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THE EFFECT OF MAGNETIC FIELD ON *IN VITRO* DEVELOPMENT OF FUNGUS-LIKE ORGANISMS *SAPROLEGNIA PARASITICA* ON SELECTED MICROBIOLOGICAL MEDIA

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ABSTRACT

The objective of the study was to determine the rate of growth and cytotoxicity of mycelium of fungus-like organisms *Saprolegnia parasitica* on media with variable form of available carbon and nitrogen (Sabouraud Dextrose Agar (SDA), Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Czapek-Dox Agar (CDA)) in magnetic field of 1, 5 and 10 mT. The growth rate of *S. parasitica* in magnetic field was most varied on SDA medium: the mycelium growth was the slowest in the field of 5 mT compared to the remaining experimental variants and to the control. On MEA and PDA media the growth rate did not vary among the tested field values and the control. The kind of medium and magnetic field had an effect on cytotoxicity of *S. parasitica* – on SDA medium the mycelium cytotoxicity was reduced to the greatest extent in magnetic field of 5 mT. The phenomenon can be used to increase the effectiveness of stocking material production.

Key words: *Saprolegnia parasitica*, fungus-like organisms, magnetic field, microbiological medium, cytotoxicity.

INTRODUCTION

The Earth's magnetic field is a constant component of biosphere; it protects organisms from harmful cosmic and solar radiation. Magnetic fields of different values have an effect on many vital functions of organisms [4, 9, 20, 46]. The effect may be reinforced due to magnetic and electromagnetic pollution of biosphere which is a still underestimated consequence of technical and technological progress. This effect is not indifferent for the functioning of practically any living organism [4, 9, 10, 34, 47]. The effect of the Earth's magnetic field on the behavior of organisms [22] of various taxa, from bacteria [23], fungi [33], invertebrates [21, 27, 28], through fishes [9, 16, 24, 32, 35, 36, 41] and birds [44, 46], to mammals has been demonstrated to date; their reactions are diverse and specific, they also depend on the stage of ontogenetic development [29], sex, condition etc.

Magnetic field has also an effect on the biology and reproduction of hydrobiots. Among those, fungi-like organisms (FLO), representing kingdom Chromista or Stramenopila, are very little known. In water bodies they play an important part in maintaining ecological equilibrium, since being chlorophyll-less and heterotrophic, they derive their carbon and mineral salts which are necessary for their development from hydrolysis of live or dead organic matter. This ability contributes to self-purification of waters, guarantees circulation of elements and limits the harmful effect of heavy metals which are accumulated in the hyphae [1]. Some aquatic fungus-like organisms, especially of the genus *Saprolegnia,* are also very serious pathogens which pose a great threat to fishes during the whole of their ontogeny [2, 3, 6, 7]. Though till recently it was believed that mass occurrence of aquatic parasitic fungus-like organisms was rare, and that they caused epidemics among their hosts only

sporadically, now they are increasingly often recognized as biological factors which determine the health condition of various developmental stages of fishes, especially in hatcheries [42]. Species of genus *Saprolegnia* isolated from diseased fishes are most often circumstantial parasites which infect fish only under favourable (or unfavourable) conditions, that is upon wounding, egg damage, or physiological weakening [39, 43]. The first destructive effect of FLO of the genus *Saprolegnia* on the Atlantic salmon *Salmo salar* was described in the 19th c. In Japanese fish farms *S. parasitica*, caused death of 50% of population of the Pacific salmon *Oncorhynchus kisutch* [17, 18]. There are known cases of complex saprolegniosis evoked by *S. parasitica* and *S. ferax*; it led to death of 40% of population of adult perch (*Perca fluviatilis*) in Swiss lakes [30].

Differences in the intensity of fish mycoses result from both the host's sensitivity and the pathogenicity of strains of *Saprolegnia*. The properties of *S. parasitica* depend on many biotic [8] and abiotic factors [25, 40, 42], among which the effect of magnetic field is the least known. Hence we undertook *in vitro* studies in order to determine the rate of growth and cytotoxicity of mycelium of *S. parasitica* on media of varied form of available carbon and nitrogen Sabouraud Dextrose Agar (SDA), Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Czapek-Dox Agar (CDA) in magnetic field of 1, 5 and 10 mT.

