
ORIGINAL RESEARCH ARTICLE

Mechanism of growth inhibition effect of aquatic plants on water cyanobacteria

Lei Cheng, Xiujuan Cao, Xinchun Yu

College of Bioengineering, Weihai Agricultural University, Shangdong, China

ABSTRACT

Environmental pollution caused by industrial and agricultural production has recently become a serious problem. One of the most significant ones is the phenomenon of cyanobacteria bloom caused by eutrophication of water body. Cyanobacterial bloom not only pollutes the water, but also secretes microcystins, endanger the health of animals and humans, as well as carcinogenic effects. In the research field of ecological management of cyanobacteria, it has opened a great concern on allelopathy. In this paper, the total phenolic and tannin contents in four common aquatic plants such as lotus were determined, and their different algae inhibition effects were compared. The mechanism of algae suppression was analyzed, and suppression ability of the difference of algae was analyzed, these provided evidence for scientific control of cyanobacteria. The results showed that the correlation coefficient between the total phenol and the growth of the cyanobacteria was 0.81122, and the phenolic substance was the effective anti-algae. Among them, total phenol content in Iris is the highest and the most effective in algae suppression.

Keywords: *aquatic plants; cyanobacteria; allelopathy; inhibition mechanism*

COPYRIGHT

Copyright © 2017 by author(s) and EnPress Publisher LLC. This work is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0). <http://creativecommons.org/licenses/by/4.0/>

Introduction

Cyanobacteria is one of the most serious hazards in freshwater. Because it is frequently happened, within long duration, and mostly poison producing, it is difficult to be removed, and thus people give much attention on it. China's Taihu Lake region and the Yunnan region had several outbreak of cyanobacteria bloom. It is seriously affecting the company production and social life, so the relevant departments of China has been studying the effective method of controlling cyanobacteria^[1]. At present, the world's main method of controlling cyanobacteria is based on biological management, with the integration of physical and chemical methods, especially the allelopathic inhibition effects of aquatic plants has many advantages in controlling eutrophication and blooming. In recent years, the theory and technology research of this field have been receiving attention from world-wide. Tannic acid, also known as tannic acid, the chemical name is 3, 4, 5-trihydroxybenzoic acid, is a polyphenol, widely found in natural plants. Tannin can form precipitation complex with protein, vitamins and minerals, and it has antibacterial and antioxidant properties^[2]. In this study, we investigated the effects of total phenolics and tannins on the allelopathy of plants by studying the correlation between total phenolics and tannin and cyanobacteria growth, and revealed the mechanism of aquatic plants inhibiting on the growth of cyanobacteria, these provide theoretical basis and practical guidance for the research on allelopathy.

Characteristics of cyanobacteria

Cyanobacteria are also known as blue-green algae, or cyanophyta, including microcystis, spirulina and other organisms. Although it is traditionally believed that cyanobacteria belong to algae, but the cell has no nucleus and this is similar to the bacteria, thus it is classified as bacteria. As an ancient prokaryote, cyanobacteria has been on earth for about 3 billion years, is the earliest photosynthetic oxygenated organisms, it played

a great role in the formation of the Earth's aerobic environment^[2]. There are about 2,000 species of cyanobacteria known in the world, and about 900 species of cyanobacteria in China. According to the morphology it can be divided into Chloococcales, Pleurocapsales, Oscillatoriales, Nostocales and Trichosanthos^[3].

Basic biological characteristics of cyanobacteria

Cyanobacteria are generally single cells or in colony in water. When the cyanobacteria gather too much, the cells arranged to form filamentous, and they are not branched, fake branched or true branches, no flagellum, no swimming cells, and some filaments can stretch and swing around. Cyanobacteria have lack of cellulose, mainly composed of peptides, the cell wall is surrounded by glial, has only nucleoid. Cell contains not only chlorophyll and carotenoids, but also contains phycocyanin, some species contains phycoerythrin. The secondary metabolism of cells produces microcystins^[3,4].

Growth and reproductive characteristics of cyanobacteria

Cyanobacteria can reproduce by fission, and also reproduces through spores formation. It grows rapidly in the high temperature and under strong light. The growth of cyanobacteria is very sensitive to temperature. During the autumn and winter season, the growth of cyanobacteria will be inhibited when the water temperature is below 17 °C. When the water temperature rises above 28 °C in summer, the cyanobacteria will grow rapidly, the longer the hot weather, and the longer time for cyanobacteria to grow. Water pH is alkaline. Cyanobacteria is suitable to grow at pH = 9.0 ~ 9.5 alkaline water environment^[5].

