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Plant derived compounds for the control of toxic cyanobacterial blooms: a comprehensive mini-review

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Abstract:
Cyanobacteria are photoautotrophic prokaryotes with potential to form harmful blooms under eutrophic conditions. Cyanobacterial bloom removal is critical due to the detrimental effects caused by blooms and the cyanotoxins they release as secondary metabolites, to the aquatic environment. Numerous physical, chemical and mechanical methods have been tested for this purpose and have found to possess serious cons like lack of specificity, expensive, and so on. Biological methods as efficient alternatives for the physical and chemical treatment techniques have been reviewed in this paper. Though Biological techniques are specific and has shown high efficiency, it continues to be expensive. This Review emphasizes on the use of plants for the control of cyanobacterial blooms by exploiting allelochemicals released by them and promotes the development of cost- effective, efficient, and safe treatment technique using terrestrial plants taking allelopathy of cyanotoxins against aquatic plants into consideration.
Keywords: cyanobacteria, cyanobacterial blooms, cyanotoxins, control measures, allelopathy, allelochemicals

1. Introduction

Cyanobacteria are morphologically diverse group of photoautotrophic prokaryotes possessing both beneficial and detrimental features. They are important primary producers and nitrogen-fixing species with high nutritive value and contributing to water and soil fertility worldwide (Rai; 1990). However, abundant growth of cyanobacteria on water bodies or in water treatment work creates serious problems. Increased growth of a single or few species of cyanobacteria on surface waters wherever temperature, light and nutrient source is conducive is called cyanobacterial bloom (or cyanobacterial harmful algal bloom) (Chorus and Bartram; 1999).

Eutrophication is the most prominent cause for bloom formation. Eutrophication occurs when excess of nutrients such as phosphorus and nitrogen enters the water system due to industrial or agricultural runoff. Cyanobacterial blooms can lead to a wide range of problems as depicted in figure 1.

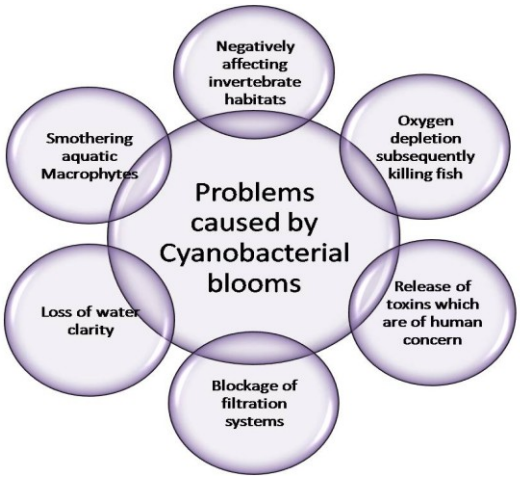

Figure 1: Problems created by cyanobacterial blooms in the environment.

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Many cyanobacteriae produce toxic secondary metabolites which has the potential to cause acute intoxication in mammals (including humans) thereby affecting hepatic, nervous, digestive, endocrine, and dermal systems (Charmichael; 2001, Christoffersen *et al*; 2002). Table 1 represents the common cyanotoxins and genera producing them.

Cyanotoxin	Target organ in Mammals	Genera of cyanobacterial bloom
Mycrocystin	Liver	*Mycrocystis, Anabaena, Nostoc, Anabaenopsis, Oscillatoria, Gleotrichia, Planktothrix, Plectonema,Rivularia, Arthrospira,Phormidium, Tolputhrix.*
Anatoxin-a and Homoanatoxin-a	Nerve synapse	*Anabena, Arthrospira, Cylindrospermum, Oscillatoria.*
Nodularin	Liver	*Nodularia, Nostoc.*
Saxitoxin	Nerve axons	*Aphanizomenon, Cylindrospermopsis, Anabaena, Lyngbya.*
Cydrospermopsin	Liver	*Cylindrospermopsis, Raphidiopsis, Anabaena, Aphanizomenon.*

Table 1: Major cyanotoxins and the genera producing them

By taking all the negative effects caused by the blooms into consideration, they have to be removed efficiently. Tools presently available to mitigate or control harmful algal blooms arrive with limited effect and use, because they are either of high cost to be used constantly, or have drawbacks to be applied in large scale (Barrett, 1994; Caffrey and Monahan, 1999).

