COMMON SEAWEED DISEASES AND TREATMENT METHODS IN KHANH HOA PROVINCE, VIETNAM

Nguyen Thi Tuyet¹, Pham Truong Giang¹, Thai Ngoc Chien¹, Truong Van Toan¹

ABSTRACT

Khanh Hoa is one of the largest seaweed producers in Vietnam. However, seaweed disease is increasing serious problems that caused decreasing production, especially Ice-Ice, epiphytes diseases on Kappaphycus alvarezii, and parasitic disease on Sea grapes (Caulepa lentillifera). In this study, these diseases were treated with different chemicals such as iodine, formalin, My Hao washing liquid, KMnO₄, erythromycine, and ciprofloxacin with different doses. The result shows that My Hao washing liquid at 15- 20.10³ ppm and 10-15 minutes had the highest efficiency for treating parasitic disease on C. lentillifera. A combination of iodine (1.5 ppm) and ciprofloxacin (2 ppm) had the highest efficiency for treating Ice-Ice disease on K. alvarezii. My Hao washing liquid at 2.10³ ppm for 15 minutes had the highest effectiveness for treating seaweed epiphytic disease on K. alvarezii.

Keywords: Seaweed disease, Kappaphycus alvarezii, Caulepa lentillifera, Ice-Ice disease, epiphytes, parasitic disease

I. INTRODUCTION

The marine algae are one of the most important marine resources in the world and widely used as human food, animal feed and raw material for many industries (Mikhail et al., 2013) especially in the Asia-Pacific region seaweed cultivation is a major industry in many countries particularly Japan, Korea, and China (Morita et al., 2003). In contrary, the disease now is big problem that especially of Ice-Ice disease has not only affected the seaweed farmers but also the nation as a whole. Latest statistics show that because of ice-ice disease, farming in Zamboanga City suffered the drastic decline in aquaculture production with negative growth of 42.8 percent as a result of the ailing seaweed sector. Ice-Ice disease and epiphyte infestation dropped in annual seaweed yield by 22 percent (BAR, 2003). There are two kinds of diseases in seaweed: infectious and non-infectious type. The former involves a transmissible infectious agent (bacteria, fungi, virus, etc.) while the latter is induced by physiogenic factors such as extremes of temperature, salinity, light intensity or pollution. Other than those in economic seaweeds, most of what is known to be diseases in seaweed are the types that are generally less threatening to the natural seaweed population.

There are many causative effects on seaweed such as environmental stress, e.g., high temperature, low irradiance and low salinity, induced the disease in cultivated seaweeds (Largo et al., 1995a). For example Ice-Ice disease which is generally caused by unfavorable environmental conditions in the planting site. Addressing any of these factors is considered a management intervention strategy. Aside from these abiotic factors, the marine bacteria Vibrio and Cytophaga-
Flavobacterium groups have also showed pathogenic activity and produced the disease in *Kappaphycus alvarezii* Doty (Largo et al., 1995b). Other bacteria, e.g., *Pseudomonas*, *Xanthomonas* and *Achromobacter*, were also isolated from infected seaweeds (Uyenco et al., 1981). Several marine fungi have also been reported to cause diseases in green, brown and red algae (Raghukumar 1986, 1987, Hyde et al., 1998, Ramaiah., 2006). The parasitization such colonization by *M. membranacea* is reduced the rate of photosynthesis by reducing pigment concentration (Hepburn et al. 2006), decreasing ammonium uptake rates in algal tissues (Hurd et al., 2000), and reducing spore release from fertile blades (Saier and Chapman, 2004). In addition, epizootic colonization of the seaweed may lead to changes in biochemical composition at the protein level of the host.

In Vietnam, the seaweed study substantially started in 1923 by the Institute of Oceanography, Nha Trang. There are approximately 1,000 seaweed species (Pham, 1969; Huynh and Nguyen, 1998) of which about 638 species identified including 269 species of Rhodophyta, 143 species of Phaeophyta, 151 species of Chlorophyta and 76 species of Cyanophyta (Nguyen and Huynh, 1998).

