Iraqi Journal of Biotechnology, 2016, Vol. 15, No. 1 , 97-102

Evaluation the Activity of Rosemary (*Rosmarinus officinalis* L.) Essential Oil Against Some Cyanobacteria

Ali M. Najem, Ibrahim J. Abed, Alaa M. Dh. AL-Haidari

Department of Biology, College of Science, University of Baghdad

Received: November 8, 2015 / **Accepted**: January 28, 2016

Abstract: Freshwater resources are threatened by harmful algal blooms (HABs) internationally. The HABs are sometimes direct result of eutrification status caused by pollution entering water bodies, such as partially treated nutrient-rich effluents discharged in the rivers, in addition to fertilizers and animal wastes. The anti-cyanobacterial activity of *Rosmarinus officinalis* essential oil on *Microcystis aeruginosa* and *Chroococcus minor* was evaluated. The essential oil was extracted by steam distillation method, the yield of extraction was 1 % (v/w),and the concentrations of this oil 0.05, 0.1 and 0.15% were prepared. Results revealed very strong antagonistic effects of the essential oil on *M. aeruginosa* and *C. minor* growth rates by measuring the amount of chlorophyll- a of each alga. Generally growth rates of both algae severely decreased as the time and the concentrations of the essential oil increased comparing with the growth rate of control (algae without added essential oil) which exponentially increased along with time. *M. aeruginosa* was more sensitive to the allelopathic effect of the oil.

Keywords: Essential Oil, *Rosmarinus officinalis*, Cyanobacteria.

Corresponding author: should be addressed (Email: Ibrahimabed95@yahoo.com)

Introduction

Toxic cyanobacteria are found worldwide coastal water environments. At least 46 species have been shown to cause toxic effects in vertebrates (1). The most common toxic cyanobacteria in fresh water are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. *Oscillatoria*) *rubescens*, *Synechococcus* spp., *Planktothrix* (syn. *Oscillatoria*) *agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Schizothrix* spp. and *Synechocystis* spp. (2). Cyanobacteria, mostly dominated by *Microcystis*, *Nostoc* spp., *Oscillatoria* spp., *Chroococcus* spp. *Lyngbya* spp. *Microcystis aeruginosa* is one of the world wide and most frequently encountered bloom-forming cyanobacteria in freshwater bodies, several of its strains produce powerful toxins—microcystins (3).

Cyanobacteria are amongst the most successful bloom forming algae. These algae can convert and use different forms of C, N, P, and S help in occupying almost all kinds of aquatic habitats. Moreover, they grow well in shaded light, show resistance against grazing pressure and release allelochemicals to out-compete co-occurring organisms. Presence of gas-vacuoles facilitates their migration in the water column to ensure enough light and nutrient availability. Cyanobacterial blooms adversely affect water quality, structure and composition of biological communities and a range of ecological services. Many of the bloom forming cyanobacteria produce toxins responsible for mass mortality of aquatic and exposed vertebrate populations (4). Cyanobacterial scum can represent thousand-fold to million-fold concentrations of cyanobacterial cell populations (5).

Mechanical and physiochemical methods have been devised in attempts to manage cyanobacterial blooms with limited success. The most direct control method involves the use of chemical treatments such as algicides, including copper, Reglone A (diquat, 1, 1-ethylene-2, 2-dipyridilium dibromide), potassium permanganate, chlorine and Simazine (2-chloro-4,6- bis (ethylamino)-s-triazine (6). Copper sulphate or organo-copper compounds have been used successfully to control harmful algal blooms in raw water supplies intended for human consumption (6). These chemicals induced cyanobacterial cell lysis, followed by the release of toxins into surrounding water. New and alternative approach to control the algal blooms involves the use of plant extracts exactly the essential oils. The aim of this study was to find out alternative method for prementioned control by using the essential oil of Rosemary to evaluating the allelopathic activity against *Microcystis aeruginosa* and *Chroococcus minor.*

Materials and Methods

Culture of Algae

Chrococcus minor and *Microcystis aeruginosa* unialgal isolates were obtained from Ministry of Science and Technology laboratories, cultured in BG11 media up to the exponential growth phase (7 days following inoculation) to obtain a high biomass. The microalgae were cultivated to the exponential growth phase for use. The density was monitored every two days.

