ALLELOPATHIC ACTIVITY OF Myriophyllum spicatum L. ON NATURAL PHYTOPLANKTON ASSEMBLAGES

ABSTRACT: Widespread eutrophication of the water bodies and consequential occurrence of toxic algal blooms is one of the most serious environmental problems. Considering that aquatic macrophytes and microalgae compete for nutrients and light, allelopathic inhibition of algal growth is considered to be an effective macrophyte competitive strategy against algae that can bloom and thus significantly decrease an amount of light that reaches macrophytes. Three different concentrations of Myriophyllum spicatum ethanolic extract were tested for their inhibitory allelopathic activity on natural phytoplankton assemblages. After applying the extract, the average biomass of 3 replicates was measured during the experimental time. All the three concentrations of the M. spicatum extracts showed inhibitory effect to a certain extent. The maximal inhibitory effect was achieved with the 5g/50 ml concentration of extract at first sampling time. The inhibitory effect of extracts is evident within all recorded algal phyla. Phylum Cyanobacteria is found to be the most sensitive to applied extracts compared with Chlorophyta and Bacillariophyta.

KEYWORDS: allelopathy, antialgal activity, extract, Myriophyllum spicatum L., natural phytoplankton assemblages

INTRODUCTION

Living in the same water habitat with limited amount of resources, photoautotrophic organisms, such as plants and microalgae compete for nutrients and especially for light. Therefore, light is the most important factor which determines the type and intensity of interaction between submerged plants (and macrophytes in general), epiphyton and phytoplankton. This happens because those epiphytic and especially blooming planktonic algae consequently decrease the amount of light that reaches the plants (Hilt, 2006).

* Corresponding author. E-mail: B3001_2016@stud.bio.bg.ac.rs
During their long-time evolution, plants, as well as algae have developed numerous competitive strategies where allelopathy takes an important place.

Allelopathy includes the release of organic compounds by plants or bacterial species that affects other plants or bacterial species, which is regarded as a form of interference competition (Jiang et al., 2014). The chemical compounds produced in this biological phenomenon are named allelochemicals.

The production and excretion of allelochemicals provide to aquatic macrophytes an effective defense strategy against other photosynthetic organisms competing for light, e.g. other macrophytes, algae, and cyanobacteria (Gross et al., 1996).

The numerous allelochemicals with algicidal and algistatic activity produced by plants have been discovered until now, but only a few of them are structurally elucidated. The reported allelochemicals with the antialgal activity in literature include mainly polyphenols, fatty acids, terpenoids alkaloids, and polyethers (Meng et al., 2015).

*Myriophyllum spicatum* (L.) (family Haloragaceae), known as Eurasian milfoil, is a submerged species, native to Europe, Asia and northern Africa. Milfoil is a very invasive aquatic plant which propagated fast in eastern USA and Canada after its introduction from Europe at the end of the last century (Chambers et al., 1993). Beside that, it may displace the native vegetation, and milfoil-dominated lakes usually have low phytoplankton densities (Gross et al., 1996).

*M. spicatum* (L.) contains up to 30% polyphenols based on dry weight in apical meristems and exhibits a strong inhibitory action against various cyanobacteria and algae, which is mainly based on the polyphenol tellimagrandin II (Bauer et al., 2009). Further, gallic and ellagic acid, which caused algal inhibition, are allelochemicals found in crude milfoil extracts, too (Gross et al., 1996). There are several modes of inhibition for some allelochemicals: linkage with extracellular algal proteins (e.g. alkaline phosphatase) which makes them inactive (Gross et al., 1996), as well as inhibition of photosystem II (Leu et al., 2002).

The basic idea in this experiment was to study allelopathic activity of the different concentrations of the *Myriophyllum spicatum* extracts on the structure and dynamics of natural phytoplankton assemblages in *ex situ* conditions. The results would potentially provide insight into the patterns of using this plant allelochemicals for algal bloom control, given that control and elimination of harmful algal blooms became crucial in the management and mitigation of aquatic ecosystems (Zhang et al., 2014).
MATERIALS AND METHODS

Sampling

The natural phytoplankton community was collected from the Sava Lake on 20 May 2016 by a plankton net (net frame 25 cm, mesh size 22 µm). Thereafter, the fresh *M. spicatum* macrophytes were collected from the same lake.

Phytoplankton samples preparation

In sterile conditions (laboratory), 50 ml of the collected phytoplankton samples were added in each of 12 pre-autoclaved 250 ml flasks filled with 150 ml of BG-11 medium (Rippka et al., 1979). These flasks were sealed with a sterile gauze tampons coated with aluminum foil and cultured for 16 hours in an incubator using a light intensity of 400 lux, at 20±3 °C and photoperiod 15 L : 9 D, for proper acclimation.

