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Article

Saturated and unsaturated fatty acids as potential allelochemicals for aquatic ecosystems rehabilitation

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Abstract. Cyanobacterial blooms (HCBs) in water bodies adversely affect aquatic ecosystems. In laboratory experiments, metabolites-allelochemicals of aquatic macrophytes, nonanoic – a monobasic saturated and palmitoleic – an Omega-7 monounsaturated fatty acids effectively inhibit the development of cyanobacteria *Synechocystis aquatilis* Sauvageau, strain No. 1336 of CALU (Collection of Algae of Leningrad University). In contrast to palmitoleic acid (Suppression Index (SI) within 3.5), nonanoic acid at the highest tested concentrations of 1–1.8 mg/l (SI above 20) had more pronounced effect. Nonanoic acid can be referred to an algaecide of a new generation based on aquatic macrophyte metabolites to prevent and attenuate HCBs.

Keywords: nonanoic acid, palmitoleic acid, cyanobacteria, allelopathy, algaecide of a new generation, macrophyte metabolites

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Научная статья

Насыщенные и ненасыщенные жирные кислоты как потенциальные аллелохемики для реабилитации водных экосистем

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Аннотация. Опасное цианобактериальное цветение (ОЦЦ) водоемов может приводить к значительным отрицательным последствиям. В лабораторных экспериментах показано, что метаболиты-аллелохемики водных макрофитов, нонановая кислота (одноосновная предельная жирная кислота) и пальмитолеиновая кислота (Omega-7 мононенасыщенная жирная кислота), способны эффективно подавлять развитие цианобактерии *Synechocystis aquatilis* Sauvageau, штамм № 1336 коллекции CALU (Collection of Algae of Leningrad University). Воздействие нонановой кислоты при наибольших из исследованных концентраций (1 и 1.8 мг/л) было более выраженным (коэффициенты подавления (SI) более 20), чем пальмитолеиновой кислоты (SI не более 3.5). Нонановая кислота может быть рекомендована для включения в состав альгицидов нового поколения на основе метаболитов водных макрофитов, используемых для предотвращения и ослабления ОЦЦ.

Ключевые слова: нонановая кислота, пальмитолеиновая кислота, цианобактерии, аллелопатия, альгициды нового поколения, метаболиты макрофитов

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Introduction

Hazardous cyanobacterial blooms (HCBs) are often a man-made phenomena that may threat aquatic ecosystems as a whole, aquatic organisms, near-water animals and humans. For instance, the exposure to cyanotoxins can cause both local and global degradation of water resources (Huisman et al. 2018; Šulčius et al., 2017).

The problem of preventing and mitigating the consequences of HCBs is especially topical for a great number of small reservoirs widely used for various types of water consumption (fisheries, aquaculture, water supply, recreation, etc.). It brings to the fore the search and implementation of the novel biotechnological methods able to hinder and suppress the excessive development of cyanobacteria, being at the same time safe for other components of aquatic ecosystems.

Such methods primarily include the convergent nature-like technologies, i.e. the approaches based on natural mechanisms responsible for particular effects. Modern environmental management should focus exactly on these technologies in order to ensure the sustainable development of the society and nature (Kovalchuk and Naraikin, 2017; Nature-like..., 2019; Zhironkin et al., 2019).

Characteristic of terrestrial and aquatic ecosystems, allelopathy is a natural phenomenon of plant inhibiting (or stimulating) the development of other organisms through specific allelochemical compounds (Chemical Ecology..., 2002). The experience in studying allelopathy and low-molecular-weight organic compounds (LOC) in aquatic ecosystems indicates that this phenomenon can be very useful for the effective prevention and attenuation of HCBs development in water bodies (Fink, 2007; Gurevich, 1953, 1973; Hu and Hong, 2008; Kurashov et al., 2014; Kurashov et al., 2021; Macías et al., 2008). Macrophytes-synthesized allelochemicals can suppress cyanobacteria and prevent both the emergence and development of HCBs (Asif et al., 2021; Mushtaq et al., 2020; Śliwińska-Wilczewska et al., 2021).

