Application of Green Algae for Biodegradation and Utilization of the Organophosphorus Insecticide Malathion

Wael M. Ibrahim1, Asmaa A. Adawy2 and Mohamed A. Karam2

Abstract—Malathion is a highly toxic organophosphorus insecticide to aquatic organisms. In the present study, green algal strains; *Chlorella vulgaris* and *Scenedesmus quadricuda* were used to remove malathion from contaminated water. The growth of both algal strains was decreased as malathion concentration was increased. *C. vulgaris* was recorded as a more tolerant strain than *S. quadricuda*. Treatment of algal strains with malathion affected total carbohydrate and protein contents. To study the ability of algal strains to utilize malathion as a sole phosphorous source, algal strains were subjected to grow under P-limitation in absence and presence of malathion. The algal growth under P-limitation recorded a very poor level. When the P-limited medium was amended with malathion, the algal growth and phosphorus content of cells were increased significantly. *C. vulgaris* was recorded as the highest efficient strain (99.7%) to remove malathion. This study clarified that, *C. vulgaris* is considered as new biotechnology for remediation of pesticide.

Keywords—Biodegradation, Green algae, Malathion insecticide, Phosphorous limitation.

I. INTRODUCTION

As a result of human impact, the levels of organic compounds found in surface water have increased in the recent decades. Of these organic compounds, pesticides are most commonly detected in all aquatic environments [1]. These pesticides are mainly used for agricultural purposes [2]. They enter the aquatic environment via runoff after being sprayed in agricultural fields and can potentially reach groundwater [3].

Malathion is a non-systemic, wide spectrum organophosphorus pesticide (OP), used to control insects on field crops, fruits, vegetables and also extensively used to prevent mosquitoes, flies, household insects, animal parasites, and head body lice [4]. Recent research showed that, malathion has a variety of syndromes and effects including hepatotoxicity [5], human breast carcinoma [6], genetic damage [7], and disrupted normal hormone activity [8].

The chemical and physical methods for removal of OP compounds are not only expensive and time-consuming, but also in most cases they do not provide a complete solution. Bioremediation provides a suitable way to remove contaminants from the environment. Most OP compounds are degraded by microorganisms in the environment as a source of phosphorus or carbon or both [9, 10].

Phototrophic microorganisms, such as green algae, are used for wastewater treatment to remove nitrogen and phosphorus [11]. They have potential to remove various pollutants, such as dyes [12], heavy metals [13], and pesticides [10, 14]. Therefore, this study was conducted to investigate the growth and tolerance of some green isolates *Chlorella vulgaris* and *Scenedesmus quadricuda* with different concentrations of malathion, as well as evaluating their efficiency for removing this pesticide from contaminated wastewater.

II. MATERIALS AND METHODS

2.1. Algal Strains

The algal strains; *Chlorella vulgaris* and *Scenedesmus quadricuda* were isolated from different water samples collected from Al-Fayoum Governorate, Egypt.

2.2. Chemicals

The organophosphorus insecticide is commercially available as Malathion. It was obtained from Kafr Elzayyat company, Egypt (98% active ingredient).

2.3. Experimental Design

The algal isolates were batch-cultured in 500 mL Erlenmeyer flasks. Into each flask, 200 mL of liquid culture MBL medium was added [15]. The initial inoculum was approximately $5 \times 10^4$ cell/mL. Malathion was added to the culture medium to the final concentrations 0.02, 0.2, 2, 20, 50 and 100 ppm. The flasks were incubated in a culture room $23 \pm 1 \text{°C}$ under continuous illumination (40 $\mu$Em$^{-2}$ s$^{-1}$). Samples were taken after every four-days intervals up to fifty-two days for the estimation of the growth in terms of cell count. After 20 days, 50 mL of algal cultures was filtrated by centrifugation. The algal filtrate was used to determine malathion residues in the culture medium.

To obtain phosphorus-limited cultures, the growing algal cells were inoculated into flasks containing medium with...
1/10th of the original phosphorus concentration. The phosphorus-limited cells were cultured in a medium with and without malathion. Samples were taken after 20 days for estimation of cell count and phosphorus content in the different algal cells.

