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Effect of some saprotrophic soil fungi on the embryonic development of *Ascaris suum* (*Nematoda*)

WANDA KUŹNA-GRYGIEL [1], LIDIA KOŁODZIEJCZYK [1], KRYSTYNA JANOWICZ [2], and KINGA MAZURKIEWICZ-ZAPAŁOWICZ [3]

[1] Chair of Biology and Medical Parasitology, Pomeranian Academy of Medicine in Szczecin
Powstańców Wielkopolskich 72, PL-71-110 Szczecin, Poland
e-mail: kuzgryg@sci.pam.szczecin.pl
[2] Chair of Applied Entomology, Agricultural Academy in Szczecin
Słowackiego 17, PL-71-434 Szczecin, Poland
[3] Chair of Zoology, Agricultural Academy in Szczecin
Judyma 20, PL-71-466 Szczecin, Poland

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Effect of *Penicillium frequentans* and *Stachybotrys chartarum* fungi on the embryonic development of *Ascaris suum* were studied in the present paper. In eggs that were incubated with fungi, significant delay of initiation of zygote division, as well as retardation of the development of individual stages of embryogenesis, was given a closer insight. Additionally, the following phenomena were observed: vacuolisation of zygote and disturbances in the distribution of yolk, non-synchronous and unequal divisions of blastomere, deformations of the blastula, gastrula, and larval stages. The above changes were more distinct in eggs that were incubated with *P. frequentans*. In the cultures with *P. frequentans*, a significantly lower number of larvae, as well as their earlier mortality were observed.

Key words: soil fungi, embryogenesis, *Ascaris suum*.

INTRODUCTION

Soil plays an important role in the epidemiology of soil-born parasitic diseases, both human and animal as well. Species of the genus *Ascaris*: *A. lumbricoides* (parasite of human) and *A. suum* (parasite of swine) are nematodes of which embryonic development until the stage of the infective larva proceeds in the soil. Eggs of these nematodes are introduced to the soil with the cesspool or manure. They retain their infective properties for as many as 15 years (Krasnonos 1978).

A. suum constitutes a problems predominately in veterinary parasitology, although humans can also become accidental hosts. In the latter case, however, the parasite reaches only the stage of larva migrans.

Research on limiting the number of infective eggs of geohelminths in the soil by the inhibition of their embryonic development has been carried out for many years. Effect of numerous physical and chemical factors on the development and viability of *Ascaris* sp. eggs has been relatively well investigated (Grzyb and Szydłowska 1964; Mizgajska 1993; Szkudliński 1998). Chemical substances, however, like pesticides, destroy the other elements of the soil biocenosis sooner than they can destroy the eggs of geohelminths (Lysek 1979).

As early as in the 1960s efforts were made to use soil organisms in the biological control of geohelminths. The most significant role in limiting the number of nematode eggs, among others also *Ascaris*, has been attributed to saprotrophic fungi that are common in the ecosystem (Lysek 1967; Jarnicka-Stanios and Wawrzkiewicz 1974; Lysek 1979; Mizgajska 1994). The studies carried out by Lysek (1963, 1967, 1979, 1982), and also by Jarnicka-Stanios and Wawrzkiewicz (1974) showed different degrees of destructive effect of ovicidal fungi on eggs of geohelminths.

A study on the effect of saprotrophic soil fungi and their metabolites on *Globodera rostochiensis*, a nematode parasitic on crops showed that *Penicillium frequentans* and *Stachybotrys chartarum* significantly diminished the viability of larvae of the above nematode (Mazurkiewicz-Zapałowicz et al. 1999).

The aim of the present study was to determine the effect of *P. frequentans* and *S. chartarum* on the course and pace of the embryonic development of *Ascaris suum*.

MATERIALS AND METHODS

Adult females of *Ascaris suum* were collected from the Municipal Slaughterhouse at Szczecin. Fertilised eggs were dissected from the terminal portion of the nematode uterus.

Saprotrophic fungi – *Penicillium frequentans* and *Stachybotrys chartarum* – were isolated from the soil and cultured on the standard PDA-Difco medium, at 25°C.

