Polish Journal of Environmental Studies Vol. 14, No. 1 (2005), 49-55

Original Research

Aquatic Fungi Growing on Seeds of Plants in Various Types of Water Bodies of Podlasie Province

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Received: 11 February, 2004
Accepted: 19 May, 2004

Abstract

The author investigated aquatic fungi developing on the seeds of some plants in various types of water bodies of Podlasie Province. The bait method was used to isolate the fungi. Seventy-four aquatic fungal species were found on seeds of three species of plants: *Cannabis sativa* hemp-seeds 59, *Fagopyrum esculentum* buckwheat-seeds 55 and on *Vicia sativa* vetch-seeds 44 in water of four various bodies. The mould species belonged to Blastocladiales (5), Chytridiales (6), Monoblepharidales (1), Hyphochytriales (1), Lagenidiales (5), Leptomitales (4), Peronosporales (14), Saprolegniales (37) and Plasmodiophorales (1). The most common were *Achlya americana*, *Achlya polyandra, Aphanomyces laevis*, *Pythium rostratum*, *Saprolegnia ferax* and *Saprolegnia parasitica*. The following phytopathogens were determined: *Achlya racemosa*, *Phytophthora infestans*, *Pythium butleri*, *Pythium debaryanum* and *Pythium myriotylum.*

Keywords: aquatic fungi, seeds, plants, physical and chemical study, spring, river, pond, lake, Podlasie Province

Introduction

Fungi are the predominant factors causing diseases in plants. Apart from being involved in decomposition of organic substances, they are major etiological factors of plant diseases. Plants are usually infected by spores, which mechanically perforate the epithelium by pressure induced with the infectious hyphae. Some fungi are capable of mechanical perforation of the skin and simultaneous secretion of enzymes that break down the cuticulus. Fungi penetrate tissues in various ways – through the undamaged skin, via natural openings such as tracheal apparatus, stomatal pores, slits in the cuticles, gynoecia, androecium, sprouts, root hairs or through the skin injured mechanically or previously infected with other pathogens. Aquatic and soil environments play an essential role in the process of mycotic infection in plants. Considerable air humidity, frequently in the form of condensed water vapour, even in a microscopic quantity, stimulates germination of fungal spores on plants, promoting mycotic infections. Fungi grow on dead plant remains and decompose cellulose. Mycelia frequently appear on seeds, fruits, petals, leaves, twigs and other elements of plants fallen into water [1,2].

The main aim of the present study was to establish the biodiversity of micromycetes found in various types of water bodies of Podlasie Province, to determine or exclude potential etiological factors of mycotic infections in plants and to demonstrate the effect of physical and chemical factors on the growth of aquatic fungi.

Material and Methods

Water for the experiment was collected from four water bodies in Podlasie Province in 2001-03.

Cypisek Spring, limnokrenic type, width 0.41 m, depth 0.17 m., discharge 2.4 dm ^{3} s^{-1} , is in the northern part of Białystok without trees.

The Bug River has its source in Gołogóry in Ukraine and falls to the Vistula River as its largest tributary at 813 km

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Table 1. Chemical composition in mg l^{-1} of different water samples.

Specification	Cypisek Spring	Bug River	Dojlidy Pond	Komosa Lake
Temperature (^{0}C)	11.2	9.2	15.2	13.0
pH	7.00	7.93	7.12	7.4
Oxidability	9.60	9.97	14.53	9.36
CO_2	37.2	8.8	34.2	18.8
Alkalinity in $CaCO^3$ (mval l^{-1})	4.7	4.0	4.9	3.4
$N\text{-}NH_4$	0.251	0.500	0.284	0.243
$N\text{-}NO_2$	0.016	0.013	0.122	0.021
$N\text{-}NO_3$	0.284	0.290	0.197	0.075
$P\text{-}PO_4$	0.934	0.590	1.524	0.802
Cl	40.2	39.0	69.7	35.6
Total hardness in Ca	111.9	75.6	105.7	105.1
Total hardness in Mg	21.73	19.80	19.60	20.65
$S\text{-}SO_4$	33.8	40.7	54.3	54.20
Fe	0.36	0.28	0.46	0.56
Dry residue	176.0	218.0	450.5	470.0
Dissolved solids	149.0	191.0	420.4	451.0
Suspended solids	37.0	27.0	30.1	19.0

long. Three parts of the main riverbed can be distinguished within Polish borders, depending on river appearance, the nature of banks and bottom, and primarily on the fall level.

