon that fungi accumulate is equal to or exceeds the amount of carbon in plant roots (RITZ & YOUNG 2004). The importance of fungi in soil is also related to their diverse and complex interactions with other components of the soil environment. From an ecological point of view, the role of soil fungi as saprophytes participating in the decomposition of dead organic matter is particularly valuable. Additionally, some fungi species are a natural control for plant pathogens, and researchers are investigating those that could be used in biological plant protection (MOOSAVI & ZARE 2012; JYOTI & SINGH 2016). One important avenue in this field is the search for soil fungi which exert inhibitory effects on populations of parasitic nematodes of humans and animals, as soil infestation by the dispersive stages of intestinal parasites is a serious epidemiological problem in many regions of the world.

Nematodes *A. lumbricoides* and *A. suum* are the most common intestinal parasites of humans and pigs. It is estimated that infection by *A. lumbricoides* affects more than 1 billion people and causes 20,000 deaths annually (HOPKINS 1992). The reproductive potential of female *A. lumbricoides* is very high, as evidenced by the fact that one female can lay up to 250 thousand eggs per day. Eggs of *Ascaris* sp. show high resistance to most methods of inactivation (BROWNELL & NELSON 2006). *Ascaris* egg shells are composed of four layers: the inner lipoprotein layer (ascaroside layer), the chitin/protein layer, the lipoprotein vitelline layer, and the outer acid mucopolysaccharide/protein uterine layer. The ascaroside layer, which is responsible for the impermeability of the shell, consists of a mixture of 25% protein and 75% lipid-containing ascarosides. The chitinous layer contains chitin spindles in a protein matrix, and both the vitelline and uterine layers contain protein (WHARTON 1980; BROWNELL & NELSON 2006).

The prevalence of *A. suum* on pig farms varies depending on country, population, method of pig breeding, pig nutrition, and pig age. The difficulty of controlling *A. suum* at production plants is related to the longevity of invasive eggs, the high fecundity of female nematodes, and the resistance of eggs to harmful environmental factors (NOSAL 1996). Pig slurry is often contaminated with *A. suum* eggs and plays an important role in the spread of these nematodes. Parasitic larvae in stored slurry are resistant to disinfectants. Animals kept in open systems (at organic farms) are even more often exposed to parasite infections (ROEPSTORFF & NANSEN 1994). EIJCK and BORGSTEEDE (2005) found *A. suum* in 21.0% of free range pigs, but in only 3.22% of conventionally-raised pigs. In Denmark, CARSTENSEN *et al.* (2002) showed a prevalence of *A. suum* in the range of 18.0-38.0% in piglets, around 20% in sows, and 33% in porkers. ROEPSTORFF *et al.* (1998) found that pigs in the Nordic countries showed *A. suum* prevalence of 11.3%. In Poland, the extensity of *A. suum* infection in pigs is even higher, ranging from 30% to 60% (NOSAL & ECKERT 2005; KNECHT *et al.* 2011, 2012).

Many species of fungi have demonstrated an ovistatic and/or ovicidal effect on the embryonic development of *Ascaris* sp., including *Metacordyceps chlamydosporia* (=*Verticillium chlamydosporium*) (LÝSEK & KRAJCI 1987; LÝSEK & STĚRBA 1991), *Penicillium frequentans, Stachybotrys chartarum* (KOŁODZIEJCZYK *et al.* 2001; KUŹNA-GRYGIEL *et al.* 2001), *Isaria fumosorosea* (=*Paecilomyces fumosoroseus)*, *Fusarium culmorum, Trichothecium roseum, Aspergillus niger, Trichoderma viride* (JABOROWSKA 2006), *Metarhizium flavoviride, M. anisopliae* (JABOROWSKA *et. al.* 2006), *Duddingtonia flagrans*, *Monacrosporium sinense*, *Pochonia chlamydosporia* (ARAÚJO *et al.* 2008; FERREIRA *et al.* 2011), *Aspergillus fumigatus, A. terreus, Penicillium citrinum, P. expansum, Fusarium oxysporum, Trichothecium roseum* (BLASZKOWSKA *et al.* 2013), *Acremonium alabamense, Alternaria chlamydospora, Cladosporium herbarum, Fusarium solani, Paecilomyces variotii, Paecilomyces viridis,* and *Penicillium verruculosum* (BLASZKOWSKA *et al.* 2014).