A. suum eggs were embryonated in four cultures in Petri dishes, in physiological solution (0.9% NaCl), at 26°C. One of them without fungi (served as control), and the three experimental ones with fragments of mycelium (ca 130 mm) from 21-day-old cultures. Mycelium of *Penicillium frequentans* was placed in the experimental culture I, mycelium of *Stachybotrys chartarum* was placed in experimental culture II, and mycelia of both fungi species were placed in culture III. The surface area of each mycelium amounted to 65 mm^2.

The cultures were grown at a constant humidity and the constant temperature of 26°C.

The embryonic development of eggs from control- and experimental cultures were monitored daily, throughout 70 days of the experiment, in the evaluation of the ovicidal or ovistatic effect of the fungi studied: changes in the distribution of yolk, vacuolisation and granulation of cells of embryo, morphological disorders in the structures of zygote, blastomeres, morula, blastula, gastrula, and larvae, damages to the egg shells, mycelium penetration into the eggs.

RESULTS

The duration of cleavage, gastrulation and organogenesis of *Ascaris suum*, in control and experimental eggs is described in Table 1.

In all experimental cultures development of the majority of eggs was delayed in comparison to the control, at the stage of zygote. In eggs incubated with *P. frequentans*, this stage was observed up to 25th day. In three experimental cultures, the period of cleavage (from 2 blastomeres up to blastula) was significantly prolonged. In eggs incubated with *P. frequentans* embryos, with 2 and with 4 blastomeres were observed as late as on day 25 of the culture. From day 26 on, the pace of divisions intensified. The majority of eggs in the cleavage phase (ca 60%), mostly the morula and blastula stages, were observed within the period of 29–31 days.

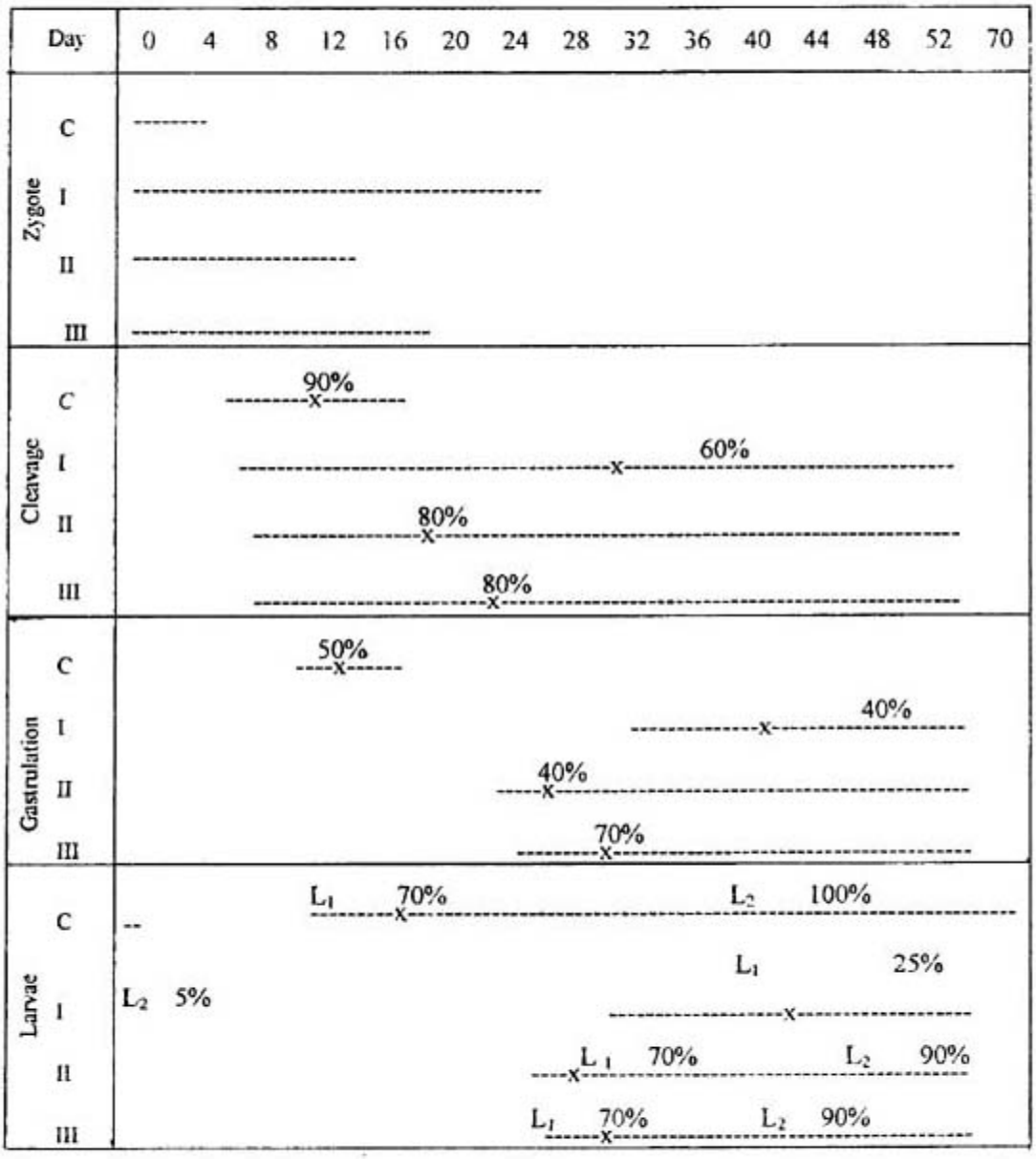
Also in the culture with *S. chartarum*, the first divisions of zygote were delayed, although not as much as in culture I. Up to day 12, only 2-blastomere embryos were observed, while later on – within 17–19 days of the experiment as many as 80% of eggs were at the stage of morula or blastula. Similar results were obtained during observation of the cleavage process in eggs that were incubated with both species of fungi at the same time. Cleavage in eggs of control group was completed not later than day 11, whereas zygote divisions in cultures with fungi in 10% of eggs were observed as late as on day 50.

