Ukrainian Journal of
Veterinary and Agricultural Sciences
http://ujvas.com.ua

The Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv

original article | UDC 619.615.5 | doi: 10.32718/ujvas6-3.10

Volume 6
Number 3

Determining the toxicity of the biomass of *Cladophora sp.*

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Article info
Received 13.10.2023
Received in revised form 16.11.2023
Accepted 17.11.2023

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Abstract

Finding new or alternative feeds, feed additives, and raw materials safe for health and the environment is significant in intensifying livestock production. At the same time, the fodder base obtained from aquatic ecosystems – algae – deserves special attention. The advantage of using algae as fodder is using small areas for their cultivation and a high reproduction rate. In addition, algae are grown where other plants cannot grow, and the productivity of algae is several times higher than that of higher plants. Algae can produce beneficial compounds and biomass, which are also used to increase the nutritional value of food products. Biomass obtained from algae is a source of necessary vitamins, minerals, proteins, polyunsaturated fatty acids, antioxidants, and other necessary nutrients. The composition of algae depends on the environment from which this biomass is collected, the conditions of algae cultivation, the season, the species of algae, and many other factors. In addition, it should be noted that some algae, out of the total amount, are toxic when consumed. That is why it is essential to research their toxicity. The article presents the results of studying the toxicity of the biomass of *Cladophora sp.* on the body of guppy fish. When studying the effect of biomass *Cladophora sp.* on the body of guppy fish, it was established that its use for 96 hours in doses of 5, 10, 25, 50, and 100 mg/l did not cause their death, and its LC_{50} is higher than 100 mg/l. In addition, no changes were detected in the behavior of the fish; they were active, mobile, and actively responding to external stimuli. The fish of the experimental groups were not distinguished from those of the control group.

Keywords: algae; biomass *Cladophora sp*; toxicity; LC_{50}; guppy.

Citation:
Kushnir, V. I., Kosenko, Yu. M., Patereha, I. P., Kabanets, A. S., Shkilnyk, O. S., & Gutyj, B. V. (2023). Determining the toxicity of the biomass of *Cladophora sp*. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 6(3), 52–55.

1. Introduction

Animal husbandry is one of the most important and leading branches of agriculture, the main task of which is to provide the population with food products (Bryk, 2018). Intensification of livestock production can lead to a shortage of traditional protein components of feed and an increase in cost. Therefore, it is essential to find new or alternative feeds, feed additives, and raw materials that are safe for health and the environment (Motlagh et al., 2019). The fodder base obtained from aquatic ecosystems – algae – deserves special attention.

The advantage of using algae as fodder is using small areas for their cultivation and a high reproduction rate. In addition, algae are grown where other plants cannot grow, and the productivity of algae is several times higher than that of higher plants. Algae can produce beneficial compounds and biomass, and they are also used to increase the nutritional value of food products (Kovač et al., 2013).

Biomass obtained from algae is a source of necessary vitamins, minerals, proteins, polyunsaturated fatty acids, antioxidants (Heiba et al., 1997; Laungsuwon & Chulalaksananukul, 2014; Michalak & Messyasz, 2021) and other necessary nutrients that can be used in feed production (Bruneel et al., 2013; Konkol et al., 2018; Wan et al., 2019), which will help to reduce or replace the shortage of feed materials.

Under these conditions, the algae Cladophora sp deserves special attention. Cladophora belongs to a group of macroscopic green algae with more than 183 species (Munir et al., 2019). Algae of the genus Cladophora sp. is a rich source of biologically active compounds containing many carbohydrates, minerals, and proteins (Srimaroeng et al., 2015).

Algae biomass Cladophora sp. is used as fertilizer, biostimulants for plant growth, animal feed, nutraceuticals, pharmaceuticals, and cosmetic products, as well as for wastewater treatment and biofuel production (Mihranyan, 2011; Zulkifly et al., 2013).

The composition of algae Cladophora sp. depends on the environment from which this biomass is collected, the conditions of algae cultivation, the time of year, the species of algae, and many other factors. After all, the appearance of this one-year filamentous macroalgae is associated with an increase in the content of nitrogen and phosphorus in water, which is the result of intensive agriculture (use of mineral fertilizers), the use of detergents containing phosphorus, an increase in the population, sewage treatment plants, etc. (Parker & Maberly, 2000; Mihranyan, 2011).

However, some of the algae in the total amount are highly toxic when consumed (Vasconcelos, 1999; Ouellette & Wilhelm, 2003; Oberholster et al., 2004). That is why it is essential to conduct studies to assess their safety, particularly their toxicity.

To establish the safety of using fodder for feeding (Kotsiumbas et al., 2006), which includes these components, studying their effect on the body of guppy fish is necessary.

Fish of this species demonstrate opportunistic, benthopelagic, omnivorous feeding behavior (Lawal et al., 2012).

The purpose of the research was to study the impact (harmlessness) of the biomass of *Cladophora sp.* on the body of guppy fish.

2. Materials and methods

A study of the toxicity of the biomass of Cladophora sp. conducted according to the OECD test No. 203, using young, healthy guppy fish of the same age and size (OECD, 2019). The essence of the method is the poisoning and death of guppy fish with toxins that enter through the gills directly into the blood, bypassing the protective mechanisms of the digestive system.

Guppy is a small fish from the Pecilia family. The average size of a male's body is 3 cm, and a female's is 6 cm. The scales appear like a rhombic grid, so the fish received the species epithet from the Latin “reticulum” – net.