MATERIALS AND METHODS

Material. The strain of fungus-like organisms *Saprolegnia parasitica* used in the studies was obtained from infected eggs of the Baltic whitefish (*Coregonus lavaretus*) (Fig. 1). Initial cultures of *S. parasitica* were sown from sporangium onto PDA medium and incubated at 12–15°C, relative humidity 85–90%, during 21 days. Then the inoculum with mycelium of *S. parasitica* of 10 mm diameter was placed in the centre of Petri dish of 100 mm diameter, with one of the standard microbiological media: CDA (Czapek-Dox Agar), PDA (Potato Dextrose Agar), SDA=SAB (Sabouraud Dextrose Agar) produced by Scharlau, and MEA (Malt Extract Agar) produced by Merck, prepared according to the producers' recommendations.

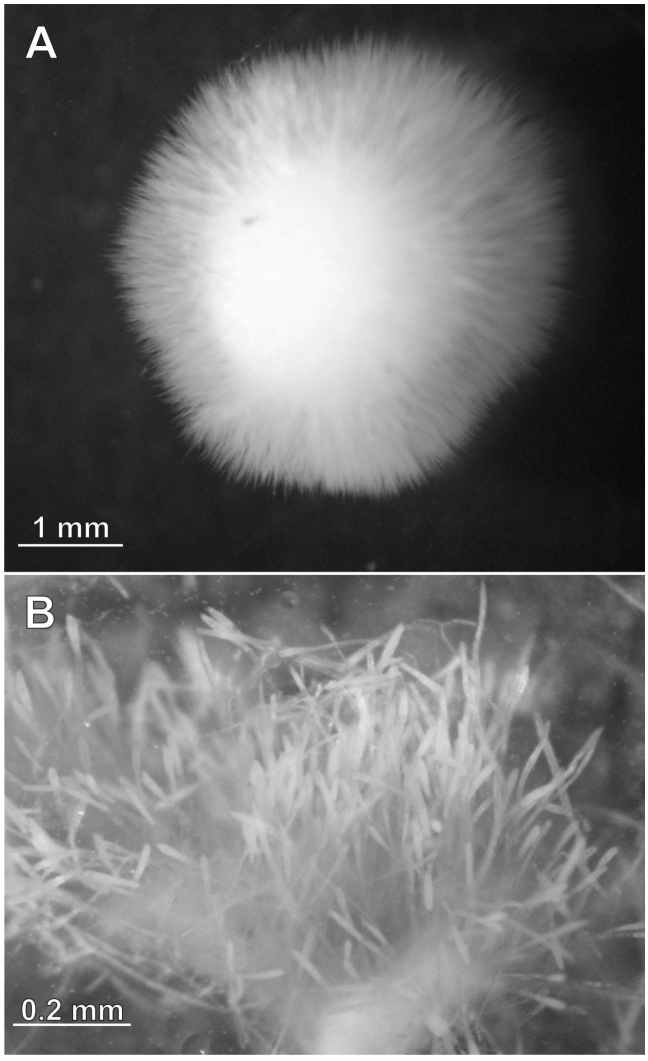


Fig. 1. Egg of Baltic whitefish (*Coregonus lavaretus*) parasitised by mycelium of *Saprolegnia parasitica* (A). Sporangia (B)

The cultures of *S. parasitica* were placed in magnetic field of 1, 5 and 10 mT and outside magnetic field (control). All the variants were within the natural geomagnetic field. The incubation conditions were adjusted to the optimum of development of mycelium of *S. parasitica* in the environment, hence the cultures were incubated at 8–10°C, relative humidity 90–95%. Mycelium cultures on each of the tested media and for each value of magnetic field (1, 5 and 10 mT) were run for 9 days. Each variant was done in five replicates.

Linear growth of mycelium was recorded every 2 days, by measuring the diameter of mycelium in the widest and narrowest place of its growth. For each variant the mean diameter of linear growth was calculated for each date of measurement. The growth rate on the tested microbiological media at different values of magnetic field was photographically documented on the 7th day of incubation.

