Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.26832

219 (2021) 157–163
April

Biological control of cyanobacterial bloom by leaf biomass

Zarina Zainuddin[a], Japareng Lalung[b,*], Tengku Nadiah Yusof[b], Mohd. Rafatullah[b], Norli Ismail[b]

[a]*Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia*
[b]*School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia, email: japareng@usm.my (J. Lalung)*

Received 15 August 2020; Accepted 23 November 2020

ABSTRACT

Cyanobacteria are able to grow rapidly when induced by suitable conditions and form blooms. These cyanobacterial blooms can lead to wide ranges of water quality problems which include depletion of dissolved oxygen and subsequent fish kills and unsafe drinking water. To a greater extent, cyanobacteria can severely degrade aquatic habitats, decrease the utilisation of water bodies as potable water supply, limit recreational activities and reduce in-lake fisheries. Previous researches conducted showed that plant leaves released anti-cyanobacterial compounds. Hence this study investigated the abilities of 15 terrestrial wild plant leaves leachates from Penang, Malaysia to inhibit the growth of 8 isolated cyanobacteria. The results showed that most leaves effectively controlled all cyanobacterial growth but at different rates, depending on the species of cyanobacteria and the plant leaves used. The outcomes suggest that the wild plant terrestrial leaves released effective anti-cyanobacterial substances, giving new insight to terrestrial leaves as natural biological controls of cyanobacterial bloom.

Keywords: Cyanobacteria; Bloom; Leaf biomass; Growth

1. Introduction

Cyanobacteria, or also known as blue-green algae, are prokaryotic microorganisms that carry out photosynthesis and grow in warm, eutrophic surface water [1]. It is reported that cyanobacteria populations are expanding and dominating many environments, particularly freshwater lakes, basins, rivers, irrigation channels, brackish, sea waters and salty lakes [2]. Some cyanobacteria species are capable of producing cyanotoxins, which can cause a hazard to humans and animals [3]. Accumulations of cyanotoxins in the environments lead to poisonings and chronic effects that can hardly be diagnosed and prevented [4]. It was found that cyanobacteria cover 90% of total algae during bloom periods in Lake Taihu, one of the widest shallow eutrophic freshwater lakes in China [5]. Thus, this incidence draws great interest to researchers and public health authorities [6]. Cyanobacterial blooms can cause harmful effects on the environment and humans. In the United States, cyanobacterial blooms are estimated to have caused losses of recreational, drinking and agricultural water resources with a value of more than $2 billion a year [2]. Highly visible cyanobacterial blooms are harmful to the environment by causing loss of water clarity that leads to suppression of aquatic macrophytes and negative effects on invertebrates and fish habitats [7]. The decay process of the cyanobacterial blooms utilises oxygen and creates hypoxic conditions which lead to plant and animal die-off. Certain cyanobacterial species are able to produce toxic secondary metabolites, known as cyanotoxins when the conditions are favourable for light and nutrients [8]. It is found that *Microcystis* cell concentrations were higher and more uniform in the

* Corresponding author.

tropical reservoir than in subtropical and temperate areas due to high temperature, light and nutrient conditions [9].

Due to increasing concern on toxic cyanobacteria and unpleasant blooming, researchers take several initiatives to control cyanobacteria by several initiatives which include chemical and physical treatments. Chemical treatment is widely used for its easy access, cheap and fast. One of the most successful compounds to control cyanobacteria growth, copper sulphate has been used to treat cyanobacteria blooming since 1904 [10]. However, the chemical has non-specific interaction; reducing the diversity of other organisms, and could lead to secondary pollution of aquatic environments [11]. In addition, the treatment reportedly has less affectivity for dense bloom biomass and immediate toxic bloom collapse leads to the release of highly concentrated cyanotoxin into the water environment due to cell damage [10]. Different physical approaches to reduce cyanobacteria biomass has been conducted, including removal of nutrients for bacterial growth [12] or directly removing cyanobacterial cell [11]. Physical treatment is generally expensive and has low efficiency compared to chemical treatment. Even though secondary pollution is less likely as per the chemical approach, it may cause injury to the non-target organism [11]. Hence, more researchers are investigating the potential of biological treatments as a way to control cyanobacterial bloom.

Both aquatic and terrestrial plants are known for producing allelopathy chemicals, secondary metabolites that affect the surrounding organisms such as microbes either harmfully or beneficially [13]. Several researches indicate that the chemical may be the natural inhibitor of cyanobacterial growth [14]. A number of active compounds released from plants have been successfully isolated and characterized in previous researches, which include polyphenol [15], terpenoid [14] and fatty acid [16]. These compounds inhibit growth via different pathways, such as inhibition of photosynthesis, disruption of cellular structure, and inactivation of enzymatic and non-enzymatic functions [15]. However, plant allelochemical activities also depended on temperature, plant maturity. In addition, most aquatic photoautotrophs are surrounded by water, which requires the allelochemicals released into the water need to be sufficiently hydrophilic and reach their target organisms in effective concentrations [17]. Besides that, higher plants also release carbon-based organic compounds and dissolved organic nitrogen compounds [17]. These compounds, therefore, may in turn stimulate the growth of cyanobacteria. Even so, many researches showed the effectiveness of plant biomass as cyanobacterial bloom management. Therefore, the aim of this study is to examine the potential of terrestrial leaves in Malaysia to inhibit cyanobacterial bloom.