Macrophyte extract preparation

Representative plants with fresh shoots were chosen and rinsed carefully with tap-water to remove all impurities, epiphytic algae and other organic materials. The chosen plant material was dried at 65 °C during 24 hours and powdered with lab ceramic mortar and pestle. One gram, 5 g and 25 g of powdered plant material were added to three different flasks (250 ml) containing 50 ml of 40% ethanol. The flasks were covered to prevent evaporation and vibrated for 13 hours at room temperature. After 13 h, each flask solvent was filtered with a vacuum pump and 1.2 µm cellulose filter to remove insoluble residue, giving three different concentration ethanol extracts of *M. spicatum*: A (1 g/50 ml), B (5 g/50 ml) and C (25 g/50 ml).

Plant extracts adding and experimental sampling

One milliliter of each extract of certain concentration was added to 3 experimental flasks which resulted in 4 series marked as: A, B, C, and K named after the concentrations of the added plant extract (1; 5 and 25 g/50 ml, respectively). The fourth, control series (K) was prepared only with 1 ml of ethanol (Figure 1). After the extract adding, the experimental flasks were returned to the incubator where the experiment took place.

During the experiment, 15 ml of microalgae suspension was taken four times from each flask: before extract application (0 state), 4 h (state I), 8 h (state II), and 24 h after adding extracts. It is noteworthy that the plant extracts
were added only once, at the beginning of the experiment. Collected sub-samples were taken in sterile conditions, fixed with Lugol’s solution and kept in the dark at a room temperature.

**Figure 1.** Experimental design

**Qualitative analysis of taken sub-samples**

Detailed analysis of phytoplankton population structure was made by Carl Zeiss AxioImager M.1 microscope equipped with digital camera AxioCam MRc5 and AxioVision 4.8 software. For the identification of the particular taxa, standard taxonomic literature was used (Hofman et al., 2013; Huber-Pestalozzi et al., 1983; Starmach, 1974, 1983, 1985; Popovský and Pfiester, 1990; Ettl, 1978; Komárek, 2013; Komárek and Anagnostidis, 1998, 2005).

**Quantitative analysis of taken sub-samples**

For quantitative analysis of phytoplankton, the Utermöhl’s method (Utermöhl, 1958) was applied using an inverted-microscope Leica DMIL. For counting individuals were used 10 ml volume Hydro-Bios plankton chambers. The phytoplankton biomass was estimated from the approximate geometric volume of each taxon (Hillebrand et al., 1999) and expressed in microgram per liter (µg/l).
Statistical analysis

CANOCO for Windows, version 5.0 (ter Braak and Smilauer, 2012) was used for statistical analysis of experimental results where particular phylum biomass was set as response variable, while extract concentrations and experimental sampling time were set as explanatory variables.

RESULTS AND DISCUSSION

A total of 67 algal species classified in 8 phyla (Cyanobacteria, Bacillariophyta, Chlorophyta, Chrysophyta, Cryptophyta, Dinophyta, Euglenophyta, and Xanthophyta) were detected by qualitative analysis of sub-samples. More than a half recorded species were members of Chlorophyta phylum, while some of the phyla (Xanthophyta, Cryptophyta, Euglenophyta) were represented by very few species, as well as individuals.

The results of quantitative analysis of the sub-samples were expressed by the average value of the total series (K, A, B, C) biomass (from 3 flasks that belong to the same series in the particular sampling time), and their dynamics are shown in Figure 2.

![Figure 2. The effect of three different concentrations (A, B, C) of the M. spicatum extracts on the total biomass of the particular series during the experimental time](image-url)
Figure 2 shows that, according to the expectations, the total biomass in the control series increased during the experimental time (with a slight exception of the first sampling time), while all the three concentrations of the *M. spicatum* extracts inhibited the algal growth and caused biomass decrease to a certain extent.

The maximal growth inhibition after the extract adding was recorded in most cases for the first sampling time and concentration B of the extract: 25 g of powdered plant material in 50 ml of 40% ethanol in the first sampling time showed the most powerful inhibitory effect although that was expected for concentration C. The explanation of this phenomenon is that the extraction efficiency has its own maximum which depends on the amount of powdered plant, as well as on the volume of solvent (ethanol). The extract with concentration B was obviously closer to reach that maximum compared to the extract with concentration C which most likely exceeded the maximal amount of powdered plant material for the ethanol volume, so it decreased its inhibitory activity.