Particularly interesting are studies on the nematostatic activity of *Fusarium* and *Penicillium* sp. due to their hydrolytic activity (STÖCKLEIN *et al.* 1993; RAPP 1995; PEKKARINEN *et al.* 2000; DAHOT 2001; BARATA *et al.* 2002; FACCHINI *et al.* 2015) and cytotoxicity (CETIN & BULLERMAN 2005; TWARUŻEK *et al.* 2018). Studies regarding the influence of *Fusarium* and *Penicillium* sp. on the development of *A. suum* eggs are rather scarce and therefore, the aim of this study was to determine the effect of selected common saprophytic soil fungi on the development of *A. suum* eggs, particularly, in relation to their cytotoxicity and enzymatic activity.

Materials and Methods

Fungi

Strains of 4 species of saprophytic fungi obtained from soil were selected for the study: *Fusarium oxysporum* Schltdl. (CCM F-358, isolated from rice field soil, Vietnam), *Fusarium sulphureum* Schltdl. (KH 1808, isolated from an arable field, Poland), *Fusarium verticillioides* (Sacc.) Kuntze = *Gibberella fujikuroi* (Sawada) Wollenw. (KH 230,

isolated from an arable field, Poland), and *Penicillium expansum* Link (CCM F-576, isolated from orchard soil, Poland). Fungal nomenclature is based on the Index Fungorum (http://www.indexfungorum.org). Single-spore cultures of the strains were grown on PDA medium (Merck). Discs (ø 4 mm) were cut out of the 3-week old mycelium from these cultures and placed on PDA medium. Incubation was carried out in the dark at 25°C for 21 days and then new discs (ø 4 mm) were cut and transferred to Petri dishes (ø 50 mm) containing a suspension of *A. suum* eggs.

Ascaris suum eggs

Fertilized eggs of *A. suum* were obtained from the uteri of female nematodes (n=60) derived from the intestines of pigs from organic (n=30) as well as traditional farms (n=30). The obtained eggs (2 ml), from organic and traditional farms, respectively, were mixed and centrifuged in distilled water (3x1000 rpm) for 3 minutes. Two ml of *A. suum* eggs were mixed with 48 ml of distilled water containing 0.05% formalin, 0.05% streptomycin sulphate, and 0.01% chloramphenicol (ARAUJO *et al.* 1995; BLASZKOWSKA *et al.* 2013).

50 ml of *A. suum* egg suspension was divided into 5 parts (10 ml each). A control culture (without fungi) and 4 experimental cultures (with fungi), containing 10 ml suspension of *A. suum* eggs each, were incubated in Petri dishes (ø 50 mm) at 26°C, 80% RH for 28 days.

Every week (on day 7, 14, 21 and 28 of incubation), 0.1 ml of *A. suum* egg suspension was collected from the control and experimental cultures and microscopic observations of embryonic development were carried out (Olympus CX21, Japan; x10; 40).

In 100 randomly observed eggs, the following development stages were determined: zygote, 2-8 blastomers, morula/blastula, gastrula, and larva. Microscopic observations of 100 randomly collected eggs from the control and experimental cultures were carried out 3 times (3 x 100 eggs).

Enzymatic activity of fungi

The API-ZYM test (bioMerieux, Lyon, France) was used to semi-quantitatively determine the activity of 19 hydrolytic fungal enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosoaminidase, α-mannosidase, and α-fucosidase. The tests were performed as per the manufacturer's instructions. The API-ZYM strips were inoculated with mature (7 days) fungal cultures grown on PDA (transferred into a sterile physiological saline solution) and then incubated at 37°C for 4 h. Hydrolytic activity was determined in nanomoles of hydrolyzed substrate, on a color scale from 0-5 provided by the manufacturer, where: 0 – negative reaction, 1 – 5 nM, 2 – 10 nM, 3 – 20 nM, 4 – 30 nM, and 5 – 40 nM or more. The 1-2 was interpreted as a low, 3 as a moderate and 4-5 as high enzymatic activity.

