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Incidence of Ice-Ice Disease Associated with *Kappaphycus alvarezii* in the Seaweed Farms in Zamboanga Peninsula, Mindanao, Philippines

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ABSTRACT

Background: The ice-ice disease is one of the major problems in the cultivation of seaweeds. This problem seems to have worsened in recent years without any significant solution and central production sites of seaweed like in the Zamboanga Peninsula are affected.

Methods: This present study investigated the current status of incidence and degree of infection of ice-ice disease associated with *Kappaphycus alvarezii* brown and green during July to September 2018 in two varying seaweed farms in the Zamboanga Peninsula, Mindanao, Philippines. Ecological parameters known to affect the occurrence of ice-ice disease were obtained in each seaweed farm such as temperature, salinity, and pH.

Results: In this study, incidence (%) and degree of infection (%) of ice-ice disease found to be highest in the seaweed farm in Mampang, Zamboanga City compared in Lapuyan, Zamboanga del Sur. Results of One-Way Analysis of Variance (ANOVA) on the incidence (%) and degree of infection (%) of ice-ice disease revealed significant differences ($p= 5.141E-09$ and $p=6.094E-06$ at $\alpha= 0.05$, respectively) between the two selected farms.

Conclusion: Occurrence of this disease could be attributed to the unfavorable environmental conditions. This incidence and infection of ice-ice disease are indicative of the varying resistance of *K. alvarezii*, thus this study may be significant with an implication to management strategies of the current status of the seaweed farms in Zamboanga Peninsula, Mindanao, Philippines.

Key-words: Ice-ice disease, *K. alvarezii*, Management strategies, Seaweed farms, Unfavorable conditions

INTRODUCTION

Philippines is one of the top producers of seaweeds in the world that can produce about 1.5 million metric tons valued at US$ 136.8 [1] where Zamboanga Peninsula is considered as the third major seaweed-producing region and contributing about 13 percent to the total national production and 20 percent of Mindanao produce [2].

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Seaweeds in the Zamboanga Peninsula are one of the flagship and champion products identified by the Regional Development Council. However, Philippine Statistics Authority (PSA) data show seaweeds production in the Zamboanga Peninsula posted decreases from 258,131 metric tons in 2010 to 206,161 metric tons in 2014 [3].

Management strategy in seaweed farming is an important opportunity that can be used as a guide for the development of the seaweed industry in order to achieve dramatic growth of seaweed production. In Mindanao, specifically in Zamboanga City, seaweed farming suffered the drastic decline in aquaculture production with negative growth of 42.8 percent because of some diseases associated with seaweeds [4].

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Seaweed disease is one of the several problems faced by the seaweed farmers [5]. It affects the normal structure, function and alters the growth rate, appearance of the seaweeds and possibly could result in poor product quality [6].

Kappaphycus alvarezii (Rhodophyta: Solieraceae) is a marine macroalgae cultivated in several Asia countries including the Philippines since the 1960s [7]. It is also known as the *cottonii* type which is a source of the semi-refined or Philippine natural grade (PNG) carrageenan [8] and recommended by the National Academy for Agriculture for commercial production of carrageenan, which has several pharmaceutical and industrial applications [9]. However, this seaweed species face a threat of a type of disease commonly known as the ice-ice disease [10].

The ice-ice disease is one of the major problems in the cultivation of seaweeds. This disease is non-infectious and could be triggered by unfavorable environmental conditions such as extreme temperature, pH and salinity, and opportunistic bacterial pathogens like Vibrio species [11]. Accordingly, the ice-ice disease leads to a significant decrease in seaweed production [12] and can lead to the decrease of carrageen a yield, viscosity and gel strength of infected thalli [13].

The problem of ice-ice disease associated with seaweeds seems to have worsened in recent years, without any significant solution, while on the other hand people in coastal areas who rely on seaweed farming, especially in the central production sites like in the Zamboanga Peninsula are affected [14].

Specifically, seaweed farmers in Zamboanga City and Zamboanga del Sur face large threat in the rise of ice-ice diseases in their seaweed farms, thus, this study was conceptualized to assess the current status of *K. alvarezii* in selected seaweed farms in the Zamboanga Peninsula, Philippines; Barangay Mampang, Zamboanga City and Barangay Danganan, Lapuyan, Zamboanga del Sur in terms of the incidence and degree of infection of ice-ice diseases associated with *K. alvarezii*.

MATERIALS AND METHODS

This study was done during July to September 2018 in selected two farms in the Zamboanga Peninsula, Mindanao, Philippines; Mampang Seaweeds Planters Association, Zamboanga City and Aquasilviculture Technology Demonstration Project of Barangay Poblacion Fisherfolk Association, Lapuyan, Zamboanga del Sur. Both seaweed farms are considered as one of the major seaweed-producing farms in the region.

