

Inhibitive effects of three compositae plants on *Microcystis aeruginosa*

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Abstract Based on common phenomena of biochemical interaction between plants and microorganisms, the inhibitive effects of three common terrestrial compositae plants, namely *Artemisia lavandulaefolia* DC., *Conyza canadensis* (L.) Cronq., and *Kalimeris indica* (L.) Sch.-Bip. on the blue algae *Microcystis aeruginosa* was studied. Live compositae plants are co-cultivated with algae in two different inoculation doses for 10 days in 5-pools incubators, in order to exclude the influence of bacteria and nutrients. The results show that *Artemisia lavandulaefolia* DC has the most inhibitive potential among the three plants as evidenced by the most drastic decrease in optical density (OD_{680}) of the algae. The inhibition rate is 93.3% (with initial inoculation dose of 2.0×10^6 Cells/mL) and 89.3% (with initial inoculation dose of 4.0×10^6 Cells/mL) respectively on the 10th day of cultivation. The average inhibition rate during the later half of the experiment is 0.76 (with initial inoculation dose of 2.0×10^6 Cells/mL) and 0.71 (with initial inoculation dose of 4.0×10^6 Cells/mL), respectively. Logistic model analysis shows that compositae plants such as *A. lavandulaefolia* DC. causes the reduction of the habitat's carrying capacity of algae. ANOVA analysis is used to determine the similarity and differences between every experimental group and an average inhibitive rate model is used to evaluate the inhibition effects. The results show that *A. lavandulaefolia* DC., which grow well in the aquatic environment, may have a great potential in controlling algae bloom in eutrophic water.

Keywords allelopathy, compositae plants, *Microcystis aeruginosa*, inhibition rate, logistic model analysis

1 Introduction

Harmful algal blooms (HABs), as a global phenomenon, have been expanding over the last few decades [–4]. It is

estimated that the economic loss of a serious HAB event is usually over millions of US dollars [5]. Moreover, about 2000 cases of human-poisoning resulting from algal toxins are reported each year [6]. In order to mitigate the harmful effects of HABs efficiently, it is imperative to develop new prevention methods for HABs [7]. Recently, allelopathy and ecological chemistry method is applied in eutrophication control as an economical and effective approach.

Molisch (1937) firstly used allelopathy to describe either positive or negative biochemical interactions between all plant types. Rice (1979), in his definition, included microorganisms and restricted the conceptual content of allelopathy exclusively to negative effects arising from the production and excretion of chemical compounds originating from plants and microorganisms in his book *Allelopathy* [8]. After the allelopathy effect is discovered to be a common phenomenon, inhibitive effect has become a hotspot of international research in many fields such as botany, ecology, agriculture, soil science, and horticulture [9] as a kind of safe bio-inhibitor.

Currently, aquatic plants are broadly studied in eutrophication control. They not only help in drawing out the excessive nutrition from the water body, but also inhibit algae growth [10]. As secondary metabolites of the plants, the inhibitive chemicals can usually degrade naturally and safely [11]. The successful use of aquatic plants in eutrophication control includes *canna* and *acorus* [12].

Many terrestrial plants also have strong allelopathy effect, together with the stronger ability to absorb nutrition than many aquatic plants. Another advantage is that their growths are not affected by the mass algae in the eutrophic water body [13]. Some terrestrial plants have already been used to control eutrophication through artificial floating-islands, as these plants have a high biomass due to their fast growth, which can help with nutrition extraction. A typical example of this is rice *Lolium multiflorum* Lam [14]. However, there are still no reports about the use of terrestrial plants in eutrophication control

concerning both their superiority on biomass and their inhibitive effect. And it can be inferred that the combination of the two effects might greatly improve the inhibitive effect of algae.

The terrestrial plant family compositae is the most diversified family in the world, in which at least 39 genera have been confirmed to have inhibitory substances [15]. The current studies of compositae mostly focus on its inhibition of terrestrial species such as invasive alien weeds and fungi, and have been shown to have obvious inhibitive effects [13], while their utilization on inhibition of algae, which might be promising, has not been studied yet. Moreover, decades of studies about soilless cultivation suggest that theoretically, any plant can adapt to soilless culture if treated properly [16], and the many successful cases have also provided reliable basis for the co-cultivation experiment [17–19].