Dojlidy Pond, area 34.2 ha, max. depth 2.85 m., while the south shore borders a coniferous woods and western part of this pond with the town of Białystok, this part of the pond is used as a beach. It is a fish breeding pond.

Komosa Lake, 12.1 ha, max. depth 2.25 m, supplied by the river Pilnica, is located in the Knyszyńska Forest and Knyszyński Landscape Park. The lake is stocked with fish for breeding.

For the culture of aquatic fungi, the experiment used seeds of the following plants: *Cannabis sativa* L., *Fagopyrum esculentum* M., and *Vicia sativa* L. ssp. nigra (L.) Ehrh. Water samples, in which the seeds were placed, were collected from water reservoirs (Cypisek Spring, Bug River, Dojlidy Pond, Komosa Lake) of varied trophicity located in Podlasie Province.

Water samples for physical and chemical analysis were obtained at 0.20 m under water surface using a Ruttner's apparatus (2.0 dm^3 capacity). Temperature and pH were measured on the spot. The remaining physical and chemical parameters, i.e. oxidability, carbon dioxide, total alkalinity, sulphates, ammonium nitrogen, nitrate nitrogen, nitrite nitrogen, phosphates, total iron, chlorides, calcium, magnesium, dry residue, dissolved and suspended substances were determined in the laboratory. Water analysis was performed using methods recommended by Greenberg et al. [3].

Bait method using seeds as baits was applied to isolate fungi. The baits were placed in one-litre containers and filled with water from the respective site. The containers were covered with glass plates to protect the water, at least partly, from penetration by bacteria. The samples were stored for approximately one month at a temperature approaching that noted in the river, pond and lake. At that time, lighting and warming were regulated to resemble natural thermal and light conditions. Microscopically determined mycelia were removed from the seeds and transferred to sterilized Petri's plates containing distilled water and seeds of some plant species. Microscopic examination of the mycelia was repeated after a few days. Several microscopic preparations were done each time. Identification of aquatic fungi involved vegetative organs, including shape and size of the hyphae, asexual reproduction organs - shape of sporangium and spores, and generative organs - the structure of the oogonium, oosporangium and antheridium. Fungi were identified according to the works of Batko [1], Bedenek [4], Dick [5], Pystina [6], Seymour and Fuller [7], and Waterhouse [8].

Results

The results of physical and chemical analysis of water have been presented in Table 1. Physical and chemical analysis revealed that water of Dojlidy Pond and Komosa Lake had the highest content of dry residue and dissolved solids.

Table 2. Aquatic fungi found on the studied seeds of plants (in water: Bug River-b,Cypisek Spring-c, Dojlidy Pond-d, Komosa Lake-k).

Taxa	*Cannabis sativa*	*Fagopyrum esculentum*	*Vicia sativa*
Chytridiomycetes			
Blastocladiales			
Blastocladia globosa Kanouse	c,k	c,k	b,c
Blastocladiopsis parva (Whiffen) Sparow	c	d	c,k
Catenaria anguillulae Soroki	b,k	c	b,c
Catenaria verrucosa Karling	c,d	b,k	b,c,k
Catenophlyctis variabilis (Karling) Karling	b,k	c,k	
Chytridiales			
Karlingia rosea (de Bary et Woronin) Johanson	c	c,d,k	b,c,d
Nowakowskiella elegans (Nowakowski) Schroeter	b,d	k	b,c,k
Phlyctochytrium aureliae Ajello	b,c	c,k	
Rhizophydium globosum (Braun) Rabenth	b	b	
Rhizophydium keratinophilum Karling	b,k	b	
Septochytrium variabile Berdan	c	d	k
Monoblepharidales			
Gonopodya polymorpha Thaxter	b,k	d	c,d
Hyphochytriomycetes			
Hyphochytriales			
Rhizydiomyces apophysatus Zopf	b		
Oomycetes			
Lagenidiales			
Lagenidium destruens Sparrow	b		
Lagenidium humanum Karling	b		
Olpidiopsis achlyae McLarty			k
Olpidiopsis saprolegniae (Braun) Cornu			b
Olpidiopsis varians Shanor		b	k
Leptomitales			
Apodachlya pyrifera Zopf	c,d	c,k	d
Leptomitus lacteus (Roth) Agardh	b,c		
Rhipidium americanum Thaxter	d	d,k	c,d,k
Rhipidium partenosporum Kanouse			b,d,k
Peronosporales			
Phytophtora gonapodoides Buisman	b	c,d,k	c,d
Pythium aquatile Höhnk			k
Pythium artotrogus de Bary			b,d
Pythium butleri Subram	b	c,k	
Pythium catenulatum Matthews		b	b
Pythium debaryanum Hesse	c	d,k	