*K. alvarezii* is a high-value species and popularly cultivated in the Middle and South of Vietnam (Tsutsui et al., 2005). It has been cultivated in large scale and become an important economic seaweed since 1995 after which was imported from Philippines in 1993. In main season (October to March) temperature was lower 30°C, the growth rate reached 6-8% per day, and harvest crop during 40-50 days. Opposite trend tempreature higher from April to September (especially from May to June temperature reached 32-34°C) Ice-Ice disease that characterized by whitening and softening of the algal thallus (Doty and Alvarez, 1975) occurred on large scale which has decreased growth rate of 3-4%/days. This disease leads to a significant decrease in seaweed production and decrease in carrageenan yield compared to the healthy crop ranging from 25 to 40% (Trono, 1993). Besides the epiphytic disease on *K. alvarezii* is also quite dangerous disease after Ice - Ice disease.

*Caulerpa lentillifera* is also an introduced seaweed species from Japan in 2007. This species is high value and consumed by domestic market and foreign export to Japan. However, the disease leads to reduce its quality and production.

In Vietnam, little study about the causative agents disease and method treatment is reported. The aim of this study is to introduce treatment method which can be easily applied to recure some common seaweed diseases on *Kappaphycus alvarezii* and *Caulerpa lentillifera* in Khanh Hoa Province. In the field, seaweed farmers have been used My Hao washing liquid to treat the epiphytes on *Kappaphycus alvarezii*. My Hao liquid is an effective compound that eliminates surface stains because it consists of LAS (C₆H₁₂SO₃: Sodium lauryl benzene sulfonate is a surfactant, acidic detergent) and SLES (Sodium Laureth Sulfate: a foaming agent combining with Alcohol Ethoxylates that eliminates clingings). However, the farmers do not know the dose to use and its mechanism. This study will provide the pricipals and knowlegde to the farmers.

II. MATERIAL AND METHOD

1. Experimental design

Disease seaweed samples were collected from farms that located on Cam Ranh, Ninh Hoa Districts, Khanh Hoa Province and gently rinse by seawater to remove wastes, dusts etc, but avoid from its damages before starting experiment.
These diseases were treated with common chemicals such as iodine, formalin, My Hao washing liquid, KMnO$_4$, with different doses. Each experiment was carried out in wet lab of RIA3 with 3 replications (V= 2.5 liters/box): 3 controlled boxes; 3 treatment boxes. The boxes were placed under fluorescent lighting system (light intensity of 3000 – 4.000 luxes in the C. lentillifera experiment area, 6.000 – 7.000 luxes in the K. alvarezii experiment area) the seaweeds were supplied oxygen for its photosynthesis. Everyday nutrient was supplied for normal growth and water temperature was controlled by air conditioner at 25°C.

1.1. Experiments for treatment method of parasitic disease on Caulepa lentillifera (specific name: dragonfly larvae)

This experiment was conducted with different kinds of chemicals with different doses and treatment time (Table 1). The experiment was taken 10 days.

Table 1. Experiments for each treatment methods of parasitic disease
(Each experiment has 3 replications for each dose)

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Dose (ppm)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine</td>
<td>0 (Control)</td>
<td>4.5</td>
</tr>
<tr>
<td>Formalin</td>
<td>0 (Control)</td>
<td>0.5</td>
</tr>
<tr>
<td>My Hao washing liquid</td>
<td>0 (Control)</td>
<td>10</td>
</tr>
<tr>
<td>KMnO$_4$</td>
<td>0 (Control)</td>
<td>2</td>
</tr>
</tbody>
</table>

1.2. Experiments for treatment method of Ice-Ice disease on K. alvarezii

Six experiments were set up with 6 treatments: (I) Iodine (immersed for 15 minutes, then placed in seawater for 3 days with light and oxygen supplement as stated above, the procedure was repeated for every three days) (E) Erythronycine (immersed during 3 days); (C) Cipprofloxacin (immersed during 3 days); (IE1) a combination of Iodine (1.5 ppm within 5 minutes) and rinsed by seawater for 1 minute and then immersed in erythromycin (5, 10 and 15 ppm) for 1 day; (IE3) a combination of Iodine (1.5 ppm immersed in 15 minutes) and immersed in erythromycin (5, 10 and 15 ppm) for 3 days; (IC) A combination of Iodine (1.5 ppm immersed in 15 minutes) and then ciprofloxacin (2, 4 and 6 ppm) for 3 days. (Table 2). All experimental procedures were repeated for every three days and lasted for 21 days.