This paper studied the inhabitation of three terrestrial plants on *Microcystis aeruginosa*—one of the blue algae which cause the water algae bloom. It provides a scientific basis for the possibility of controlling bloom by compositae and the development of a new algaecides chemical.

2 Materials and methods

2.1 Compositae plants and algae

Three plants: *Artemisia lavandulaefolia* DC. (abbreviated as A.L.D.C.), *Conyza canadensis* (L.) Cronq. (abbreviated as C.C.C.), and *Kalimeris indica* (L.) Sch.-Bip (abbreviated as K.I.S.B.) collected in the roadside, in Luoja Hill and flower bed, respectively were tested for their inhibitive effect on blue alga, here specified as *Microcystis aeruginosa* which is provided by FACHB-collection (Freshwater Algal Culture Collection of Institute of Hydrobiology) of the Chinese Academy of Sciences [20]. The detailed information of the tested plants are listed in Table 1.

Prior to the experiment, the algae is cultured for 7 days up to the exponential growth stage in BG-11 medium with a light flux of 4000 Lux (light: dark cycle, 16 h: 8 h) at 25°C, and a high biomass with cell concentration of 5.0×10^6 Cells/mL is obtain at the end of incubation.

2.2 Experiment device

Structural diagram of the incubator for microenvironment simulation is shown in Fig. 1 [12]. The Incubator is made by plexiglas, which is transparent to ensure sufficient light for photosynthesis. Each incubator comprised of five pools. The larger pool in the center is used for the cultivation of compositae and the smaller ones around for algae. The round holes between them are covered with 0.45 μm of microporous filter membrane and gauze, in order to prevent the disturbance from the rhizosphere bacteria and protozoa that adhere to the plant's roots [21]. The nutrients in the medium and the inhibitive chemicals can freely exchange within the five pools.

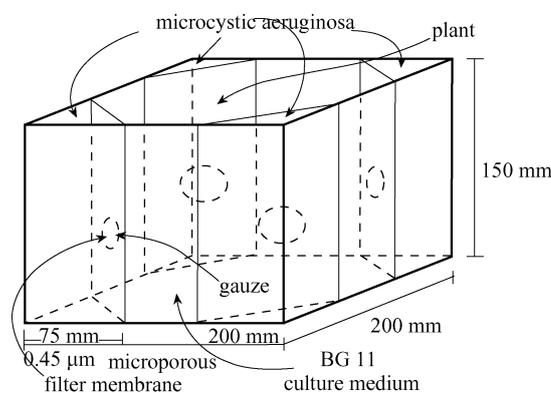


Fig. 1 Diagram of the experimental device

2.3 Research methods

2.3.1 Pretreatment of plants

Selected healthy plants with intact root system are botanized carefully. Then the roots are soaked and rinsed in tap water several times to remove the adhered soil until no soil remained in the water at the last wash. The old leaves and some flowers are pruned; the plant is cut at a length of 30–60 cm at a weight of about 100 g, which ensured normal photosynthesis. Then the roots are dipped into 4 g/L of Strengthen Rooting Medium (Xinhai Haida Environmental Protection Science and Technology Co.,

Table 1 Information of the three compositae plants

No.	genus name	species name	growth cycle	collection sites	habitat	medicinal parts/functions
1	<i>Artemisia</i>	<i>Artemisia lavandulaefolia</i> DC.	perennial herb	roadside in campus	valley, grassland, shrub roadside	whole grass/urinary tract infection therapy
2	<i>Conyza</i>	<i>Kalimeris indica</i> (L.) Sch.-Bip	annual herb	roadside in campus	farmland, roadside, furrows, wild field and surrounding of the habitant area	whole grass/unknown
3	<i>Kalimeris</i>	<i>Conyza canadensis</i> (L.) Cronq.	annual herb	Luoja Hill and roadside in campus	hillside and roadside	whole grass and roots/ Qingrejiedulishi therapy

Ltd, Ningxia, China) for 1–2 min to promote the growth of roots. Finally, the plants are cultivated in the 4 times diluted BG-11 culture medium [22] for 3–4 d to make them adapt to the aquatic environment gradually. Before co-cultivation, the roots are soaked in 0.5% KMnO_4 solution for about 30 min to eliminate bacteria adhering to the roots. The remaining potassium permanganate on the roots is rinsed by distilled water for several times. Then the plants are put into the central larger pool of the incubator, and fixed with adhesive tape.