The growth rate is affected by the content of nutrients in the water, and is closely related to the content of nitrogen and phosphorus and its ratio. When the total phosphorus concentration exceeds 0.1 mg/L (if the phosphorus is a limiting factor) or the total nitrogen concentration exceeds 0.3 mg/L (if nitrogen is a limiting factor), the algae will reproduce excessively.

The OECD proposed three indicators for Eutrophic Lake, they are the average total phosphorus concentration is greater than 0.035 mg/L; the average chlorophyll concentration is greater than 0.008 mg/L; the average transparency is less than 3m. In the closed system, the algae biomass and the

phosphorus concentration are periodically changed, and when the biomass exceeds a certain value, the blooms are exploded, and then the biomass undergoes the first reduction, then increases, and then reduces and this circulation until the equilibrium state is reached^[6].

Cyanobacteria

A common presence of neurotoxin BMAA (β -N-methylamino-L-alanine) in cyanobacteria can produce biomagnification through the accumulation of food chains. BMAA has been proven that to be highly toxic, will accelerate the animal brain degradation, limb muscle atrophy and so on. It also can induce myofascial sclerosis, Parkinson's disease and Alzheimer's disease^[7,8].

Cyanobacteria endotoxin microcystin also has a very strong toxicity, animals will be diarrhea, anorexia, vomiting and other symptoms, and even death after drink water containing microcystin. Human drinks or directly contact with the blooming water can cause skin allergies, diarrhea, gastroenteritis and other symptoms, long-term drinking it may lead to liver cancer^[8-10]. Different cyanobacteria contain different toxins, the difference can be divided according to the role of toxins on target organs such as liver toxins, neurotoxins, rash toxins and cytotoxins, etc., it can be divided into microcystins, Anatoxin, aphantoxin, etc. according to the type of cyanobacteria^[3,11].

In addition, cyanobacteria also have the ability to enrich heavy metal ions in water which may cause heavy metal poisoning^[12].

Cyanobacteria bloom and its hazards

Water blooms are algae deposits floating on the surface of water, also known as 'water bloom' or 'algae bloom'. When the water body is in eutrophic state, it is possible to promote the excessive growth of some cyanobacteria in the freshwater body as long as proper temperature and light are favorable for the natural geographical conditions of algae breeding. Which caused the water color changes, and the formation of uneven thickness of the green surface or other colors of algae floating debris, this phenomenon is known as the outbreak of blooms^[7].

Cyanobacterial blooms often lead to ecosystem disorders, aquatic biodiversity is destroyed, it is an environmental pollution phenomenon which it destroy the ecological balance, endangers human health, affecting the drinking water quality.

Research Status of Cyanobacteria Control in China and Abroad

Physical control of cyanobacteria bloom

(1) Salvaging cyanobacteria bloom is the most direct way to remove cyanobacteria out of the water, can reduce the number of cyanobacteria in water, to restore the balance of the number of biological species in the water population.

(2) The addition of activated carbon to the water can provide a good adsorption effect on the cyanobacterial toxin in the water. The removal rate of cyanobacterial toxin in the aqueous solution by kaolinite and montmorillonite is 80%. With the addition of ozone has better effect on algae removal but the corresponding costs will increase.

(3) Ultrasonic has a strong ability to kill algae and inhibit the growth of algae, algae species decreased, the total density decreased and the removal of dead algae, the transparency of the lake increased, the water sensory was significantly improved. Other prevention methods on the eutrophication of water body has not yet been taken, there is no algae outbreaks in the water body and no phenomenon of silt uplift, it will be always maintain in a relatively stable state.

(4) Reverse osmosis (RO) and membrane filtration technology. Algae removed through the membrane filter principle.

Chemical control of cyanobacteria bloom

(1) Chlorination is the earliest and most extensive method of oxidizing algae removal. It is commonly used in the pretreatment process of water source, so that algae and algal toxins are easy to be removed in the subsequent conventional water treatment process. Tsuji et al. confirmed that the removal effect was mainly determined by the free chlorine content in the water. After treatment with 60 mg of free chlorine at a concentration of 0.7 mg / L, only 35% of the microcystins (MC) was removed, and when the free chlorine concentration increased to 2.8 mg/L for 30 min, the removal rate of MC was 99%. The algae removal effect depends on the free chlorine content in the water and the pH value^[13].

