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The Inhibition Effects of Alkoxyl Thiourea Derivatives toward the Growth of Isolated *Oscillatoria* sp. from Kenyir Lake, Terengganu

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Abstract: In controlling eutrophication phenomenon, there were conventional methods which lead to negative effects to aquatic environment. This study was aimed to investigate the usage of synthesized thiourea compounds to inhibit the growth of *Oscillatoria* sp. in Kenyir lake, Terengganu, Malaysia. The inhibition effects of four eco-friendly alkoxyl thiourea derivatives compounds named as N-((4-(decyloxy)phenyl)carbamothioyl)-4-methyl benzamide, N-((4-(decyloxy)phenyl)carbamothioyl)-4-nitro benzamide, 4-chloro-N-((4-(decyloxy)phenyl)carbamothioyl) benzamide and N-((4-(decyloxy)phenyl)carbamothioyl) benzamide were examined onto the growth culture of *Oscillatoria* sp. These compounds were tested in 30 mL of *Oscillatoria* sp. cultures with different concentration of 16 $\mu g \cdot mL^{-1}$, 18 $\mu g \cdot mL^{-1}$, 20 $\mu g \cdot mL^{-1}$, 24 $\mu g \cdot mL^{-1}$ and 28 $\mu g \cdot mL^{-1}$ respectively. The treatment flasks were supplied with an aerator for 24 hours under continuous illumination at 25 ^{o}C. Chlorophyll-*a* concentration were extracted to calculate the inhibition percentage of each treatment. Overall, all these compounds showed inhibition effects towards the growth of *Oscillatoria* sp., with the highest inhibition of 37% by N-((4-(decyloxy)phenyl)carbamothioyl)-4-methyl benzamide at the concentration of 18 $\mu g \cdot mL^{-1}$. The methyl group that attach to the synthesized compound may contribute to the effectiveness of the compound which act as an algae inhibitor. However, extensive studies still need to be conducted in order to investigate the mechanism on how this compound reacts with *Oscillatoria* sp..

Key words: Eutrophication, alkoxyl thiourea, *Oscillatoria* sp., chlorophyll-*a*, inhibition effect.

1. Introduction

Nowadays, the aquatic environment has been greatly affected by various type of environmental problem. These environmental problem are closely related to the anthropogenic effects which mainly contributed by human activity. Water pollution becomes a major environmental problem that comes into concern to many of the community world widely. There are various sources of pollutants that contribute in increasing the concentration of the chemical contaminants in water resources such as industrials, constructions, agricultures and aquacultures [1]. The excess nutrient or contaminant from these activities may lead to the massive growing up of toxic blue green algae either in freshwater or seawater. One of the water pollution consequences is eutrophication problem. Eutrophication is commonly caused by the over enrichment of nutrients such as phosphate and nitrate which contribute to the depletion of oxygen in water [2, 3]. This condition will then leading to the rapid growth of the toxic blue green algae or known as cyanobacteria which will caused Harmful Algal Blooms (HABs) phenomenon. HABs occurred when there is rapid increase of the growth of toxic blue green algae when there are excessive nutrients available in the water bodies. The increment of nutrients loading into water bodies causing the eutrophication will enhances the primary productivity which result in high rates of microbial

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activities [4] and finally, lead to human health problems. There are numbers of toxic blue-green algae species that potentially produce harmful toxins. As an example, *Lyngbya robusta* is one of the cyanobacteria species that can release hepatoxin and cyanotoxin once its blooming [5]. The blooming of toxic blue green algae producing cyanotoxin in certain concentration level can be very poisonous and lead to kill animals and humans community [6]. To overcome this problem, there are conventional approaches to treat HABs such as the usage of acidification, copper sulphate and barley straw methods [7-9]. As a novel method to treat HABs problem, many researches proved that the thiourea compound was successfully used as antimicrobial and antifungal agent [10-12]. Therefore, this study was aimed to investigate the potential usage of alkoxyl substituted thiourea derivatives namely N-((4-(decyloxy)phenyl) carbamothioyl)-4-methyl benzamide (compound 1), N-((4-(decyloxy)phenyl) carbamothioyl)-4-nitro benzamide (compound 2), N-((4-(decyloxy)phenyl) carbamothioyl)-4-chloro benzamide (compound 3), N-((4-(decyloxy)phenyl) carbamothioyl) benzamide (compound 4) to inhibit the growth of toxic blue green algae, *Oscillatoria* sp. which was isolated from Kenyir lake, Terengganu, Malaysia and could be further used as anti-algae agent. However, due to the uniqueness and fair conjugation properties of thiourea derivatives of compounds 1 and 2 have been reported before in previous occasions within our group [13, 14].

