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Original Communication

Antagonistical interactions between garlic *Nitellopsis obtusa* (Charales) and mycotal organisms

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ABSTRACT

The authors investigated the influence of the garlic *Nitellopsis obtusa* on the occurrence of aquatic fungus species in the water of eutrophic lake, Blizno. More fungi were found to grow in the control containers (Co) than in the containers with garlic (No) on all sites of the lake. The mean ratio of Co/No ranged from 1.94 (site 3) to 2.22 (site 2). Therefore, garlic *Nitellopsis obtusa* limits the growth of the fungus species in the water bodies.

KEYWORDS: garlic, *Nitellopsis obtusa*, fungi, interactions, eutrophic lake, hydrochemistry

INTRODUCTION

There are many representatives of Charales in all types of water bodies, especially in the lymnological types of lakes [1, 2]. Some species of these algae are characteristic of particular lakes. Therefore, in eutrophic lakes the occurrence frequency of *Nitellopsis obtusa* is high [1, 3]. In this lake type *Nitellopsis obtusa* formations occur on the wide submerse meadows [4, 5]. Eutrophic lakes are the most common in the European Lowland [6]. This species of garlic occurs in Europe [7] and in some parts of Asia [2]. In the waters of Poland, *Nitellopsis obtusa* abundance occurs in eutrophic lakes of Pomerania [2, 8, 9], Mazury Lakeland [10] and Suwalki Lake District [11, 12]. Similarly as in other algae, thalli of this garlic are incrustated by calcium carbonate and belong to euryhaline species [13, 14]. In the alkaline lakes with muddy bed, *Nitellopsis obtusa* occurs at 1.5-7.0 m depth [1, 3, 11]. Therefore, in hydrobiological investigations, it is separated from *Nitellopsidetum obtusae* plant association [15-17] with relative zonation and stability [18].

Our ecological research of interactions among prokaryotes [19-23], cryptogam plants [24, 25], macrophytes [26-28], some species of water animals [29-32] and fungus species showed different types of interactions between them.

In this context, we have decided to focus on the interactions between *Nitellopsis obtusa* and zoosporic fungi in eutrophic lake Blizno where these alga formations are found on the wide submerses meadows.

MATERIALS AND METHODS

About 15 g of the garlic *Nitellopsis obtusa* (Desv. in Lois.- Deslong J. Groves, 1999) obtained from three sites of Lake Blizno (Fig. 1) were placed in 1- liter containers and spilled with water collected from the relevant water bodies. The whole 15 g of thalli of the garlic were rewashed several times with distilled water to remove fungi from their surface. 19 parameters were determined from physical and chemical characteristics of the water using standard methods [33].

Water samples (800 ml each) were placed in 1- liter containers. For each location, three containers with water from that particular site were used. The fourth container served as a control containing only baits. The seeds of buckwheat (*Fagopyrum sigittatum* Gilib.), hemp (*Cannabis sativa* L.), Persian clover (*Trifolium resupinatum* L.), white