2. Materials and methods

2.1. Cyanobacteria and leaves samples

Eight cyanobacterial species namely *Limnothrix* sp., *Microcystis* sp., *Ocsillatoria* sp., *Planktothrix* sp., *Pseudoanabaena* sp., *Synechococcus* sp. (from Ayer Itam reservoir), *Synechococcus* sp. (from Teluk Bahang dam) and *Synecocystis* sp. that have been identified morphologically and molecularly were obtained from Dr. Japareng Lalung's laboratory, School of Industrial Technology, Universiti Sains Malaysia (USM), Penang, Malaysia. All the cyanobacterial species were maintained on BG11 liquid medium under the continuous light conditions on incubation shaker at 95 rpm at room temperature.

15 different plants leaf were collected and identified using leaf morphologies observation and molecular approaches. The selected and identified leaves tested for their abilities to inhibit cyanobacterial growth were: *Phyllanthus* sp., *Croton* sp., *Brackenridgea* sp., *Disporum* sp., *Andira inermis*, *Millettia pinnata*, *Mesua ferrea*, *Cerbera odollam*, *Kopsia fruticosa*, *Morinda elliptica*, *Syzygium campanulatum*, *Clitoria fairchildiana*, *Pterocarpus indicus*, *Polyalthia longifolia* and *Millettia atropurpurea*

2.2. Leaves preparation for cyanobacterial bioassays

Leaf leachate of each terrestrial plants was prepared by drying the freshly collected leaves under direct sunlight for two weeks. In standard leaf bioassay, 1–10 g L^{-1} of small cut (<1 cm × 1 cm) dried leaves were added into 100 mL of cyanobacterial culture, whereas for mini bioassay, 1 g of the dried plant leaf was transferred into 250 mL conical flask containing sterile 100 mL BG11 medium. The leaf compounds were allowed to leach into the medium for 7 d at room condition before the leaf leachates bioassays were conducted.

2.3. Cyanobacterial growth in standard bioassay test

Standard bioassay test was conducted by transferring 100 mL medium into sterile 250 mL conical flasks in triplicate. Then 1–2 mL, depending on the concentration of the cyanobacterial stock culture was added. 1–10 g L^{-1} of dried leaves to be tested were then added into the flask. The triplicates were placed in a controlled-environment cabinet at room temperature (27°C) with a light intensity provided by cool white fluorescent tubes (~23 μmol m^{-2} s^{-1}). The flasks were shaken constantly using a shaker at 95 rpm for 30 d. Cells were harvested periodically between 24–72 h for extraction of chlorophyll-a.

2.4. Cyanobacterial growth in mini scale bioassay test

Anti-cyanobacterial activity for each leaf was observed by aseptically transferring 10 mL BG11 media containing leaf leachate into a sterile 28 mL universal bottle. Depending on cell density, 100 – 200 μL cyanobacterial stock culture was added into the bottle. The bottle was then capped loosely for aeration. Each leaf bioassay was conducted in triplicates under a controlled-environment cabinet at room temperature (28°C) under white fluorescence light (~23 μmol m^{-2} s^{-1}). The culture bottles were shaken constantly at 95 rpm for 15 d. For each replicate, 1 mL of culture was taken on day 7 and 15 of incubation for chlorophyll-a extraction.

2.5. Cyanobacteria growth measurement

Cyanobacterial growth was measured based on the concentration of chlorophyll-a. For the extraction of chlorophyll-a for each cell harvest, 1 mL of cell culture was

centrifuged for 2 min at 10,000 rpm and 0.5 mL of supernatant was removed. The remaining sample was further centrifuged for 2 min at the same speed. Afterwards, the rest of the supernatant was completely removed. Chlorophyll-a reading was taken by re-suspending harvested cells in 1 mL of 90% methanol containing 10 mg L^{-1} of magnesium carbonate ($MgCO_3$) and incubated for 1 h at room temperature in the dark. After the incubation, extracted chlorophyll-a was centrifuged for 5 min at 10,000 rpm. The absorbance of the supernatant was measured at 665 nm using UV-Visible Spectrophotometer (Shimadzu) and 90% methanol containing 10 mg L^{-1} of $MgCO_3$ acts as reference blank. The chlorophyll-a content was calculated using the following formula by [18]:

$$\text{Chlorophyll-a content (mg L}^{-1}) = \text{OD}_{665} \times 12.9447 \quad (1)$$

where OD_{665} = absorbance at 665 nm and 12.9447 = constant.