Cytotoxicity of fungi

Cytotoxicity was determined by using tetrazole salt MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma Aldrich). The study was performed with the use of the swine kidney cell line (SK). The MTT colorimetric assay for cytotoxicity is based on the transformation of yellow-coloured tetrazole salts into violet-coloured, water-insoluble formazan crystals. The salt reduction takes place exclusively in the mitochondria of live and metabolically active cells. If cells are damaged or destroyed by the toxin, the salt will not transform and the yellow colour of the tetrazole salts will be retained (HANELT *et al.* 1994).

The cells were cultured in a medium supplemented with an antibiotic solution (penicillin and streptomycin, Sigma Aldrich) and fetal calf serum (Sigma Aldrich) in a CO_2 Hera Cell incubator (Heraeus, Germany) (5% CO_2, 37 °C, 98% humidity). The number of swine kidney cells was 2.2×10^5/ml.

Extracts were prepared from the strains of fungi grown in the Petri dishes (PDA medium). The strains of fungi were transferred to a sterile Stomacher bag and extracted twice with 50 ml of chloroform in laboratory paddle blender for 4 min (BagMixer® 400, Intersciences). The chloroform was then poured through a fluted filter into a round bottom flask and evaporated to dryness in a vacuum evaporator. Next, the evaporation residue was dissolved into 2 ml of chloroform using an ultrasound bath and pipetted into vials. Lastly, the extracts were evaporated under a stream of nitrogen. All extraction procedures were done in a chemical fume hood.

The MTT cytotoxicity assay was performed on microtitration plates. The control (agar medium without mycelium inoculum) and experimental cultures with soil fungi on PDA were diluted in MEM (Minimum Essential Medium Eagle, Sigma Aldrich) medium containing 1.7% ethanol (POCH) and 0.3% DMSO (Merck) as 1:2. Consecutive dilutions were transferred onto MTT assay plates containing SK cells suspended in medium with ethanol, DMSO, and 10% FCS (fetal calf serum, Sigma Aldrich).

During the next 48 hrs, the plates were in a CO_2 Hera Cell incubator (Heraeus, Germany) (5% CO_2, 37 °C, 98% humidity). Next, 20 µl MTT solution in PBS (Merck) was added to the wells, and the whole set was incubated for another 4 hrs. Following liquid removal from the wells, 100 µl DMSO was added as a solvent for the formazan crystals. After 5 min of shaking (shaker Titramax 101, Heidolph), cytotoxicity was quantified with a micro-plate spectrophotometer (Ledetect 96 Microplate Reader, Labexim Products, Lengau, Austria) coupled with MikroWin 2010 OEM version (Mikrotek Laborsysteme GmbH, Overath, Germany), based on absorbance measured at 510 nm wavelength, which corresponded to maximum absorption of the formazan derivative. In the case of absorbance lower than 50% of the cell division activity, all analyzed samples were considered toxic.

Cytotoxicity IC 50 (sample concentration at which cell proliferation is inhibited by 50% compared to control cells) was determined based on consecutive dilutions according to the scale of HANELT *et al.* (1994) (Table 1). The cytotoxicity of the analyzed fungal strains was reduced by the IC 50 value of the control.

Statistics

Statistical analyses were performed using Statistica version 12 software (StatSoft. Inc). In order to determine differences between the two groups (control vs experimental), the t-test for independent samples was applied. Differences with $p<0.05$ were considered significant.

Results

The influence of soil fungi on the embryonic development of *Ascaris suum*

The presence of mycelium of the tested fungal species in the experimental cultures of *A. suum* eggs influenced the course of embryogenesis in different ways (Fig. 1).

On the 7th day of incubation, all examined species of fungi demonstrated an inhibitory effect on the embryogenesis of *A. suum*. The inhibitory effect on the development of *A. suum* eggs (expressed as a higher percentage of eggs in the zygote stage, compared to the control, $p<0.05$) was observed in the following fungal species: *F. sulphureum*, *P. expansum*, *F. verticillioides,* and *F. oxysporum* (in order from the strongest to the weakest inhibitory effect). Simultaneously, a significantly lower percentage of *A. suum* eggs at the morula/blastula stage ($p<0.05$) was found in cultures incubated with fungi in comparison to control culture.

On the 14th day of embryogenesis, a significantly higher percentage of *A. suum* eggs in the zygote stage was found only in cultures containing *F. oxysporum* compared to the control culture. A higher percentage of eggs in the morula/blastula stage were found in cultures incubated with *F. verticillioides* and a lower percentage in those incubated with *F. oxysporum* ($p<0.05$). In the same period, a significantly lower percentage of eggs in the larval stage was found only in *A. suum* cultures incubated with *F. verticillioides*.