2. Material and Methods

Toxic blue green algae, *Oscillatoria* sp. was successfully sampled at four selected sampling sites using a mesh size of 25 μm phytoplankton net from Kenyir Lake, Terengganu, Malaysia (Fig. 1). In the laboratory, these toxic blue green algae species were isolated and identified based on their taxonomic identification key under light microscope [15, 16]. After a series of sub-culturing process, pure culture of *Oscillatoria* sp. was obtained. Using BG-11 agar

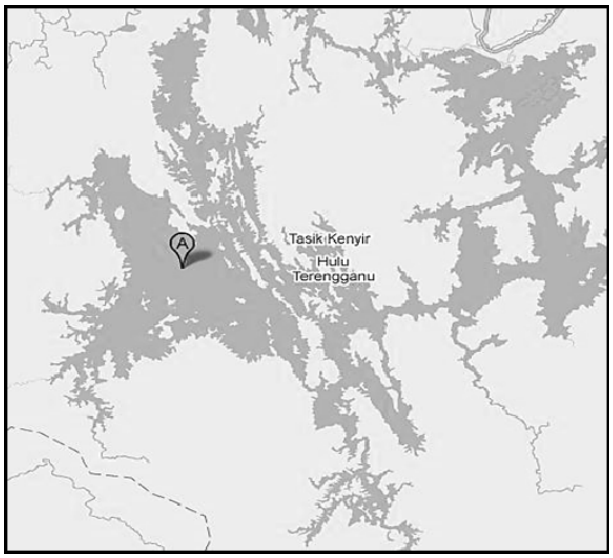
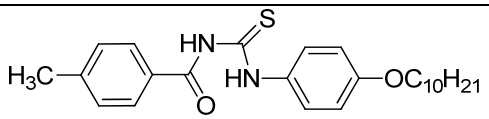
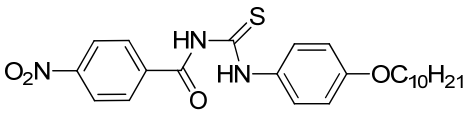
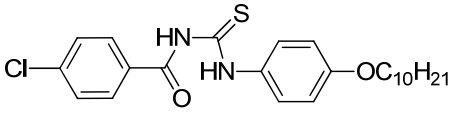
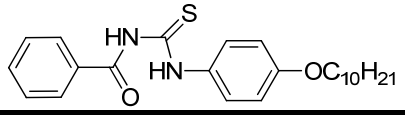


Fig. 1 Sampling location of toxic blue green algae at Kenyir lake, Terengganu, Malaysia.

media, this species was grown and maintained in pure culture. For inhibition treatment purposes, the pure culture of *Oscillatoria* sp. was transferred onto BG-11 liquid medium for mass culturing in 2 L conical flask. The mass culture of pure species was then harvested during exponential phase (6-8 days) for the inhibition effects treatment using four different alkoxyl thiourea derivatives compounds.

Four alkoxyl thiourea derivatives compounds were used throughout this study which was synthesized by Department of Chemical Sciences, School of Fundamental Science, Universiti Malaysia Terengganu (Table 1). All compounds (Table 1) were firstly diluted by adding 1,000 μg of each compounds with 1 mL of dimethyl sulfoxide (DMSO) to obtain a total concentration of 1,000 $\mu g \cdot mL^{-1}$. Two fold of serial dilution were carried out on five different concentrations resulted in final concentration of 16 $\mu g \cdot mL^{-1}$, 18 $\mu g \cdot mL^{-1}$, 20 $\mu g \cdot mL^{-1}$, 24 $\mu g \cdot mL^{-1}$ and 28 $\mu g \cdot mL^{-1}$ respectively. Two milliliter of each compounds were added up into 30 mL of *Oscillatoria* sp. growth cultures in 100 mL conical flask. Controlled treatment was also set up by replacing the compound solution with distilled water. Finally, all the treatment cultures were incubated for 24 hours under continuous illumination at 25 ^{o}C. The inhibition

Table 1 Molecular structure of four different types of alkoxyl thiourea derivatives.