2.6. Cyanobacterial inhibition efficiency measurement

Chlorophyll-a data were collected and inhibition efficiency (%) of leaf leachates was analysed based on the formula:

$$\text{Inhibition efficiency}\left(\%\right) = \left[\frac{\left(\text{Control} - \text{Treatment}\right)}{\text{Control}}\right] \times 100 \quad (2)$$

where control = chlorophyll-a value of cyanobacteria growth assay in BG11 without leaf leachates, treatment = chlorophyll-a value of cyanobacterial growth bioassay in BG11 with leaf leachates [19].

2.7. Statistical analysis

Univariate analysis of two-way analysis of variance (ANOVA) was conducted to analyse and compare inhibition efficiency of two factors: cyanobacteria species and plant species on 15 d of culture. Tukey's HSD test was conducted to analyse homogeneity between species. Statistical data was conducted using IBM SPSS statistic version 22.

3. Results and discussion

3.1. Control of cyanobacterial growth by terrestrial leaves

To test the ability of wild terrestrial plants as natural cyanobacteria bio-control, 30 d of bioassays were conducted on 5 isolated cyanobacterial species: *Synechococcus* sp. (TB), *Planktothrix* sp., *Synechocystis* sp., *Pseudoanabaena* sp., *Microcystis* sp. and mixed species of cyanobacteria from Penang Botanical Garden. Leaves used in the bioassays were the *Phyllanthus* sp., *Croton* sp., *Brackenridgea* sp. collected from the Botanical garden and *Disporum* sp. from Air Itam dam to see the influence of the leaves to the growth of cyanobacteria.

Based on Fig. 1, at the concentration of 10 g L^{-1}, all of the four leaves in the study able to inhibit the growth of cyanobacteria *Synechococcus* sp. (TB), *Planktothrix* sp., *Synechocystis* sp., *Pseudoanabaena* sp., *Microcystis* sp. and the mixed cyanobacterial culture from Botanical Garden Penang. The results show that the leaves potential in releasing anti-cyanobacteria compounds and act as the natural cyanobacterial bio-control.

3.2. Control of cyanobacterial growth by terrestrial leaves in mini scale bioassay

Table 1 shows leaf inhibition efficiency on 8 cyanobacterial species on day 7 and day 15. On day 7, growth inhibition of cyanobacteria by most leaf leachates were low, especially on five isolates; *Microcystis* sp., *Synechoccoccus* sp. Ayer Itam isolation (AI), *Synechocystis* sp., *Ocsillatoria* sp. and *Pseudoanabaena* sp. In addition, on day 7, the cyanobacterium *Synechocystis* sp. growth was enhanced when cultured in BG11 media containing *Disporum* sp., *Phyllanthus* sp. and *C. fairchildiana* leaf leachates, while *Pseudoanabaena* sp. growth increased in media containing *Brackenridgea* sp., *Phyllanthus* sp. and *M. ferrea* leaf leachates, whereas *Synechococcus* sp. (AI) growth was enhanced in media containing *Disporum* sp., *Phyllanthus* sp. and *Croton* sp. The results may likely due to the release of nutrient and organic compounds from plants leaves, which at balances with anti-cyanobacterial compounds, resulting in growth enhancement in few cases and low inhibition efficiency.

Whereas, after 15 d of incubation, all leaf leachates were generally able to inhibit growth of cyanobacteria, except *Pseudoanabaena* sp., having high resistance to most of the leaf leachates and the growth was enhanced in medium containing *M. ferrea* leaf leachates. It was also observed that *M. ferrea* as well as *P. longifolia*, *Phyllanthus* sp. and *M. atropurpurea* leaves have lower ability to inhibit growth of most cyanobacterial species compared to other plants leaf while *M. elliptica*, *M. pinnata* and *S. campanulatum* leaf leachates have high inhibition efficiency. On the other hand, cyanobacteria *Pseudoanabaena* sp., followed by *Synechocystis* sp. have high resistance towards plant leaf leachates and *Synechococcus* sp. isolated from Teluk Bahang was the most sensitive to the treatments.

However, it should be noted that all leaf leachates were able to effectively inhibit growth of toxic *Microcystis* sp., which is an important cyanobacterium to be controlled for bloom management. It was also seen that higher inhibition of cyanobacterial growth on day 15 compared to day 7 of incubation, indicating that anti-cyanobacterial compounds released from the leaves may have counteract nutrient and organic compounds, thus successfully controlled cyanobacterial growth.