In control eggs, all individual developmental stages were normal (Figs 1–4). Abnormalities were observed at the cleavage stage of eggs incubated with fungi, most of them – in the yolk distribution and in the size of blastomeres.

In the majority of zygotes, the polar and equatorial yolk aggregation and vacuolisation of the protoplast (Fig. 5) were initially observed, however, distribution of the yolk in most eggs came to normal before the division. In initial cleavage divisions, also multinuclear blastomeres were observed (Fig. 6). Some of embryons (from 2 blastomeres to morula) consisted of blastomeres of different size (Fig. 7). Most of eggs with this kind of morphological disorders were found in the culture with *P. frequentans* (ca 30%), and ca 10% – in the other experimental cultures.

Table 1

Effect of *Penicillium frequentans* and *Stachybotrys chartarum* on embryonic development of *Ascaris suum*



Explanations: x – highest percentage of *Ascaris suum* eggs in a given developmental stage; C – control culture of *Ascaris suum* eggs; I – experimental culture of *Ascaris suum* eggs with *Penicillium frequentans*; II – experimental culture of *Ascaris suum* eggs with *Stachybotrys chartarum*; III – experimental culture of *Ascaris suum* eggs with *Penicillium frequentans* and *Stachybotrys chartarum*

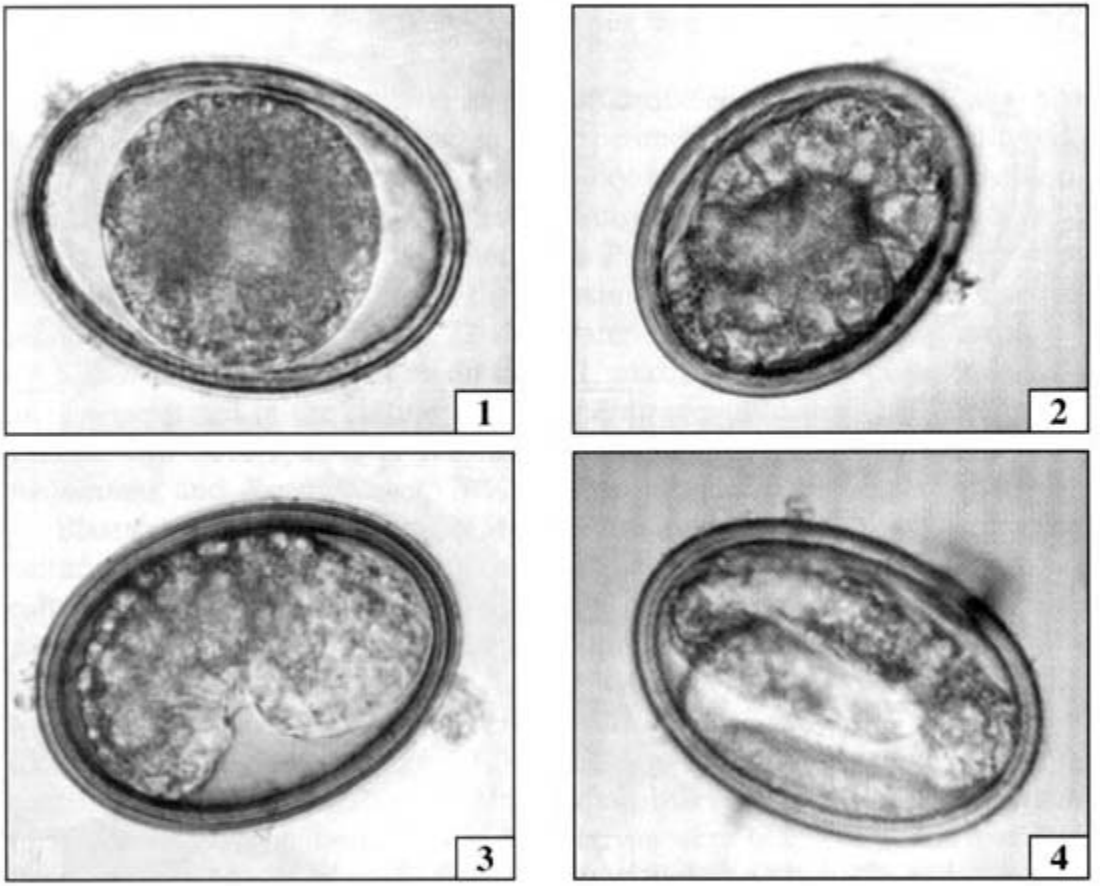


PLATE I

Fig. 1. Egg of *Ascaris suum* at the zygote stage from control culture. × 1000
Fig. 2. Egg of *Ascaris suum* at the blastula stage from control culture. × 1000
Fig. 3. Egg of *Ascaris suum* at the gastrula stage from control culture. × 1000
Fig. 4. Egg of *Ascaris suum* containing L_2 larva from control culture. × 1000