(2) Add potassium permanganate, ozone and other strong oxidants to kill cyanobacteria, while strong oxidants can neutralize MC toxicity^[14]. Ozone oxidation capacity is very strong, in the sixties of 20th century, ozone began to be used in pre-oxidation of raw water, currently widely used in drinking water

treatment. Himberg's results show that 0.2 mg/L of ozone can eliminate 16% to 60% of the algal toxin, concentration is 0.6 mg/L, the removal rate is 83% or more^[15].

Biological control of cyanobacteria bloom

Although there are many ways to control cyanobacteria, but the chemical method is often only short-term effect, and induced side effects. Construction of sewage interception works or dredging sediment or other methods are often very expensive. The use of aquatic organisms to control cyanobacteria bloom is important, simple and one of the fundamental way, to make some water body within a few years to be reverted into the initial state^[16].

(1) use of oxidizing bacteria, nitrifying bacteria and denitrifying bacteria to degrade nitrogen and organic matter in water, change the nutritional status of water, and thus inhibit cyanobacteria reproduction.

(2) controlling of zooplankton on cyanobacteria: zooplankton inhibits the reproduction of cyanobacteria by ingesting them while changing the light intensity and nutritional conditions which indirectly affect the reproduction of cyanobacteria. In order to use zooplankton to control cyanobacteria, it is necessary to increase the biomass of zooplankton and introduce the type of zooplankton that can inhibit cyanobacteria.

(3) control of other aquatic animals on the cyanobacteria: adjust the community structure of fish in the lake, reduce the consumption of fish in zooplankton, increase phytoplankton feeding fish (such as silver carp, tilapia, etc.). Snails on the removal of water cyanobacteria also have a significant effect. Snail by feeding cyanobacteria, so that the lake decolorization, transparency increases, and can remove the water nitrogen, phosphorus, to control the purpose of cyanobacteria water.

(4) Control of cyanobacteria by higher aquatic plants: the mechanisms action of higher aquatic plants to inhibit algal growth by competing the light and mineral nutrition, and secrete organic substances into water body that inhibit cyanobacteria^[17].

Allelopathic inhibition of aquatic plants

Lotus and other four common aquatic plants

Lotus (*Nelumbo nucifera*) is also known as lotus, bloom, hibiscus, Yuhuan and so on. It belongs to Water lily family, perennial aquatic herb.

Underground stems are long and plump, with long section, and has round peltate leaves. Flowering is from June to September, single flower grow at the top of the pedicel, petals majority, embedded in the nursery hole. The fruits are in oval-shaped with oval seeds.

Water lily (*Nymphaea tetragona*) is a meridian lotus. It belongs to Water lily family and perennial aquatic herbs. Leaves are floating on the water, horseshoe-shaped, with long stalk. Autumn flowering, white flower which floating on surface and open in the afternoon, closed in the evening, can be opened and closed for three or four days. It can self-grew or be cultivated in pond.

Water peanut (*Alternanthera philoxeroides*) is also known as *Alternanthera philoxeroides*. Perennial Herbaceous. Stem base creeping, upper extensible, hollow, branched, branches axillary sparsely pilose. Leaves opposite, oblong-obovate or obovate-lanceolate, apex rounded, apex, base narrowing, leaves surface consists hairs and the edge has lash. Flowering period is from May to November.

Iris tectorum, also know as alias blue butterfly, wish the British flower. It belongs to *Iris* family, with rhizome perennial flowers. It is about 80 cm high. Leaves are sword-shaped, thin and light green. Flowering from April to May, flower grow out from the leaves and large and beautiful. The fruit has 6 edges. Its origin is China and Japan. It favors to grow in wet and fertile place, mainly in the Central Plains, Southwest and East China area. *Iris* is beautiful and used as potted flower, cut flowers and parterre flowers. Its bitter, flat, toxic; rhizome can be used as medicinal.

Allelopathic inhibition of aquatic plants

The term allelopathy was first proposed by the scientist Molisch in 1937 to express all plant biochemical interactions, including microbes. Rice did not define plant allelopathy in 'Allelopathy' in 1984 as 'a direct or indirect beneficial or adverse effect of a plant on the release of chemicals from the environment to other plants, including microorganisms^[18]. Allelopathy research will be a combination of theory and application of the comprehensive development stage, covering the research methods, mechanism and development of applications and other fields^[19].

