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SHORT COMMUNICATION

Effects of environmental pollution on algal suppression of *Alternanthera philoxeroides* (Mart.) Griseb: Field and laboratory observations

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ABSTRACT

Under the conditions of aquatic environment pollution, algicidal effects of *Alternanthera philoxeroides* were analyzed by field and laboratory observations. In the alkaline habitat of *A. philoxeroides*, the mean BOD_5 and COD were 4.38 and 42.76 $mg \cdot L^{-1}$, respectively, and the phytoplankton growth was suppressed by 31%. In laboratory trial *A. philoxeroides*, inhibited the *Microcystis aeruginosa* (52 %) and *Chlorella pyrenoidosa* (70%) but had a declining inhibitory effect in order of *C. pyrenoidosa*, *M. aeruginosa* and natural phytoplankton. *A. philoxeroides* inhibited the growth of *C. pyrenoidosa* and *M. aeruginosa*, which was closely related to the inhibition of phytoplankton observed in the study. In addition, environmental factors also had algicidal effects of *A. philoxeroides* in the form of a conic curve. The three principal components, COD, BOD_5, and Chla showed stimulation of 37%, 33%, and 13%, respectively. In short, aquatic pollution enhanced the algicidal effects of *A. philoxeroides*.

Key words: Algicidal effect, *Alternanthera philoxeroides*, aquatic habitat, BOD, COD, environmental pollution, inhibitory mechanism

INTRODUCTION

Recently the increasing nutrients levels in various water bodies have led to frequent outbreaks of water bloom on surface waters. It has direct negative effects on the aquatic ecosystems and threatens the safety of potable water for human beings and animals (8). *Microcystis aeruginosa* is the dominant species found in harmful algal blooms in eutrophic water bodies throughout China, whereas, *Chlorella pyrenoidosa* is main phytoplankton in clean and shallow lakes (15). Thus, the control of cyanobacteria outbreaks, particularly these two problematic algal species, is a serious problem that needs to be addressed to ensure continued human health and well-being.

Alternanthera philoxeroides G. (Alligator weed, Photograph (i)) is a perennial and invasive plant originated in Paraná River, South America (7). It has several unique

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characteristics [high capacity for asexual reproduction and phenotypic plasticity (4)], which facilitate its wide ecological range and extreme stress tolerance (34, 14). It is problematic invasive plant species in many countries and. in Australia, it is "weed of national significance" due to its actual and potential impact (2). *A. philoxeroides* can grow in a range of habitats from dry terrestrial to aquatic. In aquatic habitats, it can root in shallow water or it can remain free-floating as an emergent macrophyte in oligotrophic, eutrophic, or severely-contaminated water bodies (6).

Photographs: (i) Alligator weed (Left), (ii).Polluted Chaohu Lake study site (Right).

It is highly competitive for nutrients, water and light and utilizes the powerful adaptive strategies to combat disturbance and grazing. It readily invades other habitats and has acquired a wide range of niches (1). The allelopathic potential of *A. philoxeroides* had been reported (11, 13), who suggested that allelochemicals mediate the invasiveness and algicidal effects of alien plants. Saponin has been extracted from *A. philoxeroides*, which inhibits the seedling growth of deep water rice cultivars (*Oryza sativa* L. cv. Bao) (10). In the polluted Chaohu Lake (Photograph (ii)), as we discovered in the field investigation, the phytoplankton leading to water bloom battled with *A. philoxeroides*. However, the allelopathic inhibition of harmful algae by *A. philoxeroides* under conditions of environmental pollution has never been reported. Thus, this study aimed to (i). test the algicidal effects of aqueous extracts of *A. philoxeroides* derived from 10-different habitats on the growth of two algal species, (ii) test the correlation between environmental pollution and algicidal activity and (iii). test the effects of environmental pollution on the allelopathic inhibition of algae by *A. philoxeroides*.

MATERIALS AND METHODS

I. *A. philoxeroides* and water bloom algae

A. philoxeroides samples were collected during 2008 from ten sampling sites in the watershed of Chaohu Lake in Southern China (117°16′54"-117°51′46"E and 31°25′28"-31°43′28"N). Three water samples were collected at the same time from each sampling sites. In our previous investigation, there were 10-different aquatic habitats (15), hence, the plant samples were classified into 10-categories according to their sampling sites. Plant material was washed to remove any debris before it was freeze-dried. Lyophilized material was finely ground and stored in air-tight containers at -5 °C in dark. Prior to the experiment, 100 g of each dried powdered plant specimen was added to flasks containing 1000 mL of distilled water and the flasks were placed on a thermostatic (25 °C) shaker for 48 h. Thereafter the samples were filtered through two layers of gauze. The supernatant was then filtered through qualitative filter paper and passed successively through 0.45 μm and 0.22 μm filters under low vacuum (-200 to -400 mbar) to remove any suspended particles and bacteria. Ten aliquots of these water extracts were diluted with sterile distilled water to produce a final concentration equivalent to 100 mg mL^{-1} of plant dry weight.