The mechanism of production, accumulation and release of LOC by aquatic and semi-aquatic plants, as primary and secondary metabolites, is essential in interaction with other aquatic organisms (Fink, 2007; Li et al., 2020). Besides, these metabolites are of critical importance for the formation of hydrobiological communities acting as the regulatory agents in aquatic ecosystems (Antioxidants in Plant-Microbe..., 2021; Koksharova, 2020; Kurashov et al., 2021).

There are numerous methods to control cyanobacteria (Burford et al., 2019), however, many of them do not effectively solve the problem of HCBs without damaging other ecosystem components since they have undesirable adventive effects on aquatic organisms and aquatic ecosystems as a whole (Huisman et al., 2018; Zhu et al., 2021). Imitation of the allelopathic impact on cyanobacteria through LOC-allelochemicals is the effective and safe (for other hydrobionts) alternative to existing methods designed for HCBs suppression. To date, the possibility of successful plant-derived allelochemical use to control HCBs has been proved (Kurashov et al., 2021; Nezbrytska et al., 2022; Zhu et al., 2021). The aquatic plant allelochemicals-based approach to combating HCBs makes it possible to maintain and restore the water quality in the reservoirs and exploit them for multifunctional purposes. In addition, natural (or synthetic) allelochemicals can effectively replace the existent algaecides (Hu and Hong, 2008; Kurashov et al. al., 2014; Mohamed, 2017).

In aquatic ecosystems, allelochemicals are extremely diverse: aldehydes, ketones, esters, terpenes, terpenoids, phytoecdysteroids, fatty acids, sulfur-containing compounds, nitrogen-containing compounds, alcohols, lactones, quinines, phenols, coumarins, flavonoids, etc. (Kurashov et al., 2014; Li et al., 2010; Nakai et al., 2012).

It is obvious that fatty (carboxylic) acids are most promising for producing algaecides of a new generation and corresponding technologies for HCBs control (Kurashov et al., 2019; Nakai et al., 2005; Zhu et al., 2021). As allelochemicals, aquatic macrophytes actively synthesize both saturated and unsaturated fatty acids (Kurashov et al., 2018). Meanwhile, the inhibitory ability of all potential allelochemicals in the fatty acid series has not been tested experimentally yet.

This study is aimed at revealing probable anti-cyanobacterial effects of nonanoic and palmitoleic acids (as a part of LOC metabolites of aquatic macrophytes) for their further inclusion into composite algaecides of a new generation meant for combating HCBs at the rehabilitation of aquatic ecosystems.

Material and methods

To confirm the anti-cyanobacterial effect of nonanoic ($C_9H_{18}O_2$, monobasic saturated carboxylic) and palmitoleic ($C_{16}H_{30}O_2$, omega-7 monounsaturated fatty) acids experimentally, we used planktonic cyanobacteria *Synechocystis aquatilis* Sauvageau, strain No. 1336 from the collection of cultures of cyanobacteria, algae and algal parasites (CALU) provided by the Resource Center “Cultivation of Microorganisms” of the Science Park of St. Petersburg State University. The strain *S. aquatilis* was isolated from a water sample taken in the Gulf of Finland near the city of Sosnovy Bor.

The strain was maintained as the growing culture on synthetic nutrient medium No. 6 grown in the Laboratory of Microbiology of the Leningrad State University (Gromov and Titova, 1983) in similar (like in our experiments) containers and conditions. Periodically (every 2 weeks), the culture was reseeded into a clean medium to ensure its active growth.