2.3.2 Determination of the inhibitive effect on algae

Examination of the inhibitive effects of the three plants on the blue algae *Microcystis aeruginosa* is conducted using the 5-pool incubator as described above; each incubator is used exclusively for one type of plant. 100 g of plants are cultivated in the central large pool with a volume of 2600 mL, and the four small pools with a volume of 250 mL for algae cultivation. Two initial inoculation doses, i.e., 2×10^6 Cells/mL and 4×10^6 Cells/mL are set and each has a duplicate. Superfluous nutritional salt is added to preclude the possibility of nutrition limitation affecting algae growth. Erlenmeyer flasks inoculated with 150 mL of algae with the same cell concentration served as controls and also two parallels for each concentration is run.

The algae optical density is determined every 24 hours by UV-Vis spectrophotometer. The TN and TP are determined on the 10th day. At the same time, the morphology of algae cells are observed under a light microscope (Eclipse E200, Nikon USA) at $50 \times$ magnification and photographed when significant changes appeared on each group with a digital camera (Megapixel Firewire Camera PL-A662, Canada).

2.4 Analysis methods

2.4.1 Determination of the cell concentration

Cell concentration of algae is determined by spectrophotometry [23], based on the linear relationship between the algae concentration and its optical density. The algae's maximum absorption wavelength is 680 nm. The absorbency can be converted into the cell concentration through a working curve (Fig. 2) which is obtained as follows:

2.4.2 Determination of total nitrogen (TN) and total phosphorous (TP)

TN is determined by persulfate oxidation-UV spectrophotometry (with Unico UV2000, Unico Instrument Co., Ltd., Shanghai, China); TP is determined using the method of molybdenum antimony-spectrophotometry (with Unico UV2000, Unico Instrument Co., Ltd., Shanghai, China) [22].

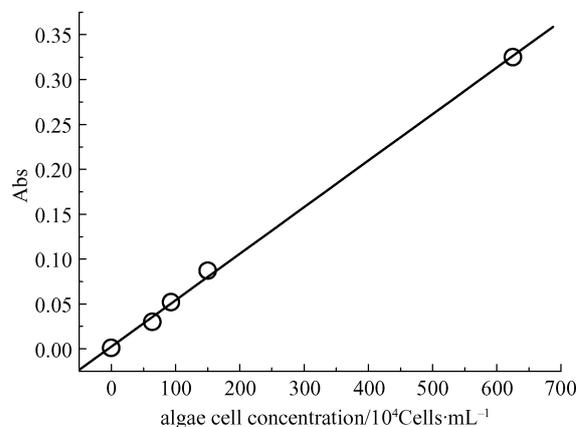


Fig. 2 Working curve used to convert the absorbency into the cell concentration
[where: absorbency = $0.00241 + 5.2 \times 10^{-4} \times$ (cell concentration), $R = 0.9994$, $P < 0.0001$]

2.4.3 Fitting analysis of the algae growth data

There are two types of growth curves for algae [24], namely the J-curve and S-curve. In this experiment, the growth of cells is affected by many factors such as space, nutrition, protozoa, and inhibitive chemicals. Therefore, it is impossible for algae to grow following a J-curve. However, a logistic curve (S-curve) can be used to describe the growth of the cell under a complicated environment:

$$C_t = \frac{K}{1 + ae^{-rt}}, \quad (1)$$

when

$$a = e^{a_0}, \quad C_t = \frac{K}{1 + e^{a_0 - rt}}, \quad (2)$$

$$t = 0, \quad C_0 = \frac{K}{1 + a}, \quad (3)$$

where K is the habitat's carrying capacity (10^4 Cells/mL), r is instantaneous growth rate (day^{-1}) and a is a constant that is related to the initial concentration C_0 and K . Equations (1) and (2) are two types of the logistic model, and Eq. 1 is used in our study. There is a positive correlation between $(a + 1)$ and K when C_0 is definite (Eq. (3)).