The allelochemicals produced by large aquatic plants can inhibit the growth of harmful algae in water. However, the current research is focused on the allelopathic effects of submerged plants and a

few floating plants on algae, and the study of the allelopathy of large aquatic plants (water plants and floating plants) is rarely reported. At the same time, the existing literature reports on the allelopathic effects of living aquatic plants on algae, and the study of whether or not the dry aquatic plants have a allelopathy after harvest^[20].

Plant allelochemicals are mainly secondary metabolites, which are distributed in various parts of plants. There are four common release methods: volatile, leaching, root secretion and decay release. The allelopathic suppression principle mainly affects algae photosynthesis by allelochemicals, destroys the cyanobacteria cell membrane, affects its enzymatic activity, affects its sub-microstructure, protein synthesis and changes the nucleic acid metabolism to achieve the inhibitory effect.

There are three main ways of aquatic plants allelopathy to control algae growth in water :

(1) Aquatic plants are cultivated to the treated water body, and the algae are inhibited by the allelochemicals released by the living plants;

(2) The death and dry plants put into the treated water body, the use of its decay release of allelochemicals inhibit algae;

(3) The application of allelochemicals extracted from plants put into water body to inhibit algae^[21].

The study on the allelopathic effect of aquatic plants on algae is of great significance to control algae explosive growth by ecological method, which is the main direction of future research on cyanobacteria blooming.

Total phenol and tannin

Phenol, the chemical formula $ArOH$, is a class of aromatic compounds where hydrogen in the aromatic hydrocarbon ring is substituted by hydroxyl ($-OH$). The simplest class of phenolics is phenol. There are more than 2,000 kinds of phenolic substances in nature, they are plant metabolites, it play important role in plant growth and development, immunity, antifungal, photosynthesis, respiratory metabolism and other vital activities. Phenolic substances combine with the protein in the cell to form insoluble proteins that cause the cells lose their activities.

Tannin (tannin), also known as tannic acid, chemical name 3,4,5-trihydroxybenzoic acid, is a polyphenol. Tannins are highly polymerized polyphenols that form complexes which are insoluble in water when combine with proteins

and digestive enzymes^[22]. Tannin is not a single compound, chemical composition is complex, can be divided into two kinds, one is condensed tannin, is a flavan alcohol derivatives, the first element of the flavanols through the carbon bond with catechol or pyrogallol. Another is hydrolyzed tannins, molecules containing ester bonds. In the 1950s, the application prospect of the tannins and proteins, polysaccharides, alkaloids, microorganisms, enzymes, metal ions and its antioxidant, free radicals, antibacterial and derivatization reactions were revealed, and the application prospect expanded rapidly^[23].

Purpose and meaning of research

Water eutrophication is one of the most serious environmental pollution in the world. More than 85% of China's lakes contain excess nitrogen and other chemical constituents, and more than 80% of the 532 major rivers are exposed to excessive nitrogen pollution. Every year the nitrogen enter the Yangtze River and the Yellow River, there are 92% and 88% are from chemical pollution, respectively. Cyanobacterial bloom management has become a serious social problem, including Yunnan, Taihu Lake and other areas with cyanobacteria pollution receiving country's attention.

In this study, the effects of total phenol-tannin content on the growth of cyanobacteria in Taihu Lake were studied. The relationship between the total phenol-tannin and the algae inhibition was studied, and the mechanism of algae inhibition was studied. This opens a new direction towards the allelopathy research on aquatic plant and to provide guidance for cyanobacteria management.

Materials and methods

Materials

Plant material samples

(1) Cyanobacteria from the Taihu Lake waters which is natural growth, obtained it after laboratory purification and cultured.

(2) Lotus leaves was obtained in three different period which are on June 20, July 20, August 20 in Huzhou City Lotus pond, ready to be used after brush and wash clean.

(3) Water lily was obtained in three different period which are on June 20, July 20, August 20 in Huzhou City Lotus pond, ready to be used after brush and wash clean.

(4) Water peanuts and its leaves and stem were obtained in three different period which are on June 20, July 20, August 20 in Huzhou Teachers College East Campus artificial, stem was selected from the bud to the end of 15 knots which submerged in the water; leaves taken from the selected those above the water level, ready to be used after brush and wash clean.