3.3. Statistical analysis on inhibition efficiency at day 15

A two-way ANOVA analysis on the day 15 data collected was conducted to analyze and compare the interaction between the 15 different leaf leachates on the 8 cyanobacterial species. The statistical analysis is summarized in Table 2. The p-value of the two factors and the interaction are $p < 0.0001$, indicating that growth inhibition differed considerably depending on both the cyanobacterial species and plants. Thus indicates that different cyanobacterial species have different sensitivities towards anti-algae compounds released and that plant species were likely to release different compounds at different concentrations.

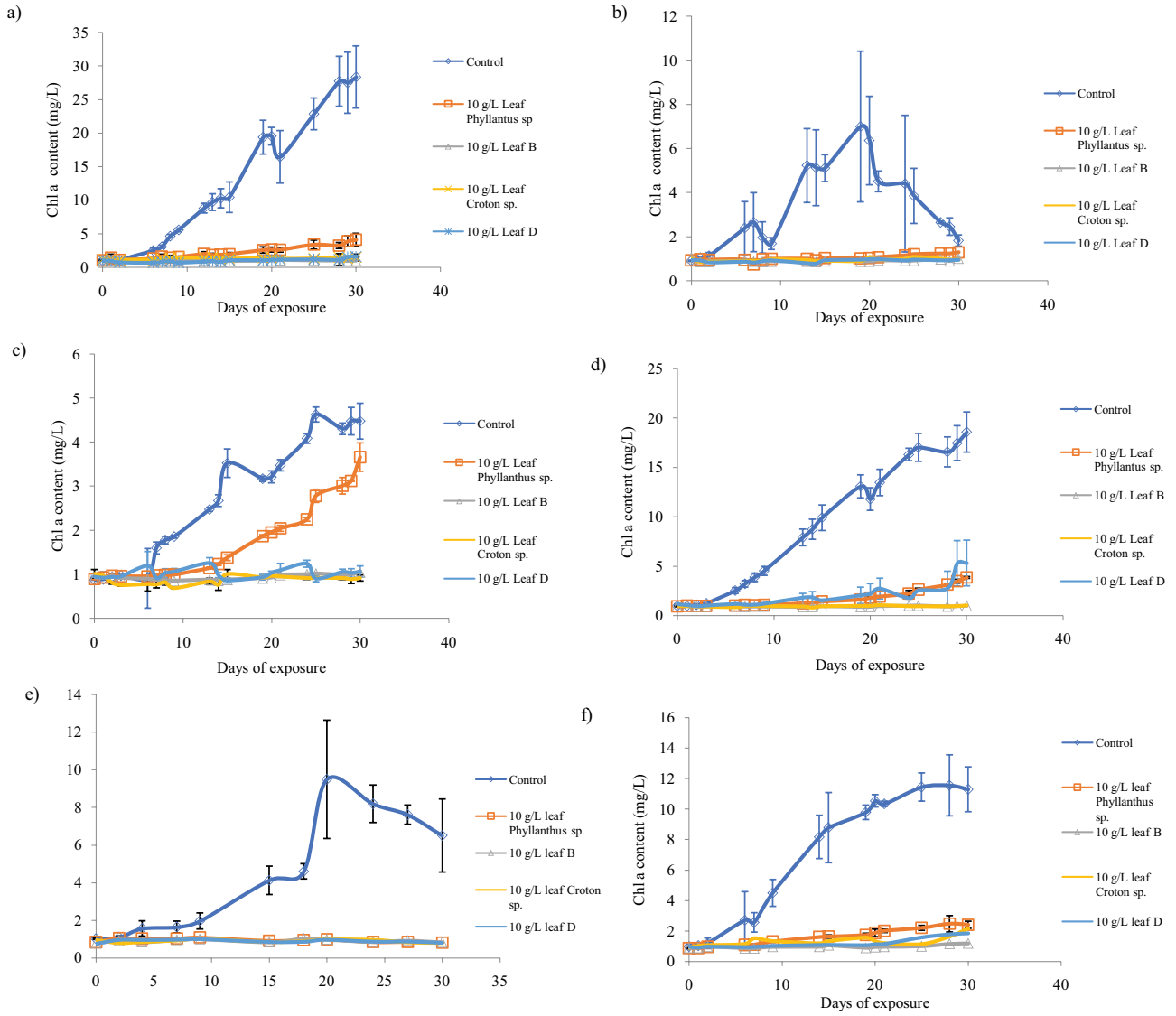

Fig. 1. Growth of (a) *Synechococcus* sp. (TB), (b) *Planktothrix* sp., (c) *Synechocystis* sp., (d) *Pseudoanabaena* sp., (e) *Microcystis* sp. and (f) mixed culture of cyanobacteria on the application of different leaves.