Different parameters and fit curves can be obtained after fitting, using *Origin 7.5* software (OriginLab Corporation, USA). These parameters can be used to evaluate the inhibitive effect of different plants.

The parameter t_x ($0 < x < 1$) is used to predict the time to grow to xK (x of K), and the inhibition can be evaluated by comparing these calculated parameters $t_{0.5}$, $t_{0.75}$, $t_{0.9}$, $t_{0.99}$ (Eqs. (4)–(7)) between the treatment and the control. For example, $t_{0.99}$ represents that it takes $t_{0.99}$ to reach 99% of the habitat's carrying capacity K .

$$t_{0.5} = \frac{\ln a}{r}, \quad (4)$$

$$t_{0.75} = \frac{\ln a}{r} + \frac{\ln 3}{r} = t_{0.5} + \frac{\ln 3}{r}, \quad (5)$$

$$t_{0.9} = \frac{\ln a}{r} + \frac{\ln 9}{r} = t_{0.5} + \frac{\ln 9}{r}, \quad (6)$$

$$t_{0.99} = \frac{\ln a}{r} + \frac{\ln 99}{r} = t_{0.5} + \frac{\ln 99}{r}, \quad (7)$$

2.4.4 Calculations of inhibition rate and average inhibition rate model analysis

The inhibition rate which is calculated using the modified equation (Eq. (8)) [25] and average inhibition rate model (Eq. (9)) built under the independent assumption of IR_t values during the last half of the experiment period are as follows:

$$IR_t = \frac{C_t^c - C_t^e}{C_t^c}, \quad (8)$$

$$\bar{IR}_{half} = \begin{cases} \frac{2}{T} \sum_{t=\frac{T}{2}+1}^T IR_t, & T : \text{even}; \\ \frac{2}{T-1} \sum_{t=\frac{T}{2}+\frac{3}{2}}^T IR_t, & T : \text{odd}, \end{cases} \quad (9)$$

where IR_t is the ratio between the treatment's cell concentration and the control's cell concentration at the time of t , C_t^e is the treatment's cell concentration at the time t , C_t^c is the control's cell concentration at the time t , and \bar{IR}_{half} is average inhibition rate during last half of the experiment period. When $\bar{IR}_{half} < 0$, the plant has a promoting effect; when $\bar{IR}_{half} = 0$, the plant has no effect; when $0 < \bar{IR}_{half} < 0.25$, the plant has less inhibitive effect; when $0.25 \leq \bar{IR}_{half} < 0.5$, the plant has inhibitive effect; when $0.5 < \bar{IR}_{half} \leq 0.75$, the plant has more inhibitive effect; when $\bar{IR}_{half} \geq 0.75$, the plant has the most inhibitive effect.

2.4.5 ANOVA analysis

ANOVA is used to test whether the difference between the means of the two groups is statistically significant or not, and to offer analysis of the difference in significance between several groups. ANOVA analysis and average inhibition rate model (built by author) analyses are both found to be good in evaluating the inhibitive effect of the plants. While SPSS software is utilized to do multiple comparisons between different treatment groups at the

same inoculation dose.

3 Results and discussion

3.1 Phenomena analysis

It seems that the inhibitive effects of three compositae plants A.L.D.C., C.C.C., and K.I.S.B. are distinct compared with the control under an initial inoculation dose of 2.0×10^6 Cells/mL (Fig. 3). The inhibition on algae growth started to show after 2 days of co-cultivation with the three plants. After 7 days of co-cultivation, the population decreases sharply compared with that of the control; the culture medium is nearly colorless in the algae pool of the experiment device. This indicates that the algae are completely inhibited. The inhibition rate of A.L.D.C. and K.I.S.B. on the 7th day is 74.6% and 41.9% respectively. The inhibition of the C.C.C. is relatively weak. After 10 days' incubation, the inhibition capacity of the tested three plants can be ranked as A.L.D.C. > K.I.S.B. > C.C.C..