Axenic strains of *C. pyrenoidosa* and *M. aeruginosa* were obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences. Before the experiments, cultures of *C. pyrenoidosa* and *M. aeruginosa* were inoculated into 2000 mL Erlenmeyer flasks containing 1600 mL of autoclaved BG11 and SE medium. The microalgae were cultured to the exponential phase before used in subsequent experiments (initial algal cell density = c. 2.35×10^6 cells mL^{-1} after inoculation) (15).

Ⅱ. Phytotoxicity assessment and water quality analysis

We inoculated 5 mL microalgae into 250 mL flasks containing 45 mL culture medium. Exponential growth microalgae were then inoculated using 2 mL water extracts of *A. philoxeroides* sampled from 10 different aquatic habitats. Controls were prepared by inoculating microalgae with an equivalent amount of autoclaved distilled water, rather than water extracts. Six replicates were conducted for each treatment.

All experiments were done thrice. Microalgae were cultured in an illuminated incubator at irradiance of 3000-4000 μmol/m^2·s, with a light: dark photoperiod of 12:12 h and temperature cycle of 25:20 °C. Flasks were shaken thrice daily at set times. We stained 1 mL of algae sample with Lugol's iodine solution and cell numbers were counted using a hemocytometer (a 40× objective and a 10× eyepiece). The phytoplankton assemblages sampled from Chaohu Lake were enumerated using the same method. The algae species of the assemblage have been identified by Zuo *et al*. (15). It consisted mainly of cyanobacteria and green algae. Nine parameters [pH, turbidity, colour, suspended solids (SS), dissolved oxygen (DO), biochemical oxygen demand for five days (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC), and chlorophyll a (Chla)] were determined for each water sample category as per the Chinese norms (MEPC, 2002). These parameters were determined by following methods: pH as the acidity; turbidity using a spectrophotometric method with Nessler's reagent; colour by comparison with platinum and cobalt; SS by filtration and weighing; DO using an electrochemistry probe; BOD_5 by dilution and inoculation; COD using the dichromate method; TOC using the combustion/oxidation non-diffusive infrared absorption method; and Chla using a spectrophotometer. Based on International Rules, surface water quality is divided into 5-levels. The water quality in first and second levels indicates very clean and safe condition. The polluted surface water is in fourth and fifth levels. The water bodies in third level are fragile facing the pollution resources. The third level of lake water quality was considered as control (9).

Ⅲ. Statistical analysis

The algal inhibitory rate (IR) was calculated as under:

$$IR= (1 - N/N_0) \times 100\%,$$

Where, IR: Inhibitory rate at specific cell densities (%), while N and N_0 are the cell densities of treatment and the control (cells mL^{-1}), respectively.

$$\text{Level of Environmental Pollution} = (\text{Treatment} - \text{Control})/\text{Control},$$

Where, Treatment was derived from the sample measurements, whereas the Control values used for BOD_5 and COD were 4.0 and 20 mg L^{-1}, respectively.

All data were tested using an ANOVA in SPSS 14.0 with a significance level of $P < 0.05$. We also tested the correlation between environment pollution indices and the algal inhibition rate of *A. philoxeroides* treatments. Moreover, due to concerning about many environmental factors simultaneously interacting, Principal Component Analysis (PCA) was conducted to yield higher resolution.

RESULTS AND DISCUSSION

Pollution levels in aquatic habitat of *A. philoxeroides*

The aquatic habitat of *A. philoxeroides* was alkaline, with a mean turbidity of 4.46. The water colour in different habitats ranged from 11.50 to 25.00, with a mean of 16.65. The SS content varied at sample sites in range 40-90, with a mean of 65. The DO content was always >5.0, indicating that *A. philoxeroides* had a high oxygen demand. The BOD_5 and COD were greater than control, indicating that Chaohu Lake was polluted. At sampling points 1, 7, 8, 9, and 10, the BOD_5 exceeded the control level by 47.50%, 5%, 40%, 32.5%, and 27.5%. At all sampling sites, the COD level was 0.53 to 2.69-folds than control level. The mean COD level was 1.14 folds than control level, indicating that Chaolu Lake was significantly contaminated. The TOC content differed in various habitats and the maximum level was 1.96-folds than minimum. The maximum Chla level was found at sampling point 5, while the minimum was at sampling point 1 and the difference between the two extremes was 0.42 mg L^{-1}(Table 1).