No.	Name	Molecular structure
1.	N-((4-(decyloxy)phenyl) carbamothioyl)-4-methyl benzamide	
2.	N-((4-(decyloxy)phenyl) carbamothioyl)-4-nitro benzamide	
3.	N-((4-(decyloxy)phenyl) carbamothioyl)-4-chloro benzamide	
4.	N-((4-(decyloxy)phenyl) carbamothioyl) benzamide	

treatments were conducted during the exponential growth of *Oscillatoria* sp. (6-8 days, Fig. 3).

After 24 hours treatment periods, the control and treated *Oscillatoria* sp. cultures with four different types of alkoxyl thiourea derivatives were filtered through 0.45 µm Whatman filter paper. The filtrates were washed off using 5 mL to 10 mL of 100% acetone in 50 mL centrifuge tubes for chlorophyll-*a* (Chl-*a*) determination. The centrifuge tubes were then sealed with aluminum foil and left overnight at 4 °C in the dark. After 24 hours, the samples were then centrifuged for 10 minutes at 3,000 rpm. The supernatant was taken out and measured at 665 (OD_{665}), 645 (OD_{645}) and 630 (OD_{630}) using UV-Vis spectrophotometer. The chl-*a* content for each centrifuge tubes was calculated using the formula as Eq. (1):

$$\text{Chl-}a\ (\mu g \cdot L^{-1} \text{ or } mg \cdot m^{-3}) = \frac{Ca \times Va}{Vc} \quad (1)$$

Where,

Ca was $11.6 \times OD_{665} - 1.31 \times OD_{645} - 0.14 \times OD_{630}$; *Va* was volume of acetone (mL) used for extraction and *Vc* was volume of culture (L).

Furthermore, the inhibition effect in terms of percentage of compounds 1-4 to the growth of *Oscillatoria* sp. cultures were calculated using Eq. (2):

$$\text{Inhibition effect (\%)} = \frac{Cc - Ct}{Cc} \times 100 \quad (2)$$

Where,

Cc was Chl-*a* concentration ($mg \cdot m^{-3}$) of control (untreated), *Ct* was Chl-*a* concentration ($mg \cdot m^{-3}$) of treated culture.

3. Results and Discussion

Samples of *Oscillatoria* sp. were successfully isolated from Kenyir Lake, Terengganu, Malaysia. The fresh specimen of *Oscillatoria* sp. was observed under light microscope based on their specific morphological structures. The filamentous and cylindrical morphology of *Oscillatoria* sp. was determined (Fig. 2). The thallus of *Oscillatoria* sp. was cylindrical and filamentous, while the colour was bright to dull blue green. The filaments were branched falsely [17]. Besides, *Oscillatoria* sp. showed simple filament which consist of mucilaginous and plasma membrane sheath. The cell wall of *Oscillatoria* sp. lies between plasma membrane and mucilaginous sheath. The innermost layer was peptidoglycan layer. In most toxic blue green algae, the peptidoglycan varies between 1 nm and 10 nm, but in *Oscillatoria* sp., it can reach up to 200 nm [17]. This species does not showed any heterocyst leading to be known as non-heterocystous blue green algae [18].