(5) Iris leaves were obtained in three different period which are on June 20, July 20, August 20 in Huzhou education college. 1/3 part of the middle part of leaves was taken, ready to be used after brush and wash clean.

2.1.2 Experimental reagents

See Table 1 for the relevant test reagents.

2.1.3 Experimental equipment and instruments

See Table 2 for related test equipment and instruments.

Methods and procedures

Preparation of the relevant solution

(1) Preparation of 0.1M of ferric chloride solution 1L (containing 0.1 NHCl): with an electronic balance weighed iron chloride hexahydrate 27.03g, hydrochloric acid 10.13g, add a small amount of distilled water to dissolve and adds distilled water up to 1000mL, stored into the jar and put in the refrigerator at 4 °C.

(2) Preparation of 0.008M potassium ferricyanide solution 1L: with an electronic balance weighed potassium ferricyanide 2.634g, add a small amount of distilled water to dissolve and adds distilled water up to 1000mL, stored into the jar and put in the refrigerator at 4 °C.

(3) Preparation of 0.2M sodium chloride solution 1L: with an electronic balance weighed sodium chloride 11.7g, add a small amount of distilled water to dissolve and adds distilled water up to 1000mL, stored into the jar and put in the refrigerator at 4 °C.

Determine the dry weight of 1 g of fresh tissue per plant tissue

(1) 1g sample selection: The sample of the plant material taken was cut into pieces of about 5 mm x 5 mm in size, and 1 g was weighed on an electronic balance. According to this method, 1 g of seven fresh samples of lotus leaf, lotus stems, water lily leaves, water lily stems, water peanut leaves, water peanuts stems and iris leaves were selected, and each sample was repeated 4 times.

Table 1. Major Chemicals and Reagents

Name	Source
Catechol	china Pharmaceutical Group Shanghai Chemical Reagent Company
Methanol	Quzhou huge reagent Co., Ltd.
Sodium chloride	Ningbo Chemical Reagent Factory
Hydrochloric acid	Nanjing Chemical Industry Co., Ltd
Ferric chloride	Guangdong Shantou Xilong Chemical Factory
Potassium ferricyanide	Guangdong Shantou Xilong Chemical Factory

Table 2. Major instruments and equipment

Name	Source
Double - sided clean bench	Suzhou purification Limited
Electronic Balance (0.01-0.0001)	METTLER TOLEDO Instruments (Shanghai) Co., Ltd
Centrifuge	Hunan Kaida Scientific Instrument Co., Ltd
722N UV - Visible Spectrophotometer	Shanghai Precision Science Instrument Co., Ltd
Electric constant temperature drying oven	Shanghai Pudong Rongfeng Scientific Instrument Co., Ltd
Shaker	Wuhan sea sound of equipment Limited

(2) The fresh samples were placed into the oven and bake-to-dry until constant weight: All samples were placed in an electrothermal oven, dried at 80 °C, dried at high temperature for 5 days to constant weight, and the dry weight of the sample was weighed with an electronic balance.

Determination of tannin and total phenolics in plant tissues

(1) 1g sample selection: The sample of the plant material taken was cut into pieces and 1 g was weighed on an electronic balance. According to this method, 1 g of fresh samples of lotus leaf, lotus stems, water lily leaves, water lily stems, water peanut leaves, water peanuts stem and iris leaves were selected, and each sample was repeated 4 times.

(2) Preparation of supernatant: Take 1g of fresh sample, add 8ml anhydrous methanol, grinds with a mortar until into a homogenate, placed in the shaker and agitate for 30 min, then add quartz sand to adjust the concentration balance at 13000 r/m of speed centrifugation for 15 min, the supernatant was taken out after centrifugation and each sample was repeated 3 times.

(3) Determination of tannin and total phenol in the sample: Diluted with 1 ml supernatant + 93 ml distilled water, then add 3 ml of 0.1M FeCl₃ solution which dissolved in 0.1N HCl and mixed for 3 min, from the time of adding 3 ml 0.008M K₃Fe (CN)₆ solution to 10 min, the OD value of the solution was measured at 720nm using the UV - visible spectrophotometry and each sample was repeated three times.

Blank control 1: 1ml anhydrous methanol + 93ml distilled water, then add 3 ml of 0.1M FeCl₃ solution which dissolved in 0.1N HCl and mixed for 3 min, from the time of adding 3 ml 0.008M K₃Fe (CN)₆ solution to 10 min, the OD value of the solution was measured at 720nm using the UV - visible spectrophotometry, and the total phenol content (%) was calculated according to the standard curve.