To avoid contamination of the cyanobacteria culture, we used special 0.5 l containers constantly aerated by a bacterial filter “BIOFIL Syringe Filter” with a pore diameter of 0.22 μm. Each experiment was performed in three replications. The culture of cyanobacteria *S. aquatilis* at the active growth phase was placed into experimental containers as a suspension of the initial culture. One series of experiments with palmitoleic acid lasted for10 days, while two series with nonanoic acid at different concentrations of an acting allelochemical – 18 and 23 days, respectively.

In the course of experiments, a special Biodesign T8 lamp with T8 FRESH WATER aquarium lamps provided a constant luminous flux of 1500 lm per 1 m. The day/night regime was set by an adjustable timer Feron TM50, 3500 W/16 A 230 V.

To study the anti-cyanobacterial effect of nonanoic and palmitoleic acids, their purified analogues produced by Acros Organics BVBA were used in concentrations characteristic of natural reservoirs: 0.01, 0.018, 0.1, 0.18, 1 and 1.8 mg/l (own data). The compounds were added to the containers with the cyanobacteria culture in the required quantities. Free-from-allelochemicals containers served as the reference specimens.

To assess the suppression of cyanobacteria development, we used the Suppression Index (SI) (Kurashov et al., 2020) defined as the ratio of cyanobacteria density in the control and experimental (with allelochemicals) cultures. In this work, SI values were additionally calculated from the changes in chlorophyll *a* and phycocyanin concentrations.

In different experiments, the development of cyanobacteria cultures was monitored with an interval of 2–9 days using a light microscope (Zeiss Axio Lab A1) for counting cells number in the Nageotte chamber, as well as by the changes in chlorophyll *a* and phycocyanin concentrations recorded by a multiparameter probe AquaTroll 500 (In-Situ Inc.). The temperature during the experiments remained in the range of 25–26 °C.

Results and discussion

In the first series of experiments with nonanoic acid, the following concentrations of allelochemicals were used: 0.01, 0.1 and 1 mg/l. The results of changes in *S. aquatilis* abundance are presented in Fig. 1. In all cases, a reduced number of cyanobacteria cells in experimental containers (compared to the control ones) was observed. The greatest suppression of cyanobacteria (average SI = 21.2) (Table 1) was noted towards the end of the experiment at the use of the peak tested concentration (1 mg/l).

An additional assessment of the culture state in the experimental containers based on the changes in chlorophyll *a* and phycocyanin concentrations (Fig. 2, 3) also showed a pronounced inhibitory effect of nonanoic acid.

In the second series of experiments with nonanoic acid at concentrations of 0.018, 0.18 and 1.8 mg/l, a strong suppression of cyanobacteria was recorded as well. Towards the end of the experiment, the median value of SI made up 19.9 at the highest concentrations (Fig. 4, Table 1).

The significant inhibitory effect of nonanoic acid on the development of *S. aquatilis* culture was also traced through the changing concentrations of chlorophyll and phycocyanin (Fig. 5, 6, Table 1). It is worth noting that the second series of experiments was longer than the first one (23 days versus 18). By the end of the second series, the stimulating effect of nonanoic acid was recorded at the lowest concentration (0.018 mg/l) (Fig. 4–6). Interestingly, its stimulating effect set in from day 4 to 11. On day 14, cyanobacteria growth slowed down as compared to the control sample, but then resumed at a faster rate. This suggests that under certain conditions, macrophyte allelochemicals are capable of not only suppressing, but also stimulating the development of cyanobacteria. Hence, the response of cyanobacteria to some allelochemicals may vary depending on concentrations, exposure time, and environmental conditions. This effect must be taken into account when using a new generation of algaecides in the whole aquatic ecosystem.

