

Original papers

Comparison of the effect of the chosen species of saprotrophic fungi on the development of *Toxocara canis* and *Ascaris suum* eggs

Kinga Mazurkiewicz-Zapałowicz¹, Magdalena Jaborowska-Jarmoluk², Lidia Kołodziejczyk³, Wanda Kuźna-Grygiel

¹Department of Hydrobiology, Ichthyology and Biotechnology of Reproduction, West Pomeranian University of Technology, K. Królewicza 4, 71-550 Szczecin

²Laboratory of Epidemiology, Voivodship Sanitary-Epidemiological Station, Spedytorska 6/7, 70-632 Szczecin

³Chair and Department of Biology and Medical Parasitology, Pomeranian Medical University, Powstańców Wlkp. 72, 70-111 Szczecin; Poland

Corresponding author: Kinga Mazurkiewicz-Zapałowicz; e-mail: kmazurkiewicz@zut.edu.pl

ABSTRACT. The study aim was to compare the antagonistic interaction between saprotrophic soil fungi and embryonic development of geohelminths *Toxocara canis* and *Ascaris suum*. The experimental cultures were fertilized eggs of *T. canis* and *A. suum* incubated together with mycelium of strains: *Fusarium culmorum*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, *Trichoderma viride* and *Trichothecium roseum*. In the control cultures the eggs of both nematode species were incubated without fungi. The experiment was conducted at temp. 26°C for 60 days. Compared with the control, all of the tested species of fungi significantly extended the embryonic development of both *T. canis* and *A. suum*. Most inhibitory effect on the rate of embryonic development of *T. canis* and *A. suum* had three fungal species: *P. fumosoreus*, *M. anisopliae* and *T. viride*. Compared with the control, on the 60th day of incubation in the presence of each of the tested fungal species, a larger percentage ($p < 0.05$) of morphological abnormalities was stated in developing embryos of *T. canis* (49–69%) than in *A. suum* (15.1–67.7%). Among the examined fungal species, only incubation with *P. fumosoroseus* resulted in significantly greater ($p < 0.05$) incidence of embryonic malformations (embryopathies) in *T. canis*, as compared with *A. suum*. Also the percentage of dead larvae of *T. canis* in the control and in cultures with fungi (12% and 100%, respectively) was significantly higher in comparison with *A. suum* (0.5% and 10.3–36%, respectively). The highest percentage of non-viable larvae of *A. suum* was found in the presence of *P. fumosoroseus*, and the lowest in the presence of *M. anisopliae*. Findings may indicate that *T. canis* eggs are more sensitive to antagonistic interaction of the examined fungal strains than *A. suum* eggs.

Key words: *Toxocara canis* eggs, saprotrophic fungi, antagonistic interaction

Introduction

Soil contamination with eggs of geohelminth parasites, mainly *Ascaris lumbricoides*, *Ascaris suum*, *Toxocara canis*, *Toxocara cati* and *Trichuris trichiura*, poses a serious threat to public health [1,2]. Eliminating eggs of these worms from the soil is a serious and still unresolved epidemiological problem. Since the use of ovicidal chemical and physical means is associated with side effects detrimental to the soil ecosystem homeostasis, it is necessary to search for biotic factors in order to

reduce the population of parasitic geohelminths [3,4].

In the light of existing research, the role of such bioregulators can be performed saprotrophic fungi coexisting in the soil with developmental forms of parasites [5,6]. On the one hand, the fungi mineralize dead organic matter, and on the other – they may interact antagonistically with organisms of soil biocenosis [7]. The effect of such interspecies interaction depends on many abiotic and biotic factors [8].

The aim of the present work was to determine the impact of selected species of soil fungi on the development of *Toxocara canis* eggs, compared to our previously obtained results for antagonism of the same fungal strains in relation to *Ascaris suum* [9–11].