Remedial measures for the removal of cyanobacterial blooms when they are grown extensively and has started controlling the phytoplankton. A number of physical, chemical and biological treatment methods have been exploited. Physical and chemical methods used for the removal of cyanobacterial blooms and their limitations have been summarized in the table 2.

Biological strategies to control cyanobacterial blooms have been summarized in this review.

2. Biological remediation of cyanobacterial blooms:

Biological techniques used for the remediation is considered safe because of the low environmental impact it leaves and free from the side effects such as fish kills and degradation of plant life. Viruses, bacteria, actinomycetes, waste biomass, aquatic macrophytes, terrestrial plants and protozoa are the potential biocontrol agents employed to control cyanobacterial blooms.

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Treatment technique	Type of treatment	Discription	Limitation	Reference
Copper Sulphate	Chemical methods	Copper sulphate is directly added to water. Once dissolved, they lyse cyanobacterial cells.	Copper sulphate lacks specificity and are proven bioaccumulators.	Van Hullebusch et al.;1993
Pesticides	Chemical method	Dispersed into water by spraying directly. Rapidly kills the cyanobacteria.	Can persist in water for weeks and cause negative effects in amphibians and higher plants.	Erturk and Walker; 2003
Ferric salts	Nutrient limitation	Once dosed into water, phosphorous precipitates with ferric salts in the sediments hence reducing its availability to the blooms	Temparory and many cyanobacteria can withstand extreme nutrient fluctuations.	Gulati and Donk; 2002
Alum	Nutrient limitation	Like ferric salts, binds to phosphorous in water and limits its availability to cyanobacterial cells	Effect reduces gradually and the blooms increase to its initial level	Van Hullebusch et al.; 2003
Filtering	Mechanical method	Pumping water through fine filters and returning back to source after removal of cyanobacteria	Not all cyanobacteria are removed and requires large space.	Naghavi and Melone; 1986.
Surface mixing	Mechanical method	Active mixing of water in order to prevent blooms to s=reside on the surface thereby reducing their accessibility to light.	Mixing brings up nutrient from depth to top hence increasing the cyanobacterial growth.	Visser et al.; 1996
Reed beds	Nutrient and light limitation	Filters light and nutrients and use them. Hence controlling blooms	Cannot be used as a primary technique for the removal	Garbett; 2005

Table 2: Commonly used techniques to control cyanobacterial bloom

2.1 Bacterial agents:

Large number of bacteriae are found to lyse cyanobacterial cells rapidly decline cyanobacterial blooms. Cyanobacterial lysis by bacteria can take place by three modes: Contact lysis, production of extra-cellular compounds, and entrapment lysis (Sigee *et al.*; 1999). Reim et al. (1974) found that culture of *Bacillus spp.* could lyse seven genera of cyanobacterial (including *Anabaena* and *Microcystis*) . In a study conducted by Jabulani and co-workers in 2009 showed that *Bacillus mycoides* had lytic effect on *Microcystis aeruginosa* along with *Pseudomonas stutzer* . A similar study conducted by Wang and his group in 2012 found that *Rhodococcus* sp. Strain p52 inhibited 93% of *M.aeruginosa* after 4 day incubation. Many bacteriae have been tested and proved for their abitlity to lyse cyanobacterial cell. The use of bacterial agents is expensive and requires skilled personnel hence making it less effective technique. Table 3 summarizes the bacteria used to lyse cyanobacterial, and the compounds involved.

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Bacteria	Cyanobacteria used in the study	Mode of action	Reference
Bacillus cereus	*Microcystis*	Contact lysis	Nakamura et al; 2005
Bdellovibrio-like bacteria	*Microcystis aeruginosa*	Endoparasitism	Caiola and Pellegrini; 1984
Saprospira albida	*Microcystis aeruginosa*	Not specified	Ashton and Roberts; 1987
Bacillus spp	*Anabaena variabilis*	Not specified	Wright and Thompson; 1985
Streptomyces neyagawaensis	*Microcystis aeruginosa*	Contact lysis	Choi et al; 2005
Bdellovibrio bacteriovorus	*Phormidium luridum*	Ectoparasitism	Burnham et al.; 1976
Myxobacteria fulvus	*Phormidium luridum*	Entrapment	Burnham et al.; 1984
Myxobacteria xanthus	*Phormidium luridum*	Entrapment	Burnham et al.; 1981
Cytophaga	*Microcystis*	Contact lysis	Rashidan and Bird; 2001
Xanthomonas	*Anabaena, Oscillatoria*	Not specified	Walker and Higginbotham; 2000
Flavobacterium flexilis	*Oscillatoria williamsii*	Contact lysis	Sallal; 1994