The experiment with palmitoleic acid was evidence of a pronounced inhibitory effect on the development of *S. aquatilis* defined due to changes in cyanobacteria cell number, as well as from chlorophyll *a* and phycocyanin concentrations (Fig. 7–9). All tested concentrations inhibited the cyanobacteria development. As compared to nonanoic acid, this process was not significant at the highest concentrations of palmitoleic acid. The estimates of inhibition by nonanoic and palmitoleic acids via different methods (direct cell counting, chlorophyll *a* and phycocyanin concentrations) turned out to be mostly close (Table 1). Note that by the end of our experiment, SI obtained through cyanobacteria exposure to peak concentrations (1 and 1.8 mg/l) of nonanoic acid in two series were higher than those for palmitoleic acid in all options, except for chlorophyll evaluation in the 2nd series of experiments. In two series of experiments, when nonanoic acid concentrations were growing (from 0.1 to 1 mg/l and from 0.18 to 1.8 mg/l, respectively), SI increased as well. The inverse process occurred with palmitoleic acid (Table 1). At the highest concentrations of nonanoic acid, SI values estimated via direct counting and from phycocyanin were high (from 19 to 25). This clearly demonstrates the prospects for using the allelochemical as a component in producing algaecides of a new generation.

According to S. Nakai et al. (2006), nonanoic acid can inhibit the growth of cyanobacteria *Phormidium tenue* and *Microcystis aeruginosa*. Previously, SI for *S. aquatilis* were established for other saturated and unsaturated fatty acids by (Kurashov et al., 2020). At different concentrations, linoleic, tetradecanoic, hexadecanoic, heptanoic and octanoic acids (3–12.5; 7.5–14.5; 10.4; 1.9 and 3, respectively) had the highest SI. Thus, among the experimentally tested fatty acids, nonanoic acid showed the best suppression of *S. aquatilis*.

Table 1. Median values of SI at allelochemical concentrations causing profound inhibition of cyanobacteria development. Estimates are based on abundance (N), chlorophyll a (Chl) and phycocyanin (Pc). Above the line: concentrations of nonanoic acid in the 1st series of experiments; below the line: nonanoic and palmitoleic acids in the 2nd series.

Allelochemical	Estimate from N		Estimate from Chl		Estimate from Pc	
	$\frac{0.1}{0.18}$	$\frac{1}{1.8}$	$\frac{0.1}{0.18}$	$\frac{1}{1.8}$	$\frac{0.1}{0.18}$	$\frac{1}{1.8}$
Nonanoic acid (experiment 1)	1.8	21.2	1.1	37.5	1.5	24.0
Nonanoic acid (experiment 2)	4.3	19.9	1.5	1.9	9.1	25.0
Palmitoleic acid	3.1	3.4	3.7	3.5	2.0	2.2

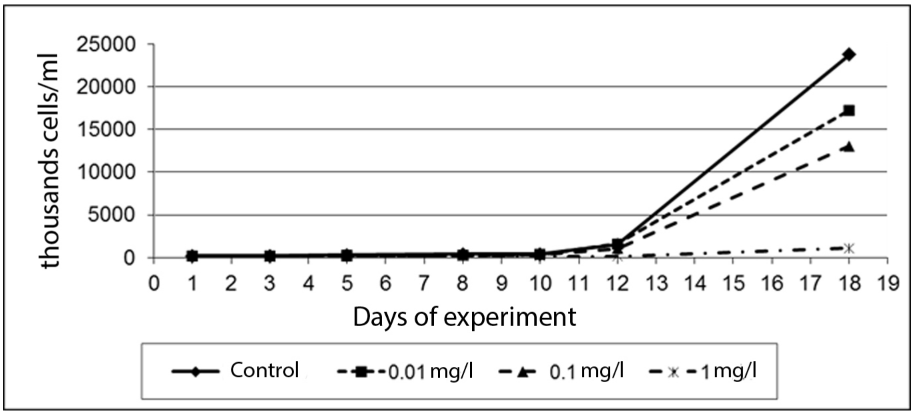

Fig. 1. Median abundance of *S. aquatilis* culture exposed to nonanoic acid (concentrations of 0.01, 0.1 and 1 mg/l) in the 1st series of experiments.