Algicidal effects of aqueous extracts of *A. philoxeroides*

Extracts of *A. philoxeroides* sampled from different habitats inhibited the propagation of *C. pyrenoidosa*, *M. aeruginosa*, and the phytoplankton assemblage. The plant species displayed the largest algicidal effect at Site 8, whereas lowest at Site 7 (Fig. 1). The algal density of *M. aeruginosa* was 34-70% (IR) lower (mean = 52%). *C. pyrenoidosa* was also significantly suppressed and the IR ranged from 42-96% with a mean of 70%. However, the inhibition of the phytoplankton assemblage by

Table 1. Pollution levels in the aquatic habitat of *A. philoxeroides* (mg·L^{-1}) study sites

Study Sites	pH	Turbidity	Color	SS*	DO*	BOD_5*	COD*	TOC*	Chla*
1	7.8 b	2.9 e	25.0 a	58.5c	4.4d	5.9a	45.2b	1583.3e	0.5 a
2	7.6 bc	3.2 d	17.5 b	49.0d	5.6a	3.6c	33.9d	1805.7d	0.2d
3	7.8 b	3.6 d	14.5 d	75.0bc	5.2b	3.5c	35.9cd	1852.3d	0.2 c
4	8.3 ab	4.1 cd	14.5 d	57.0c	5.5a	3.5c	33.3d	2207.7c	0.3 bc
5	7.8 b	4.3 c	16. 0 bc	80.0b	5.6a	3.2d	40.6c	2002.7c	0.1 d
6	7.4 c	4. 5 bc	19.5ab	80.0b	5.1b	3.9c	31.9e	2594.0bc	0.1 d
7	7.3 c	4.8 bc	11.5 e	71.0bc	5.2b	4.2b	34.5d	2725. 0b	0.3 b
8	7.2 c	5.1 b	17.5 b	40.0e	4.8c	5.6a	30.6e	2688.0b	0.3 b
9	8.8 a	5.9 ab	15.5 c	90.0a	4.9c	5.3a	73.9a	3110.0a	0.3 b
10	8.9a	6.3 a	15.0 cd	50.0d	5.1b	5.1ab	67.7ab	3012.7ab	0.1 d

*: Significant differences at $P < 0.05$ are indicated by lowercase letters (SD and SE are omitted)

Table 2. Relationship between pollution indicator parameters and the algal inhibition rate (IR) of *A. philoxeroides*

Y	X	Function	R^2	P	IR range	Y range
pH	IR	$y = -55.54x^2 + 60.72x - 8.19$	0.9577	0.015	0-1	0-8.50
Turbidity		$y = -14.77x^2 + 19.64x - 1.59$	0.9219	0.023		0-4.98
Color		$y = -147.59x^2 + 158.29x - 24.47$	0.9914	0.045		0-18.20
SS		$y = -883.32x^2 + 900.87x - 157.41$	0.9262	0.019		0-72.00
DO		$y = -1.78x^2 + 1.50x + 4.85$	0.9012	0.016		4.8-5.6
BOD_5		$y = 3.97x^2 - 1.33x + 3.99$	0.9785	0.020		4.0-6.2
COD		$y = -1278.40x^2 + 1381.80x - 318.93$	0.9894	0.011		0-52.78
TOC		$y = 5901.00x^2 - 4792.30x + 3220.20$	0.9793	0.008		2300-2700
Chla		$y = 4.09x^2 -4.26x + 1.32$	0.9625	0.035		0.2-0.4

x : Inhibition rate of harmful algae by alligator weed (IR), *y* : specific environmental parameters such as pH, SS and DO.

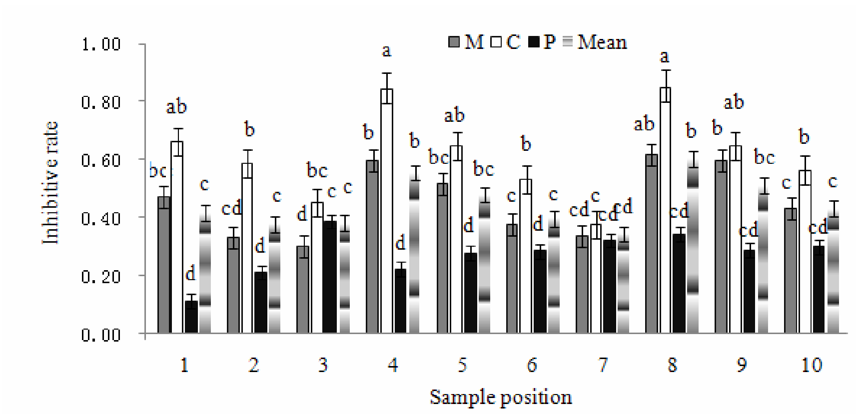

Figure 1. Algal inhibition by *A. philoxeroides* collected from different habitats. M: *Microcystis aeruginosa*; C: *Chlorella pyrenonidosa*; P: phytoplankton assemblage, Mean: the mean inhibitive rate, 1/3*(M + C + P). *: Significant differences at $P < 0.05$ are indicated by lowercase letters (SE is marked by the error bars).

