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Allelopathic activity of *Spirogyra* sp.: stimulating bloom formation and toxin production by *Oscillatoria agardhii* in some irrigation canals, Egypt

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It has often been observed that irrigation canals in Egypt that contain Spirogyra are covered with blooms of Oscillatoria during the summer season. These blooms do not occur in the canals not containing Spirogyra. Thus, two irrigation canals, one containing Spirogyra and another not containing Spirogyra, were chosen to study the possible allelopathic activity of Spirogyra on the growth of Oscillatoria agardhii. The growth of O. agardhii and other associated cyanobacterial species was followed in these two canals during the period May–September 2000. The results revealed that O. agardhii was dominant and formed blooms in the canals containing Spirogyra, while it had a moderate/rare occurrence in those not containing Spirogyra. To confirm the stimulatory allelopathic activity of this green alga on the growth of and microcystin production by O. agardhii, a laboratory experiment was run by growing O. agardhii with different concentrations of Spirogyra aqueous extract. The results showed that the growth of and microcystin production by O. agardhii increased with increasing concentrations of Spirogyra aqueous extract. This finding demonstrates the allelopathic activity of the green alga Spirogyra stimulating the growth of and toxin production by O. agardhii. Such a biotic factor should be taken into consideration when cyanobacterial blooms are monitored in freshwater bodies.

INTRODUCTION

In nature, microorganisms rarely grow in isolation, but, rather, with multispecies communities. These communities can have complex structures, as in biofilms; individual cells can change their morphology, physiology and biochemistry as they become part of the community (Costerton *et al.*, 1995). It is now realized that complex genetic and chemical systems regulate the interactions of individual organisms within communities (Swift *et al.*, 1994). Chemicals that regulate community structures would encompass both positive and negative interactions, either encouraging or discouraging growth and function of neighboring cells. These chemicals are secondary metabolites called allelochemicals (Zahner, 1979).

The occurrence of cyanobacterial blooms in eutrophic lakes under natural conditions is determined by biotic and abiotic factors. The environmental or abiotic factors influencing toxic bloom development are temperature, pH, light intensity and nutrient concentration, especially nitrogen and phosphorus (Skulberg *et al.*, 1984; Watanabe and Oishi, 1985; Carmichael, 1986; Reynolds, 1988; Sivonen, 1990; Mohamed, 1998). Biotic factors include grazing of other phytoplankton by zooplankton (Lehman and Sandgren, 1985), successful resource competition (Tilman, 1981) and possible allelopathic activity of macrophytes (Jasser, 1995) and other algae (Maestrini and Bonin, 1982).

Cyanobacteria are known to produce a variety of toxins that can be lethal to livestock, pets, wildlife as well as humans upon consumption of water contaminated with toxic cells or toxins. The cyanobacteria incriminated in producing these toxins are *Anabaena* and *Aphanizomenon*, for example, which synthesize neurotoxins, whereas species of *Microcystis*, *Nodularia* and *Oscillatoria* mostly generate hepatotoxins (Carmichael, 1992). These toxins are

released into the water column both during collapse of a bloom (Berg *et al.*, 1987) and from actively growing cyanobacterial populations (Sivonen *et al.*, 1990).

The term 'allelopathy' covers chemical interactions between plants (from bacteria to higher plants), including both stimulatory and inhibitory activities (Rice, 1984). Algal allelopathy has received less attention than that of higher plants, in spite of reports on the presence of such phenomena in cyanobacteria and other classes of algae (Keating, 1977, 1978; Patterson *et al.*, 1979; Wolf and Rice, 1979; Wium-Anderson *et al.*, 1982; Rice, 1984). Although studies on the interaction between cyanobacteria and other algae, both in natural habitats and in the laboratory, suggested new opportunities for natural control of the symptoms of eutrophication, there has been little progress in the use of natural bioactive compounds for lake management and ecotechnological treatment of eutrophication (Smith and Doan, 1999). Thus, understanding allelochemical interactions between cyanobacteria and other algae might be useful in a more limited way to control specific nuisance cyanobacteria.