A.L.D.C. shows the highest inhibition effect among the

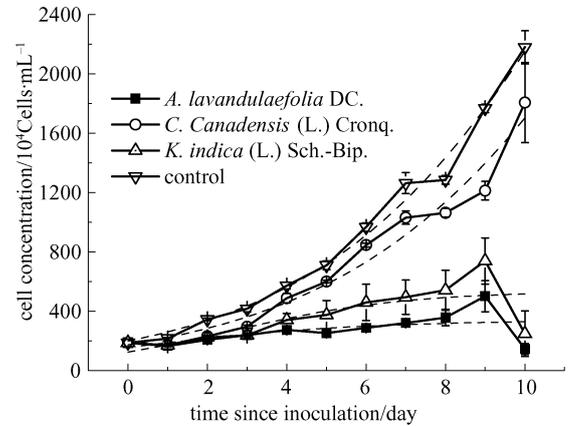


Fig. 3 Algae growth curves(symbol and line) and logistic fitting curves (dashed) of three compositae plants (*A. lavandulaefolia* DC., *C. Canadensis* (L.) Cronq., and *K. indica* (L.) Sch.-Bip.) under the initialization concentration of 2.0×10^6 Cells/mL during 10 days.

tested plants in the trial with an initial inoculation dose of 4.0×10^6 Cells/mL (Fig. 4). After 4 days, the population density of algae maintained at the level of 4×10^6 Cells/mL, with the color of the culture turning brown and the algae agglomerating. After 10 days' co-cultivation, the inhibition rate of this trial reaches 89.3% with a population density of 2.0×10^6 Cells/mL.

However, the algae growth tendency in the trial of co-culture with C.C.C. does not change, which is similar to the result of the lower initial inoculation dose. Finally, the IR_{10} value is 4.9%, only a little different from the control. So it can be concluded that different inoculation concen-

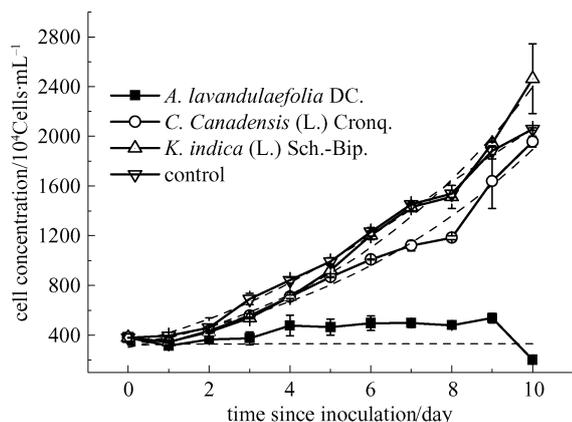


Fig. 4 Algae growth curves (symbol and line) and logistic fitting curves (dashed) of three compositae plants (*A. lavandulaefolia* DC., *C. Canadensis* (L.) Cronq., and *K. indica* (L.) Sch.-Bip.) under the initialization concentration of 4.0×10^6 Cells/mL during 10 days

trations do not affect the inhibition of the C.C.C. on the growth of the algae.

In the trial with higher initial inoculation dose, K.I.S.B. shows a great difference from that of lower initial inoculation dose. The plant begins to promote the growth of algae on the 9th day. According to the descriptions above, it can be concluded that with high initial inoculation dose, the inhibition capacity of the tested three plants can be ranked as A.L.D.C. > C.C.C. > K.I.S.B..

According to the analysis of the Figs. 3 and 4, it is found that A.L.D.C. has the same effect under a different initial inoculation dose. The cell number changed greatly on the 10th day compared with the values days before. After long-term accumulation of the inhibitive chemicals in the culture medium, a large number of algae are killed in a very short time because the resistance of *Microcystis aeruginosa* collapses. C.C.C. shows no inhibitive effect at all, indicating that little inhibitive chemicals are produced or the concentration of the inhibitive chemicals produced is too low to inhibit cell growth.

Under light microscope, it is discovered that the algae

inhibited by A.L.D.C. has non-uniform distribution and the cells are bigger than that of the control group's. Sun et al [7] studied the mechanism of harmful algal bloom mitigation by use of sophorolipid which is produced by some plants. They found the thecae of the cell are damaged by that chemical. The phenomenon described in Sun's study is similar to this study.

3.2 Logistic model analysis

The parameters of logistic growth curves are obtained by fitting the experiment data in the logistic equation (model) (Eq. (1)) (Table 2). The parameters of the control group are the reference values of the treatment group.