A. philoxeroides was lower and the IR fluctuated in the range 13-74% with a mean of 31%. The field tests suggested that the more complex natural conditions reduced the algicidal effects of *A. philoxeroides*. There was a significant correlation among the algicidal effects on *M. aeruginosa*, *C. pyrenoidosa* and the phytoplankton assemblage ($P < 0.05$).

The effects of aquatic habitat pollution on the algicidal potential of *A. philoxeroides*

The IR of *A. philoxeroides* on microalgae was correlated closely with the nine indicators of environmental pollution in the aquatic habitat, in the form of conic curve ($P < 0.05$). The relationship was divided into two categories with quadratic functions where the parabola widened when a > 0 whereas it dropped when a < 0. For BOD_5, TOC, and Chla, a > 0, whereas a < 0 for the remaining parameters. In conditions of environmental pollution, *A. philoxeroides* inhibited the growth of harmful algae and different parameters had variable utility as predictors of the algicidal effects (Table 2). *A. philoxeroides* grew well in acidic or weak alkaline environment and it exhibited strong allelopathy against the water bloom algae. Turbidity, colour, and SS ranged from 0-5, 0-18, and 0-72, respectively. The DO, TOC and Chla results suggested that algal inhibition by *A. philoxeroides* had a high oxygen demand, indicating that this activity resulted from synergistic effects and the high productivity of the aquatic habitat. The COD and BOD_5 results suggested that low pollution enhanced the algicidal effects of *A. philoxeroides*. It should be highlighted that three principal components, COD, BOD_5, and Chla showed an influential percent of 37%, 33%, and 13%, respectively (Table 3).

In our field investigation, *A. philoxeroides* grew well in alkali environments and it improved the turbidity, colour, and SS content of its aquatic habitat. In high oxygen conditions, *A. philoxeroides* was highly effective in suppressing the harmful algae that grew in eutrophic aquatic ecosystems. In pollution at low concentration, *A. philoxeroides* displayed great resistance and it released an abundance of allelochemicals that were responsible for algal inhibition.

Table 3. Principal component analysis of environmental factors concerned with algal inhibition rate (IR) of *A. philoxeroides*

Indicator	Initial eigen value	Variance (%)	Cumulative (%)
Chemical oxygen demand (COD)	3.3078	36.7533	36.7533
Biochemical oxygen demand for 5-days (BOD_5)	2.9931	33.2563	70.0096
Chlorophyll a (Chla)]	1.1331	12.5901	82.5997
Suspended solids (SS)	0.8634	9.5934	92.1931
Total organic carbon (TOC)	0.5385	5.9838	98.1769
Turbidity	0.0866	0.9627	99.1396
Colour	0.0583	0.6480	99.7876
pH	0.0175	0.1946	99.9822
Dissolved oxygen (DO)	0.0016	0.0178	100

Note: While initial eigen-value >1 and cumulative percent > 80%, the corresponding indicator would be screened as the principal component.

The algicidal effects of *A. philoxeroides* were significantly correlated with nine indicators of environmental pollution in the form of a quadratic curve. This close relationship indicated that *A. philoxeroides* had a consistent potential to inhibit the algae, which did not depend on the algae species, habitat features, or the allelopathy bioassay format. Water extracts of alligator weed from different habitats produced different algicidal effects. This was possibly because the alligator weed from different habitats absorbed different substances from the water, including poisonous chemicals. Based on our statistical analysis, it was concluded that *A. philoxeroides* inhibited the growth of harmful algae in normal or low-pollution water bodies. Interestingly, an appropriate COD or BOD_5 stimulated the algicidal effects of *A. philoxeroides*. Yu *et al*. (12) confirmed the allelopathic effects of *A. philoxeroides* on *Chlamydomonas reinhardtii* and discovered that this effect was not as strong as that of *Eichhornia crassipes*. They suggested that the allelopathic effect of higher aquatic plants on algae, including that of invasive plants, was a common phenomenon that might play an important role in the formation and succession of aquatic ecosystems.

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