Growth curve of *Oscillatoria* sp. was plotted within 9 days (Fig. 3) showed that the maximum growth rate was up to 9 day's incubation periods. The lag phase of

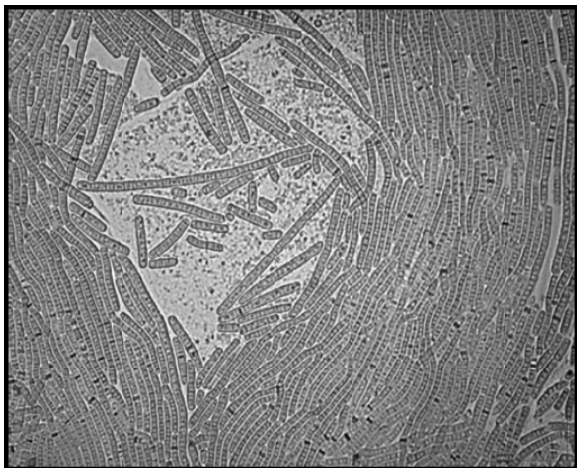


Fig. 2 Morphological structure of *Oscillatoria* sp. captured by microscope eyepiece camera, Dino-Eye under 40X magnification.

Oscillatoria sp. was between 1 days and 6 days in order for this species adapted to the new culture environment. The exponential phase was observed between 6 to 8 days, where the highest growth rate showed the most rapid growth. In comparison, *Microcystis aeruginosa* has longer exponential phase ranged between 6 days and 11 days [19]. It was important to determine the exponential phase of each isolated toxic blue green algae species for inhibitory treatment due to their maximum ability to consume nutrient sources for growth.

Four compounds of alkoxyl thiourea were used as candidates to be potential inhibitor towards the growth of toxic blue green algae, *Oscillatoria* sp. (Table 1). In general, it was proven that alkoxyl thiourea compounds were effectively acted as growth inhibitor for *Oscillatoria* sp. at the concentration of not less than 16 $\mu g \cdot mL^{-1}$. Among all four compounds, the best candidate of alkoxyl thiourea compound that can inhibit the growth of *Oscillatoria* sp. was compound 1 at the concentration of 18 $\mu g \cdot mL^{-1}$ with 37% of inhibition percentage (Fig. 4). However, at lower concentration (16 $\mu g \cdot mL^{-1}$), compound 4 showed the highest inhibition percentage with up to 30% as comparing to the other compounds. In addition, similar amount of inhibition percentage was observed for compound 3 at the concentration of 20 $\mu g \cdot mL^{-1}$. The treatment with compound 2 showed fluctuate trend where the highest inhibition percentage of 26% was observed at the concentration of 16 $\mu g \cdot mL^{-1}$.

The leaving group that attached to alkoxyl substituted thiourea compounds showed different impacts to the inhibition percentage. It was observed that methyl group that attached to compound 1 showed the highest impact to inhibit the occurrence of HABs. As the results, this study showed different pattern of inhibition toward different concentration and different compounds. This might due to the effectiveness of inhibition percentage which definitely depends on the substituent that attach to the compounds. As an overall finding of this study, it was proven that all those compounds of alkoxyl subtituted thiourea derivatives showed the potential of inhibition effect towards the growth of *Oscillatoria* sp.. Thiourea

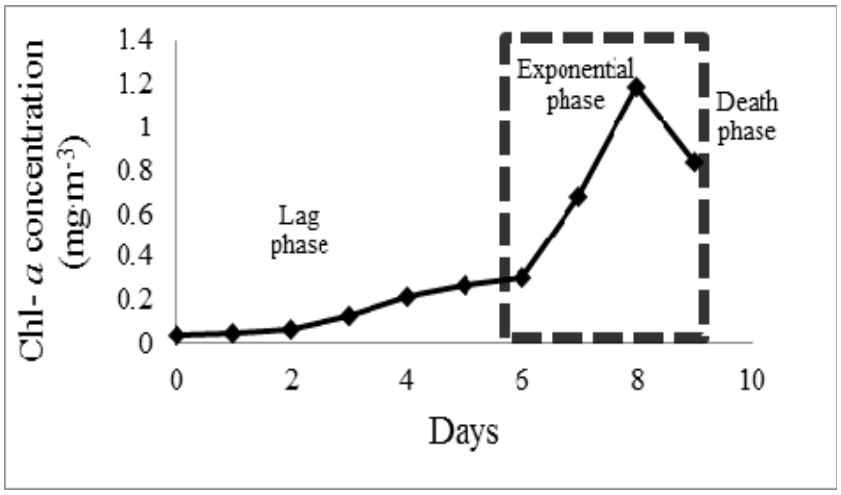


Fig. 3 Growth curve of *Oscillatoria* sp. determined by chl-*a* concentration from day 0 to day 9.