It indicates that the environmental capacity of the algae population decreases under the stresses of inhibitive chemicals; hence the algae's growth is limited. The K value of the C.C.C. trial is close to or larger than that of the control, showing that the plant has a promotive effect on algae instead of an inhibitive effect. In this model, the fluctuation of instantaneous growth rate r is caused by inhibitive chemicals. In the three trials, some r values fluctuate drastically (relative deviation 130%–200%) (Table 2, column 5), and some values are even negative, and algae grow unstably. Other r values have limited change ranges (relative deviation 11%–22%) (Table 2, column 5); therefore, the algae grow stably.

The results of the logistic model analysis are in accordance with those of phenomena analysis. These confirm the stress caused by A.L.D.C. and K.I.S.B. Different t_x for 50% of K , 75% of K , 90% of K , and 99% of K , are calculated using these parameters (Eqs. (4), (5), (6), and (7), Table 2) $t_{0.5}$, $t_{0.75}$, $t_{0.9}$, and $t_{0.99}$ (Table 2, column 6, 7, 8, and 9). These are used to evaluate whether the algae number reached the habitat's carrying capacity. The analysis of t_x indicates that algae population in the promotion group and the control group do not reach the habitat's carrying capacity.

Table 2 Parameters K , a , and r of logistic growth curves

compositae plants	cell concentration $K/10^4$ Cells·mL ⁻¹		A	r/day^{-1}	t_x/day			
	10^4 Cells·mL ⁻¹				$t_{0.5}$	$t_{0.75}$	$t_{0.9}$	$t_{0.99}$
<i>A. lavandulaefolia</i> DC.	200*	340	1.16 ± 1.01	0.36 ± 0.53	0.4	3.5	6.6	13.3
	400**	331	0.04 ± 0.06	1.08 ± 2.18	–	–	–	1.3
<i>C. Canadensis</i> (L.) Cronq.	200	6887	38.64 ± 58.25	0.25 ± 0.07	14.4	18.7	23.0	32.5
	400	11802	35.72 ± 62.45	0.19 ± 0.04	18.6	24.3	30.1	42.5
<i>K. indica</i> (L.) Sch.-Bip.	200	533	3.29 ± 2.03	0.46 ± 0.27	2.6	5.0	7.4	12.6
	400	10712	34.42 ± 27.76	0.23 ± 0.03	15.4	20.2	25.0	35.4
control	200	6889	33.49 ± 26.99	0.27 ± 0.06	12.9	16.9	21.0	29.8
	400	3169	8.64 ± 1.17	0.28 ± 0.03	7.8	11.7	15.7	24.4

Notes: The algae growth data of three compositae plants under the same concentration is fitted by logistic equation utilizing Origin 7.5 fitting function. The parameters R -square (R^2) is very important, for it is used to evaluate whether the fitting is good or not. In the table above * $R^2 = 0.9677$, ** $R^2 = 0.9888$, $n = 10$, the fitting is very good. "–" shows that the values are not reasonable.

3.3 ANOVA and average inhibition rate model analysis

According to the multiple comparisons principle, if the result of variance equal test shows that the significance possibility $p > 0.05$, it indicates that the variance has no significant difference and is equal at $\alpha = 0.05$ level (Table 3). For the trial with initial inoculation dose of 2.0×10^6 Cells/mL, significance possibility $p = 0.185 > 0.05$, it indicates that the difference of 10-day-average inhibition rates between A.L.D.C. and K.I.S.B. are insignificant in this trial. They belong to a group caused by a similar factor. There is still a certain difference of $p = 0.185$, indicating that the degree of effect of this factor is different. There is significant difference in the 10-day-average inhibition rate between A.L.D.C. and C.C.C. And there is significant difference in the 10-day-average inhibition rate between K.I.S.B. and C.C.C. The results in the trial with an inoculation dose of 4.0×10^6 Cells/mL show that the difference of the 10-day-average inhibition rate between K.I.S.B. and C.C.C. is insignificant. They belong to a group caused by a similar factor; however, there is still a certain difference of $p = 0.082 > 0.05$. The difference of 10-day-average inhibition rate between A.L.D.C. and C.C.C. is significant, and so is the difference of 10-day-average inhibition rate between K.I.S.B. and A.L.D.C.. Hence, it is concluded that the growth of C.C.C. is influenced by another factor of inhibition in both trials as analyzed above.