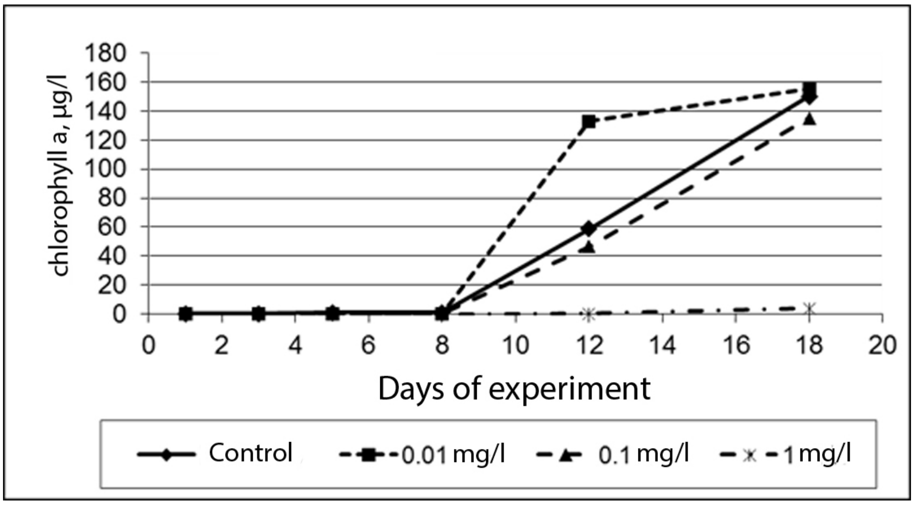

Fig. 2. Median concentration of chlorophyll *a* exposed to nonanoic acid (0.01, 0.1 and 1 mg/l) in the 1st series of experiments.

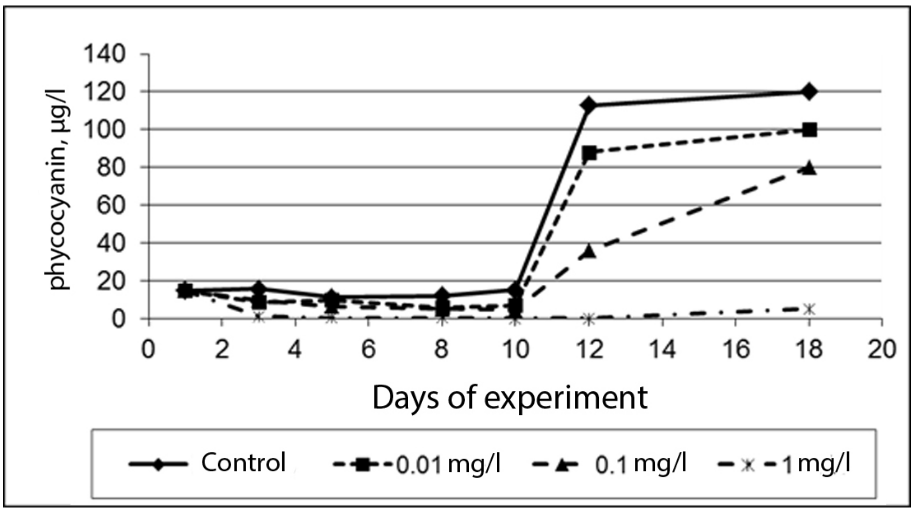

Fig. 3. Median concentration of phycocyanin exposed to nonanoic acid (0.01, 0.1 and 1 mg/l) in the 1st series of experiments.