On the 21st day of incubation, the percentages of particular developmental stages of *A. suum* eggs did not show statistically significant differences between control and experimental variants.

On the 28th day of incubation, the highest percentage of eggs in the zygote stage with the lowest percentage of eggs in the larval stage was found in cultures containing *P. expansum*. A higher percentage of eggs in the morula/blastula stage ($p<0.05$) compared to the control culture was found only in the presence of *F. verticillioides* (Fig. 1).

Table 1

The scale of cytotoxicity

Step	1	2	3	4	5	6	7	8	9	10	11	12
IC_{50} (cm^2/ml)	31.25	15.625	7.813	3.906	1.953	0.977	0.488	0.244	0.122	0.06	0.03	0.015
Cytotoxicity degree*	(+)			(++)				(+++)				

*(-) – no cytotoxicity; (+) – low cytotoxicity; (++) – moderate cytotoxicity; (+++) – high cytotoxicity

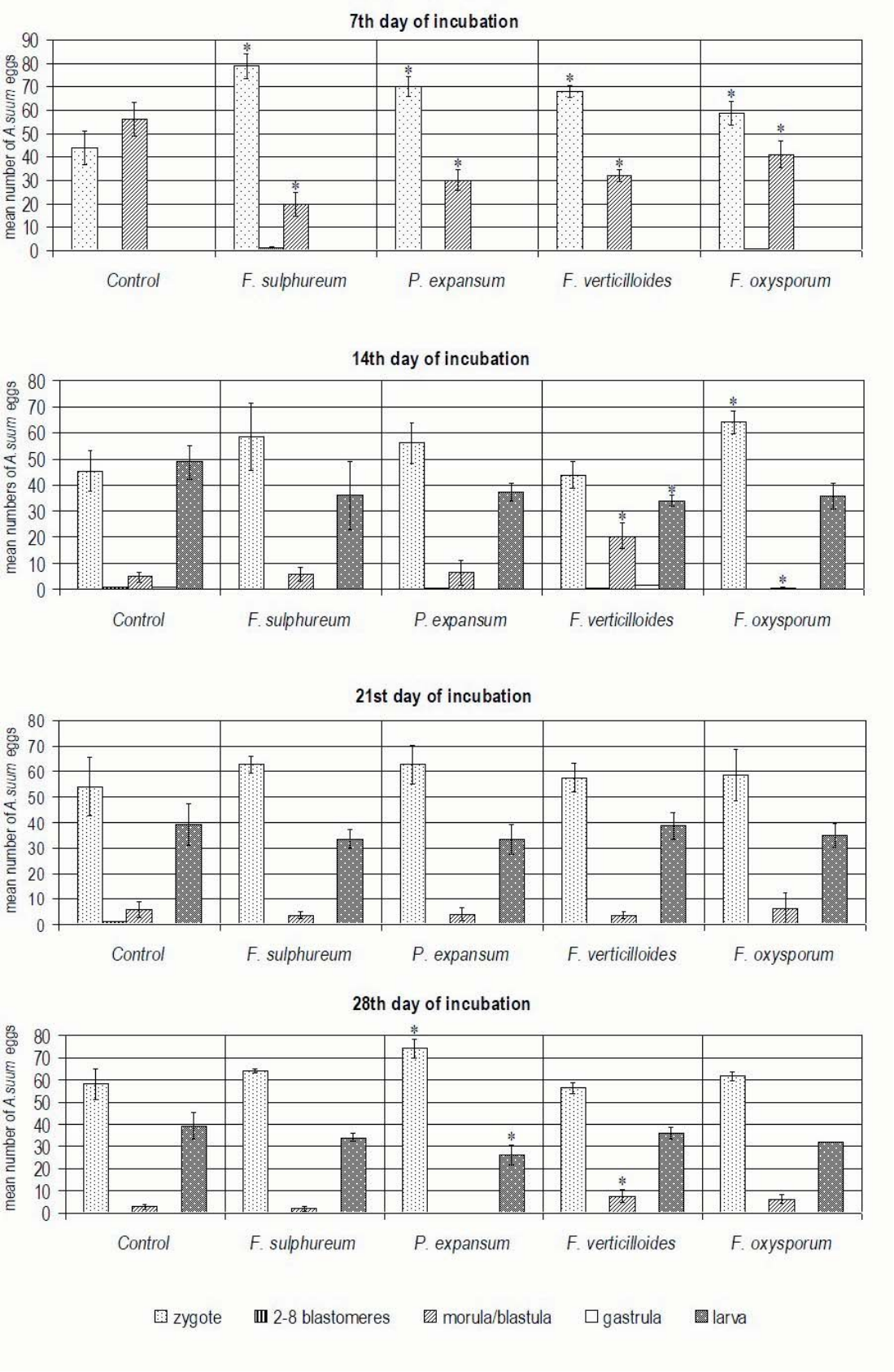

Fig. 1. The mean number (mean ± SD) of *A. suum* egg zygotes, 2-8 blastomers, morula/blastula, gastrula, and larva at the 7th, 14th, 21st, and 28th day of incubation in the control culture and in the presence of fungi: *Fusarium sulphureum, Penicillium expansum, F. verticillioides,* and *F. oxysporum*. Bars – means, whiskers – SD, * – differences statistically significant at $p<0.05$ between the control and experimental group.

Table 2

The production of 19 hydrolases by soil fungal species in the API-ZYM test

	Enzyme	*F. sulphureum*	*P. expansum*	*F. verticillioides*	*F. oxysporum*
1	Control	0	0	0	0
2	Alkaline phosphatase	5	3	0	3
3	Esterase (C4)	3	1	1	3
4	Esterase lipase (C8)	5	1	1	3
5	Lipase (C14)	0	0	0	0
6	Leucine arylamidase	1	3	0	1
7	Valine arylamidase	1	0	0	0
8	Cystine arylamidase	0	0	0	0
9	Trypsin	0	0	0	0
10	Chymotrypsin	0	0	0	0
11	Acid phosphatase	5	5	1	5
12	Naphtol-AS-BI-phosphohydrolase	5	5	1	5
13	α-galactosidase	4	3	0	0
14	β-galactosidase	0	3	0	0
15	β-glucuronidase	0	0	0	0
16	α-glucosidase	0	0	0	0
17	β-glucosidase	5	5	3	5
18	N-acetyl-β-glucosaminidase	5	4	3	5