Cytotoxicity of the strain of *S. parasitica* grown on media CDA, MDA, PDA and SDA under the effect of magnetic field of 1, 5 and 10 mT and in the control 1 (C1) was determined after 21 days of incubation in thermal and humidity conditions identical with those adopted for the linear growth cultures.

Cytotoxicity was determined with the use of tetrazole salt MTT (3[4,5-dimetylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma Aldrich) with addition of pig kidney cells (SK), subject to chloroform extraction. The agar culture medium without mycelium of *Saprolegnia parasitica* was control 2 (C2).

The MTT colorimetric test for cytotoxicity is based on 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 the cell is damaged or destroyed by the toxin, the salt will not transformed and the yellow color of tetrazole salts will be retained [11, 15].

The MTT cytotoxicity test was done on microtitration plates. Controls 1 (agar medium with mycelium inoculum outside magnetic field) and control 2 (agar medium without mycelium inoculum) and each experimental variant (culture of *S. parasitica* on CDA, MEA, PDA and SDA in 1, 5 and 10 mT) 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 test plates containing SK cell suspended in medium with ethanol and DMSO and addition of 10% FCS (foetal calf serum, Sigma Aldrich).

During the next 48 h the plates were incubated at 37°C; then MTT solution in PBS (Merck) was added to the wells, and the whole set was incubated for another 4 h. Following liquid removal from the wells, DMSO was added as solvent for the formazan crystals. After 5 min of shaking (shaker Titramax 101, Heidolph), absorbance was read using reader of microtitration plates (Mikrotek Laborsysteme, ASYS Hitech GmbH). The change of color intensity determined at wave length of 510 nm was the measure of the degree of toxicity.

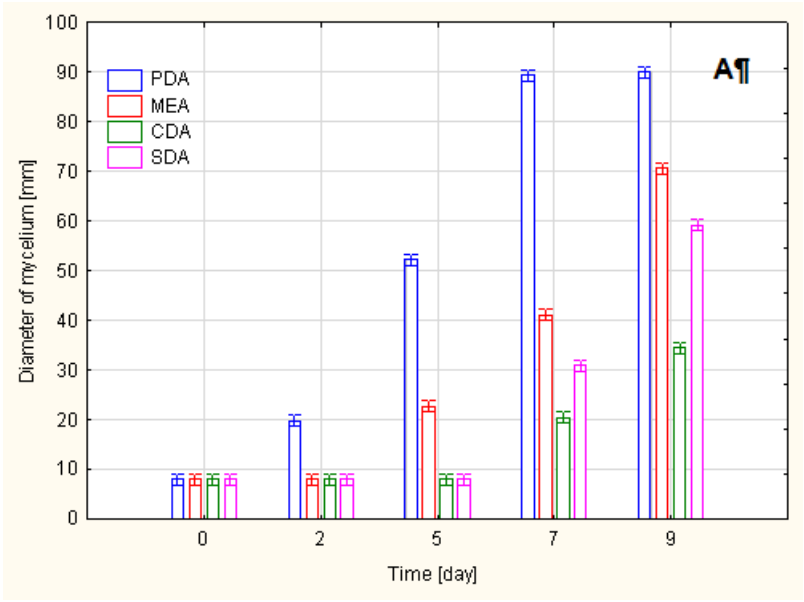
Cytotoxicity IC 50 (sample concentration at which the cell proliferation is inhibited by 50% compared to control cells) was determined based on consecutive degrees of dilution according to the scale of Hanelt [15].

Magnetic field. Sets from each experimental variant were separately exposed to static magnetic field of 1 mT, 5 mT and 10 mT value. The field was generated with permanent magnets. The control was not exposed to magnetic field. Both experimental and control series were within the natural Earth's magnetic field. The values of magnetic field were measured with teslometer TH 26 (Wrocław Technical University).

Statistical analysis. The results were analysed using Statictica PL. v. 10.0 software. The data were subject to variance analysis (ANOVA). All analyses were performed at significance levels of $p \leq 0.05$. All values were expressed as mean values with confidence intervals.