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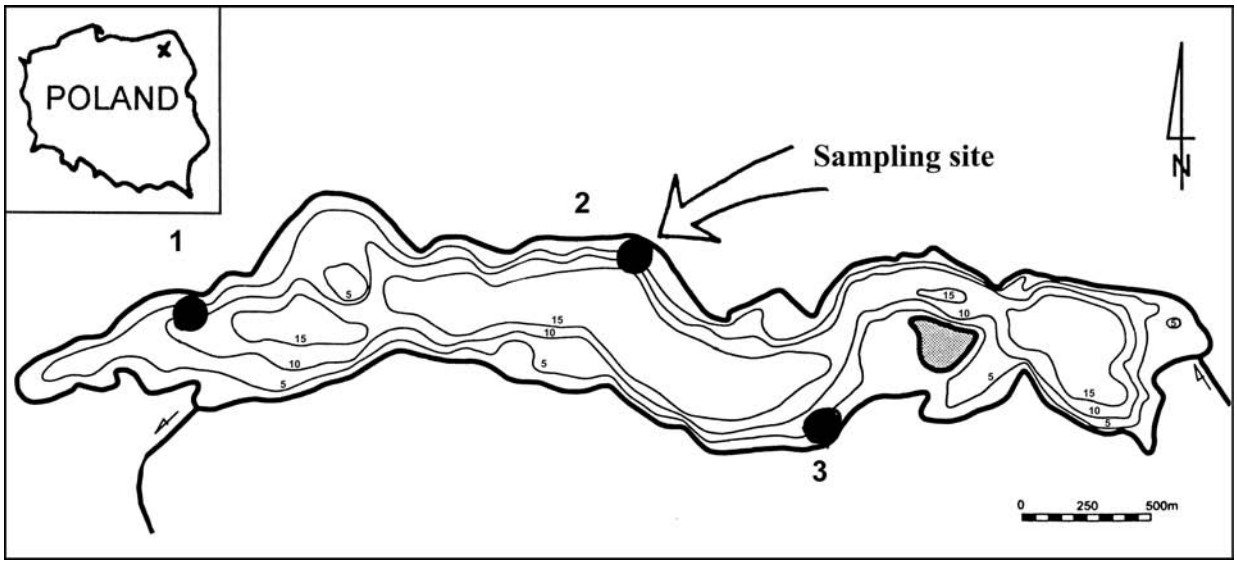

Fig. 1. Study area and sampling sites (1-3) at Lake Blizno.

clover (*Trifolium repens* L.) and snake exuviae (*Natrix natrix* L.) were used as baits (in containers with garlic and controls) in accordance to the general principles of culture [34]. All containers were enclosed in Petri dishes and were stored at 15±4°C, with access to daylight resembling natural conditions and following recommended instructions [35]. The analyses of water and experiments were carried out in three parallel repetitions.

After one month of exposure, the surfaces of baits were examined under a light- microscope. The baits were observed every 3-4 days. Morphological structures (zoospore, antheridia and oogonia) of aquatic fungi growing in particular containers were recorded. For determination of particular species of fungi, the following keys were used: Batko [36], Karling [37], and Pystina [38]. The systematics of straminipilous organisms was used according to Dick [39] and Johnson *et al.* [40], of zoosporic fungi according to Barr [41] and James *et al.* [42] and higher Fungi to Blackwell *et al.* [43]. Some results were tested for significance with ANOVA and the results were evaluated by the S-Scheffe test [44].

RESULTS

Chemical analysis of water from the Lake Blizno showed its differences in content of particular parameters at particular sites (Table 1). The water from the third site, has been characterized by minimum content of COD_5, CO_2, all forms of nitrogen, phosphates, chlorides, calcium, magnesium and iron. Water from the second site was the richest in nitrogen, phosphates, chlorides, calcium and magnesium and it showed the maximal oxidability and alkalinity indicators whereas the oxygen and sulphates occurred in minimal amounts.

Forty-five zoosporic fungi species including 36 belonging to *Straminipila*, 8 to true Fungi and one to the *Plasmodiophoromycetes* were found to grow on the baits in water used in experiment from particular sites of Lake Blizno (Table 2). The most of fungi species were isolated on the baits from site 3 and the fewest in the water of site 2. The most common species were *Catenophlyctis variabilis*, *Rhizophydium pollinis-pini*, *Aphanomyces laevis* and *Saprolegnia ferax*. The most fungi species occurred in control containers, and the fewest in containers with garlic *Nitellopsis obtusa* (Table 3). During the investigation, the lower number of fungal species was found in containers with garlic than inside the control containers (Table 4). The mean Co/No ratio oscillated between 1.94 (site 3) and 2.22 (site 2).

Table 1. Chemical and physical properties of water in particular sites of lake Blizno.