Table 3 Multiple comparisons of the inhibition rate between three plants

cell concentration	plant (I)	plant (J)	mean difference (I-J)	sig.
200×10^4 Cells·mL ⁻¹	A.L.D.C.	C.C.C.	0.395*	0.000
	A.L.D.C.	K.I.S.B.	0.100	0.185
	C.C.C.	K.I.S.B.	0.295*	0.000
400×10^4 Cells·mL ⁻¹	A.L.D.C.	C.C.C.	0.390*	0.001
	A.L.D.C.	K.I.S.B.	0.492*	0.000
	C.C.C.	K.I.S.B.	0.101	0.082

Notes: * The mean difference is significant at the 0.05 level. a. LSD analysis, b. Tamhane analysis

The inhibitive potential is analyzed with the average inhibition rate model (Eq. (9), Table 4, Figs. 5 and 6). In the trial with the inoculation dose of 2.0×10^6 Cells/mL, the IR of the C.C.C. keeps fluctuating at 0.19,

Table 4 \bar{IR}_{half} value of three compositae plants and their inhibitive potentials

	A.L.D.C.	C.C.C.	K.I.S.B.
2.0×10^6 /Cells·mL ⁻¹	0.76	0.19	0.67
inhibitive potentials	++++	+	+++
4.0×10^6 /Cells·mL ⁻¹	0.71	0.16	-0.03
inhibitive potentials	+++	+	-

Notes: ++++ represents the most inhibitive effect; +++ represents more inhibitive effect; ++ represents inhibitive effect; + represents less inhibitive effect; 0 means no effect, - represents promotion.

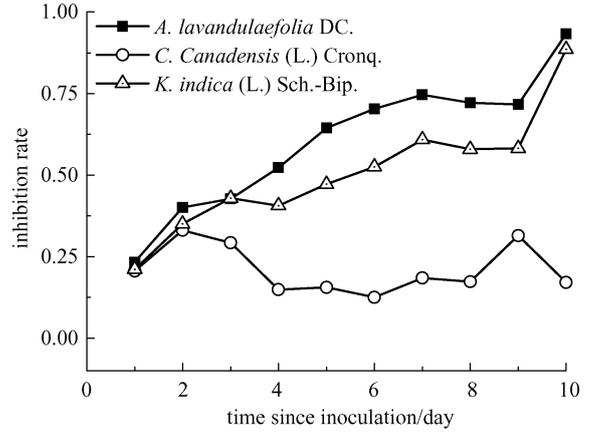


Fig. 5 Inhibition rate curves(symbol and line) and evaluation curves (dashed) of three compositae plants (*A. lavandulaefolia* DC., *C. Canadensis* (L.) Cronq., and *K. indica* (L.) Sch.-Bip.) under the initialization concentration of 2.0×10^6 Cells·mL⁻¹ during 10 days

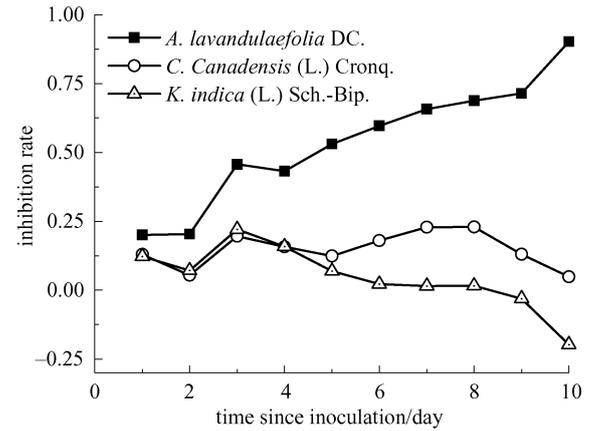


Fig. 6 Inhibition rate curves(symbol and line) and evaluation curves (dashed) of three compositae plants (*A. lavandulaefolia* DC., *C. Canadensis* (L.) Cronq., and *K. indica* (L.) Sch.-Bip.) under the initialization concentration of 2.0×10^6 Cells·mL⁻¹ during 10 days

while the IR of other plants shows a tendency to go up; the algae cell division of the A.L.D.C. group is quicker than that of the K.I.S.B. group. In the trial with the initial inoculations dose of 4.0×10^6 Cells/mL, the distances between the three IR curves are large after 10 days' cultivation; therefore, the difference in the inhibitive effect of these three plants is significant at this inoculation level. According to the data in Table 4, the A.L.D.C. exhibits the highest inhibitive potential on algae growth at both inoculation levels. While the K.I.S.B. shows growth-promoting tendencies at a higher concentration.