19	α-mannosidase	1	1	0	0
20	α-fucosidase	0	0	0	0

The enzymatic activity of fungi

In the API-ZYM test (Table 2), the activity of peptide hydrolases (leucine arylamidase, valine arylamidase) was low/moderate (1-3) or undetectable (cystine arylamidase, trypsin, chympotrypsin). Low activity of leucine arylamidase was observed in *F. oxysporum* and *F. sulphureum*, while moderate activity was observed in *P. expansum* (3). Low activity of valine arylamidase was observed only in *F. sulphureum*, while none of the strains gave a positive reaction for cystine arylamidase.

In all fungi, activity of esterase (C4) and lipase esterase (C8) was observed, while none of the strains tested produced lipase (C14).

All strains showed moderate/high activity of β-glucosidase (3-5) and N-acetyl-β-glucosaminidase (3-5). Alkaline phosphatase activity was found in *F. oxysporum*, *P. expansum* (3), and especially in *F. sulphureum* (5), whereas in *F. verticillioides* activity of this enzyme was undetectable. High acid phosphatase activity was observed in *F. oxysporum*, *F. sulphureum*, and *P. expansum* and low activity was seen in *F. verticillioides*. High activity of naphthol-AS-BI-phosphohydrolase was observed in *F. oxysporum*, *F. sulphureum*, and *P. expansum*, and low activity was found in *F. verticillioides*. α-galactosidase activity was demonstrated in *F. sulphureum* and *P. expansum*, whereas β-galactosidase was produced only by *P. expansum*. No activity of β-glucuronidase or α-fucosidase was observed in any of the strains studied. Low activity of α-mannosidase was observed in *F. sulphureum* and *P. expansum*.

Cytotoxicity of soil fungi in MTT test

The MTT test performed on the selected fungal strains showed various levels of cytotoxicity: high (IC_{50} 0.015 cm^2/ml) for *F. sulphureum*, moderate for *F. verticillioides* and *P. expansum* (IC_{50} 0.488 cm^2/ml and IC_{50} 3.906 cm^2/ml respectively), and low (IC_{50} 31.25 cm^2/ml) for *F. oxysporum* (Table 3).