Materials and Methods

Mature females of *Toxocara canis* were obtained from de-wormed puppies from the Shelter for the Homeless Animals in Szczecin. Fertilized eggs were obtained from final uterine parts and placed in phosphate buffered saline (PBS). In the suspension prepared for the experiment there were approx. 10^4 eggs /ml.

The experiment was conducted with five species of fungi isolated from the soil: *Fusarium culmorum* (W.G.Sm.) Sacc., *Metarhizium anisopliae* (Metschn.) Sorokin, *Paecilomyces fumosoroseus* (Wize) A.H.S.Br&G.Sm. (present synonym *Isaria fumosorosea* Wize), *Trichoderma viride* (Pers.) and *Trichothecium roseum* (Pers.) Link. Strains of these fungi were grown in standard CDA medium at $24\pm 2^\circ\text{C}$ for 21 days. 40 mm diameter mycelial discs cut out of the tested species were placed in 50 mm diameter Petri dishes. *T. canis* egg suspension (10 μl) was added to each culture. For each fungal strain the experiment was carried out in triplicate. For each culture group the mean values of the obtained data were estimated. Control cultures of *T. canis*

eggs were conducted in PBS without fungi. Experimental and control cultures were carried out for 60 days at 26°C .

Samples of egg suspension (10 μl) were collected for microscopic observation and their developmental stage was determined. The day of incubation, in which all eggs in respective cultures were at the stage of larvae, was recorded. This day was considered to be the end of embryonic development. On the 60th day of the experiment the percentage of embryos with morphological abnormalities and dead larvae was assessed. Iodine staining was used for evaluation of eggs viability [12].

Evaluation of the significance of differences between frequencies was made using the chi-squared test for independence or the chi-squared test for independence with Yates correction. Differences were deemed statistically significant at $p < 0.05$.

Results

The present and previous studies [9–11] revealed that all tested species of fungi significantly extend the embryonic development of both *T. canis* and *A. suum*. Among the studied fungal species most inhibitory effect on the rate of development of *T. canis* and *A. suum* eggs was exerted by *P. fumosoroseus*, *M. anisopliae* and *T. viride* (Table 1).

Compared with the control, incubation of *T. canis* eggs with *P. fumosoroseus* extended embryogenesis by 42 days, and of *A. suum* by about 34

Table 1. A comparison of the duration of development, percentage of deformed embryos and dead larvae of *Toxocara canis* and *Ascaris suum* in the control and experimental fungi cultures

	Development in days		Deformed embryos at 60 day of incubation [%]		Dead larvae at 60 day of incubation [%]	
	<i>Toxocara canis</i>	<i>Ascaris suum</i> ^A	<i>Toxocara canis</i>	<i>Ascaris suum</i> ^A	<i>Toxocara canis</i>	<i>Ascaris suum</i> ^A
CONTROL	16	15	8	5	12 a	0,5
<i>Fusarium culmorum</i>	50 *	28*	58*	54*	100* a	13*
<i>Metarhizium anisopliae</i>	57*	43*	49*	40*	100* a	10*
<i>Paecilomyces fumosoroseus</i>	58*	49*	54* a	15*	100* a	36*
<i>Trichoderma viride</i>	55*	36*	69*	68*	100* a	13*
<i>Trichothecium roseum</i>	48*	29*	55*	50*	100* a	19*

Explanations: ^A results published in papers: [9–11]; * significantly different from control ($p < 0.05$); a significantly different from *Ascaris suum* ($p < 0.05$)