Study sites- Seaweed farm in Mampang, Zamboanga City is associated with mangrove area (6.9139°N, 122.1462°E) using the fixed off-bottom method. The water depth in this farm ranged from 0.3-0.5 meters at low tide up to 2 meters at high tide. Therefore, this farm is exposed to light intensity during low tide. In Lapuyan, Zamboanga del Sur (7°38'11"N, 123°11'45"E), floating monolines method was used and located away from the shore. Fig. 1 shows the map of Zamboanga Peninsula, Mindanao, Philippines and the actual site of the two selected seaweed farms; Mampang, Zamboanga City (A) and Lapuyan, Zamboanga del Sur (B).

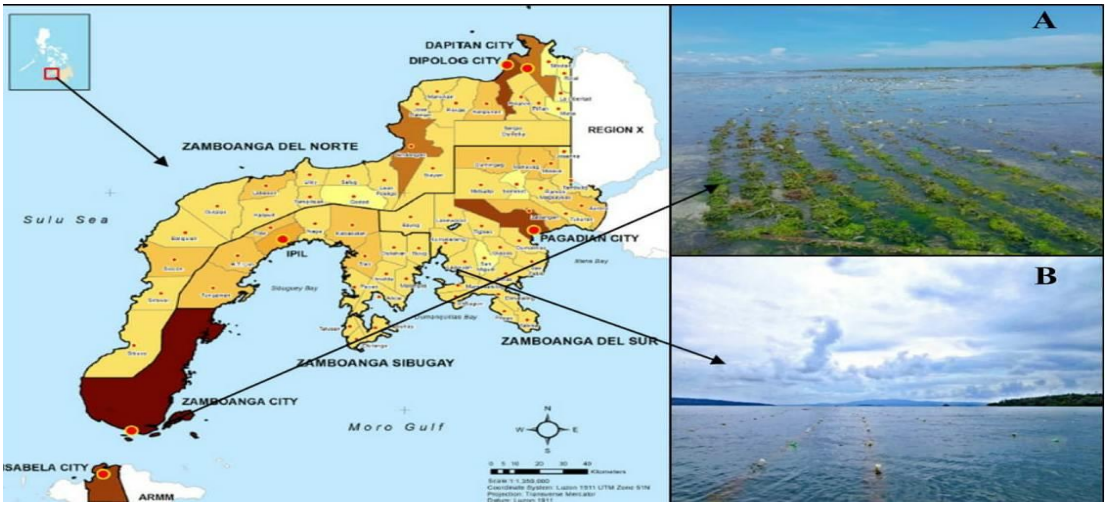

Fig. 1: Map shows the geographical location of Zamboanga Peninsula, Mindanao, Philippines and the actual site of Mampang, Zamboanga City (A) Lapuyan, Zamboanga del Sur (B)

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Sampling Design- In this study, the sampling design was based on the study of Tisera and Naguit [15]. In each of the two selected two farms in Zamboanga Peninsula; Barangay Mampang, Zamboanga City and Lapuyan, Zamboanga del Sur, cultivating ropes where the bunches of seaweeds are being tied measure for about 500 meters and approximately 500 seaweeds were planted. In each site, ten cultivating ropes were randomly chosen, five ropes for *K. alvarezii* brown and five ropes for *K. alvarezii* green. Additionally, ten bunches were sampled for *K alvarezii* brown per cultivating rope and ten bunches of *K. alvarezii* green were also sampled. Therefore, there are 100 bunches from each site monitored, fifty bunches for *K. alvarezii* brown and fifty bunches for *K. alvarezii* green.

Assessment of infected seaweeds- For the computation of the % incidence and % degree of infection of ice-ice disease, the formula of Tisera and Naguit [15] was developed.

To compute for the incidence, the number of the infected bunches will be divided by the total number of bunches observed for every farm.

Incidence (%)= No. of infected bunches/ Total no. of bunches x 100

To compute the degree of infection, the number of infected branches was divided by total branches in the bunch-

Degree of Infection (%)= No. of infected branches/ Total no. of branches x 100

Determination of Physico-chemical parameters- Water temperature was determined using a digital thermometer by placing the end of the thermometer into the sea water for 2–3 minutes. The pH level of water was determined using pH meter, pH meter was dipped into the seawater and the measuring button was pressed to begin reading for 2–3 minutes. The water salinity was obtained using a Refractometer by getting 1ml of water sample then placed it in the testing part of Refractometer to begin reading for 2–3 minutes.

Statistical Analysis- The data were summarized using mean and standard error. One-Way Analysis of Variance (ANOVA) was utilized, to test if there is a significant difference in the mean incidence and the degree of infection of ice-ice disease between the two selected farms using Paleontological Statistics (PAST) Software 3.7 version.