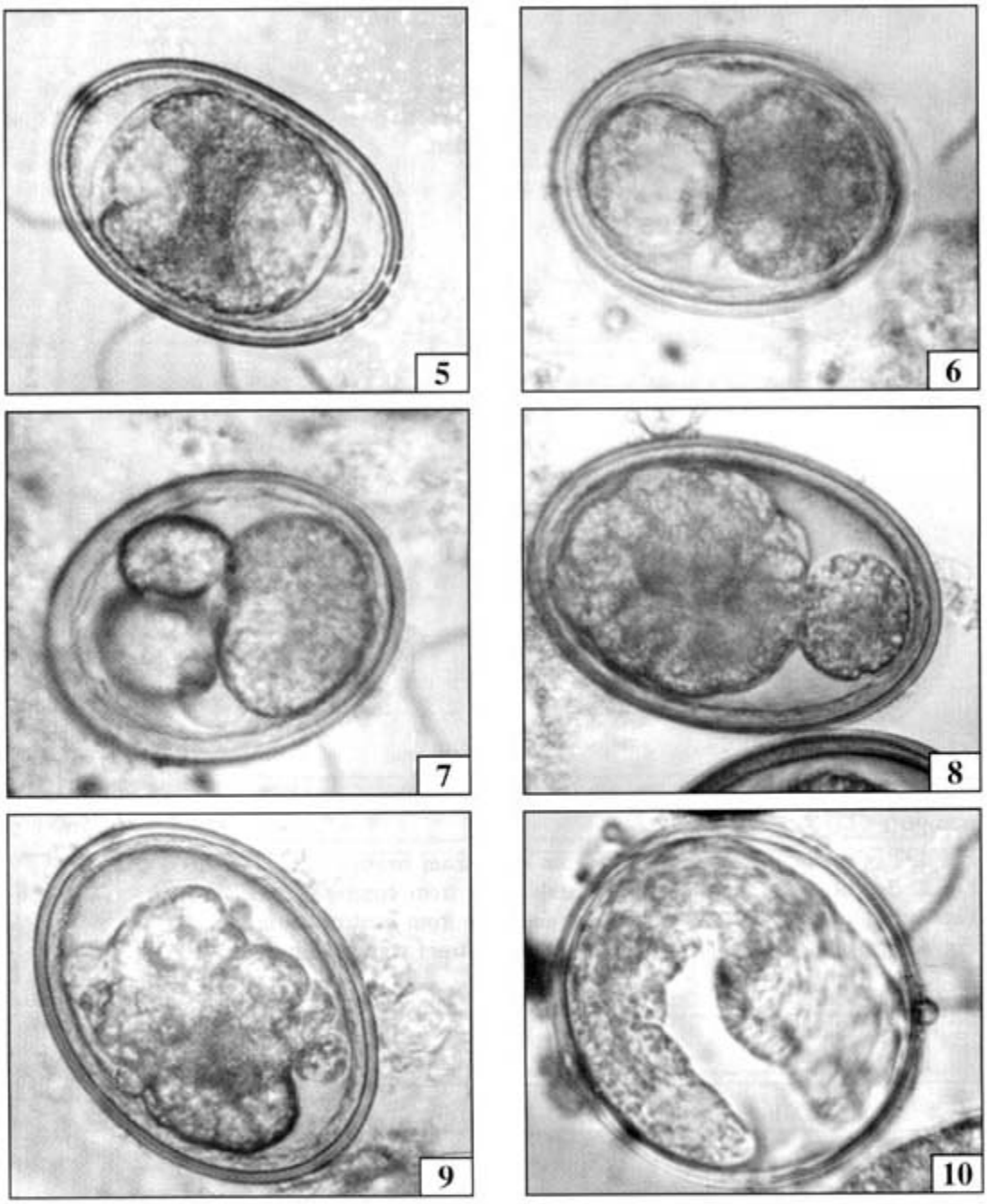


PLATE II

Fig. 5. Aggregation of yolk and vacuolisation of the zygote in *Ascaris suum* egg incubated with *Penicillium frequentans*. × 1000

Fig. 6. Blastomere with a big vacuole and multinuclear blastomere in *Ascaris suum* egg incubated with *Penicillium frequentans*. × 1000

Fig. 7. Unequal divisions of blastomeres in *Ascaris suum* egg incubated with *Stachybotrys chartarum*. × 1000

Fig. 8. Blastula and non-divided blastomere in *Ascaris suum* egg incubated with *Stachybotrys chartarum*. × 1000

Fig. 9. Deformation of gastrula in *Ascaris suum* egg incubated with *Penicillium frequentans* and *Stachybotrys chartarum*. × 1000

Fig. 10. Deformed larva in *Ascaris suum* egg incubated with *Penicillium frequentans*. × 1000

In comparison with control, also significant delay and elongation of the gastrulation process was observed in the experimental cultures. This embryonic stage was observed in control eggs from day 11 to day 16 of the cultivation. The highest percentage of eggs in gastrula stage (ca 60%) were stated on day 14 of the experiment. In eggs incubated with *P. frequentans*, first gastrulae were observed as late as on day 28 and their maximum number (ca 40%) were stated as late as on day 36, that is, 22 days later as compared with control.