Table 3

The cytotoxicity of fungal strains in the MTT test

No	Soil fungi	Step	IC_{50} (cm^2/ml)	Degree of cytotoxicity
1	Control	2	15.625	+
2	*F. sulphureum*	12	0.015	+++
3	*P. expansum*	4	3.906	++
4	*F. verticillioides*	7	0.488	++
5	*F. oxysporum*	1	31.25	+

Discussion

The ovistatic effect of the tested fungal strains (demonstrated as a delayed initiation of zygote division and reaching of the morula/blastula stage) manifested itself mainly on the 7th day of incubation for all tested fungi: *F. sulphureum*, *P. expansum*, *F. verticillioides,* and *F. oxysporum*, and corresponded with their cytotoxicity in the MTT assay (high – *F. sulphureum*, moderate – *P. expansum* and *F. verticillioides,* and low – *F. oxysporum*). None of the tested strains showed an ovicidal effect on the eggs of *A. suum*. Microscopic observations of *A. suum* eggs incubated in the presence of fungi up to the 28th day of development did not reveal any symptoms of destruction of egg shells and/or penetration of the fungi into the eggs. This is also confirmed by the results of BLASZKOWSKA *et al.* (2013) under similar conditions, where hyphal penetration by *F. oxysporum* and *P. expansum* into the *A. suum* eggs was observed in 10-20% and 10-15% of eggs, respectively, only after 28, 48, and 60 days of incubation. In our study, only *F. verticillioides* and *F. oxysporum* at day 14 and *P. expansum* at day 28 of incubation showed an inhibitory effect on the embryogenesis of *A. suum*. This may indicate a weakening of the antagonistic effect of the examined fungi during incubation, which is contrary to the results of BLASZKOWSKA *et al.* (2013), where this effect increased with the length of exposure (up to 60 days). Very high ovicidal activity of *F. oxysporum* on *Toxocara canis* eggs was demonstrated by CIARMELA *et al.* 2010. In our study, the highest ovistatic effect on *A. suum* up to day 7 was shown by *F. sulphureum*, which may be related to its high enzymatic activity and cytotoxicity. In a study by CIARMELA *et al.* 2010, *F. sulphureum* demonstrated an ovicidal effect on the eggs of *T. canis*. These differences seem to confirm that *T. canis* eggs are more sensitive to the antagonistic effects of fungal strains than *A. suum* eggs, as demonstrated by comparative studies in the presence of *Fusarium culmorum, Metarhizium anisopliae, Paecilomyces fumosoroseus, Trichoderma viride,* and *Trichothecium roseum* (MAZURKIEWICZ-ZAPAŁOWICZ *et al.* 2014). These conflicting results in the studies of different authors concerning the same fungal species may be due to differences in the metabolic activity of different strains of a given species.

A surprising finding was the high percentage of *A. suum* eggs in the control culture (more than 50%) that remained in the zygote stage even on the 28th day of embryogenesis. The *A. suum* eggs used for the tests (both in experimental and control cultures) were obtained from 60 nematodes collected from pigs from organic (n=30) and traditional (n=30) farms. On organic farms, one of the methods of reducing parasitic infections is the use of feed additives in the form of medicinal plants containing active substances that reduce the level of parasitic infections in the host organism. These substances affect the physiological functions of the parasite, such as mobility, food absorption, or reproductive processes (MASAMHA *et al.* 2010). In our study, at the organic farm from which the *A. suum* were obtained, feed contained a mixture of herbs such as garlic (*Allium sativum*), pumpkin (*Cucurbita pepo*), thyme (*Thymus vulgaris*), mugwort (*Artemisia vulgaris*), and fennel (*Foeniculum vulgare*). These plant species show different levels of antagonistic activity against nematodes (COSTA *et al.* 2008; EL SHENAWY *et al.* 2008; BURKE *et al.* 2009; FEITOSA *et al.* 2013; GIARRATANA *et al.* 2014). The presence of these herbal species in the host's diet was most likely the cause of the inhibition of the embryonic development of *A. suum* (to the same degree in the zygote stage), both in the control and experimental cultures.