RESULTS

Seaweed farm conditions- In this study, physicochemical parameters vary between the two selected farms in the Zamboanga Peninsula, Mindanao, Philippines in terms of water temperature, salinity and water pH level (Table 1). The water temperature and pH levels of water during the study were higher in Mampang, Zamboanga City compared in Lapuyan, Zamboanga del Sur. Only salinity was higher in Lapuyan, Zamboanga del Sur.

Table 1: Mean physico-chemical parameters of the two selected farms in Zamboanga Peninsula, Mindanao, Philippines

Study Sites	Physico-chemical Parameters		
	Temperature (°C)	Salinity (ppt)	pH
Mampang, Zamboanga City	34	24	7.9
Lapuyan, Zamboanga del Sur	28	33	7.7

The ice-ice disease was identified based on standard symptoms of whitening of the branches and initiated with the color changes of thalli which appeared transparent [11] (Fig. 2).

Incidence of Ice-Ice Disease- The mean incidence of ice-ice disease was observed to vary between the two selected farms in terms of strains and farm. In terms of the farm, the highest mean incidence was observed in Mampang, Zamboanga City in *K. alvarezii* brown (39.78±2.45) and *K. alvarezii* green (33.40±2.62) compared in Lapuyan, Zamboanga Del Sur (25.11±1.70) in *K. alvarezii* brown and (21.85±1.53) in *K. alvarezii* green (Table 2).

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Fig. 2: Actual image of the representative of *K. alvarezii* green (A) and *K. alvarezii* brown (B), showing the thalli conditions as observed in the seaweed farms in Mampang and Lapuyan, Zamboanga Peninsula, Mindanao, Philippines with wide-scale whitening of ice-ice

Table 2: Mean % incidence of ice-ice disease in *K. alvarezii* green and *K. alvarezii* brown between the two selected farms in Zamboanga Peninsula, Mindanao, Philippines

Study Sites	***K. alvarezii* strains**	
	***K. alvarezii* green (%±SE)**	***K. alvarezii* brown (%±SE)**
Mampang, Zamboanga City	33.40±2.62	39.78±2.45
Lapuyan, Zamboanga del Sur	21.85±1.53	25.11±1.70

Degree of Infection- The degree of infection is the variation in infectious rate which represents whether the disease level is increased or decreased [11]. The mean degree of infection of ice-ice disease observed in *K. alvarezii* varied in terms of strain and farm. In terms of farm, the highest mean degree of infection was observed in Mampang, Zamboanga City both in *K. alvarezii* brown (29.36±1.16) and *K. alvarezii* green (28.72±3.12) compared in Lapuyan, Zamboanga del Sur in *K. alvarezii* brown (21.2±0.50) and *K. alvarezii* green (19.4±1.12) (Table 3).

Statistical Analysis- One-Way Analysis of Variance (ANOVA) result shows a significant difference (p=5.141, E-09 at α= 0.05) in terms of the incidence of ice-ice disease between the seaweed farms in Mampang, Zamboanga City and Lapuyan, Zamboanga del Sur (Table 4).

Table 3: Mean % degree of infection of ice-ice disease in *K. alvarezii* green and *K. alvarezii* brown between the two selected farms in Zamboanga Peninsula, Mindanao, Philippines

Study Sites	***K. alvarezii* strains**	
	***K. alvarezii* green (%±SE)**	***K. alvarezii* brown (%±SE)**
Mampang, Zamboanga City	28.72±3.12	29.36±1.16
Lapuyan, Zamboanga del Sur	19.4±1.12	21.2±0.50

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Table 4: One-Way Analysis of Variance (ANOVA) results of the % incidence of ice-ice disease between the two selected seaweed farms in the Zamboanga Peninsula, Mindanao, Philippines

	Sum of square	DF	Mean square	F	p value
Between groups	8600.29	1	8600.29	37.36	5.141E-09*
Within groups	45585.4	198	130.229		
Total	54185.7	199			

***Significant at α=0.05**

As shown in Table 5, degree of infection of ice-ice disease between the seaweed farms in Mampang, Zamboanga City and Lapuyan, Zamboanga del Sur show a significant difference (p=6.094E-06 at α= 0.05) based on One-Way Analysis of Variance (ANOVA) results.

Table 5: One-Way Analysis of Variance (ANOVA) results of the % degree of infection of ice-ice disease between the two selected seaweed farms in the Zamboanga Peninsula, Mindanao, Philippines

	Sum of square	DF	Mean square	F	p value
Between groups	381.93	1	381.93	39.73	6.094E-06*
Within groups	173.04	18	9.61		
Total	554.98	19			

***Significant at α=0.05**

The mean % incidence and degree of infection of ice-ice disease were substantially different between the two selected farms in the Zamboanga Peninsula, Philippines. Incidence and degree of infection of ice-ice disease in Lapuyan, Zamboanga del Sur were considerably lower than in Mampang, Zamboanga City, indicating varying resistance to the ice-ice disease. It was in accordance with Tisera and Naguit [15], who stated that the seaweed farm with the least mean incidence and degree of infection were the most resistant to ice-ice disease.