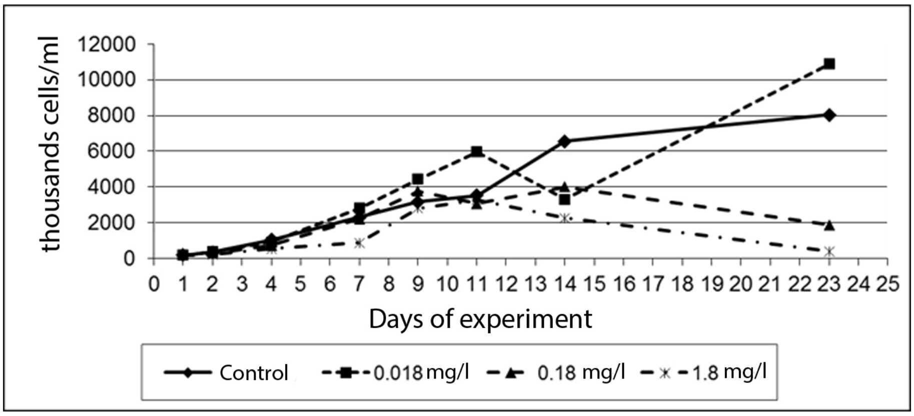

Fig. 4. Median abundance of *S. aquatilis* culture exposed to nonanoic acid (concentrations of 0.018, 0.18 and 1.8 mg/l) in the 2nd series of experiments.

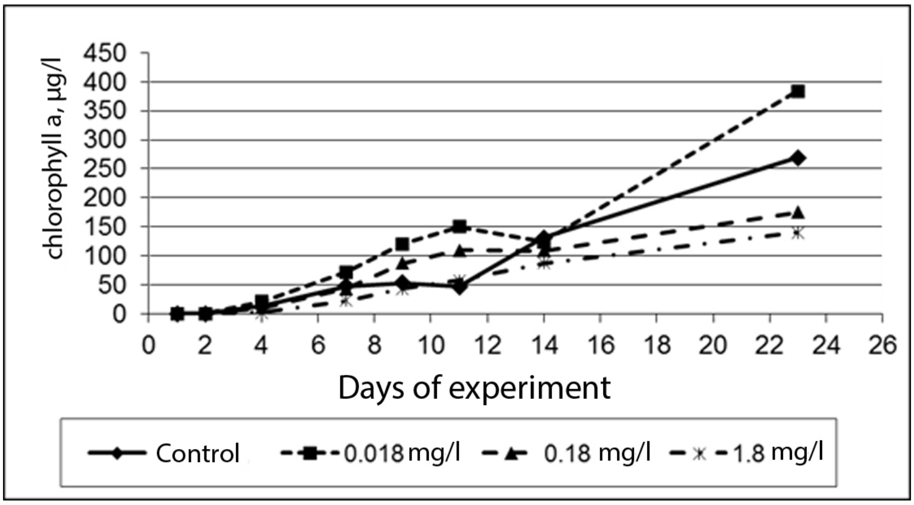

Fig. 5. Median concentration of chlorophyll *a* exposed to nonanoic acid (0.018, 0.18 and 1.8 mg/l) in the 2nd series of experiments.