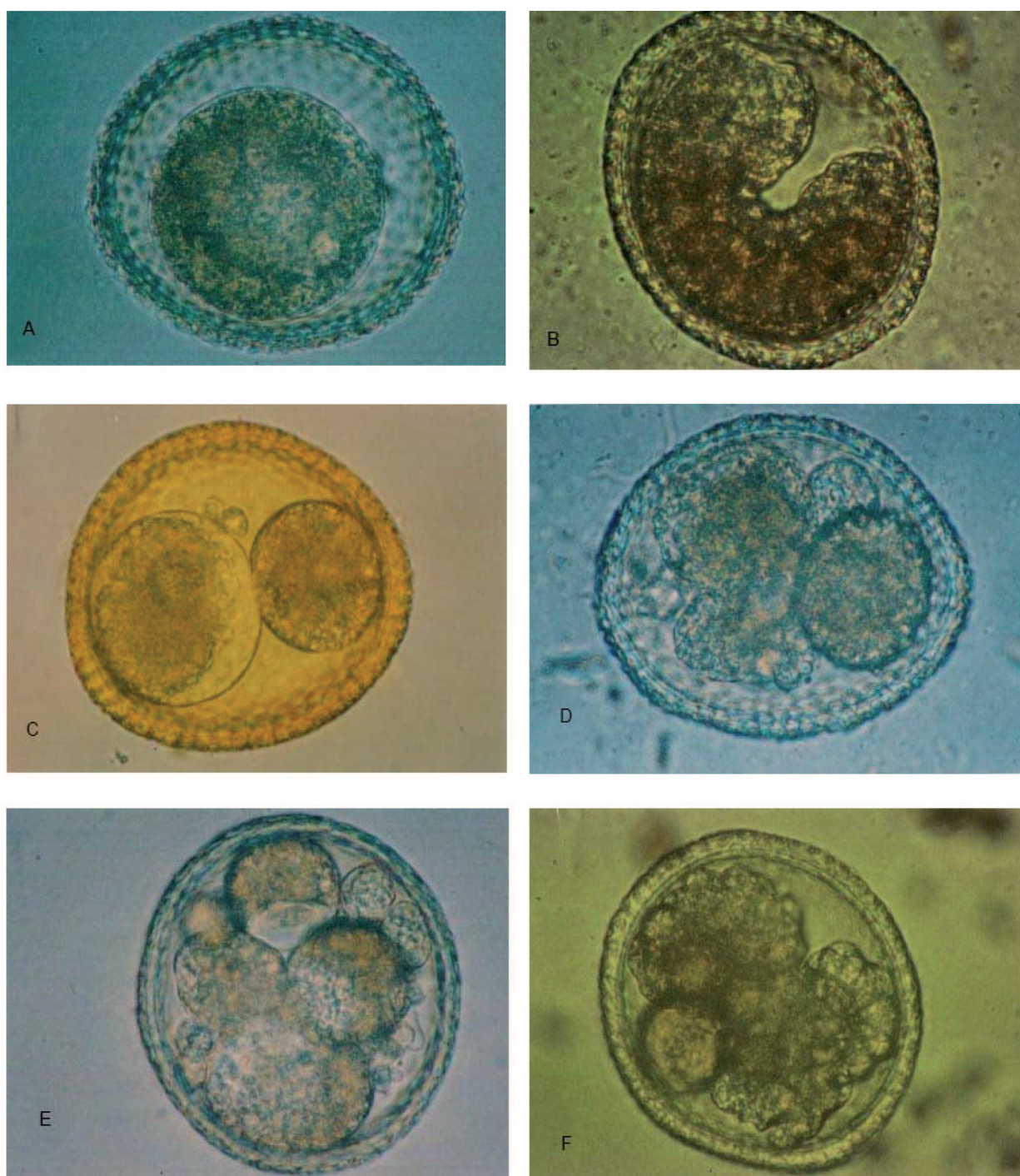


Fig. 1. Eggs of *Toxocara canis* in the course of the respective stages of embryogenesis in the control and experimental cultures with fungi. A – zygote stage from the control culture; B – gastrula stage from the control culture; C–E – blastula stage with blastomere of unequal size from experimental cultures with: C – *Trichothecium roseum*, D – *Fusarium culmorum*, E – *Metarhizium anisopliae*; F – deformed gastrula in *Toxocara canis* egg incubated with *Trichoderma viride* (1000×)