RESULTS

The results demonstrate that the *in vitro* development of mycelium of fungus-like organisms *S. parasitica* was possible on all the tested media (CDA, MEA, PDA and SDA), but its rate and mode differed (Fig. 2). On CDA medium in control conditions and in magnetic field *S. parasitica* did not produce hyphae of mycelium. This medium yielded also the widest variation in the growth rate of *S. parasitica* (Fig. 2C). In the 5 mT variant the mycelium was the fastest to develop, while at 10 mT the growth was the slowest. On the remaining media (MEA, PDA and SDA) formation of aerial mycelium was observed during the growth of *S. parasitica.*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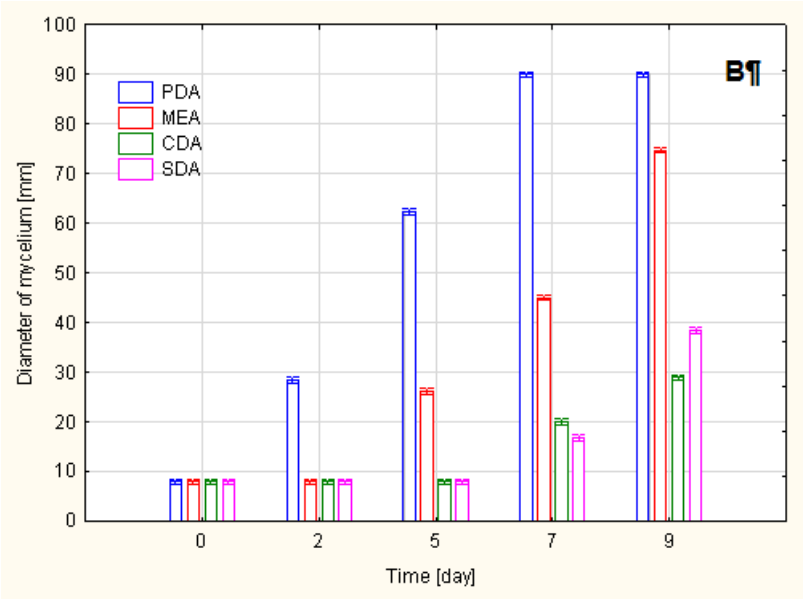
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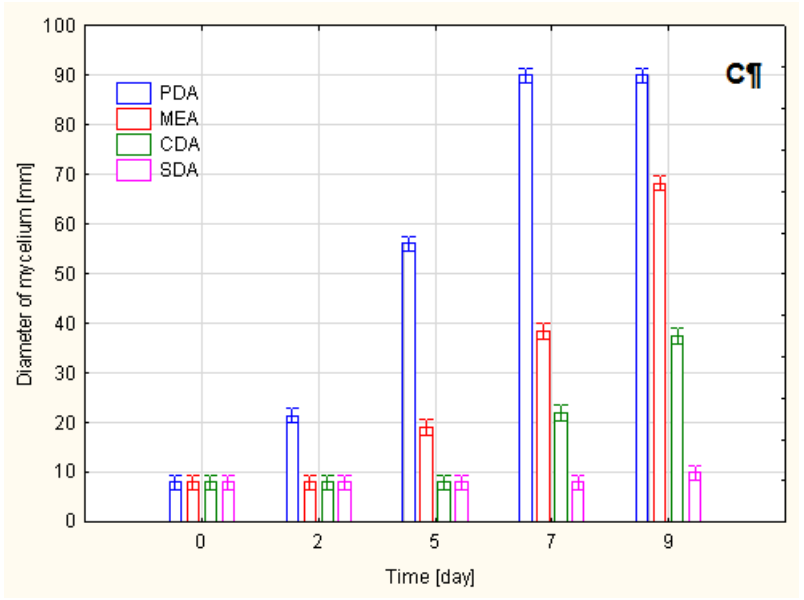
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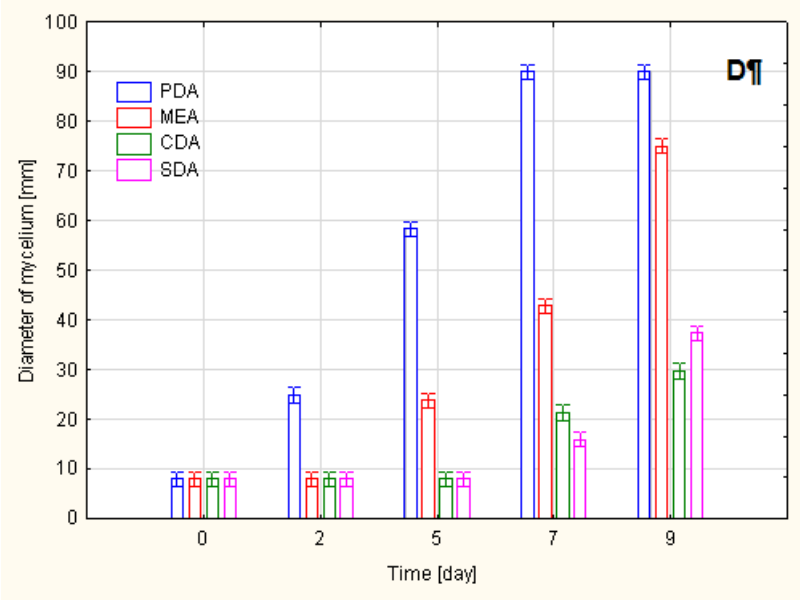