2.4. Analytical Analysis

From each algal culture, 10 mL was placed on vials containing 0.1 mL Lugol’s solution [16]. Cell count was carried out using a standard haemocytometer under an Olympus BH-2 light microscope. The total phosphorus content in the algal biomass was measured according to Pierpoint [17]. Hewlett Packard Agilent GC System (Gas Chromatograph, Model 6890, USA) was used for determination of malathion residues in the culture medium.

III. RESULT AND DISCUSSION

3.1. Effect of malathion on growth of tested algal strains

Data in Figures (1 and 2) demonstrated the effect of different malathion concentrations on the growth of two green algal strains, recorded significant increase in growth of *C. vulgaris* with lower concentrations from 0.02 to 2.0 ppm of malathion during all experimental period, while, treatment of *C. vulgaris* with higher concentrations (50 and 100 ppm) of malathion caused a very high significant decrease in cell count in the first 28 days, afterward very high significant increase was estimated. The lower concentrations of malathion reduced the growth of *S. quadricuda* and the higher concentrations stimulated the growth after 24 days. Regression lines (Figure 3) showed that *C. vulgaris* was more tolerant to different concentrations of malathion than *S. quadricuda*. Highest tolerance of *C. vulgaris* could be as a result of its highest ability to biodegrade malathion (Figure 4).

In consistent with our results Shizhong et al. [18] studied the growth response of *C. vulgaris* to dimethoate, an organophosphorus pesticide. They found that, low concentration of dimethoate can accelerate growth of *C. vulgaris* by increasing cell density, chlorophyll and protein content. In this respect, extensive studies have been made concerning the inhibitory effects of organophosphorus pesticides on the cell count of different algal species [10, 19, 20, 21].

The inhibitory effect of malathion could be attributed to the adsorption of this compound on the rich-lipid plasma membranes of the algal cells, thus, varying the membranes permeability [22] and decreasing photosynthetic activity [23] as well as enhancing reactive oxygen species [24].

3.2. The ability of algal strains to utilize malathion as phosphorus source

Algal strains were grown under phosphorus limitation condition in absence and presence of malathion in order to investigate their ability to utilize malathion as a sole phosphorus source. In absence of malathion, the growth of cells was markedly dwindled under phosphorus limitation recording a decrement in the total cell count by 94% in case of *C. vulgaris* and 83% in case of *S. quadricuda* compared with the unlimited cells (Table 1). In the other hand, the algal strains that were cultured under phosphorus limitation conditions and amended with malathion were able to grow and record a very high significant increase in the cell number.

The ability of algal strains to use malathion as phosphate source was confirmed by analyzing the internal phosphorus content of algal biomass. Data in Table (2) revealed that the total phosphorus content of the cells that were cultured in media with P-limitation was very minor. When the limited culture was amended with malathion, the mounts of total phosphorus were increased to the same range spotted in the unlimited cells.

In accordance with such results, Ibrahim and Essa [14] studied the effect of malathion on the growth of *A. oryzae* under phosphorus limited conditions. They found that the growth of *A. oryzae* under P-limitation recorded a very poor level and a massive enhanced growth was obtained when the P-limited medium was supplemented with malathion. The simulative effect of malathion on growth could be as a result of the increment of the available phosphorus, resulting from degradation of this compound by algal strains [10].

The growth enhancement of algal strains that were grown in the presence of malathion could be attributed to their capability to utilize this compound as sole sources of phosphorus in the absence of inorganic phosphate from the medium [10, 25].

IV. CONCLUSIONS

A general and collective conclusion that we can withdraw from our work, is that microalgae such as *C. vulgaris* and *S. quadricuda* certainly represent a great hope and an attractive candidate for treating contaminated water. They are considered so because of their ability to bioremoval this insecticide, and cheap easy culturing in the laboratory. Hence, work in this regard should continue to characterize the genetic and enzymatic components responsible for the utilization of malathion and other organophosphorus pesticides of this strain in order to evaluate its efficiency for the bioremediation of these environmental pollutants.