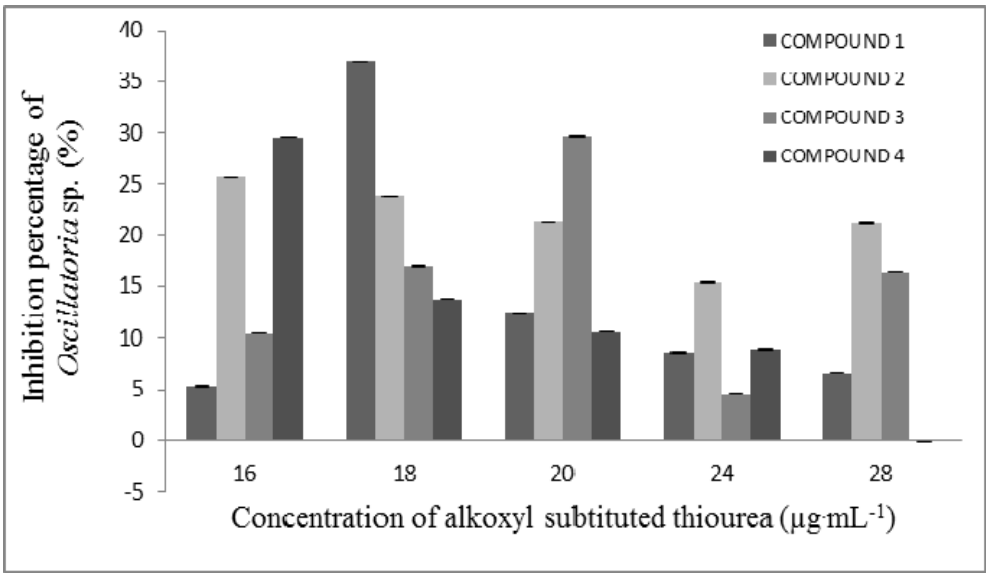


Fig. 4 Overall results showed the inhibition percentage of four different alkoxyl substituted thiourea compound, based on their chl-*a* concentration of control and treated cyanobacteria, *Oscillatoria* sp, data mean ± standard deviation from three replicates.

derivatives of carboxymethyl chitosan showed inhibition effect toward the growth of three different types of bacteria species, *Bacillis subtilis*, *Staphylococous aureus* and *Escherichia coli*.. It was expected that inhibition of those bacteria were occurred through binding of chitosan with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis via penetration of chitosan into the nuclei of the microorganisms. As an assumption toward the inhibition role of four different compounds of alkoxyl subtitued thiourea towards the growth of *Oscillatoria* sp., it possibly due to a significant changes in the cyanobacteria biomass, where the containing chl-*a* in the cyanobacteria play an important role in the photosynthesis process. This assumption was made based on the reduction of chl-*a* concentration for the determination of the inhibition percentage. The inhibition of alkoxyl substituted thiourea derivatives would distract the photosynthetic process. Consequently, the cyanobacteria will die due to lack of nutrients. On the other hand, the gamma radiation might give an adverse effect on the photosynthetic pigments. The level of carotenoids in other toxic blue green algae, *Microcystis aeruginosa* cells was reduced considerably in response to increasing doses of radiation [20].

4. Conclusions

Four compounds of alkoxyl thiourea derivatives were successfully showed an inhibition effects toward the growth of toxic blue green algae, *Oscillatoria* sp.. With the highest inhibition percentage at low amount of concentration, it can be concluded that N-((4-(decyloxy)phenyl)carbamothioyl)-4-methyl benzamide (compound 1) can be used as potential candidate for toxic blue green algae growth inhibitor. Even though the inhibition percentage was considerably low with less than 50%, there was still sign of inhibition that need to be investigated. In conclusion, alkoxyl thiourea derivatives have high potential to be developed as the algaecidal agent but further study on this system should be conducted towards their mechanisms of inhibition.

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