Most allelopathic studies have dealt with inhibitory effects among organisms, but to the author's knowledge stimulatory effects among algae are not well studied. Thus, the aim of this study was to demonstrate the stimulatory influence of the green alga *Spirogyra* on bloom formation and toxin production in *Oscillatoria agardhii* in some irrigation canals containing heavy growths of *Spirogyra*.

METHOD

Field study

Water samples were collected during the period May–September 2000 with a 25-μm-mesh net from two irrigation canals at Sohag district. One of these canals is inhabited by a large biomass of *Spirogyra*, while the other almost does not contain this green alga. Cyanobacterial species present in these samples were identified according to Prescott (Prescott, 1978), and then counted by haemcytometer.

Laboratory study

Preparation of Spirogyra aqueous extract

Spirogyra mats were collected from irrigation canals, washed six times with distilled water, and then oven-dried at 80°C for 24 h. A known weight of the dried algal material was extracted twice in a known volume of distilled water. The extract was centrifuged, and the supernatant was diluted with distilled water to different concentrations (1, 2, 5 and 10 g/l). The toxicity of this alga was tested by an *Artemia salina* assay. The alga is nontoxic at a concentration of 10 g/l.

Experiment

To predict the optimal time for growth and toxin production in *O. agardhii*, a few filaments of this cyanobacterium were grown in filtered, sterilized, canal water for 3 weeks at 30 ± 2°C and illuminated with fluorescent light at 24 μmol m^{-2} s^{-1} in a 16 h:8 h light:dark cycle. The concentration of chlorophyll (Chl) *a* was determined according to Talling and Driver (Talling and Driver, 1963). Microcystin content was determined by enzyme-linked immunosorbent assay (ELISA) according to An and Carmichael (An and Carmichael, 1994).

To start the experiment, phytoplankton samples were collected in June from the irrigation canal without *Spirogyra* and passed through 350 μm nylon mesh to remove as much zooplankton as possible to avoid the grazing factor in this experiment. The filtered phytoplankton samples were combined and placed in 15 one-litre glass conical flasks, 500 ml each. Except for three, which were used as controls, the flasks were dosed with different concentrations of *Spirogyra* aqueous extract (1, 2, 5 and 10 g/l) in triplicates. Both control and treated flasks were incubated for 2 weeks at 30 ± 2°C and illuminated with fluorescent light at 24 μmol m^{-2} s^{-1} in a 16 h:8 h light:dark cycle.

Analysis

Investigation and identification of species, Chl *a*, microcystin content and LC_{50} of the *A. salina* assay were performed in the 2 week harvested cells. Chlorophyll *a* was determined according to the method of Talling and Driver (Talling and Driver, 1963). Microcystin content was determined by ELISA according to An and Carmichael (An and Carmichael, 1994). *Artemia salina* assay was determined by the method of Kiviranta *et al.* (Kiviranta *et al.*, 1991).

RESULTS

The results of the field study revealed that *O. agardhii* was dominant, and formed blooms in the irrigation canal containing the green alga *Spirogyra* during the period May–September 2000, while it had a moderate/rare occurrence in the canal not containing *Spirogyra* (Table I).

Growth and toxin production curves of *O. agardhii* showed that the optimal time for its growth and toxin production is 2 weeks (Figure 1). Therefore, the laboratory experiment was terminated at that time.

As shown in Table II, the number of *O. agardhii* filaments increased by increasing the concentration of *Spirogyra* extract, reached its maximum at 5 g/l, and then decreased when the concentration was raised to 10 g/l. The numbers of other associated cyanobacterial species were not changed significantly. On the other hand, a few

Table I: Occurrence of cyanobacteria in irrigation canals containing and not containing Spirogyra sp. during the period May–September 2000

Species	Canal containing *Spirogyra*					Canal without *Spirogyra*				
	May	June	July	Aug.	Sep.	May	June	July	Aug.	Sep.
Anabaena variabilis	+	+	–	+	+	–	–	–	–	–
Gomphosphaeria lacustris	++	++	++	+	–	++	++	++	+	–
Merismopedia incerta Lemm.	++	++	++	++	++	++	++	++	++	++
Lyngbya limnetica	+	+	–	–	–	++	+	+	–	–
Oscillatoria agardhii Gom.	++++	++++	+++	+++	+++	++	++	+	+	+

–, absent; +, rare; ++, moderate; +++, high; ++++, bloom.