To examine the significant differences between each factor, Tukey's HSD test was also conducted (Table 3). Based on statistical analysis, cyanobacteria *Pseudoanabaena* sp., followed by *Synechocystis* sp. have a high resistance towards plant leaf leachates, and *Synechococcus* sp. isolated from Teluk Bahang and *Limnothrix* sp. cyanobacterial were the most sensitive to the treatments. Meanwhile, *M. ferrea*, followed by *Phyllanthus* sp. and *P. longifolia* and *Disporum* sp. have a low cyanobacterial growth inhibition efficiency mean compared to other leaf leachates, while *S. campanulatum*, followed by *M. pinnata*, *M. elliptica*, *P. indicus* and *Brackenridgea* sp. leaf leachates have a high inhibition efficiency.

Although the study showed a high inhibition effect of the leaves leachates to cyanobacterial growth, inhibition activity may change with environmental conditions, such as temperature, water, photoperiod, and research methodologies, such as initial concentration, sun exposure and maturity of plant leaves [20]. Additionally, the inhibitory effect by the substances are also dependent on time, concentration and exposure days [14], which evidently observed in this study that low inhibition efficiency occurred at day 7 in compare to day 15. And unlike showed in this study, in environmental condition, the allelopathic substances may require longer time to inhibit cyanobacterial growth [14]. Furthermore, if the released substances are polyphenols, the effectiveness of the compound may be declined with the increasing of pH value [21]. Therefore, laboratory scale experiment may not reflect the actual environment.

Even so, interestingly to note, no severe cyanobacterial blooms were observed in 2014 to 2015 in Ayer Itam reservoir, Mengkuang and Teluk Bahang dam, Penang, Malaysia although as a tropical country, cyanobacterial blooms possibly occurred all year round due to favourable light intensity and temperature for cyanobacterial growth [22].

Table 1
Effect of 15 individually leaf leachate on the growth of 8 cyanobacterial