Table 3: Lysis of Cyanobacteria with different bacterial strains.

2.2 Viral Agents:

Viruses are non-living obligate parasites dependent on the host for replication. Viruses specific to cyanobacterial are collectively called cyanophages. Safferman and Morris in 1963 studied the cyanophages for the first time. Cyanophages attack the blooms when the nutrients available for the blooms run low. Three recognized familiesof ds-DNA viruses consists cyanophages, Myoviridae (contractile tails), Styloviridae (long non-contractile tails) and Podoviridae (short tails) (Researchgate; Accessed on April 30, 2015). Cyanophage Ma- LBP has been found to lyse *Micrcystis* by Stephen and Peter in 2005. Much earlier to that, Kozjakov and his friends in 1972 stated long tailed virus A-1 to be active against *Anabaena variabilis* . There has been a number of studies carried out to know the efficiency of cyanophages to remove cyanobacterial blooms. However, use of cyanophages as a routine to remove the blooms is still in debate because of the host- specific nature of many viruses (Frazier et al.; 2007).

2.3 Fungal Agents:

Fungal agents are less studied for their role in control of cyanobacterial blooms because of the allelopathic relationship they share. Among very few fungi experimented, Wang et al., in 2010 found that white-rot fungus *Lopharia spadicea* could inhibit *Microcystis aeruginosa,* and *M. flos-aquae.* Similar results were observed by JiaO and co-workers when fungal strain *Trichaptum abietinum* 1302BG was co-cultured with the above mentioned cyanobacterial species'. The same team isolated *Penicillium* from lakeshore soil and studied its ability to inhibit M.aeruginosa in 2010 and observed favorable results. However, ability of the cyanotoxins (secondary metabolites of cyanobacterial) to inhibit fungal growth has hindered their efficacy. 60 Terrestrial fungi have been analysed for their anti-cyanobacterial activity by Han et al. in

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2011. The results showed that 13 of them could inhibit efficiently.

2.4 Waste Biomass:

The most common biomass studied for the control of cyanobacterial blooms are Barley straw, rice straw and leaf litter. Rotten barley straw has been used by the farmers to control cyanobacterial blooms since 1970s (Welch et al.; 1990). Gibson et al.;(1990) and Pillinger et al.; (1992) investigated inhibition of eight different algae by both barley straw and hay. Park and co-workers in 2006 investigated the efficacy of rice straw to inhibit *M.aeruginosa* and concluded that rice straw can inhibit *M.aeruginosa* in 8 days. Similar studies were carried out with leaf litter by Kumiko and group in 2005 and concluded that *Rhus trichocarpa* Miq., *Quercus variabilis* Blume *and Mallotus japonicus* (Thunb.) could inhibit *M.aeruginosa* by allelopathy exhibited by leaf litter.

Waste biomass being cost effective and easily available is still not the most accepted remedy to control cyanobacterial. Barley straw is however being employed in few fields changes the taste and gives odor to the treated water hence disturbing the water aesthetically. Both barley straw and rice straw are found to be algistatic rather than algicidal (Su et al.; 2013). Leaf litter exhibits allelopathy hence been affected by cyanotoxins hence releasing toxic substances and worsening the condition.