REFERENCES


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http://dx.doi.org/10.1603/ME12068


Fig. 1: Effect of different concentrations of malathion insecticide on growth of C. vulgaris in terms of cell count.

Fig. 2: Effect of different concentrations of malathion insecticide on growth of S. quadricuda in terms of cell count.

Fig. 3: Regression lines for cell count of the two tested strains.
Fig. 4: Efficiency of different algal strains to biodegrade malathion insecticide. Error bars represent the standard errors of the means.

**Table I**

GROWTH OF ALGAL STRAINS, EXPRESSED AS CELL COUNT (NO. OF CELLS X10^4/ML), UNDER UNLIMITATION AND P-LIMITATION CONDITIONS WITH THE ADDITION OF MALATHION

<table>
<thead>
<tr>
<th>Algal strains</th>
<th>C. vulgaris</th>
<th>S. quadricuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlimitation</td>
<td>687±27.1</td>
<td>119±15.7</td>
</tr>
<tr>
<td>P-limitation</td>
<td>37***↓±2.4</td>
<td>20**↓±1.2</td>
</tr>
</tbody>
</table>

P-limitation with malathion (ppm)

<table>
<thead>
<tr>
<th>P-limitation with malathion (ppm)</th>
<th>C. vulgaris</th>
<th>S. quadricuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>232***↑±14.7</td>
<td>88****b±9.7</td>
</tr>
<tr>
<td>0.2</td>
<td>142****↑±9.3</td>
<td>62****b±6.1</td>
</tr>
<tr>
<td>2</td>
<td>143****↑±10.7</td>
<td>46****b±5.3</td>
</tr>
<tr>
<td>20</td>
<td>108****↑±9.8</td>
<td>36****b±3.5</td>
</tr>
<tr>
<td>50</td>
<td>278****↑±11.2</td>
<td>24a+b±2.3</td>
</tr>
<tr>
<td>100</td>
<td>491****↑±28.8</td>
<td>8****b±0.6</td>
</tr>
</tbody>
</table>

Values are means of three replicates ± standard errors.

*Significant decrease compared with unlimitation condition .

*Significant increase compared with P-limitation condition

**Table II**

TOTAL PHOSPHORUS CONTENT (MG/G DRY WEIGHT) OF ALGAL BIOMASS, UNDER UNLIMITATION AND P-LIMITATION CONDITIONS WITH THE ADDITION OF MALATHION

<table>
<thead>
<tr>
<th>Algal strains</th>
<th>C. vulgaris</th>
<th>S. quadricuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlimitation</td>
<td>3.2±0.1</td>
<td>6.04±0.06</td>
</tr>
<tr>
<td>P-limitation</td>
<td>1.3***↓±0.05</td>
<td>1.7***↓±0.08</td>
</tr>
</tbody>
</table>

P-limitation with malathion (ppm)

<table>
<thead>
<tr>
<th>P-limitation with malathion (ppm)</th>
<th>C. vulgaris</th>
<th>S. quadricuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>11.8****b±0.2</td>
<td>4.8****b±0.05</td>
</tr>
<tr>
<td>0.2</td>
<td>9.2****b±0.06</td>
<td>3.5****b±0.1</td>
</tr>
<tr>
<td>2</td>
<td>8.9****b±0.7</td>
<td>3.1****b±0.06</td>
</tr>
<tr>
<td>20</td>
<td>7.8****b±0.07</td>
<td>2.9****b±0.07</td>
</tr>
<tr>
<td>50</td>
<td>5.4****b±0.8</td>
<td>2.6****b±0.1</td>
</tr>
<tr>
<td>100</td>
<td>4.9****b±0.1</td>
<td>2.6****b±0.09</td>
</tr>
</tbody>
</table>

Values are means of three replicates ± standard errors.

*Significant decrease compared with unlimitation condition .

*Significant increase compared with P-limitation condition