Table 2. Experiments for each treatment method of Ice-Ice disease
(Each experiment has 3 replications for each dose)

<table>
<thead>
<tr>
<th>Experiments</th>
<th>I</th>
<th>E</th>
<th>C</th>
<th>IE1</th>
<th>IE3</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>15</td>
<td>6</td>
<td>15</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
1.3. Experiments for treatment method of epiphytic disease on K. alvarezii

This experiment was conducted for different doses of My Hao washing liquid: 0 (Control), 1. $10^3$, 2. $10^3$, 3. $10^3$ ppm (for 15 minutes, with time step is 3 days). Each treatment was conducted for 15 days.

2. Data analysis

Daily growth rate, DGR as a percentage, was calculated using the formula:

$$DGR (%) = \left( \frac{W_t}{W_0} \right)^{1/t} - 1 \times 100$$

where $W_o$ and $W_t$ were initial and final biomass at day $t$, respectively (Lignell et al., 1987).

Recovery ability is number of whitening and softening of the seaweed thallus changed on K. alvarezii and the number of parasitic chambers releasing on C. lentillifera

The data were analyzed using Microsoft Excel 2007

III. RESULTS AND DISSCUCIONS

1. Experiments for treatment method of parasitic disease on Caulepa lentillifera

Parasitic chambers consist of many dragonfly eggs which attach to seaweed thallus, during the early stage of larvae, they live inside the chambers and respire by opening chamber mouth. They close the mouth immediately in case of outside impacts.

During swimming stage, they develop the wings and swim freely in water, but get into the chambers when there are impacts from outside. After late swimming stage, they fly out of the sea water.

In the first experiment the parasite on C. lentillifera was treated with 4 chemicals. The result shows that My Hao washing liquid at 20. $10^3$ and 15. $10^3$ ppm immersing within 10-15 minutes had the highest efficiency group for parasitic disease on C. lentillifera with the chambers released reached 77% and 66% respectively, while only 44% chambers releasing by using iodine 4.5 ppm. The lower efficiency is flowing: KMnO$_4$ (2 ppm) and My Hao washing liquid (10. $10^3$ ppm) were obtained 33% chambers releasing. And then KMnO$_4$ (5 ppm) and formalin (both of dose 0.5 and 1 ppm) were 22% chambers releasing.

### Table 3. Experiment results for parasitic disease treatment on C. lentillifera

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Dose (ppm)</th>
<th>Seaweed condition (N: Normal; W: weak)</th>
<th>Chamber releasing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine</td>
<td>4.5</td>
<td>N</td>
<td>44 ± 3</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>N</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>W</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>KMnO$_4$</td>
<td>2</td>
<td>W</td>
<td>33 ± 2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>W</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>W</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>My Hao washing liquid</td>
<td>10. $10^3$</td>
<td>N</td>
<td>33 ± 3</td>
</tr>
<tr>
<td></td>
<td>15. $10^3$</td>
<td>N</td>
<td>66 ± 3</td>
</tr>
<tr>
<td></td>
<td>20. $10^3$</td>
<td>N</td>
<td>77 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>W</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Formalin</td>
<td>0.5</td>
<td>W</td>
<td>22 ± 2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>W</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>W</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>
Figure 1. Parasitic chambers on *C. lentillifera* (Photo by Tuyet, 2014)

2. Experiments for treatment method of Ice-Ice on *K. alvarezii*

One of the factors associated with high Ice-Ice occurrence in the field is the high incidence of epiphytes. Largo (1999), found that the combined effect of stress and biotic agents, such as opportunistic bacteria *Vibrio* sp. and *Cytophaga* sp. (Largo et al., 1999) are primary factors of the Ice-Ice disease. These findings suggest that the whitening phenomenon is caused by both abiotic and biotic factors acting in combination. When the seaweed is under stress, it emits a moist organic substance that attracts bacteria in the water and induces the “whitening” and hardening of the seaweed branches. Uninfected parts remain healthy while infected ones undergo depigmentation and eventually lead to plant breakage by any force of nature.