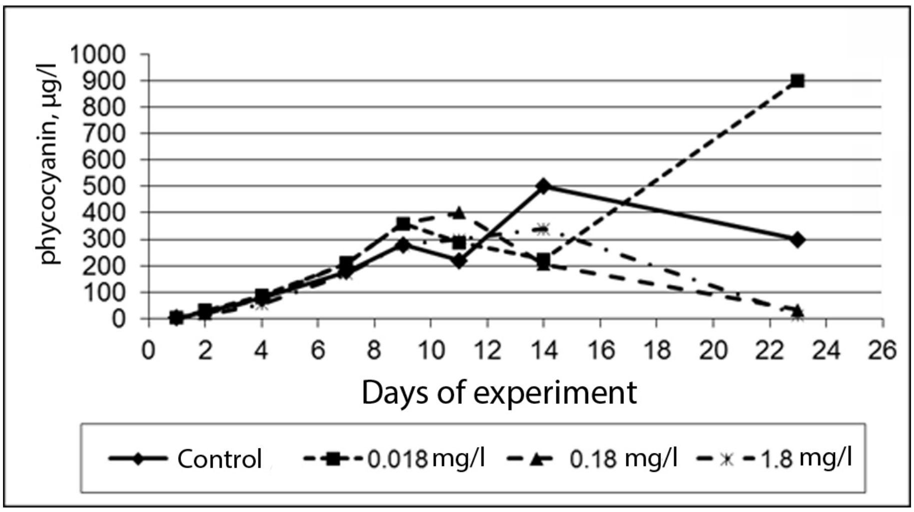

Fig. 6. Median concentration of phycocyanin exposed to nonanoic acid (0.018, 0.18 and 1.8 mg/l) in the 2nd series of experiments.

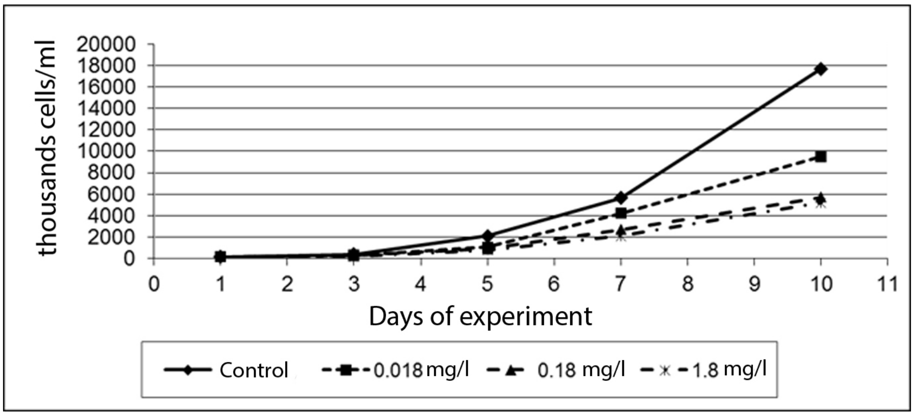

Fig. 7. Median abundance of *S. aquatilis* culture exposed to palmitoleic acid (concentrations 0.018, 0.18 and 1.8 mg/l).0.018, 0.18 and 1.8 mg/l).

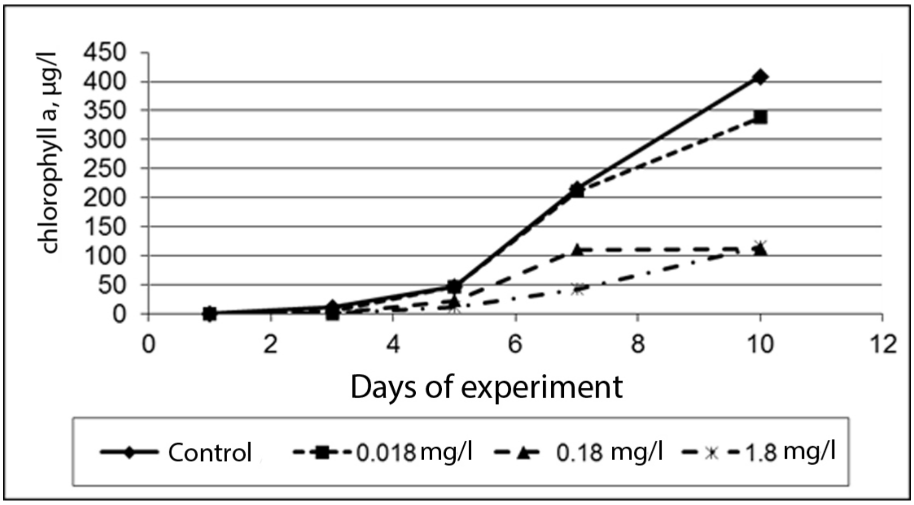

Fig. 8. Median concentration of chlorophyll *a* exposed to palmitoleic acid (0.018, 0.18 and 1.8 mg/l).