Plant species	Inhibition efficiency															
	Day 7								Day 15							
	Lim.	Mic.	Osc.	Pla.	Pse.	Syn. (AI)	Syn. (TB)	Syne.	Lim.	Mic.	Osc.	Pla.	Pse.	Syn. (AI)	Syn. (TB)	Syne.
Brackenridgea sp.	78.8 ± 1.6	46.8 ± 7.5	47.5 ± 9.5	75.0 ± 2.0	–2.4 ± 6.1	15.5 ± 11.7	73.9 ± 1.0	14.6 ± 7.0	88.9 ± 1.0	79.4 ± 1.6	86.5 ± 1.6	89.0 ± 1.6	83.0 ± 1.6	84.9 ± 2.5	93.1 ± 1.1	78.4 ± 1.2
Disporum sp.	74.8 ± 7.1	49.1 ± 6.5	19.9 ± 14.6	47.9 ± 10.5	18.5 ± 3.2	–46.4 ± 15.1	45.1 ± 3.6	–36.3 ± 3.4	86.4 ± 1.9	79.5 ± 4.1	25.0 ± 9.8	75.5 ± 1.2	82.9 ± 3.2	50.1 ± 4.0	68.8 ± 1.6	41.5 ± 6.3
Phyllanthus sp.	71.6 ± 6.1	19.6 ± 2.3	37.7 ± 11.7	39.1 ± 17.9	–94.6 ± 8.1	–5.2 ± 27.0	67.7 ± 2.0	–24.5 ± 33.1	73.7 ± 6.1	72.7 ± 5.8	75.5 ± 7.3	28.9 ± 8.9	39.8 ± 3.1	50.9 ± 21.0	79.0 ± 1.0	33.0 ± 6.4
Croton sp.	78.5 ± 3.1	32.3 ± 2.1	35.1 ± 18.0	55.2 ± 7.4	41.6 ± 1.4	–27.9 ± 12.0	49.0 ± 1.6	42.6 ± 10.1	87.7 ± 3.1	86.8 ± 1.0	74.9 ± 4.8	54.4 ± 6.9	91.4 ± 0.2	30.6 ± 7.3	68.5 ± 5.1	84.9 ± 2.2
Andira inermis	74.6 ± 2.1	38.3 ± 6.8	54.5 ± 6.0	70.5 ± 0.6	18.7 ± 1.2	30.6 ± 0.9	64.4 ± 3.8	18.0 ± 7.1	78.9 ± 3.9	73.6 ± 1.8	74.7 ± 1.7	82.2 ± 2.6	45.0 ± 3.2	81.6 ± 3.4	80.8 ± 1.5	67.9 ± 0.7
Pterocarpus indicus	76.2 ± 3.2	54.3 ± 5.4	64.6 ± 5.7	69.8 ± 2.9	41.6 ± 2.5	31.3 ± 8.9	83.6 ± 1.4	27.4 ± 14.5	85.4 ± 4.1	78.1 ± 5.4	89.0 ± 1.1	89.4 ± 1.4	88.8 ± 2.6	90.8 ± 0.4	93.6 ± 0.6	82.8 ± 0.8
Cerbera odollam	67.4 ± 9.6	34.0 ± 5.1	42.5 ± 3.7	75.2 ± 2.3	16.2 ± 0.7	41.8 ± 1.9	72.3 ± 2.9	19.8 ± 5.6	80.0 ± 3.9	74.0 ± 0.5	72.3 ± 1.2	82.9 ± 5.1	43.2 ± 3.6	85.8 ± 0.1	87.7 ± 1.2	72.0 ± 0.6
Clitoria fairchildiana	77.9 ± 6.7	41.4 ± 10.5	29.3 ± 33.9	65.9 ± 5.3	20.95 ± 2.4	40.0 ± 12.6	75.0 ± 3.3	–9.9 ± 53.3	80.5 ± 1.2	70.5 ± 4.6	73.1 ± 13.4	78.3 ± 1.7	42.9 ± 10.0	83.7 ± 1.5	87.5 ± 2.9	60.4 ± 9.8
Kopsia fruticosa	77.2 ± 1.8	51.9 ± 7.2	62.3 ± 7.5	80.8 ± 2.1	32.4 ± 2.3	55.7 ± 5.1	79.0 ± 2.5	44.1 ± 4.5	78.1 ± 4.5	70.0 ± 1.5	83.7 ± 1.0	85.8 ± 0.7	42.6 ± 3.2	86.4 ± 0.3	90.3 ± 0.4	73.6 ± 4.4
Kopsia fruticosa	77.2 ± 1.8	51.9 ± 7.2	62.3 ± 7.5	80.8 ± 2.1	32.4 ± 2.3	55.7 ± 5.1	79.0 ± 2.5	44.1 ± 4.5	78.1 ± 4.5	70.0 ± 1.5	83.7 ± 1.0	85.8 ± 0.7	42.6 ± 3.2	86.4 ± 0.3	90.3 ± 0.4	73.6 ± 4.4
Mesua ferrea	67.6 ± 4.2	46.8 ± 4.5	28.3 ± 16.2	71.0 ± 7.7	–21.8 ± 2.43	12.4 ± 12.8	51.8 ± 9.6	26.1 ± 6.3	68.7 ± 6.2	67.7 ± 4.4	55.8 ± 13.0	67.7 ± 7.4	–36.4 ± 15.7	59.1 ± 3.6	61.0 ± 4.0	33.8 ± 5.7
Millettia atropurpurea	77.7 ± 6.4	46.6 ± 10.9	52.0 ± 4.4	62.3 ± 7.1	16.5 ± 2.4	31.9 ± 13.2	72.8 ± 5.4	10.2 ± 20.9	78.6 ± 2.6	78.1 ± 5.0	81.3 ± 3.4	54.1 ± 3.5	15.8 ± 21.4	73.2 ± 8.4	86.1 ± 5.3	47.6 ± 11.1
Millettia pinnata	79.2 ± 1.8	46.6 ± 3.0	71.3 ± 3.3	73.6 ± 4.2	40.2 ± 7.6	52.4 ± 9.6	71.1 ± 0.3	35.0 ± 16.4	86.8 ± 1.7	83.6 ± 1.1	91.3 ± 0.9	85.8 ± 4.8	65.5 ± 3.0	90.9 ± 0.7	88.1 ± 2.3	84.1 ± 2.5
Morinda elliptica	77.6 ± 2.7	49.6 ± 0.6	58.2 ± 6.3	75.7 ± 3.0	29.6 ± 2.1	30.4 ± 4.7	74.9 ± 0.8	34.5 ± 1.8	87.8 ± 0.4	82.5 ± 0.8	91.7 ± 0.3	90.7 ± 0.9	67.0 ± 1.5	79.4 ± 5.2	86.9 ± 0.7	83.4 ± 0.7
Polyalthia longifolia	75.7 ± 4.7	38.1 ± 3.3	31.6 ± 7.2	64.7 ± 3.3	31.3 ± 0.7	8.2 ± 5.8	58.0 ± 6.5	18.8 ± 10.4	78.3 ± 1.7	74.2 ± 2.3	85.3 ± 2.6	61.0 ± 1.0	38.7 ± 11.6	33.8 ± 3.7	45.8 ± 2.8	30.5 ± 5.1
Syzygium campanulatum	81.3 ± 1.5	51.9 ± 2.7	65.4 ± 4.2	79.0 ± 0.8	17.3 ± 7.6	39.5 ± 9.6	81.0 ± 0.6	39.7 ± 2.8	86.6 ± 1.6	83.3 ± 0.4	90.9 ± 1.5	89.8 ± 0.2	65.3 ± 1.3	89.4 ± 0.5	94.1 ± 0.3	82.4 ± 0.3

Lim.: *Limnothrix* sp.; Mic.: *Microcystis* sp.; Osc.: *Oscillatoria* sp.; Pla.: *Planktothrix* sp.; Pse.: *Pseudoanabaena* sp.; Syn. (AI): *Synechococcus* sp. (AI); Syn. (TB): *Synechococcus* sp. (TB); Syne.: *Synechocystis* sp.