Blank control 2: fresh sample grinding by adding 8 ml 0.2M NaCl solution instead of the same amount of ethanol in the preparation of supernatant same as the preparation above. Then add 3 ml of 0.1M FeCl₃ solution which dissolved in 0.1N HCl and mixed for 3 min, from the time of adding 3 ml 0.008M K₃Fe (CN)₆ solution to 10 min, the OD value of the solution was measured at 720nm using the UV - visible spectrophotometry and then calculates the tannin content (%) based on the standard curve.

(4) Production of standard curve: A series of catechol solutions at a concentration of 0.02%, 0.04%, 0.06%, 0.08%, 0.1% were prepared with methanol. Each solution of catechol was given 2 ml each, 93 ml of distilled water was added, and add 3 ml of 0.1M FeCl₃ solution which dissolved in 0.1N HCl and mixed for 3 min, from the time of adding 3 ml 0.008M K₃Fe (CN)₆ solution to 10 min, the OD value of the solution was measured at 720nm using the UV - visible spectrophotometry (With blank control 1 as control group)

Plant material suppression algae activity experiment

(1) Autoclaving of plant material: Add 200 ml of BG-11 culture medium to the culture flask, add 0.1%

of less than 5 mm x 5 mm of fresh plant material, and then autoclave.

(2) Preparation of plant extracts: The 50 g autoclaved plant material was cut into small pieces of less than 5 mm x 5 mm. After mixing, 10 g of the mixture was boiled in 100 ml of distilled water for 2 h, used glass fiber filter (Whatman GF / C) to filtered it after cool down, and finally the filtrate was set to 100 ml (i.e., an extract at a concentration of 10%).

(3) Determination of algae activity of plant extracts: The effects of the plant extracts (the final volume of 200 ml) on the growth of microcystis were determined in a culture flask equipped with BG-11 culture medium. After adding the extract, the medium was autoclaved at high temperature and pressure, and the medium was inoculated into 4 ml of *Microcystis aeruginosa* which in the logarithmic growth phase after the medium was cooled. The treatment concentrations of the plant extract were 0.01%, 0.05%, 0.1%, 0.5%, respectively, and distilled water was used as a control. Four treatments were repeated. All the conical flask was placed on the culture rack of the Culture Room. The culture conditions were as follows: temperature was 22 °C, daylight illumination, light time was 12 h per day, light intensity was 5000 lux, shaking bottle twice a day. After incubation for 0, 1, 3, 5, 7, 9, 17, 19 days later, the OD value of cyanobacteria was measured at 665 nm using an ultraviolet-visible spectrophotometer.

(Experiment on the algae activity of plant material is completed by students, and the results are taken as the analysis reference)

Results

Correlation between total phenolics and tannin content in plants and cyanobacteria growth

Table 3 shows the correlation coefficient between the total phenol tannin content and the OD value of cyanobacteria growth. According to the catechol standard curve, the total phenol and

tannin content per kilogram of sample is expressed in terms of the corresponding amount of catechol in milligrams, using the sample material taken in June, FW representing the total phenols per kilogram of sample. The content of total phenol and tannin per kilogram of sample. The correlation between the OD value of cyanobacteria growth and the total phenol tannin content of the samples after 11 days of culture with 0.1% concentration of autoclaved plants was calculated and the correlation coefficients were obtained.

Table 3 showed that the correlation between the total phenol and tannin content in plant and the OD value of the cyanobacteria growth is negative, so the total phenol and tannin have an inhibitory effect on the growth of cyanobacteria. The correlation coefficients of the total phenol and the dry sample on the growth of cyanobacteria were -0.81122 and -0.95647, while the correlation coefficient of tannin was lower. The highest content of total phenol tannin is iris leaf, the lowest is water peanuts.

Discussion

The results showed that the total phenols and tannins in four kinds of aquatic plants such as lotus were related to the inhibition of cyanobacteria growth. Among them, the total phenol content of iris, water lily and water peanut was higher, while the tannin content of iris and water lily was higher than that of peanut. Among them, iris and water lily are not only high in total phenol tannin content, but also the proportion of total phenol tannin in its organic matter is high, and these two plants are rich in total phenol and tannin. Table 3 shows that the higher the total phenol and tannin content in plants, the more obvious the inhibition of cyanobacteria growth, can prove that the total phenol and tannin on the inhibition of cyanobacteria growth has a high correlation.