Comparing the two kinds of analyses, it is found that they supplement each other. In the analysis above, ANOVA analysis is used to distinguish the differences between them and classify them. Average inhibition rate model analysis tells the detailed difference in evaluating the inhibitive level or degree.

3.4 Effects of space, nutritional salt, and protozoa

The study shows that the cell number of the control and the promotion groups are more than that of the inhibition group, which indicates that the decrease of the amount do not result from space limitations. In natural water, the blue or green algae will over-reproduce if the TP concentration exceeds 0.2 mg/L and TN concentration exceeds 2.0 mg/L [26]. The TN and TP concentrations determined on the 10th day shows that the lowest TN (41 mg/L) of the three plants is 20.5 times higher than the limit TN value (2.0 mg/L), and lowest TP (2.4 mg/L) is 12 times higher than the limit TP value (0.2 mg/L) (Fig. 7). Therefore, it is impossible that the limited growth resulted from the lack of nutritional salts such as TP and TN. Zhang, et al [22] found that the protozoa may be one of the main causes for the inhibitive effect by barley straw extract. In our study, the 0.45 μm of microporous filter membrane is used to quarantine the protozoa that exist in the plant box from the algae, allowing chemicals to permeate. Therefore, a conclusion can be drawn that the effect of space, nutritional salts, and protozoa on the growth of the algae can be eliminated.

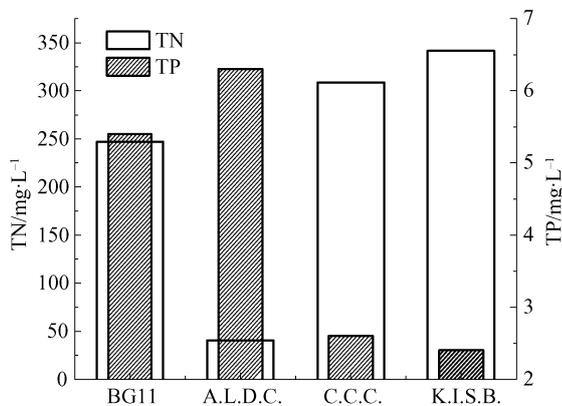


Fig. 7 TN and TP concentrations in the culture medium BG-11 after 10 days' experiment

4 Conclusions

The inhibition rates on the algae growth of the three plants *Artemisia lavandulaefolia* DC., *Conyza canadensis* (L.) Cronq., and *Kalimeris indica* (L.) Sch.-Bip are 93.3%, 17.1%, 88.6% (at initial inoculation dose of 2.0×10^6 Cells/mL), and 89.3%, -3.8%, and -30.8% (at initial inoculation dose of 4.0×10^6 Cells/mL), respectively. It can be concluded that *A. lavandulaefolia* DC. has the strongest inhibitive potential among them.

Logistic model analysis indicates that compositae plants such as *A. lavandulaefolia* DC. decrease the algae habitat's carrying capacity, resulting in a shorter time to reach the capacity value for the inhibition groups. An average inhibition rate model is utilized to evaluate the inhibitive

effect of the three plants. ANOVA analysis gives a proof of the similarity and the difference among every experimental trial. Terrestrial plant *A. lavandulaefolia* DC. grows well in the aquatic environment, thus it shows a great potential to be applied to treat eutrophic water.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 20877060), and the Project of the State Key Laboratory of Freshwater Ecology and Biotechnology (Grant No. 2005 FB06). The authors would like to thank School of Resource and Environmental Science, Wuhan University for its financial support as well (Water Environment Research & Data Sharing Platform in the Middle Reaches of the Yangtse River, Grant No. WERDSPMYR-0606).

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