Determination of Algal Growth Curve

Standard growth curve of 2 species of algae used in this study were determined and another growth curve after the volatile oil was applied on the algae to demonstrate the impact of the oil against the algae in a comparison with the standard curve as the following:

Algal culture in 30 ml was prepared as primary inoculum. 100 ml of cultured media was prepared and inoculated with algal species, and incubated with the presence of light for 14 days. Chlorophyll-a was measured by determine the O.D. at the incubation time (time=0) then after 2 days, 4, 6 until 14 days. The curve was drowning between O.D. and time of days. The same procedure repeated after adding volatile oil and the growth curve was drawed.

Extraction of Volatile Oil

Rosmarinus officionalis volatile oil were extracted by hydrodistillation with

300g dry plant powder for 5h, using clavenger apparatus, the extracted oil was kept at 4°C until testing (7,8).

Evaluation the Activity of Rosemary Essential Oil Against Cyanobacteria

According to (9) the algicidal activity of *R. officinalis* volatile oil was tested at 3 concentrations (0.05, 0.1, 0.15%) was monitored throughout 12 days against these two algae. The volatile oils were dissolved in DMSO (Dimethyl sulfoxide) and add to the flask containing 1ml of alga and 9ml of culture media (BG11). The culture condition (salinity, temperature, and light) during the experiments were the same. The highest concentration of DMSO used in the experiment equaled the volume of v. oil. The density of alga was monitored every 2 days by estimation of chlorophyll content (indirect method of cyanobacterial biomass determination), the pigment can be completely extractable in solvents like acetone and exhibits characteristic absorbance at 663nm, the estimation of chlorophyll a was estimated as follows: The cyanobacterial culture was taken and centrifuged at 5000rpm for 10min. the pellet was washed twice in D,W, thus the pellet was re-suspended in 4 ml of 80% acetone and vortexes thoroughly. Tubes were incubated in a water bath at 60°C for 1 h in dark with occasional shaking. The suspension was centrifuged at 5000rpm for 10 min and the supernatant was stored. The process was repeated to ensure complete extraction. Absorbance of the supernatant was read at 663nm in U.V. spectrophotometer against 80% acetone as blank. The amount of chlorophyll a in the sample was calculated using the formula.

$$\text{Chl.a} = \frac{A_{663} \times 12.63 \times \text{volume of acetone}}{\text{Volume of sample}} \ \mu g.ml^{-1}$$

A663: absorbance at 663nm.
12.63: correction factor and the amount were expressed as $\mu g.ml^{-1}$.

Results and Discussion

Results recorded an immense decline in the growth rate of *Chroococcus minor* and *Microcystis aeruginosa* due to the allelochemical effect of *Rosmarinus officinalis* essential oil on these microalgae, represented by massive decrease in the amount of chlorophyll-a of both cyanobacterial algae and that appeared in all tested concentrations (0.05, 0.1 and 0.15% v/v) within first time interval (after 48Hr.) of applying the essential oil and this antialgal impact progressed along with time of the experiment reaching to 0.0 µg/ml amount of chlorophyll mainly against *M. aeruginosa*. Whoever, growth rates of control (algae without added essential oil) representing by chlorophyll-a concentration were exponensially increased along with time as shown in figure (1and 2). *M. aeruginosa* was more sensitive to the essential oil than *C. minor*. The effects of the rosmary essential oil on *C. minor* and *M. aeruginosa* increased as the time and concentrations of the essential oil increased except some irregular trends *C. minor* showed a slightly resistance to 0.15% than 0.05 and 0.1% , generally uncountable with the massive drop in the amount of chlorophyll-a Figure 1 and 2 shows the anticyanobacterial activity of the rosmary essential oil on

C. minor and *M. aeruginosa* growth rate respectively and that appeared clearly the remarkable effieceny of the oil by reducing the amount of chlorophyll a of these algae comparing with that in control set along with time. Following the prementioned pattern of comparison, the essential oil highest inhibitory action was during the sixth day of the experiment (144 hr.) against *M. aeruginosa* according to the optical desity readings (formulated into chlorophyl-a concentration) then the readings slightly increased as the time proceed, which indicates that this alga developed a resistance mechanism agaist the oil. Adaptation of cyanobacteria and algae, which are the principal primary producers of aquatic ecosystems to environmental changes seems to be the result of spontaneous mutation from sensitivity to resistance that occurs randomly prior to the cells coming into contact with the selective agent (10). Results of this study showed regular reducing in the amount of chlorophyll-a of *C. minor* approaching to the highest inhibitory data during day12of the experiment (288 hr. Kobaisy *et al*. (11) examined the essential oil of *Hibiscus cannabinus* leaves against *Oscillatoria perornata* which showed slightly inhibitory effect while it had no effect against one type of green algae, algicidal activity was not observed for the oil major components when tested separately under the same conditions as the essential oil.