Fig. 2. The rate of linear growth of *Saprolegnia parasitica* mycelium on CDA, MEA, PDA and SDA (SAB) medium in control (A) and in magnetic field with values 1 mT (B); 5 mT (C) and 10 mT (D)

On PDA medium, both in control conditions and in all the tested values of magnetic field, the development of mycelium of*S. parasitica* was the earliest to start (after 2 days of incubation) – at that time no mycelium development was observed on the remaining media, even in the original fragments of inoculum. On the fifth day of incubation mycelium development started on MEA medium in the control (Fig. 2A) and on CDA medium in magnetic field of 5 mT (Fig. 2C).

The results of the measurements of linear growth of*S. parasitica in vitro* on various media show that the greatest variation in the growth rate in magnetic field occurred on SDA medium. On this medium the growth was the slowest at 5 mT, compared to the remaining experimental variants and the control; the mycelium was the latest to develop, i.e. after 7 days from inoculation. Mycelia of*Saprolegnia parasitica* cultured at 1 and 5 mT started to develop at the same time as in the control, but their size reached only ½ to ¾ of the size of the control mycelium. On media MEA and PDA no differences were observed in the growth rate between the tested field values and the control.

Both the kind of medium and the value of magnetic field had an effect on the cytotoxic properties of*S. parasitica* in the *in vitro* culture, except the mycelia cultured on PDA and CDA. In all the remaining experimental variants, both in magnetic field and in the control, mycelium of*S. parasitica* showed a small degree of cytotoxicity (Tab. 1). On MEA medium, at 5 and 10 mT, cytotoxicity of*S. parasitica* was smaller than in the control. On SDA, magnetic field of all the tested values decreased the cytotoxicity from medium to small. The effect of magnetic field on cytotoxicity was the greatest when the field's value was 5 mT, since it reduced the toxicity from step 4 to step 1, with IC 50 of 31.25 cm^2/ml; the effect of 10 mT was smaller, reducing the cytotoxicity of*S. parasitica* from step 4 to step 2 (IC 50 – 15.62 cm^2/ml) (Tab. 1).

Table 1. Results of MTT cytotoxicity test for *Saprolegnia parasitica* grown on various media (CDA, MEA, PDA and SDA) in magnetic fields of 1, 5 and 10 mT and in control

Medium	Magnetic fields	Inhibitory concentration IC 50 [cm^2/ml]	Evalution
CDA	control	7.813	+
	1 mT	15.625	+
	5 mT	15.625	+
	10 mT	15.625	+
MEA	control	3.906	++
	1 mT	3.906	++
	5 mT	7.813	+
	10 mT	7.813	+
PDA	control	–	–
	1 mT	–	–
	5 mT	–	–
	10 mT	–	–
SDA(=SAB)	control	3.906	++
	1 mT	7.813	+
	5 mT	31.25	+
	10 mT	15.625	+