After reaching the greatest rate of algal growth inhibition, most likely due to the decomposition of the active allelochemicals, it comes to the gradual recovery of the survived algal populations, so there is a slight increase in biomass noted.

![Figure 3. Redundancy analysis of the experiment results (different extract concentrations – K, A, B, C; different sampling time – 0, I, II, III)](image-url)
Redundancy analysis showed that there are statistically significant differences between the control and the treatments. The first two axes taken together display 24.3% of total variation, and Figure 3 confirms that the extract with concentration B achieved the maximal inhibitory effect on total biomass at the first sampling time. Speaking in terms of different sensitivity of algal phyla, Figure 3 also indicates that Cyanobacteria is the group most sensitive to the extract B compared with Chlorophyta and Bacillariophyta, which is in accordance with the results of Planas et al. (1981), Gross et al. (1996), Körner and Niklisch (2002), Hilt and Gross (2008), and Švanys et al. (2013).

Numerous studies (Gross et al., 1996; Nakai et al., 1999; Körner and Niklisch, 2002; Mulderij et al., 2003; Hilt, 2006; Eigemann, 2013) also pointed that there are differences in sensitivity to allelochemicals not only in particular phyla, but also in different genera, as well as species in the phylum. Although most studies show that Cyanobacteria have the highest sensitivity to the allelochemicals extracted from *M. spicatum*, some species, such as *Anabaena flos-aquae*, could be characterized as exceptions (Körner and Niklisch, 2002). Differences in susceptibility among cyanobacterial species were also established by Nakai et al. (1999), but the reasons for it still remained unknown (Eigemann, 2013).

**CONCLUSION**

The analysis of the obtained results leads to the conclusion that different concentrations of *Myriophyllum spicatum* extracts show inhibitory allelopathic effects on algal growth. The rate of inhibitory activity is correlated with the extract concentration. Because of the reduced extraction efficiency during the preparation of the extract of concentration C, the extract of concentration B at the first sampling time showed the strongest inhibitory effect on the total algal biomass, which is confirmed by the redundancy analysis of the results of the experiment.

The inhibitory effect of different concentration extracts is evident within all recorded algal phyla, where Cyanobacteria reached the maximum of susceptibility to applied extract, compared with Chlorophyta and Bacillariophyta.

Considering the trend of the worldwide and comprehensive eutrophication of water bodies accompanied by frequent and harmful algal blooms, as well as the advantage of potential allelopathic control of this natural phenomenon, the obtained results give the opportunity for a more detailed and specific research that will have an effective and sustainable management and recovery of water bodies as an ultimate goal.
REFERENCES


АЛЕЛОПАТСКА АКТИВНОСТ ЕКСТРАКТА *Myriophyllum spicatum* L. НА ПРИРОДНУ ЗАЈЕДНИЦУ ФИТОПЛАНКТОНА

Марија Н. ПЕЂИЋ, Драгана Д. ПРЕДОЈЕВИЋ
Гордана В. СУЂАКОВ СИМИЋ

Универзитет у Београду, Биолошки факултет
Институт за ботанику и Ботаничка башта „Јевремовац“
Таковска 43, Београд 11000, Србија

РЕЗИМЕ: Изражена еутрофикација водних тела која за последицу има појаву честог и интензивног цветања алги које продукују токсисне представља један од највећих проблема у управљању и одржавању водених екосистема. Насељавајући иста станишта, акватичне макрофите и алге ступају у најразличитије видове компетитивних односа за нутријенте и наорочито светлост, при чему алеопатија представља врло ефективну компетитивну стратегију против алги које цветају и на тај
начин смањују количину светлости која до макрофита доспева. У овом истраживању испитиван је инхибиторни алелопатски утицај три различите концентрације етанолског екстракта Myriophyllum spicatum на природне фитопланктонске заједнице. Након додавања екстракта, у одређеним временским интервалах израчунавана је просечна вредност биомасе фитопланктона од три реплике. Све три концентрације екстракта Myriophyllum spicatum показале су у одређеном степену инхибиторни ефекат на раст алги. Најјачи инхибиторни ефекат на заједницу алги имао је екстракт концентрације 5 g/50 ml у првом времену узорковања. Инхибиторни ефекат је, такође, запажен у оквиру свих група алги. Највећа осетљивост на додате екстракте уочена је код раздела Cyanobacteria у поређењу са осетљивошћу раздела Chlorophyta и Bacillariophyta.

КЉУЧНЕ РЕЧИ: алелопатија, антиалгална активност, екстракт, Myriophyllum spicatum L., природна фитопланктонска заједница