days. Similarly, the inhibitory effect was observed in experimental cultures in the presence of *M. anisopliae*, in which extension of embryogenesis for *T. canis* amounted to 41 days and for *A. suum* 28 days. However, in the presence of *T. viride*, the embryogenesis of *T. canis* was delayed by 39 days, and of *A. suum* by 21 days. The other two species of

fungi (*T. roseum* and *F. culmorum*) slowed embryogenesis of *T. canis* from 34 to 32 days, in the case of *A. suum* from 13 to 14 days. These differences were statistically significant when compared with the control (Table 1). However, no statistically significant differences were found between the duration of embryogenesis of *T. canis*

and *A. suum* in the presence of each of the examined fungi.

In control cultures of *T. canis* no morphological abnormalities were observed in the course of embryogenesis (Fig. 1A,B). In *T. canis* eggs co-cultured with fungi blastomeres significantly differed in size (Fig. 1C–E), and transparent granules of various sizes frequently occurred in the vicinity of dividing blastomeres (Fig. 1C). However, gastrulae in eggs incubated with fungi were deformed, often with indistinct cell structure (Fig. 1F). Under both the light and scanning microscopy, no hyphal penetration through the eggshells in *A. suum* eggs was observed, while in *T. canis* similar results were obtained only in light microscopy observations.

Microscopic observation of eggs on day 60 post-incubation with the tested fungal species showed diverse proportion of morphological abnormalities in embryos of *Toxocara canis* (49–69%) and *Ascaris suum* (15.1–67.7%). In both species of nematodes the highest percentage of deformed embryos (over 50%) was found in the presence of *T. viride*, *F. culmorum* and *T. roseum*. Although in experimental cultures with all strains of fungi the percentage of *T. canis* abnormal embryos was higher than of *A. suum*, statistically significant differences ($p < 0.05$) were found only in co-cultures with *P. fumosoreus*. In the presence of this fungus the percentage of deformed embryos of *T. canis* was three times higher in comparison with *A. suum* (Table 1).

In the control cultures on day 60 after incubation dead larvae of *T. canis* constituted 12% and of *A. suum* only 0.5%. In the experimental cultures with fungi the percentage of non-viable larvae of *A. suum* ranged from 10.3 (in the presence of *M. anisopliae*) to 36% (in the presence of *P. fumosoreus*), while the incubation of *T. canis* eggs with all the tested fungi resulted in 100% mortality of larvae. Compared with the control, the differences between the percentage of dead larvae of both *T. canis* and *A. suum* in experimental fungal cultures were statistically significant. Also the comparison of percentages of dead larvae of both nematode species in the presence of all the examined fungi showed statistically significant differences ($p < 0.05$) (Table 1).

Discussion

The results of our study, and of other authors, confirm that some species of saprotrophic soil fungi

inhibit the embryonic development of geohelminths. Delaying of *A. suum* embryonic development was observed under the influence of *Penicillium frequentas* and *Stachybotrys chartarum* [11,13], as well as *Fusarium* sp. [14], *Fusarium culmorum*, and *Trichothecium roseum* [9]. Błaszowska et al. [7] demonstrated a very high inhibitory activity of *Aspergillus terreus*, *Penicillium expansum* and *Fusarium oxysporum* on the development of *A. suum* eggs, with weaker activity of *Penicillium citrinum*, *P. fumigatus* and *Trichothecium roseum*.

Also research on the effect of fungi on the development of *T. canis* eggs indicates their diverse inhibitory activity. A significant impact on *T. canis* embryopathy was found in the presence of *Fusarium pallidoroseum*, compared with the influence of *Mucor hiemalis* [15]. A very high and high ovicidal activity on *T. canis* eggs was also demonstrated by strains: *Chrysosporium merdarium*, *Fusarium oxysporum*, *F. sulphureum* [16] and *Trichoderma viride*, *Fusarium solani*, *Acremonium* sp. [17].