Serial dilutions for isolates of *Saprolegnia parasitica* [cm^2/ml]:
(–) – lack of cytotoxicity; (+) – low of cytotoxicity; (++) – medium of cytotoxicity; (+++) – high of Cytotoxicity
CDA – Czapek-Dox Agar; MEA – Malt Extract Agar; PDA – Potato Dextrose Agar; SDA – Sabouraud Dextrose Agar

DISCUSSION

The ability of fungus-like organisms *S. parasitica* to develop on all the tested media shows that its mycelium can utilise both simple and complex carbohydrates, as well as organic (PDA, MEA and SDA) and inorganic (CDA) nitrogen sources. It is especially significant that *S. parasitica* was the fastest to develop on media PDA and MEA. The first medium contains trace amounts of nitrogen, while the second one contains both organic and inorganic forms of the element. *In vivo* conditions availability of these components is associated with their presence in the environment in the form of a wide range of compounds (salts, amino acids and polypeptides, as well as mono- and polysaccharides). The fact is likely to modify the growth rate and pathogenicity of microorganisms which develop on such substrata. Invasion of specific substratum, such as fish egg envelopes, by parasitic microorganisms depends on both the qualitative and the quantitative chemical composition of the envelopes and on their structure which depends on the species. Many authors mantion that the susceptibility of eggs to pathogenic infections may result from differences in the chemical composition and thickness of egg envelopes in various fish species [19, 45]. The envelope is built of a substance called pseudokeratin or ichthyulokeratin which chemically resembles insect cuticle. The inner layer is mainly composed of protein substances (96%) and small quantities – 3–4% of carbohydrates [13, 37]. The outer layer has a multimolecular structure; besides glycoproteids it also contains sulphosaccharides and sometimes sialic acid [12, 38]. Glycoproteids (ZP1, ZP2, ZP3) of egg envelopes are their crucial structural and functional components [5]. The composition and number of aminoacids of egg envelopes are characteristic of different fish species [14]: for example the egg envelope of *Oryzias latipes* is built of 30 aminoacids, that of *Oncorhynchus keta* contains 19 aminoacids (the dominant ones being glutamic acid and prolin) [26]. Since the aminoacid composition differs, the susceptibility of egg envelopes to mycoses will differ, if only for this reason. Eggs of some species will be colonised faster, those of other species – more slowly. This fact is reflected in the different growth rate of mycelium on media of different content of nitrogen compounds which are necessary for the normal course of the mycosis' vital processes.

Another problem is the modification of mycelium growth rate in magnetic field and in control conditions. The growth inhibition may result on the one hand from a decreased conidia formation [31], on the other from a decrease in the growth rate of hyphae. The observed decrease in cytotoxicity by as many as 3 steps under the effect of magnetic field results in a decrease in virulence of hyphae which in turn reduces their ability to occupy new space. This view is confirmed by our studies which indicate that it is the magnetic field that is responsible for changes of physiological-biochemical properties of the kingdom Chromista or fungus-like organisms which are often difficult to explain.

Only very few papers deal with the mycobiota reactions; there is no information in the effect of magnetic field on *S. parasitica*. Our studies are the first to document the sensitivity of *S. parasitica* to static magnetic field, and to show that its reactions to such field vary. The results obtained in the experiments with *S. parasitica* may constitute an incentive to extend such studies to include other species of Chromista or fungus-like organisms. Explanation of non-specific reactions of various species of fungi, which are difficult to interpret, may be used in modern biotechnology.

Identifying factors which reduce cytotoxicity of not only *S. parasitica*, but also of other mycoses-causing species, may be of practical significance. Saprolegniosis causes great losses in pisciculture, especially in hatcheries. Proper use of magnetic fields of specific value, which are safe for the developing fish embryos, may limit egg infection and thus improve efficiency of fish culture.

CONCLUSIONS

On the basis of conducted research it was stated that:

1. *S. parasitica*, similarly like other organisms, is susceptible to activity of magnetic field,
2. Magnetic field differentiate biochemical activity of *S. parasotica*,
3. Cytotoxic reduction of *S. parasitica* in magnetic field may restrain infection of spawn in fishes

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