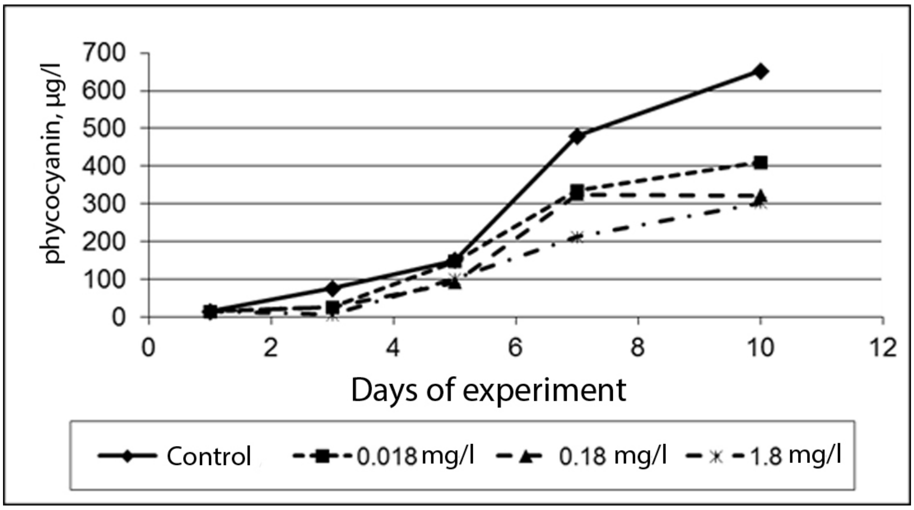

Fig. 9. Median concentration of phycocyanin exposed to palmitoleic acid (0.018, 0.18 and 1.8mg/l).

There is an opinion that the shorter the carbon chain and the number of unsaturated bonds in fatty acids, the stronger the inhibitory effect on cyanobacteria is (Tan et al., 2019; Zhang et al., 2009). Apparently, this problem is not so clear-cut. For example, among the tested fatty acids, heptanoic and octanoic ones with a minimum number of carbon atoms had the lowest SI (Kurashov et al., 2020). The present study suggests that nonanoic acid has higher SI as compared, for example, to tetradecanoic and hexadecanoic acids. At the same time, the values of SI for unsaturated acids (palmitoleic (our study) and linoleic (Kurashov et al., 2020)) do not exceed (especially for palmitoleic) those for saturated carboxylic (nonanoic, tetradecanoic and hexadecanoic) acids.

Fatty acids with an odd number of carbon atoms are reported to have better algal inhibitory effects than those with an even number (Zhang et al., 2009). Our results are consistent with this view because of higher SI obtained for nonanoic acid than for all tested acids with an even number of carbon atoms.

Conclusion

Our findings suggest that the use of fatty acids as potential agents for aquatic ecosystems protection from HCBs and their rehabilitation holds much promise. These compounds should be referred to the composite algaecides of a new generation based on aquatic macrophytes-derived allelochemicals. The natural phenomenon of allelopathy is the basis of the novel convergent nature-like technology for preventing and combatting HCBs development in water bodies. Clearly, this technology requires thorough elaboration and implementation. Among fatty acids tested in the present study, nonanoic one demonstrates the best anti-cyanobacterial inhibitory properties and can be recommended for its inclusion in the group of new algaecides.

Despite the early stage of the nature-like technology development, one can outline the most prospective ways of its elaboration. For instance, allelochemicals transport to aquatic ecosystem targets can be implemented not by adding drugs to water, but using the alginate-chitosan microcapsule technology (Ni et al., 2013, 2015) able to imitate the inhibitory effect of macrophytes on planktonic algae and cyanobacteria throughout the growing season due to allelochemical sustained-release microspheres and thereby to control and prevent HBCs.

Thus, the data on fatty acids and other LOC effects on cyanobacteria open up new promising trends in the research and practical use of allelochemicals of aquatic macrophytes at the rehabilitation of aquatic ecosystems.

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