Significantly earlier, that is on day 21, maximal number of gastrulae (ca 40%) were stated in the culture with *S. chartarum*, and somewhat later in the culture with both species of the fungi. On 26th day of incubation with *P. frequentans* and *S. chartarum*, 70% of eggs attained the stage of gastrula.

Blastula and gastrula deformations were observed in all experimental cultures (Fig. 8). The above changes appeared however, more often in eggs from cultures with *P. frequentans*. In many eggs, blastula or gastrula were accompanied by blastomeres arrested in development (Fig. 9).

In experimental cultures, as compared with control, the larval appearance was delayed 19 days in eggs incubated with *P. frequentans*, 13 days in eggs incubated with *S. chartarum*, and 17 days in eggs incubated with both fungi. In control, the highest percentage of larvae was observed on 11 and it amounted to 70%, whereas not more than 25% of larvae were observed on day 40 and their number diminished in the following days down to 8% in the experimental culture I.

In cultures II and III, the development inhibition did not affect significantly the number of larvae, because, similarly with the control, the highest percentage of L_1 larvae amounted to 70%, and until day 50—90% of eggs contain of L_2 larvae.

In all experimental cultures, disorders in the development of larvae were stated. Most of larvae in the culture with *P. frequentans* and about 5% of them in the remaining experimental cultures had deformations in their structures. Deformed larvae were most often short and with irregular thickness (Fig. 10). Also partial development of gastrula to the partial larva was observed. All the larvae with irregular constitution were always motionless. In control, up to the end of the experiment, ca 90% of larvae were moving, and in all the experimental cultures, only single larvae showed the signs of viability on day 52 of experiment. All larvae, also those without visible morphological disturbances, were dead on day 72 of experiment.

No penetration of mycelium into eggs was observed throughout entire embryogenesis. No signs of destruction of egg shells were observed, either. Only in the eggs incubated with *P. frequentans*, various rates of degradation of dead larvae and destruction of egg shells were observed, starting on day 40 of culture.

DISCUSSION

Results of the present study indicate on inhibitory effect, of mycelia of *Penicilium frequentans* and *Stachybotrys chartarum*, on the embryonic development of *Ascaris suum* the influence of *P. frequentans* being definitely stronger. The above statement was justified by the fact that most of eggs incubated with *P. frequentans* remained at the stage of zygote for the longest period (up to the 25th day). Also stages of morula and blastula persisted the longest time in most of eggs in the culture with the above fungus, that is up to day 29, and the amount of eggs in the final cleavage stage was lower by 30 percentage points in comparison with control, and by 20 percentage points in comparison with the remaining experimental cultures. The slowest development pace of gastrula and L_1 larva was observed also in the culture with *P. frequentans*. The highest number of eggs with L_1 larvae amounted to as little as 25%. By the end of the experiment that is on day 52 only ca 5% of larvae showed the signs of viability.

L y s e k (1982) determined three stages of ovicidal effect of saprotrophic fungi to eggs of nematodes: 1 – without damage to the egg shells (ovistatic); 2 – with destruction of the egg shells only; 3 – with destruction of egg shells and penetration of mycelia of fungi into eggs.

Microscope observations carried out by the authors of the present study demonstrated only the ovostatic effect of both by *P. frequentans* and *S. chartarum* on the *Ascaris* eggs.

Significant morphological disorders in the developing embryos were stated, although degradation of egg-shells was not observed. The above fact indicates teratogenic properties of fungi secretions. The described disturbances in the developing eggs can be explained by penetration of metabolites secreted by the mycelia and showing properties of mycotoxins. As demonstrated by A r t h u r and S a n b o r n (1969), B a r r e t t (1976), as well as by C l a r k and P e r r y (1980) the nematode egg shells were the most resistant biological structures, permeable only to respiratory gases, water, and soluble lipids. The lipid layer (W h a r t o n 1980) plays the most important role in protecting the embryo. It is possible that the secretions of the studied fungi species have an affinity to lipids and therefore they can penetrate through egg shell. The observed vacuolisation of the zygote can be a result of accumulation of fungi secretions, and in turn, the vacuolisation can push the yolk to one of the poles, or to the equatorial plane. Certainly, an abnormal yolk distribution can have a decisive effect on the further blastomere divisions. As it is commonly known, the way of cleavage depends on the quantity and distribution of yolk in the egg. Yolk is distributed regularly in normal eggs of *Ascaris*. Certainly, translocation of yolk as a result of vacuolisation influences seriously the abnormalities in development, as early as during the first zygote division. In a number of eggs studied, already the first blastomeres differed significantly in size, and small ones contained no yolk at all, or they contained only very small quantities of yolk.