Table 2
Two-way ANOVA results for inhibition efficiency on day 15

Source	Type III sum of squares	df	Mean square	*F*	Sig.
Plant species	56,011.244	14	4,000.803	130.242	0
Cyanobacterial species	64,232.538	7	9,176.077	298.717	0
Plant species × cyanobacterial species	58,479.927	98	596.734	19.426	0
Error	7,372.396	240	30.718		
Total	1,972,367.296	360			

Table 3
Tukey's HSD test were conducted to examine homogeneity of each factors based on mean: (a) cyanobacterial species and (b) plant species

Cyanobacterial species	Subset				
	1	2	3	4	5
Pseudoanabaena sp.	38.01				
Synechocystis sp.		63.75			
Synechococcus sp. (AI)			71.37		
Planktothrix sp.			74.19	74.19	
Oscillatoriales sp.				76.75	
Microcystis sp.				76.94	
Synechococcus sp. (TB)					80.78
Limnothrix sp.					81.72
Sig.	1	1	0.24	0.273	0.99

Plant species	Subset						
	1	2	3	4	5	6	7
Mesua ferrea	47.18						
Phyllanthus sp.	48.78						
Polyalthia longifolia		55.94					
Disporum sp.		57.73					
Millettia atropurpurea			64.38				
Croton sp.			69.55	69.55			
Clitoria fairchildiana				72.12	72.12		
Andira inermis				73.09	73.09		
Cerbera odollam				74.75	74.75	74.75	
Kopsia fruticosa					76.33	76.33	
Brackenridgea sp.						79.75	79.75
Pterocarpus indicus							83.57
Morinda elliptica							83.68
Millettia pinnata							84.52
Syzygium campanulatum							85.22
Sig.	1	0.1	0.09	0.08	0.35	0.12	0.05

The reservoirs from where the cyanobacteria isolated are situated far from industrialized area and are surrounded by forest trees. From the observation, fallen leaves from the forest trees may potentially play role in inhibiting cyanobacterial bloom formation. Thus, based on results from this study, this strengthens the theory that fallen dry leaves acts as natural cyanobacterial bloom management.

However, dynamics of cyanobacterial population in an environment involves many factors and is yet to be fully understood [23]. In the study, volume is also shown to affect cyanobacterial growth pattern. And while high phosphorus or nitrate is known to cause eutrophication, researchers also indicate that the characteristics of the water body and environmental conditions such as temperature,

light intensity and wind direction influence the distribution and formation of blooms, rather than the availability of nutrients [24]. Besides that, biotic factors such as cyanobacteria predator, bacteria and cyanophage are also able to influence the cyanobacterial population dynamics.

In addition, using biological-derived substances for cyanobacterial bloom management may arise other concerns, such as weak inhibitory effect, emerging anti-cyanobacterial compound resistance in a population pool and release of toxin during bloom collapse [11]. Besides that, as observed in this study, cyanobacterial growth inhibition is dependent on cyanobacterial and plant species. As such, a specific cyanobacterial species may be enhanced by a plant leaves, but inhibited by another plant leaves, and vice versa. Nevertheless, by planting various species of terrestrial leaves surround the water-bodies, variety of cyanobacterial species can be controlled and early prevention of cyanobacterial bloom able to prevent release of toxin due to bloom collapse.

4. Conclusion

Based on the experimentation results, it can be seen that leaves are able to inhibit the growth of cyanobacteria, indicating release of an anti-cyanobacterial compound. However, inhibition efficiency depended on the cyanobacterial and plant species. Overall, comparing to chemical and physical approaches, using plant biomass offers environmentally friendly and low cost for cyanobacterial bloom management.

Acknowledgement

The authors are grateful to Universiti Sains Malaysia for financial assistance through USM RU grant [Grant number 1001.PTEKIND.811253, 2013] and MOE ERGS grant [Grant number 203.PTEKIND.6730135, 2013] for this work.

References

[1] Y.Y. Qiu, T. Yuan, T. Kon, R. Zurawell, Y. Huang, M. Graham, S. Gabos, X.L. Pang, Rapid detection and quantitation of microcystin-producing *Microcystis* using real-time PCR, Mol. Biomarkers Diagn., S5 (2013) 1–7, 006, doi: 10.4172/2155-9929.S5-006.

[2] M. Manganelli, S. Scardala, M. Stefanelli, F. Palazzo, E. Funari, S. Vichi, F.M. Buratti, E. Testai, Emerging health issues of cyanobacterial blooms, Annali dell'Istituto Superiore di Sanità, 48 (2012) 415–428.

[3] J. Głowacka, M. Szefel-Markowska, M. Waleron, E. Łojkowska, K. Waleron, Detection and identification of potentially toxic cyanobacteria in Polish water bodies, Acta Biochim. Pol., 58 (2011) 321–333.