Principles of the research. The fish are exposed to the test substance for 96 h under static, semi-static, or flowing conditions. At the same time, the time of death of fish and visible deviations related to appearance and behavior are recorded. If possible, determine the concentration that causes the death of 50% of fish (LC50).

Equipment. The following laboratory equipment was used for the analysis:

- pH meter;
- appropriate devices for temperature control (thermometers);
- equipment for maintaining water temperature and oxygen content (heaters, compressors);
- tanks (aquariums, vessels) are made of chemically inert materials.

Keeping fish. Before conducting research, guppy fish were kept in the laboratory for nine days. The first 48 hours were the settling-in period. The fish were then acclimatized for seven days (48 hours of housing + 7 days of acclimatization = 9 days) in water similar to the test water immediately before the start of the test.

Fish were kept under the following conditions:

- Light period: day/night – 16/8 hours;
- Temperature: 21–25 °C;
- Oxygen concentration: at least 60 % of the air saturation value;
- Feeding: daily up to 24 hours before applying the researched product. Feed until saturation. Remove excess food and excrement to avoid waste accumulation.

During the acclimatization period (7 days), the death of less than 5 % of the population was recorded; under these conditions, the entire batch of fish was accepted.

For the experiment, fish without visible signs of disease and stress were used; guppies had no obvious developmental defects and did not undergo previous treatment for diseases or parasites during the last 14 days before testing.

When determining the toxicity of the studied biomass, dechlorinated drinking water was used, in which guppy fish were mobile, consumed food well, and actively responded to external stimuli.

Test solutions. The starting solutions were prepared by simply mixing the tested agent in water. When studying the toxicity of biomass on the body of guppy fish, six groups of 10 individuals each were formed according to the principle of analogs. Fish of the control group were kept in dechlorinated drinking water. Fish of experimental group I were treated with the drug at a dose of 5 mg/L, fish of experimental group II – 10 mg/L, fish of experimental group III – 25 mg/L, fish of experimental group VI – 50 mg/L, fish of experimental group V – 100 mg/L The study was conducted in 2 repetitions.

Individual measurements (weight and length) were determined at the beginning and the end of the experiment to study the effect of the biomass on the body of guppy fish.

Observation. During the first 24 hours, fish were observed every 3 hours. On days 2–4 of the experiment, all vessels with live fish were inspected twice daily (early in the morning and late in the evening to best cover 24 hours). At the same time, death and visible deviations were registered regarding:

- balance (loss of balance, raising or lowering the head, floating on the surface or diving);
- appearance (weak or dark pigmentation, exophthalmia);
- ventilation behavior (hyper, hypo, or irregular ventilation);
- behavior during swimming (increased or hypoactivity, immobility, convulsions near the surface or bottom).

At the same time, the time of occurrence and nature of intoxication, its severity, course, and time of death of fish or their recovery were noted.

At the end of the experiment, all fish of the control and experimental groups and those that survived were not used for further experimental purposes.

3. Results and discussion

When determining the toxicity of the studied biomass on the body of guppy fish, the data shown in Table 1 were obtained.

Table 1
Evaluation of the degree of toxicity of *Cladophora sp*. biomass. With the help of fish – guppies after 96 hours of the experiment (M ± m, n = 10)

№	The number of guppy fish, individuals	Drug dose, mg/L	Survived guppy fish, individuals	Died guppy fish, individuals	Guppy fish died, %
1.	10	5	10	0	0
2.	10	10	10	0	0
3.	10	25	10	0	0
4.	10	50	10	0	0
5.	10	100	10	0	0
6.	10	control	10	0	0

As can be seen from the data presented in Table 2, the use of the investigated biomass in doses of 5, 10, 25, 50, and 100 mg/L did not cause the death of fish. At the same time, no changes were detected in the behavior of the fish; they were active, mobile, and actively responding to external stimuli. The fish of the experimental groups were not distinguished from those of the control group.

Along with determining toxicity, the investigated agent's effect on weight and length was determined (Table 2).

Table 2
The effect of the studied biomass on the weight and length of guppy fish (M ± m, n = 10)

Group of animals	Before usage	After usage
The effect of the studied biomass on weight, g		
Control	0.28 ± 0.02	0.28 ± 0.02
I group	0.29 ± 0.01	0.29 ± 0.01
II group	0.30 ± 0.01	0.30 ± 0.01
III group	0.28 ± 0.01	0.28 ± 0.01
VI group	0.28 ± 0.01	0.28 ± 0.01
V Group	0.28 ± 0.01	0.28 ± 0.01
The effect of the studied biomass on the length of guppy fish, cm		
Control	2.15 ± 0.01	2.15 ± 0.01
I group	2.14 ± 0.02	2.14 ± 0.01
II group	2.16 ± 0.02	2.16 ± 0.02
III group	2.14 ± 0.01	2.14 ± 0.01
VI group	2.15 ± 0.01	2.15 ± 0.01
V Group	2.14 ± 0.01	2.14 ± 0.01

As seen from the data in Table 2, it was established that the 96-h. Exposure of the investigated biomass *Cladophora sp.* did not cause significant changes in the weight and size of the fish.

Therefore, based on the conducted research, it was established that using biomass *Cladophora sp.* in doses of 5, 10, 25, 50, and 100 mg/L did not cause death or disease of fish. At the same time, the fish were active, responding to external stimuli. In addition, it was established that the studied biomass is not toxic, and its LC_{50} is higher than 100 mg/L.

4. Conclusions

The biomass of *Cladophora sp.* is a non-toxic substance for guppy fish, and its LC_{50} is more than 100 mg/L.

Conflict of interest
The authors declare that there is no conflict of interest.

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