2.5 Plants to treat Cyanobacteria:

2.5.1 Macrophytes:

Macrophytes are aquatic plants which grow in or near water. They can be emergent, floating or submerged. Majority of the macrophtyes and cyanobacterial share allelopathic relationship which exploited for the control of blooms. Macrophytes are the most studied biocontrol agents for cyanobacterial blooms because of their existence with cyanobacteria (Hongying and Yu; 2008). The first study on the inhibition of cyabacteria using macrophytes is traced back to 1949,when Hasler and his team discovered the ability of *Elodea canadensis* (then called, *Anacharis canadensis*) and *Potamogeton perfoliatus* to inhibit cyanobacteria (Hasler et al.; 1949). Similar inhibitory effects were observed when extracts of *Ceratophyllum demersum* and *Brasenia schreberi* were added to blooms formed by *Anabaena* (Kogan and Chinnova; 1972, Elakovich and Wootan; 1987).

Early studies were focused on understanding the allelopathic relationship between macrophytes and cyanobacterial. It is only after the development of precise biochemical techniques to analyze, the components responsible for the allelopathic effect is being studied. Secondary metabolites responsible for allelopathy are collectively called allelochemicals. Common allelochemicals associated with macrophytes are organic polyphenols (Hongying and Yu; 2008). Macrophytes and their allelochemicals can destroy cyanobacterial blooms by destroying the structure of the cyanobacterial (Wang et al.; 2004)), by affecting the respiration of the cells (Pollio et al.; 1993), by disturbing photosynthesis of cyanobacterial cells (Korner&Nicklisch; 2002), or by affecting the enzymatic activity of the cells (Li & Hu; 2005). Sensitivity of cyanobacteria to allelopathy is influenced by various factors. Biological factors like growth status, species, growth stage

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of cyanobacteria and macrophyte and so on. Non-biological factors like temperature, nutrient stress and so on. Many studies have shown macrophytes to be species-specific. For example, *Myriophyllum spicatum* cannot inhibit *Anabaena,* but can inhibit *Microcystis aeruginosa, Oscillatoria,*and *Scendesmus* with inhibitory effect gradually weakening (Korner&Nicklisch; 2002). Macrophytes are not used widely because of the variance in the results observed in lab-scale and field. Competition for nutrients in the field may be one of the reasons for the discrepancy along with the allelopathy exerted on macrophytes themselves. Studies have also revealed that cyanotoxins released during stress are potential allelochemicals against macrophytes, hence making its use difficult in large scale.

The allelochemicals extracted with sophisticated techniques have been studied for its ability to inhibit the blooms, however it is expensive. A group of allelochemicals together show inhibition while individual compounds fail to do so, thus increasing the cost of the treatment procedure.

Table 4 depicts the common macrophytes, allelochemicals involved in inhibition and the cyanobacterial inhibited.

Macrophyte Species	Life style	Cyanobacteria Inhibited	Allelochemical involved	Reference
Eleocharis microcarpa	Emergent	*Anabaena flos-aquae, Oscillatoria tenuis*	3-hydroxy-cyclopentanone octadecenoic acids, 3-hydroxy-cyclopentyl eicosapentaenoic acid	Nakai et al.; 1999
Phragmites communis	Emergent	*Microcystis aeruginosa*	Vanillic acid, p-coumaric acid, syringic acid, gallic acid, ethyl 2-methylacetoacetate	Li et al.; 2005
Acorus calamus	Emergent	*Microcystis aeruginosa*	α-asarone, β- asarone, γ-asarone	Greca et al.; 1989
Typha latifolia, T. minima, T. angustata, T. domingensis	Emergent	*Anabaena flos-aquae*	Palmitic acid, cholesteryl cis-9-octadecenoate, salicylaldehyde, linoleic acid	Li,2005, Gallardo et al.; 1998
Brasenia schreberi	Floating	*Anabaena flos-aquae*	Not Identified	Elakovich, 1987
Potamogeton malaianus, P. maackianus, P. crispus, P. natans, P. pectinatus	Floating	*Microcystis aeruginosa*	Linolenic acid or isomers, lactone diterpenes, furano diterpenes, labdane diterpenes	Wu et al.; 2007
Elodea canadensis	submerged	*Microcystis aeruginosa*	Not identified	Hasler and Jones, 1949
Vallisneria spiralis	submerged	*Microcystis aeruginosa*	4-oxo-β-ionone, 2-ethyl-3-methylmaldeimide	Xian et al.; 2006,Nakai et al.; 1999
Hydrilla verticillata	submerged	*Microcystis aeruginosa*	1,2-Benzenedicarboxylic acid diisooctyl ester, di-n-butyl phthalate etc.	Wang et al.; 2006
Myriophyllum spicatum, M. alterniflorum, M. heterophyllum, M.verticillatum	submerged	*Majority of the cyanobacteria*	Ellagic acid, eugeniin, pyrogallic acid, gallic acid, (+) catechin, nonanoic acid, palmatic acid	Nakai et al.; 2000, Nakai et al.; 2005
Chara globularis, C. rudis, C. tomentosa, C. delicatula, C. fragilis	submerged	*Majority of the cyanobacteria*	4-methylthio-1,2-dithiolane, 5-methylthio-1,2,3-trithiane,dithiolane	Berger and Schagerl; 2004, Korner and Nicklisch, 2002
Najas marina	submerged	*Microcystis aeruginosa, Anabaena spp.*	Polyphenol-like compounds	Gross et al.; 2003
Nitella gracilis, N. opaca, Nitellopsis obtusa	submerged	*Microcystis aeruginosa, Porphyridium aerugineum*	Dithiolane	Berger and Schagerl; 2004