Some studies at China and abroad^[18-21] found that aquatic plants of various types of life had a growth inhibitory effect on algae. In the study about the total phenol tannin found that^[22,23], total phenol tannin have antioxidant properties, can effectively inhibit

Table 3. Correlation between total phenolics and tannin content and cyanobacteria growth in plants

Plant material	Lotus leaf	Lotus stem	Water peanut leaf	Iris leaf	Correlation Coefficient
Total phenol(FW)	7182.378	3751.592	1592.214	8373.063	-0.81122
Tannin(FW)	689.1098	532.7063	451.9819	6138.006	-0.24553
Total Phenol(DW)	27865.68	28260.58	10544.46	28050.46	-0.95647
Tannin(DW)	2673.559	4012.854	2993.258	20562.84	-0.21617
OD value (at 11th day)	0.223	0.258	0.354	0.254	Nil

algal cell metabolism, thereby inhibiting its growth and reproduction. In the experiment, four kinds of common aquatic plants such as lotus were used as experimental materials, and the algae inhibition mechanism was analyzed by measuring the contents of total phenol and tannin in different kinds of plants. Through comparative analysis, we can conclude that the total amount of phenol and tannin contained in water lily is the largest, and the total phenol and tannin content of lotus and water peanut are the least. The higher the total phenol and tannin content, the stronger the algae resistance. In 2007, Li Lei et al.^[24] found that water lilies, lotus and other aquatic plants have obvious ability to suppress algae. The content of total phenol tannin in water lily was the highest, but it did not show obvious algae inhibition in the study of the correlation with cyanobacteria growth. So we think it is due to improper operation of the experiment, resulting in data errors, so the water lily of the relevant data was processed as invalid data, research on water lily resistance to algae has to be verified in the future.

However, we can also analyze the inhibition mechanism of cyanobacteria growth by measuring the contents of total phenol and tannin in fresh and dried plant samples. Dried samples that contain more total phenolics and tannins in water lily stem and water peanut leaves can be used in its organic matter, in the practical use of its extract on the outbreak of cyanobacteria bloom water body biochemical method Governance.

What is currently reported in experiments is to use plant extracts to inhibit the growth of cyanobacteria and does not describe the mechanism by which plants inhibit the growth of cyanobacteria. The inhibitory effect of the extract on cyanobacteria depends mainly on the algae allelopathy substance. Aquatic plants of various types of life have a growth inhibitory effect on algae.

Conclusions

In this study, four kinds of common aquatic plants, such as lotus, were selected as the research object to study the inhibitory mechanism of total phenolic tannin content on cyanobacteria. The following conclusions are drawn from the experiment:

The total phenols and tannins in aquatic plants have a high correlation with the inhibition of cyanobacteria growth. The higher the content of total phenols and tannins in plants, the more obvious the inhibitory effect. By comparison, the higher the content of tannin in the plant, the better effect

of its algae allelopathy. Among the plants, iris has the highest content of total phenol tannin, and the inhibitory effect on algae was the best, and the content of total phenol tannin in water peanut was the lowest.

In this study, we studied the growth inhibition mechanism of aquatic plants by four kinds of aquatic plants such as lotus on cyanobacteria. It was proved that total phenol and tannin had some inhibitory effect on cyanobacteria. If these plants could be used reasonably and effectively, it can be the long-term effective ecological management, or be a reasonable application in the treatment of eutrophication of water body, and will achieve a good management effect. Due to the limitation of the conditions, the inhibition mechanism on cyanobacteria was not studied in this experiment. However, the related studies at China and abroad showed that the total phenols and tannins in plants have a high correlation with the inhibition of cyanobacteria growth. Therefore, the role of total phenolics and tannins on cyanobacteria should be used as a future research direction, in order to provide theoretical basis and an effective way to use of aquatic plants in cyanobacteria removal.