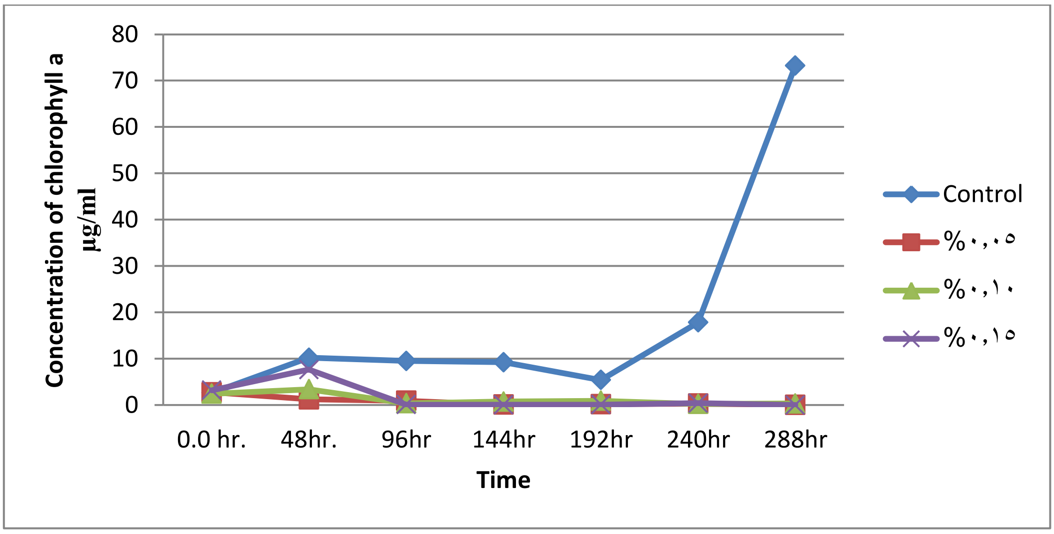

Figure 1: Effect of Rosmarinus officinalis essential oil concentrations on the growth rate of *C. minor* comparing with the growth rate of the control

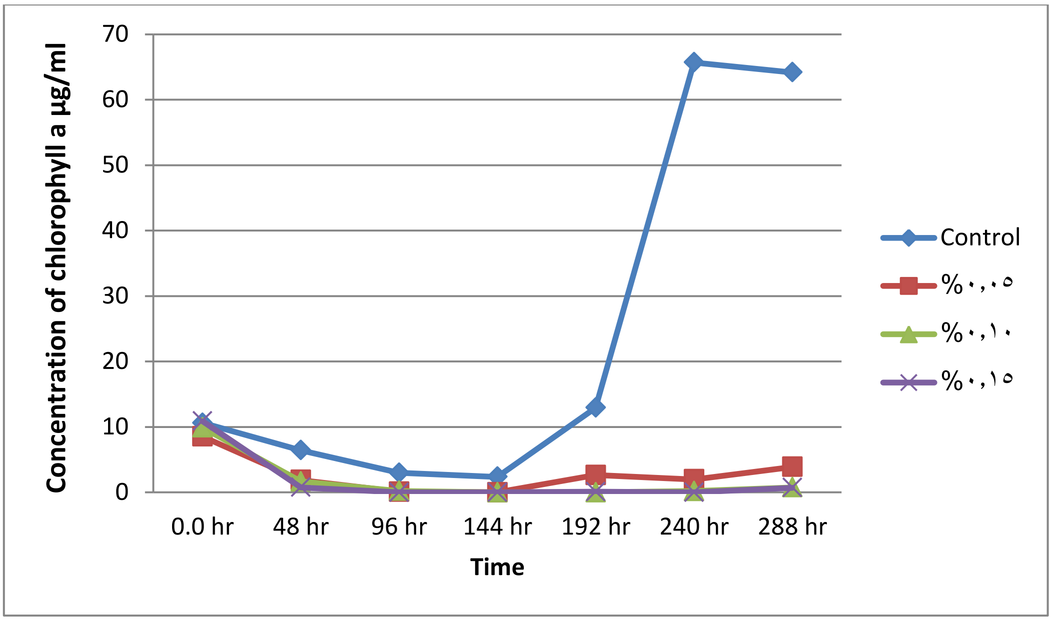

Figure 2: Effect of *Rosmarinus officinalis* essential oil concentrations on the growth rate of *M. aeruginosa* comparing with the growth rate of the control

It is difficult for reaserchers to study allelopathic effects among aquatic organisms under natural conditions, because factors such as nutrient and light competition, temperature and pH change could totally mask an allelopathic effect (12). Therefore, "fast reaction and high effectiveness of inhibitors play a key role in a successful HABs control. The possible explanation for the better activity of the essential oil could be due to easier absorption of the essential oil and lipophilic extracts into the cell body of the algae (13)". Wang *et al*. (14) studied the anti cyanobacterial activities of essential oils extracted from six submerged plants *Potamogeton cristatus*, *P. maackianus*, *P. lucens*, *Vallisneria spiulosal*, *Ceratophyllum demersum* and *Hydrilla verticellata*, which showed high potency on *Microcystis aeruginosa*.