Table 2 continues on next page...

Pythium dissotocum Drechsler	b		c
Pythium gracile Schenk	b	b	b,c,k
Pythium intermedium de Bary		b	
Pythium myriotylum Drechsler	b		
Pythium pythioides (Roze & Cornu) Rambsbottom		c	b
Pythium rostratum E.J Butler	b,c,d,k	b,c,d,k	c,d,k
Pythiogeton utriforme Minden	b,c	b,c	
Pythiopsis cymosa de Bary	c	c,d,k	
Saprolegniales			
Achlya ambisexualis Raper	b		b
Achlya americana Humphrey	b,c,d,k	b,c,d,k	b,c,k
Achlya apiculata de Bary	b,c	b,k	
Achlya bisexualis Couch	b		c
Achlya caroliniana Coker	c	b,c,d	b,k
Achlya colorata Pringhsheim	b,c	b,k	b,c,k
Achlya debaryana Humphrey	b,c	b	
Achlya dubia Coker		b,c	
Achlya flagellata Coker	b		b,c,d
Achlya klebsiana Pieters	c,d,k	b,c,d,k	
Achlya orion Coker et Couch	c	b	
Achlya polyandra Hildebrandt	b,c,k	b,c,d,k	b,c,d
Achlya prolifera Coker	b	b,k	
Achlya racemosa Hildebrandt	b		
Achlya treleaseana (Humphr.) Kauffman	b	b,c,d,k	c,k
Aphanomyces irregularis Scott	c	c,d,k	
Aphanomyces laevis de Bary	b,c,d,k	b,c,d,k	b,c
Aphanomyces phycophilus de Bary	b,c	b,d,k	
Aphanomyces stellatus de Bary	c	b,c,d,k	c
Aplanes androgymus (Archer) Humphrey	d	d	b,d
Cladolegnia unispora (Coker et Couch) Johannes	b,c	k	b
Dictyuchus magnusii Lindtstedt	b		
Dictyuchus monosporus Leitgeb	b	b,c,d	k
Dictyuchus sterilis Coker		b	
Leptolegnia caudata de Bary	d,k	b,c	b,d
Protoachlya polyspora (Lindstedt) Apinis	b	b	b
Saprolegnia anisospora de Bary		b	
Saprolegnia delica Coker		b	
Saprolegnia diclina Humphrey			b,d
Saprolegnia ferax (Gruith.) Thuret	b,c,d,k	b,c,d,k	b,c,d,k
Saprolegnia furcata Maurizio	b		k
Saprolegnia glomerata (Tiesenhausen) Lund	b	b	

Table 2 continues on next page...

Saprolegnia litoralis Coker			b,c
Saprolegnia megasperma Coker	b	b	
Saprolegnia parasitica Coker	d,k	b,d	b,c,d,k
Saprolegnia unispora Coker et Couch	c	b,k	b
Thraustotheca clavata (de Bary) Humphrey	b	b,c	b
Plasmodiophoromycetes			
Plasmodiophorales			
Woronina polycystis Cornu	b,c	c	
Total number	59: b-41 c-28 d-12 k-13	55: b-35 c-26 d-22 k-27	44: b-28 c-23 d-15 k-19