Table 4. Experiment results for Ice-Ice treatment

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (ppm)</th>
<th>DGR (%/day)</th>
<th>Recovery ability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>1.3 ± 1.1</td>
<td>13 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.1 ± 0.1</td>
<td>22 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.5 ± 1.1</td>
<td>15 ± 2.2</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.2 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>1.4 ± 0.6</td>
<td>15 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.2 ± 0.2</td>
<td>24 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.3 ± 0.5</td>
<td>23 ± 2.1</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>1.5 ± 0.6</td>
<td>26 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.4 ± 0.04</td>
<td>25 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.6 ± 1.1</td>
<td>28 ± 2.7</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0 ± 0.9</td>
<td>0</td>
</tr>
<tr>
<td>IE1</td>
<td>5</td>
<td>1.2 ± 0.7</td>
<td>21 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.3 ± 1.1</td>
<td>22 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.0 ± 0.9</td>
<td>20 ± 2.2</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.2 ± 0.09</td>
<td>0</td>
</tr>
<tr>
<td>IE2</td>
<td>5</td>
<td>1.2 ± 0.1</td>
<td>22 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.1 ± 0.04</td>
<td>21 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.4 ± 0.06</td>
<td>21 ± 0.7</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>IC</td>
<td>2</td>
<td>2.1 ± 0.01</td>
<td>40 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.0 ± 0.2</td>
<td>20 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.3 ± 0.4</td>
<td>23 ± 1.6</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>1.1 ± 0.06</td>
<td>0</td>
</tr>
</tbody>
</table>
The result shows that a combination of iodine (1.5 ppm) and Ciprofloxacin (2 ppm) expressed the highest effectiveness: growth rate was 2.1% per day and recovery ability was the highest (40%). This growth rate was higher in comparison with study by Matern (2003) that growth rate was reduced to around 1.1% per day with visible epiphyte infestation.

3. Experiments for treatment method of epiphytic disease on \textit{K. alvarezii}

Epiphytes refer to organisms, small or large, that colonize the surfaces of seaweeds. The \textit{Polysiphonia} epiphytes and diatoms are the causative agents. This result also coincides with the findings of Largo (2002). These algae create small, slightly elevated pores on the surface. These pores give “goosebumps” appearance on the thalli surface of seaweeds.

![Figure 3. Epiphytes disease caused by \textit{Polysiphonia} sp on \textit{K. alvarezii} (Thuy, 2014)](image)

Epiphytic effects are often negative, as epiphytes can decrease the host’s growth and reproduction, by limiting carbon uptake and reducing light penetration, and the host’s survival, by increasing drag (Kraberg and Norton, 2007). Some epiphytes are actually parasitic on their hosts, reducing the host’s fitness by translocating nutrients from it (Penot et al., 1993).

Table 5. Experiment results for experiment on epiphytic disease treatment of \textit{K. alvarezii}.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Dose (ppm)</th>
<th>Recovery ability (%)</th>
<th>DGR (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0 ± 0.1</td>
<td>0.8 ± 0.01</td>
</tr>
<tr>
<td>My Hao washing</td>
<td>1.10³</td>
<td>20 ± 1.1</td>
<td>1.3 ± 3.4</td>
</tr>
<tr>
<td>liquid</td>
<td>2.10³</td>
<td>70 ± 5.0</td>
<td>2.5 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>3.10³</td>
<td>40 ± 3.1</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

(Values are mean ± SD)

Daily growth rates of \textit{K. alvarezii} in three different doses (1.10³, 2.10³, 3.10³ ppm) were 1.3, 2.5 and 1.1% respectively (Table 5); the best dose was 2.10³ ppm as 70% epiphytic releasing and daily growth rate was 2.5% per day.

IV. CONCLUSION

My Hao washing liquid at 15- 20.10³ ppm and 10-15 minutes had the highest effective for treating parasitic disease on \textit{C. lentillifera}.

The Ice-Ice disease on \textit{K. alvarezii} can be controlled by using a combination of iodine (1.5 ppm for 15 minutes) and Ciprofloxacin (2 ppm)

My Hao washing liquid at 2.10³ ppm for 15 minutes had the highest effiveness for treating seaweed epiphytic disease on \textit{K. alvarezii}.

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