Specification	Site 1	Site 2	Site 3
Temperature (°C)	13.2	13.6	13.8
pH	8.02	8.1	8.3
DO (mg l^{-1})	16.42	12.64	12.14
BOD_5 (mg l^{-1})	2.81	2.76	2.18
COD (mg l^{-1})	4.12	6.24	3.06
CO_2 (mg l^{-1})	5.32	5.18	4.18
Alkalinity in $CaCO_3$ (mval l^{-1})	2.45	3.16	2.45
N-NH_3 (mg l^{-1})	0.213	0.274	0.186
N-NO_2 (mg l^{-1})	0.003	0.005	0.002
N-NO_3 (mg l^{-1})	0.025	0.030	0.018
P-PO_4 (mg l^{-1})	0.140	0.158	0.124
Sulphates (mg l^{-1})	14.08	11.02	12.12
Chlorides (mg l^{-1})	14.04	15.18	12.06
Calcium (mg l^{-1})	40.89	45.04	36.12
Magnesium (mg l^{-1})	11.34	12.13	9.66
Iron (mg l^{-1})	0.12	0.10	0.08
Manganese (mg l^{-1})	0.0	0.0	0.02
Dry residue (mg l^{-1})	182.0	196.0	186.0
Dissolved solids (mg l^{-1})	140.0	158.0	158.0
Suspended solids (mg l^{-1})	42.0	38.0	28.0

Table 2. Aquatic fungi and straminipilous organisms found in water from particular sites of lake Blizno.

Taxa	Site		
	1	2	3
Fungi			
Blastocladiomycota			
Blastocladiales			
1. *Catenophlyctis variabilis* (Karling) Karling	x	x	x
Chytridiomycota			
Chytridiales			
2. *Diplophlyctis laevis* Sparrow	x		
3. *Endochytrium digitatum* Karling	x		
4. *Polyphagus euglenae* (Bail) J. Schröt.		x	
5. *Rhizophydium globosum* (A. Braun) Rabenh.			x
6. *R. pollinis-pini* (A. Braun) Zopf	x	x	x
Spizellomycetales			
7. *Rhizophlyctis rosea* (de Bary et Woronin) A. Fish.	x	x	

Table 2 continued..

8. *Rozella septigena* Cornu			x
Protista			
Plasmodiophoromycota			
Plasmodiophorales			
9. *Woronina polycystis* Cornu	x	x	
Straminipila			
Hyphochytriomycota			
Myzocystiopsidales			
10. *Syzygangia marchalianum* (De Wild.) M. W. Dick			x
Peronosporomycota (Oomycota)			
Leptomitales			
11. *Apodachlya pyrifera* Zopf	x	x	
12. *A. seriata* Lund			x
Olpidiopsidales			
13. *Olpidiopsis saprolegniae* (A. Braun) Cornu		x	x
14. *O. vexans* Barret	x		
Pythiales			
15. *Phytophtora undulata* (H. E. Petersen) M. W. Dick	x		
16. *Pythium echinulatum* V. D. Matthews	x		
17. *P. proliferum* de Bary		x	
18. *P. rostratum* E. J. Butler			x
Saprolegniales			
19. *Achlya apiculata* de Barry	x		
20. *A. crenulata* Ziegler		x	
21. *A. debaryana* Humphrey	x		x
22. *A. hypogyna* Coker et Pember.		x	x
23. *A. klebsiana* Pieters	x	x	
24. *A. oblongata* de Barry			x
25. *A. polyandra* Hildebr.	x		x
26. *A. racemosa* Hildebr.	x		x
27. *A treleaseana* (Humphrey) Kauffman		x	
28. *Aphanomyces irregularis* W. W. Scott		x	x
29. *A. laevis* de Barry	x	x	x
30. *A. scaber* de Barry			x
31. *Aplanes androgynous* (W. Archer) Humphrey	x		
32. *Dictyuchus monosporus* Leitgeb	x	x	
33. *D. sterilis* Coker			x
34. *Isoachlya monilifera* (de Barry) Kauffman	x		x
35. *I. torulosa* (de Barry) Cejp	x		
36. *Pythiopsis cymosa* de Barry		x	

Table 2 continued..