Seventy-four aquatic fungal species were found on seeds of three species of plants: *Cannabis sativa* hemp-seeds 59, *Fagopyrum esculentum* buckwheat-seeds 55 and on *Vicia sativa* vetch seeds 44 in various types of water bodies of Podlasie Province. The mould species belonged to Blastocladiales (5), Chytridiales (6), Monoblepharidales (1), Hyphochytriales (1), Lagenidiales (5), Leptomitales (4), Peronosporales (14), Saprolegniales (37) and Plasmodiophorales (1) (Table 2, Figure 1). The most common species were *Achlya americana*, *Achlya polyandra, Aphanomyces laevis*, *Pythium rostratum*, *Saprolegnia ferax* and *Saprolegnia parasitica*. The following phytopathogens were determined: *Achlya racemosa*, *Phytophthora infestans*, *Pythium butleri*, *Pythium debaryanum* and *Pythium myriotylum.* The largest number of fungal species occurred on the seeds of *Cannabis sativa* and the fewest fungi species were observed on seeds *Cannabis sativa* in the Dojlidy Pond water (12), whereas most of the seeds of *Cannabis sativa* were expanded in Bug River water (41).

Discussion

Seventy-four aquatic fungi species were marked on plant seeds. The mould species belonged to Blastocladiales (5), Chytridiales (6), Monoblepharidales (1), Hyphochytriales (1), Lagenidiales (5), Leptomitales (4), Peronosporales (14), Saprolegniales (37) and Plasmodiophorales (1). Most of them had already been observed on the seeds, fruits, pollen of other plants [9,10,11]. Some of them, however, have been known to occur on other substrates. For instance, *Aphanomyces phycophilus* is rated in monographs as a parasite of algae [1]. In our study this fungus was found to grow on seeds of hemp and buckwheat. That mould was noticed on the hemp-seeds and buckwheat-seeds in Poland by Czeczuga et al. [12].

Phlyctochytrium aureliae was stated in water of investigated reservoirs on the hemp-seeds and buckwheat-seeds. Willoughby [13] isolated *Phlyctochytrium aureliae* on animal chitin and plant cellulose from a lake in England. Johnson [14] observed this species on a varied organical substrate from Iceland`s lakes. The other species which belonged to a *Phlyctochytrium* such as *Phlyctochytrium circulidentatum* and *Phlyctochytrium multidentatum* were first described from the pollen of a plant by Umphlett and Koch [15] and Czeczuga and Muszyńska [16].

Achlya klebsiana is the fungus that commonly appears in every plant seed in investigated water states. Lund [17] observed progress of this fungus on plant twigs in Denmark waters while Czeczuga and Godlewska [18] on the boletus mushroom in ponds waters in Białystok.

Achlya flagellata was observed in our investigations on hemp-seeds and vetch-seeds. However, Cejp [19] marked

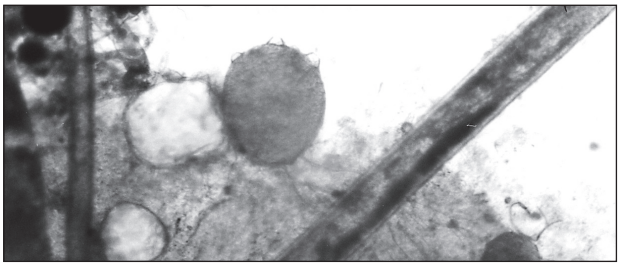

Fig. 1a. *Karlingia rosea* - sporangium.

Fig. 1b. *Pythiopsis cymosa* - hyphae from sporangium.

that species of fungi on rice and the species from rice was classified as parasite plants. He was detecting it in water from rice fields also. This fungus was first described by Zaborowska [20] from the peatbog Bocian in Poland. *Achlya flagellata* was isolated from animals: thunder-fish's spawn [21] and a crayfish and frog's croak, afterwards [22].

The most common species were *Achlya americana*, *Achlya polyandra, Aphanomyces laevis*, *Pythium rostratum*, *Saprolegnia ferax* and *Saprolegnia parasitica*. The largest number of fungal species occurred on the seeds of *Cannabis sativa*. The following pathogens were determined: *Achlya racemosa* as a parasite of rice, *Phytophthora infestans* as a parasite of tuber potatoes, *Pythium butleri* as a parasite of root systems of tobacco and potatoes, *Pythium myriotylum* and *Pythium debaryanum* as soil pathogens causing decay of seedlings of cotton, pea, cabbage, tobacco, and sugar beet [1,6].