[4] P.J. Oberholster, A.-M. Botha, T.E. Cloete, Toxic cyanobacterial blooms in a shallow, artificially mixed urban lake in Colorado, USA, Lake Reservoir Res. Manage., 11 (2006) 111–123.

[5] P. Zhang, C.M. Zhai, R.Q. Chen, C.H. Liu, Y.R. Xue, J.H. Jiang, The dynamics of the water bloom-forming *Microcystis aeruginosa* and its relationship with biotic and abiotic factors in Lake Taihu, China, Ecol. Eng., 47 (2012) 274–277.

[6] B. Lévesque, M.-C. Gervais, P. Chevalier, D. Gauvin, E. Anassour-Laouan-Sidi, S. Gingras, N. Fortin, G. Brisson, C. Greer, D. Bird, Prospective study of acute health effects in relation to exposure to cyanobacteria, Sci. Total Environ., 466–467 (2014) 397–403.

[7] H.W. Paerl, T.G. Otten, Harmful cyanobacterial blooms: causes, consequences, and controls, Microb. Ecol., 65 (2013) 995–1010.

[8] United States of Environmental Protection Agency, Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems, 2012. Available at: www.epa.gov/sites/production/files/2014–08/documents/cyanobacteria_factsheet.pdf

[9] S.H. Te, K.Y.-H. Gin, The dynamics of cyanobacteria and microcystin production in a tropical reservoir of Singapore, Harmful Algae, 10 (2011) 319–329.

[10] E.V. Hullebusch, V. Deluchat, P.M. Chazal, M. Baudu, Environmental impact of two successive chemical treatments in a small shallow eutrophied lake: Part II. Case of copper sulfate, Environ. Pollut., 120 (2002) 627–634.

[11] J.H. Shao, R.H. Li, J.E. Lepo, J.-D. Gu, Potential for control of harmful cyanobacterial blooms using biologically derived substances: problems and prospects, J. Environ. Manage., 125 (2013) 149–155.

[12] M. Lürling, E.J. Faassen, Controlling toxic cyanobacteria: effects of dredging and phosphorus-binding clay on cyanobacteria and microcystins, Water Res., 46 (2012) 1447–1459.

[13] X. Wu, H. Wu, J.Y. Ye, B. Zhong, Study on the release routes of allelochemicals from *Pistia stratiotes* Linn., and its anti-cyanobacteria mechanisms on *Microcystis aeruginosa*, Environ. Sci. Pollut. Res., 22 (2015) 18994–19001.

[14] L.X. Ni, X.Y. Hao, S.Y. Li, S.J. Chen, G.X. Ren, L. Zhu, Inhibitory effects of the extracts with different solvents from three compositae plants on cyanobacterium *Microcystis aeruginosas*, Sci. China Chem., 54 (2011) 1123–1129.

[15] L.X. Ni, K. Acharya, X.Y. Hao, S.Y. Li, Antioxidant and metabolism responses to polyphenol stress in cyanobacterium *Microcystis aeruginosa*, J. Environ. Sci. Health., Part B, 48 (2013) 153–161.

[16] S. Nakai, S. Yamada, M. Hosomi, Anti-cyanobacterial fatty acids released from *Myriophyllum spicatum*, Hydrobiologia, 543 (2005) 71–78.

[17] E.M. Gross, Allelopathy of aquatic autotrophs, Crit. Rev. Plant Sci., 22 (2003) 313–339.

[18] R.J. Ritchie, Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents, Photosynth. Res., 89 (2006) 27–41.

[19] I. Ridge, J. Walters, M. Street, Algal growth control by terrestrial leaf litter: a realistic tool?, Hydrobiologia, 395–396 (1999) 173–180.

[20] S.-L. Peng, J. Wen, Q.-F. Guo, Mechanism and active variety of allelochemicals, Acta Bot. Sin., 46 (2004) 757–766.

[21] P. Laue, H. Bährs, S. Chakrabarti, C.E.W. Steinberg, Natural xenobiotics to prevent cyanobacterial and algal growth in freshwater: contrasting efficacy of tannic acid, gallic acid, and gramine, Chemosphere, 104 (2014) 212–220.

[22] M.E. Van Apeldoorn, H.P. Van Egmond, G.J.A. Speijers, G.J.I. Bakker, Toxins of cyanobacteria, Mol. Nutr. Food Res., 51 (2007) 7–60.

[23] D.V. Baxa, T. Kurobe, K.A. Ger, P.W. Lehman, S.J. Teh, Estimating the abundance of toxic *Microcystis* in the San Francisco Estuary using quantitative real-time PCR, Harmful Algae, 9 (2010) 342–349.

[24] R.L. Oliver, G.G. Ganf, Freshwater Blooms, B.A. Whitton, M. Potts, Eds., The Ecology of Cyanobacteria – Their Diversity in Time and Space, Kluwer Academic, Dordrecht, 2000, pp. 149–194.