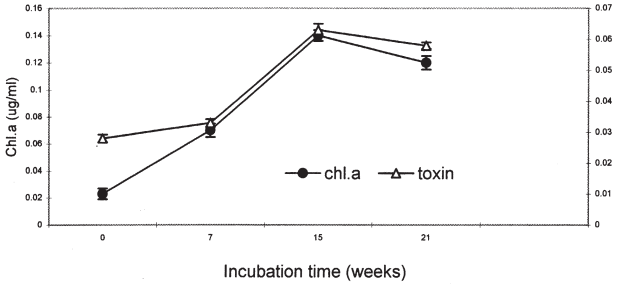

Fig. 1. Growth and toxin production curves of *O. agardhii*.

cells of green algae and diatoms were found associated with *Oscillatoria* mats (data not shown).

Both the dry weight and Chl *a* content of the non-axenic cultures, grown under the conditions of different concentrations of *Spirogyra* extract, were associated with the number of *O. agardhii* filaments. They increased with increasing concentration of *Spirogyra* extract until they reached their maxima at 5 g/l and then declined at 10 g/l (Figures 2 and 3).

Increasing the concentration of *Spirogyra* extract stimulated microcystin production by the cells dominated by *O. agardhii* even at the highest concentration (10 g/l), which inhibited the growth of these cells (Figure 4). The toxicity of the cells, as determined by *A. salina* assay, was concomitant with microcystin content within the cells. The LC_{50}s of the cells grown in high concentrations of *Spirogyra* extract were lower than those of cells grown in the lowest concentrations (Figure 5).

DISCUSSION

Field patterns as well as physiology-based model results indicate that the dominance of Oscillatoriaceae in shallow lakes is attributed to the following: (1) these shade-tolerant cyanobacteria are able to cause an increase in turbidity that favors their competitive advantage; (2) the relative

Table II: Effect of Spirogyra aqueous extract concentrations on the numbers of the cyanobacterial population grown under the experimental conditions

Species	Zero time	Concentrations of *Spirogyra* extract (g/l)				
		Control	1	2	5	10
Anabaena variabilis	115[f] ± 8	76 ± 3	92 ± 4	78 ± 5	55 ± 2	–
Gomphosphaeria lacustris	280[c] ± 16	150 ± 12	170 ± 11	186 ± 20	135 ± 13	150 ± 16
Merismopedia incerta Lemm.	208[c] ± 22	270 ± 16	200 ± 18	159 ± 13	148 ± 15	118 ± 13
Lyngbya limnetica	30[f] ± 2	–	–	–	–	–
Oscillatoria agardhii Gom.	148[f] ± 7	236 ± 27	845 ± 29	bloom	bloom	5080 ± 67

[f]filament; [c]colony.

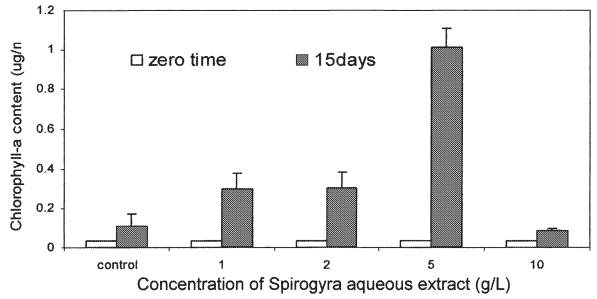

Fig. 2. Effect of the concentration of *Spirogyra* aqueous extract on Chl *a* of the non-axenic culture dominated by *O. agardhii* and grown under the experimental conditions.