The most devastating pathogen in these moulds is *Phytophthora infestans,* the organism which causes late blight of potato. The disease organism grows into the stem and leaf tissues, causing death, and most also infest the tubers, which are the part of the plant. The disease spreads rapidly under cool and damp conditions. Other species of *Phytophthora* destroy eucalyptus, avocado, pineapples, and other tropical crops [1].

We often noticed a varied kind of fungi, *Pythium*, in water reservoirs. The fungi of the genus *Pythium* have been frequently noted in various types of water bodies. These fungi belong to saprobionts and have been described as soil phytopathogens. They are also parasites of aquatic animals [23,24]. Eleven fungus species were isolated, of which *Pythium debaryanum* was known as early as in the 19th century as a phytopathogenic fungus [26, 27].

Pythium rostratum is a common saprophyte in water-soil, which occurred on all the plants [28,29]. That fungus was marked in the waters of Denmark peatbog by Lund [17] and in the waters of northeastern Poland recorded by Czeczuga [25,30,31,32].

Pythium inflatum was detected in soil on cereal corns [33] and on fragments of plants in ponds of Iceland [34]. Czeczuga [30] isolated this fungus on plants in the Węgorapa River.

Gonopodya polymorpha appeared rather often on plants in examinated water reservoirs. That fungus was noticed in Great Britain waters by Perrott [35].

The genus *Aphanomyces* contains several destructive parasites of the roots of vascular plants causing disease of sugar beets, peas, and other crops [1]. *Aphanomyces laevis* was isolated from the most of the investigated water reservoirs on seeds of examinated plants. Wolf and Wolf [36] were marked this fungus in samples of soil from Florida in the United States. Klich and Tiffani [37] isolated on a substrate of plants which were growing in water reservoirs with mud bottoms of Java. Similarly, on the hemp-seeds in the rivers Świder and Jeziorka, *Aphanomyces laevis* was marked by Stpiczyńska-Tober [38]. In the water of Gorbacz peatbog, *Aphanomyces irregularis* was identified by Czeczuga [39]. Czeczuga and Muszyńska marked this fungus on the gymnosperm pollen plants in water of varied trophic state.

Often in the examinated reservoirs at the seeds of plants appered another species from the genus *Aphanomyces*, *Aphanomyces phycophilus*. In northestern Poland its presence was noticed in springs in Białystok on seeds of hemp and buckwheat [12].

Most common on fungi species isolated from surface water Podlasie Province on the seeds was *Achlya colorata*. Czeczuga [30,40] noticed this fungus on plants in the Pisa River. Czeczuga et al. [41] were observed that fungus at a lot of types of water bodies in northeast Poland on the chitin substrate.

Achlya klebsiana appeared in water on investigation seeds of plants. Lund [17] described its growth on twigs of plants on Danish fresh water. Czeczuga [42] noted this species in the lakes of Augustów.

Among marked species of fungi on seeds were found fungi mentioned above: *Aphanomyces irregularis*, *Rozella septigena*, *Rhizidiomyces apophysatus*, *Olpidiopsis achlyae*, *Olpidiopsis aphanomycis*, *Olpidiopsis saprolegniae*, *Olpidiopsis varians* and *Olpidiopsis vexans*. These fungi are expanding on the mycelium and oogonium. Those moulds are described as parasites of genus *Achlya* and *Saprolegnia* [43,44,45].

In order of Leptomitales *Apodachlya pyrifera, Leptomitus lacteus* and *Rhipidium americanum* were marked on the seeds plants. *Apodachlya pyrifera* belongs to saprophyte growing on twigs and on algae. It was noted in the pond Moczydło of Lubelskie Province by Staniak [46] and in Szurpiły Lake by Czeczuga [32].

The physical and chemical analysis revealed that water of the Dojlidy Pond and Komosa Lake had the highest content of dry residue and dissolved solids.

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