We observed no morphological damage to the *A. suum* eggs which might suggest a biochemical basis for the antagonistic effects of the tested fungal strains. Lipases and proteases produced by

fungi may be involved in cell membrane degradation (AHMAD *et al.* 2006). In our study, the delayed embryonic development of *A. suum* may also have been caused by the secretion of mycoenzymes. Among the tested peptide hydrolases (leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, and chymotrypsin), only leucine arylamidase showed weak activity in the test fungal strains except *F. verticillioides*. Valine arylamidase activity was detected only in *F. sulphureum*. The API-ZYM test makes it possible to determine the activity of only a few select proteolytic enzymes, which in this study showed low or no activity. This observation does not exclude the participation of other proteolytic enzymes in the degradation of *A. suum* egg shells, as fungal species from genera *Fusarium* and *Penicillium* have been reported to exhibit proteolytic activity (BARATA *et al.* 2002; PEKKARINEN *et al.* 2000; DAHOT 2001).

Compared to the low activity of arylamidases, we observed high activity of N-acetyl-β-glucosoaminidase (NAG), a high molecular-weight hydrolytic lysosomal enzyme which breaks chemical bonds in the glycosides and amino sugars that form structural components in many tissues. This enzyme is necessary for the degradation and disposal of various parts of the cell, including the cell membrane, and its high activity in this study suggests that it may be involved in *A. suum* egg degradation (PUSZTAHELYI & PÓCSI 2014). High activity of alkaline and acid phosphatases was also observed in the tested fungal strains, except in *F. verticillioides*. These enzymes are involved in catalyzing the dephosphorylation of various phosphate esters, which may suggest that, together with other enzymes, they may exert an inhibitory effect on the embryonic development of *A. suum*.

LÝSEK and KRAJCI (1987) found that proteases and chitinases are involved in lysing the chitin-protein complex of the chitinous envelope layer of *A. lumbricoides* eggs. These enzymes, considering the high activity shown in the API ZYM test, may have participated in the ovistatic effect of the tested fungi on *A. suum* eggs, the shells of which are composed of chitin and a mixture of glycosides knowns as ascarosides. Exudation of some enzymes may cause degradation of diol ascarosides and acetylated aglycone that constitue 70% of ascarosides in the layers of *A. suum* egg shells, and thus increase their permeability (KUŹNA-GRYGIEL *et al.* 2001). The important role of mycoenzymes (lipases and proteases) in the destruction of eggs has been demonstrated previously in phytopathogenic nematodes whose egg shells are composed of protein, chitinous, and lipid layers (MOOSAVI & ZARE 2011). In our study, the lipolytic activity of the tested fungal strains may have caused changes in the permeability of the cell membranes of the developing embryos and interfered with the course of the embryogenesis of *A. suum* eggs.

Fungi are known to produce other metabolites such as allergens, mycotoxins, antibiotics, glucans, and volatile organic compounds (VOCs). In the present study all fungi belong to species known to produce toxic metabolites (WEIDENBÖRNER 2001). Many fungal strains produce mycotoxins, such as patulin produced by *P. expansum* (ANDERSEN *et al.* 2004), moniliformine by *F. oxysporum*, and fumonisin by *F. verticillioides* (COVARELLI *et al.* 2012). Some important secondary metabolites of the genus *Penicillium* are mycotoxins (such as patulin and griseofulvin) and antobiotics (PERRONE & SUSCA 2017), which may affect the growth and development of other organisms. A single fungus strain can produce several different mycotoxins and one mycotoxin can be produced by many different fungi.

Our results indicate that the degree of antagonistic interactions between soil fungi and *A. suum* eggs may depend on the metabolic specificity of strains within the species. Therefore, possible use of saprotrophic soil fungi in the reduction of dispersive stages of nematodes should be preceded by interdisciplinary studies of the biochemical and cytotoxic activity of individual strains.

Author Contributions

Research concept and design: L.K., K.M.-Z.; Collection and/or assembly of data: L.K., K.M.-Z., M.T., J.G., Ł.Ł., A.R., E.D., B.P.; Data analysis and interpretation: L.K., K.M.-Z. Ł.Ł.; Writing the article: L.K., K.M.-Z. Ł.Ł.; Critical revision of the article: L.K., K.M.-Z. Ł.Ł.; Final approval of article: L.K., K.M.-Z., Ł.Ł.

Conflict of Interest

The authors declare no conflict of interest.

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