References

1. Miao Xinxin. Governance cyanobacteria, we have no delay [J]. *Water Treatment Engineering and Equipment*, 2009, 7 (1): 59-60
2. Salvemini D, Cuzzocrea S. Superoxide. Superoxide dismutase and ischemic injury [J]. *Curr Opin Investig Drugs*, 2002, (3): 886-895
3. Watanabe Yoshida, Harada Kenichi, Fujimoto Hiroyuki. The emergence of cyanobacteria and its toxins [M]. Tokyo: Tokyo University Press, 1994
4. Greg Miller. Guam's Deadly Stalker: On the Loose Worldwide [J]. *Science*, 2006, 28 (313), 428-431
5. Yu Hong-man. Talking about the harm and prevention of cyanobacteria [J]. *Beijing Aquatic Products*. 2004 (1): 29
6. ZHOU Jie, ZENG Cheng, WANG Ling-ling. Nonlinear dynamics analysis of growth characteristics of cyanobacteria in Taihu Lake [J]. *Hydrobiologica Sinica*, 2009, 33 (5): 931-936
7. Huang P, et al. Effects of crude extract of cyanobacteria bloom on expression of HSP70 and GRP78 in liver of mice. *Acta Scientiae Circumstantiae*, 2009, 29 (6): 1278 (in Chinese with English abstract)
8. Dai Jinjin, Chen Dehui, Gao Yunfang, Ma Qing. Studies on the research of cyanobacterial toxin [J]. *Wuhan Botanical Research*, 2009, 27 (1): 90-97
9. Zhang Shuhua, Li Qiao, Pan Xiping, Xu Jingbo. Microcystin-LR research progress [J]. *Modern Preventive Medicine*, 2009, 36 (17): 3236-3239

10. Ding Xiaosheng, Li Xuoyu. Study on the Toxicity of Microcystins to Mouse Liver [J]. *Fisheries Science*, 2008, 27 (2): 98-100
11. C. James Hastie, Emma B. Borthwick, Louise F. Morrison, Geoffrey A. Codd, Patricia TW Cohen. Inhibition of several protein phosphatases by a non-covalently interacting microcystin and a novel cyanobacterial peptide, nostocyclin [J]. *Biochimica et Biophysica Acta*, 2005: 187-193
12. Cai Jinshun, Li Wenqi, Pang Yong, Yang Xuguang. Studies on the Relationship between Dissolution Microcystins and Microcystins in Algae in Water [J]. *Journal of Hydraulic Engineering*, 2009, 40 (3): 328-334
13. Tsuji K, Watanuki T, Kondo F, et al. Stability of Microcystins from cyanobacteria-iv. Effect of Chlorination on decomposition. *Toxicon*, 1997, 35 (7): 1033-1041.
14. GROSS. E. M, MEYER. H, SCHILLING. G. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum* [J]. *Phytochemistry*, 1996, 41 (1): 133-138.
15. Deng Peng, Xue Wentong, Hu Peng, Wang Zhaohua, Du Fangling. Study on the mechanism of microcystin toxin and its prevention and treatment [J]. *Chinese Journal of Food and Nutrition in China*, 2009, 08: 52 -53
16. Wang Yangcai, Lu Kaihong. Cyanobacteria bloom and the governance dynamics. *Fisheries Journal*, 2004, 17 (1): 90-94
17. SUN X X, Choi J K, Kim E K. A preliminary study on the mechanism of harmful algal bloom mitigation by use of sophorolipid treatment [J]. *Journal of Experimental Marine Biology and Ecology*, 2004, 304: 35-49
18. RICE E L. Allelopathy [M]. The second edition. London: Academic Press, 1984: 1-2.
19. Chinese Journal of Botany, 2003, 23 (3): 509-515 (in Chinese with English abstract) [J]. *Chinese Journal of Botany*, 2003, 23 (3): 509-515
20. Li Fengmin, Hu Hongying. Inhibitory effect of leaching solution of large aquatic plants on algae [J]. *China Water Supply and Drainage*, 2004, 20: 18-21
21. Hu Hongying, Meng Yujie, Li Fengmin. Study on the Inhibitory Effect of Plant Allelopathy on Algae Growth [J]. *Environmental Environment*, 2006, 15 (1): 153-157
22. ZHONG Yu, HU Jian, LIU Ai-jing, ZHANG Ji-shu. Physiological effects of plant tannin [J]. *Shaanxi Agricultural Sciences*, 2008 (4): 98-104
23. Theoretical study on the relationship between antioxidant activity and structure of Tannin. *Journal of Leather Science and Engineering*, 2009 (10), 19 (5): 9-13 (in Chinese with English abstract)
24. Li Lei. Study on algae growth of lotus, water lily and snail in eutrophic water [D]. *China Academy of Environmental Sciences*, 2007: 9-10