Conclusion

This study provided an alternative method by using the essential oil of Rosemary. The findings revealed allelopathic activity against *Microcystis aeruginosa* and *Chroococcus minor*. *M. aeruginosa* was more sensitive to the allelopathic effect of the essential oil.

References

1. Sivonen, K. and Jones, J. (1999). Cyanobacterial toxins. In: Chorus I, Bartram J., ed. Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. Published by E & FN Spon on behalf of the World Health Organization, pp. 41–112.
2. Chorus I., Bartram J., ed. (1999). Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. Published by E & FN Spon on behalf of the World Health Organization.

3. Abed, I. J.; Al-Hussieny, A. A; Kamel, R. F. and Jawad, A. M. (2013) .Environmental and Identification Study of Algae Present in Three Drinking Water Plants located on Tigris River in Baghdad. International Journal of Advanced Research, 2 (3): 895-900.
4. Paerl, H.W.; Fulton III, R.S.; Moisander, P.H. and Dyble, J. (2001). Harmful freshwater algal bloom, with an emphasis on cyanobacteria. The Scientific World Journal, 1: 76–113.
5. Chorus, I. and Fastner, J. (2001) Recreational exposure to cyanotoxins. In: Chorus I, ed. *Cyanotoxins, occurrence, causes, consequences*. Heidelberg, Springer, pp. 190–199.
6. Lam, A. K.; Prepas, D.; Spink, and Hrudey. S. E. (1995). Chemical control of hepatotoxic phytoplankton blooms: Implications for human health. Water Research, 29: 1845-1854.
7. Selim, S.; Adam, M.; Hassan, S. and Albalawi, A. (2014). Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (Cupressus sempervirens L.). BMC Complementary and Alternative Medicine, 14, pp:179.
8. Jamshidi, R.; Afzali, Z. and Afzali, D. (2009). Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with other countries. American-Eurasian J. Agric. & Environ. Sci., 5 (1): 78-81.
9. Kim, D.; Oda, T.; Muramatsu, T., Kim, Daeil, Matsuyama, Y. and Henjo, T. (2002). Possible factors responsible for the toxicity of *Cochlodinium polykrikoides*, a red tide phytoplankton. Comparative Biochemistry and Physiology, 132: 415-423.
10. Costas, E.; Carrillo, E.; Ferrero, L. M.; Agrelo, M.; Garcı , A.; Villada, L.; Juste, J. and Lopez-Rodas, V. (2001). Mutation of algae from sensitivity to resistance against environmental selective agents: the ecological genetics of Dictyosphaerium chlorelloides (Chlorophyceae) under lethal doses of 3-(3,4-dichlorophenyl)-1,1-dimethylurea herbicide. Phycologia, 40:391–398.
11. Kobaisy, M.; Mario, R.; Tellez, R.; Webber, C. L.; Dayan, F. E.; Schrader, K. K. and Wedge, D. E. (2001). Phytotoxic and fungitoxic activities of the essential oil of Kenaf (*Hibiscus cannabinus* L.) leaves and its composition. Journal of Agiculture and Food Chemistry, 49(8): 3768-3771.
12. Wang, R.; Xiao, H.; Wang, Y.; Zhou, W. and Tang, X. (2007). Effects of three macroalgae, *Ulava linza* (Chlorophyta), *Corallina pilulifera* (Rhodophyta) and Sargassum thunbergii (phaeophyta) on the growth of the red tide microalgae *Prorocentrum donghaiense* under laboratory conditions. Sea research, 58: 189-197.
13. Yi, Y.L.; Lei, Y.; Yin, Y.B.; Zhang, H.Y. and Wang, G.X., (2011). The antialgal activity of 40 medicinal plants against *Microcystis aeruginosa*. Applied Phycology, DOI. 10.1007/s10811-011-9703-2.
14. Wang, H.; Liang, F. and Zhang, L. (2015). Composition and Anti-cyanobacterial Activity of Essential Oils from Six Submerged Macrophytes. Pol. J. Environ. Study, 24 (1): 333-338.