37. *Saprolegnia anisospora* de Barry		x	
38. *S. diclina* Humphrey	x		
39. *S. ferax (Gruith)* Thur.	x	x	x
40. *S. glomerata* (Liesenh.) A. Lund			x
41. *S. litoralis* Coker	x		
42. *S. megasperma* Coker	x	x	
43. *S. parasitica* Coker	x		x
44. *Sommerstorffia spinosa* Arnaudov	x		
45. *Thraustotheca clavata* (de Barry) Humphrey		x	x
Total number of species	26	20	22

Table 3. Aquatic fungi and straminipilous organisms found in particular containers.

Specification	Aquatic fungi and straminipilous organisms (see Table 2)	Number of species
Only control	2,3,4,5,6,7,8,9,10,11,12,13,21,22,26,27,28,33,34,37,41,42,44	23*
Only with garlic	1,14,15,16,24,30,31,35,36,40	10*
Control and with garlic	17,18,19,20,23,25,29,32,38,39,43,45	12

*Asterisks indicate difference significant at the ≤0.05 level.

Table 4. Mean number of fungal species in control (Co) and with *Nitellopsis obtusa* containers (No).

Site	n	Co±SD	No±SD	Ratio Co/No
Site 1	6	13.5±0.94	*6.4±0.92	2.11
Site 2	4	10.0±0.86	*4.5±0.74	2.22
Site 3	8	12.6±1.02	*6.5±0.98	1.94
Mean ratio Co/No				2.09

*Differences significant at the ≤0.05 level in respect to Co.

DISCUSSION

As our investigations showed the influence on the growth of fungi and straminipilous organisms, the aquatic plants may be divided into three groups [24]. The first group determines examined species which inhibit the growth of the fungi and straminipilous organisms. The second group determines the species which occur inside the containers causing the most rapid growth of species from mycota than it takes place inside the control containers. This group of water plants is the most wide spread species in water bodies of northwestern Poland. Third, the last group, were some examined species of plants in which abundance of mycota species was almost equal in both types of containers. As this investigation showed *Nitellopsis obtusa* species belong to the first group similar as other species of the garlic.

The so-called submerse meadows may occur in lakes where the garlic species exist (depending from the light factors and depths). In creation of submerse meadows in pond and lakes the representatives of garlic participate numerously [45]. Considering that sometimes the bottom of water bodies is fully covered with garlic and in lakes the covering is even higher than 60% of bottom areas [46]. In these meadows, the occurrence frequency of *Nitellopsis obtusa*

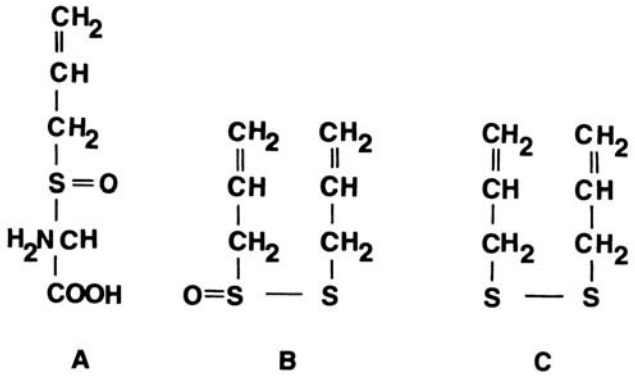

Fig. 2. Biologically active materials in garlic oil: A- alliin, B- allicin, C- diallyl disulphide.

specimens is very high. As it is well-known, all submerse flora excrete to environment (to water) both- photosynthesis products and metabolites [27, 47, 48]. Those substances have diverse chemical structures and variable physiological functions, like being a medium for other hydrobiont, especially for bacteria and fungi, or being the growth inhibitors for various organisms, both plants and animals. The algae inhibitors are sulphur compounds [49] such as alliin and derivative [50] and other polyphenols [51]. Several metabolites inhibit the growth of the cyanobacteria [52] and phytoplankton [53-55]. The metabolites of the garlic species inhibiting the growth of some animals, especially mosquito larvae [56] is known for a long time. *Nitellopsis obtusa*, similar to other garlic species, covers nearly the entire bed of some shallow ponds and lakes in northeastern Poland [57]. In these water bodies, the biological life is deficient.