Table 4: Allelopathy of macrophytes on cyanobacterial and allelochemicals involved

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2.5.2. Terrestrial plants:

Anti-cyanobacterial property of terrestrial plans is less exploited when compared to that of aquatic macrophytes. Among terrestrial plants, Chinese medicinal herbs are the most tested. Pillinger and co-workers in 1995 identified antialgal activity of brown rotten wood. A decade later, Cantrell and his team succeeded in identifying and isolation anticyanobacterial components from aerial parts of *Haplophyllum sieversii*. In the same year, Meepagala's team identified antialgal compounds in the roots of *Ruta graveolans* and synthesized their analogs (Meepagala et al.; 2005). In 2007, Jancula and his team evaluated the effect of five species of *Papaveraceae* on cyanobacterial and found that *Chelidonium majus* was significantly toxic to cyanobacterial while it showed minimal toxicity in non- target organisms. In 2009, Purcaro and fellow members revealed ethyl acetate extracts of roots of *Swinglea glutinosa* could inhibit *Oscillatoria perornata.* In the same year , 66 Chinese medicinal herbs were tested for their activity aginst *Microcystis aeruginosa,* results indicated that 16 of them could inhibit the growth. *Malaphis chinensis, Cynips gallae-tinctoriae* and *Fructus mume* were found to be highly effective than the rest (Jing et al.; 2009). Similar study conducted by Yang and coworkers in 2012 with 40 Chinese medicinal plants revealed the ability of the root of *Salvia miltiorrhiza*, rhizome of *Acorus tatarinowii* rhizome of *Polygonum cuspidatum* cortex of *Phellodendron amurense* ,and fruits of *Crataegus pinnatifida* to inhibit *M.aeruginosa.* In 2013, Zhang and team isolated and identified potential algicidal compound from *Salvia miltirrhiza* and studied the potential inhibition mechanism of *M.aeruginosa.* Four Chinese medicinal herbs were tested for their ability to inhibit *M.aeruginosa* by Ye and team in 2014, the results showed that two out them (Phellodendri chinensis cortex and Scutellaria baicalensis G.) could be an effective alternative for the removal of cyanobacteria. Hongqiang and team in 2015 revealed the ability of anticyanobacterial activity of ethyl extracts of shaddock peel, pomegranate peel and pomegranate seed and also allelochemicals from all the three were identified.

3.Conclusion

Biological treatments measures are being investigated for the control of cyanobacterial blooms considering the cost, time and negative effects caused by physical and chemical methods. Of all the biological treatment techniques, use of plants is most accepted because of the low cost involved and allelopathic relationship they share with cyanobacteria making them most efficient control agents. Aquatic macrophytes are effective only for a brief period as they succumb to the cyanotoxins released by cyanobacteria under stress. Majority of the allelochemicals in plants responsible for the inhibition of blooms are polyphenols. Extraction of allelochemicals from macrophytes is less supported as the amount produced is very less. Taking all the above aspects into consideration, extraction of allelochemicals from plants that produce them in large quantities in a cost effective manner and application of the same can be an efficient alternative to the techniques currently used.

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