Probably, blastomeres without yolk did not undergo further divisions because single, non-divided blastomeres were observed along deformed blastulae and gastrulae.

Parallel study on the activity of oxidative enzymes carried out by Kołodziejczyk et al. (2001) showed that a delay in the development of eggs incubated with fungi could be caused by weakening of the respiratory processes which fact could suggest that mycotoxins were inhibitors of respiratory metabolism enzymes.

Enhanced toxic properties of *P. frequentans* as compared with toxic properties of *S. chartarum* were confirmed also by the low rate of eggs, (25%), that reached the L_1 larva stage exposed to mycelium of *P. frequentans*. Larvae of the next stage died out in the final period of the experiment, only 5% of larvae showed the symptoms of viability. In the culture with *S. chartarum*, as many as 90% of the larvae were viable in the same final stage of experiment.

The carried out experiment showed that both fungi species caused necrosis of all larvae in ca 70th day of incubation. All larvae were found to be dead on the above day, whereas most of larvae in control eggs, (95%), were still viable. Lethal effect of the fungi on larvae could be connected with the enhanced penetrability of egg shell before hatching. A number of studies proved that the permeability of egg shell before hatching was caused by decomposition of diol ascarosides and acetylated aglycone that composed 70% of total ascarosides present in the layers of *Ascaris* egg shell (Jezyk and Fairbairn 1967; Tarr and Fairbarn 1973; Tarr and Schnoes 1973). The mechanism of the above process was not clear. However, effect of chitinase was observed in the disolving of solution of the chitinous shell. Maximum chitinase activity occured in 20-day-old eggs (Ward and Fairbairn 1972). Considering the delay in egg development in experimental cultures, also the activity of chitinase could be increased in the observed eggs. As observations described in the present paper show, damages of egg shells were observed only in eggs with dead embryos, it could be assumed that the damages resulted from the embryo necrosis and not from the ovicidal effect of mycelium.

The stated weakening of the toxic effect of *P. frequentans* in the presence of *S. chartarum* indicate vaviability of properties of their metabolites. The results of the present study one consistent with the observations of Kunert and Lysek (1987), Kunert et al. (1987), and Kunert (1992). The above authors revealed the inter-specific and even intra-specific vaviability of ovicidal properties of saprophytic fungi.

It should be assumed that studies on toxic properties of fungi and their secretions should be carried out in a complex way, as properties of fungi can change depending on the complicated interrelations in the soil biocenosis.

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Wpływ wybranych saprotroficznych grzybów glebowych na rozwój embrionalny *Ascaris suum*

Streszczenie

Badano oddziaływanie grzybów *Penicillium frequentans* i *Stachybotrys chartarum* na rozwój zarodkowy *Ascaris suum*. W jajach inkubowanych z grzybami wykazano znaczne opóźnienie inicjacji podziału zygoty i spowolnienie rozwoju poszczególnych stadiów embriogenezy. Ponadto obserwowano wakuolizację zygoty i zaburzenia w rozmieszczeniu substancji żółtkowej, niesynchroniczne i nierównomierne podziały blastomerów, deformacje stadium blastuli, gastruli i larwy, a zmiany te wyraźniej manifestowały się w jajach inkubowanych z *P. frequentans*. W hodowli z *P. frequentans* stwierdzono znacznie mniejszy odsetek larw i wcześniejsze ich obumieranie. Badania wykazały, że toksyczne właściwości metabolitów *P. frequentans* są znacznie słabsze w obecności grzybni drugiego badanego gatunku *S. chartarum*.