The main substance in garlic specimens which are inhibiting the growth of organisms is alliin and its derivatives: allicin and diallyl disulphide (Fig. 2) [49, 50]. The substances occur in all species of *Charophyta* [58, 59] and in some species of the red algae [60]. It is thought that those sulphur compounds inhibit also the growth of fungi and fungus- like organisms [24]. Also the decomposition of garlic acid and hydrolysable polyphenols is constitutively activated by the freshwater plant-associated bacteria especially from *Matsuebacter* genus [61]. The algicidal hydrolysable polyphenols occur also in the submersed macrophyte *Myriophyllum spicatum* L. [62] and according to Maksimov *et al.* [63] polyphenols occurs in small amounts in all species of the macrophytes. Some species of the submerged *Ranunculaceae* representants contain also biologically active substances - anemonine, which inhibit the growth of fungi and straminipilous organisms [28].

The bacteria and fungi play a significant role in aquatic ecosystems as a food source for many invertebrates and especially in the mineralization of the organic matter. Especially fungi and straminipilous organisms decompose such sparingly decomposable biochemical structure as chitinous carapace of crustaceans [64-66], insects [67, 68] and such keratinized structure as feathers of the birds [69] and hairs of the animals [70] and man [71].

Recent studies indicate that some species of *Streptomyces* genus [72] and white rot fungi inhibited the growth of the phytoplankton species [73] and especially of cyanobacteria including *Microcystis aeruginosa* [74] which cause the cyanobacteria blooms in eutrophic lakes. The mass development of phyto- and zooplankton in the lakes of such type is restricted by charophytes [24, 75-77]. Worth of special interest is the finding of *Rozella septigena* on other fungi specimens in Blizno lake. Nowadays, *Rozella* genus is being placed as the primary branch of the fungal kingdom [78]. The representatives of *Rozella* genus do not produce a chitin - rich cell wall during any of the life cycle stages observed and do not comform to the standard fungal body plan [79]. The *Rozella* species does not synthesize the cell wall during many phases of its life cycle [80], instead, it appears in cell wall material from its hosts during infection [81, 82]. The amoeboid

phases are also observed and seem to phagocytose the organelles of its host [81].

The *Rozella* genus and *Rozella septigena* species were described in 1872 by Cornu [83]. This genus includes approximately 22 identified and several unidentified species which parasitize freshwater aquatic *Chytridiomycetes* and *Peronomycetes*, and one species *Rozella marina* (Sparrow) Johnson is marine and parasitic in *Chytridium* species [37]. Species of the *Rozella* genus occur in all limnological types of water bodies [84-87]. In water bodies of Northeastern Poland four species of *Rozella* (*R. achlyae* Shanor, *R. laevis* Karling, *R. longicollis* Karling, *R. septigena* Cornu) occur [86, 87]. Specimens of *Rozella septigena* is parasitic in *Achlya* and *Saprolegnia* species [36]. In lake Blizno the *Rozella septigena* was observed in the cells of *Achlya polyandra* and in *Saprolegnia ferax*. The *Rozella septigena* occurs not only in fungi, but has also recently been noted in the same species of aquarium fishes [88].

At present, the *Rozella* genus is placed in *Chytridiomycota* [89-91], but in origin belongs to *Olpidiales* [36, 37] and nowadays to *Spizellomycetales* [41, 92, 93]. Phylogenetic analysis used a combination of environmental DNA sequencing and fluorescence microscopy, Jones *et al.* [80] identified a new component of the fungal tree of life and named this highly diverse clade as *Cryptomycota* (crypto, hidden; mycota, from the kingdom *Fungi*) in anticipation of formal classification.

In conclusion, the garlic inhibits the growth of the fungi and straminipilous organisms slowing down decomposition and mineralization of organic matter especially the chitinous and keratinous structures of dead organisms.

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