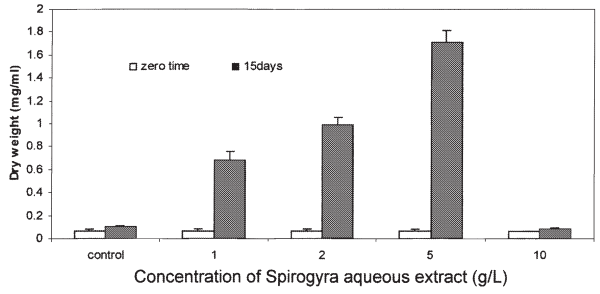

Fig. 3. Effect of the concentration of *Spirogyra* aqueous extract on the dry weight of the non-axenic culture dominated by *O. agardhii* and grown under the experimental conditions.

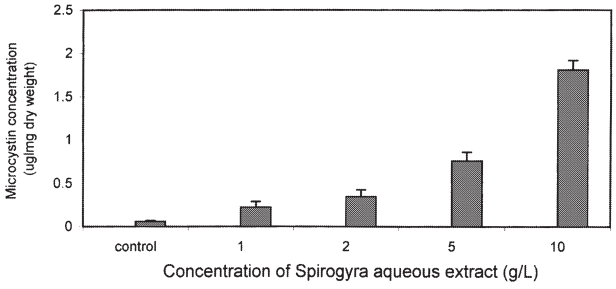

Fig. 4. Effect of the concentration of *Spirogyra* aqueous extract on the microcystin content of the non-axenic culture dominated by *O. agardhii* and grown under the experimental conditions.

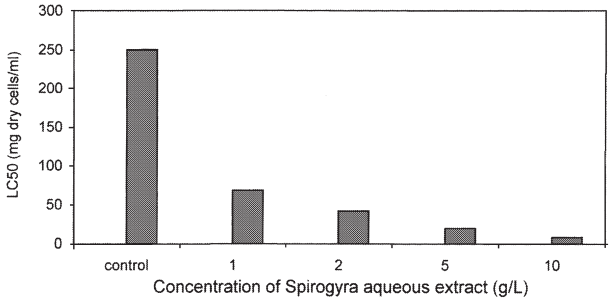

Fig. 5. Effect of the concentration of *Spirogyra* aqueous extract on the toxicity (LC_{50}) of the non-axenic culture dominated by *O. agardhii* and grown under the experimental conditions.

inedibility of filaments for zooplankton may further enhance the stability of blue-green dominance; (3) high flush rates reduce the probability of blue-green dominance because of their relatively slow growth rates; and (4) allelopathic substances from aquatic macrophytes can affect the competitive balance in favor of other algae (Scheffer *et al.*, 1997).

In the present study, *O. agardhii* was dominant in the irrigation canal containing *Spirogyra*, but of rare occurrence in the canal not containing *Spirogyra*. This is what suggested that the presence of *Spirogyra* mats in some irrigation canals in Egypt may be the most important biotic factor behind bloom formation of *O. agardhii* on the water surface of these canals. Previous studies reported that *O. agardhii* was the most bloom-forming species in temperate shallow water (Berger, 1975; Sas, 1989). This dominance was attributed to the role of seasonality, herbivorous zooplankton and the effects of allelopathic substances.

It is well known that *O. agardhii* is a member of the cyanobacterial species capable of producing microcystins (Ericksson *et al.*, 1989; Meriluto *et al.*, 1989; Bruno *et al.*, 1992; Luukkainen *et al.*, 1993). The current study revealed that *O. agardhii* exhibited high growth and microcystin production when it was grown in high concentrations of an aqueous *Spirogyra* extract. Other cyanobacterial species associated with *Oscillatoria*, however, have not been affected by this extract. This finding suggests that *Spirogyra* may produce allelochemicals stimulating growth and toxin production by *O. agardhii*. These results concur with those obtained by Jasser (Jasser, 1995), who found that the number of *Oscillatoria limnetica* grown in bags placed in an aquarium containing macrophytes was significantly higher than that in bags placed in a macrophyte-free aquarium.

In conclusion, the present study is the first to focus on the allelopathic activity of *Spirogyra* promoting bloom formation of *Oscillatoria* in shallow water. Thus, such a biotic factor should be taken into consideration when cyanobacterial blooms are monitored in freshwater bodies. Further study on the identification of stimulatory allelochemicals produced by algae is needed.

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