The degree of antagonistic activity of fungi on *T. canis* and *A. suum* eggs is not identical. This is evidenced by the results of the present study in which all tested strains of fungi caused after 60 days of incubation high percentage of eggs with embryo deformities, but the degree of their teratogenic effect on both the geohelminths varied. Most *T. canis* and *A. suum* embryopathies were induced by *T. viride* (about 70% of eggs), while significant differences between the percentages of deformed embryos of *T. canis* and *A. suum* were found only during incubation with *Paecilomyces fumosoreus*. Fungi of the genus *Paecilomyces*, owing to their strong antagonistic interaction, belong to microorganisms used in biological plant protection for controlling numerous crop pests [8,18,19]. Fungal strains of this genus were also tested in interaction with *T. canis* eggs [20]. Studies demonstrated higher ovicidal activity of *P. lilacinus* than of *Paecilomyces marquandii*, which was observed as early as 2 weeks after incubation with these fungal strains. At that time, in the presence of *P. lilacinus*, more than 80% of *T. canis* eggs were destroyed, while *P. marquandii* caused death of only about 23% of eggs. Also the results obtained in our research seem to indicate that another species of the genus *Paecilomyces*, i.e. *P. fumosoreus*, may also be effective in inhibition of *T. canis* development, since it induced deformations in more than 50% of the

nematode embryos. *P. fumosoroseus* caused the highest percentage of dead larvae of both nematodes. However, it is difficult to explain significantly weaker inhibitory effect of this strain on *A. suum* eggs, in which morphological abnormalities were found only in 15% of the embryos.

Nematode eggs shells consist of an outer protein layer, a middle chitinous layer and an inner lipid layer. The lipid layer, which may also have a very diverse structure, plays the most important role in protecting the embryo [12,21]. It can be assumed that even subtle differences in the structure of eggshells may select permeation of metabolites that are typical for each species, and even fungal strains. The specific nature of nematode-fungus interaction is dependent on many factors, which is evidenced in research by, among other things, 100% mortality of *T. canis* larvae in the presence of all the examined fungi, with a much lower percentage of mortality of *A. suum* larvae.

A common feature of nematode-fungus interaction in our research is the lack of fungal hyphae penetration inside the eggs of the examined geohelminths. This fact may suggest a similar mechanism of inhibition of nematode egg development based on the activity of fungal metabolites and their penetration of the eggshells. Transparent granules of different sizes observed inside eggs may be indicative of such mechanism of interaction. Kołodziejczyk et al. [22] observed similar granules in the *Ascaris* eggs cultured in ochratoxin A solution.

Morphological disorders of developing nematode embryos and associated retardation of their embryogenesis may be the result of impaired biosynthesis of proteins following reduction in enzyme activity under the influence of fungal metabolites. It has been shown that metabolites of mold fungi, such as aflatoxins, ochratoxins and destruxins, inhibit replication, transcription and translation [23–27]. Consequently, this may lead to deregulation of the cell cycle, abnormal cell division and slower embryonic development.

Our findings indicate that species of studied geohelminths differ in their sensitivity to fungi used in the tests. *T. canis* eggs were more sensitive to the effects of the tested fungi in comparison with *A. suum*. *Toxocara canis* differed from *A. suum* not only in extended pace of development, but also in higher percentage of deformed embryos, especially co-cultured with *P. fumosoroseus*. The results to

date do not explain in full the reasons for greater sensitivity of *T. canis* eggs to the activity of the examined fungi. The explanation of such differences in sensitivity of geohelminths requires further biochemical and cytological research.

Diverse effects of the same saprotrophic soil fungi on the rate of embryonic development of different geohelminth species, induction of developmental abnormalities and the survival rate of their invasive larvae confirm complexity of interactions between various components of soil biocenosis and point to the need for continuation of comprehensive and interdisciplinary research.

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Received 1 July 2014

Accepted 25 August 2014