DISCUSSION

The ice-ice disease is caused by unfavourable environmental factors in the seaweed farms. Unfavourable factors refer either too high or too low temperature, salinity or light intensity [11]. In Mampang, Zamboanga City, the mean incidence and degree of infection found to be highest in all observations compared in Lapuyan, Zamboanga del Sur. Seaweed farm in Mampang is associated with mangrove-estuarine where freshwater meets seawater and during low tide, the water depth ranges for about 0.5 meters where it directly exposed to the atmospheric air and sunlight. The combined effect of air and light exposure elevated the temperature to thalli and stressed the seaweeds and eventually leads into ice-ice disease [14].

Temperature plays a vital role in the growth of *K. alvarezii*, it is considered as one of the main reasons that can contribute to the occurrence of ice-ice disease in *K. alvarezii* [11]. The higher temperature was reflected in the seaweed farm in Mampang (34°C), it may be one important factor affecting the higher incidence and degree of infection of ice-ice disease on this farm. This finding was supported by Ask and Azanza [16], who stated that when the temperature reaches up to 33–35°C, this triggers the occurrence of ice-ice disease, which could eventually lead to complete damage of branches of seaweeds. The warmer temperature was observed in the seaweed farm of Lapuyan (28°C), this could be the reason why incidence and degree of infection of ice-ice disease in this farm was lower compared in Mampang.

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Brisk growth and high biomass production of *Kappaphycus* occur during months characterized by warmer temperaures (25–30°C) as quantified by Trono [17].

Salinity is another important factor that determines the growth of seaweed, the rise or decline in the level of salinity could influence the turgor pressure that will induce seaweed to do an osmotic adjustment [16]. Lower salinity was observed in Mampang's seaweed farm (24 ppt) because of its characteristic estuarine condition, which can be subjected to pronounce salinity fluctuations because of rainfall and inflow from rivers. If the cultivation ground is close to the fresh water sources like rivers and creeks, it reduces the salinity of the seawater below normal and a stressful factor to the seaweed [18]. However, salinity level of the seaweed farm in Lapuyan (33 ppt) was within the range of the normal water salinity requirement for the growth of *K. alvarezii* (33-35 ppt) [11], this could be one of the reasons why lower mean incidence and degree of infection of ice-ice disease was observed in this farm compared in Mampang.

The fixed off-bottom method was used in Mampang seaweed farm. Cultivating ropes in this method has shown to be closed with each other making them more susceptible to spreading ice-ice disease, whereas in Lapuyan, floating monolines method was used. Compare with an off-bottom method, cultivating ropes in floating monolines method were established farther from each other which lessens the prevalence of the spreading of ice-ice disease. These findings are supported by the study of Trono [12] and Uyenco *et al.* [19], who stated that crowding of plants due to high planting density may produce an artificial shading effect that could eventually lead to ice-ice disease because of the presence of epiphytes in the immediate vicinity of each thallus.

According to Largo *et al.* [18] another factor that contributes to the development of ice-ice disease is the combined effects of stress and biotic agents such as opportunistic pathogenic bacteria from the complex *Cytophaga-Flavobacterium* and the *Vibrio-Aeromonas* are the suspected causative agents in the development of the symptoms. The infection of the seaweed by pathogens was found by Largo *et al.* [20] to depend initially on the bacteria's ability to establish them on the seaweed surface. The rough thallus surface of *K. alvarezii* enables the microorganisms to easily attach to it and later will penetrate to the cortex and medullary layers of thalli, making the thalli to become weaker, when the seaweed is under stress, it emits a moist organic substance that attracts bacteria in the water and induces whitening and hardening of the branches, which will undergo depigmentation and eventually lead to breakage [15].

The highest mean incidence of ice-ice disease as recorded in this study was lower than what was obtained by Tisera and Naguit [15] in Jose Dalman, Zamboanga Del Norte. The highest mean incidence of ice-ice disease in Tisera and Naguit [15] study was 66.67%±4.55 for *K. alvarezii* brown, while in this study, the highest mean incidence of ice-ice disease was 39.78%±2.45 for *K. alvarezii* brown in Mampang, Zamboanga City. This increase could be related to the changes of environmental conditions in Jose Dalman, Zamboanga Del Norte mainly, increasing amount of pollution due to increasing human activities near the farms and increasing temperatures as an effect of global warming [15]. However, the mean degree of infection of ice-ice disease as recorded in this study was higher than what was obtained in the same site in the study of Tisera and Naguit [15]. In this study, the mean degree of infection of ice-ice disease in Mampang, Zamboanga City was 29.36%±1.16 compared in Jose Dalman, Zamboanga Del Norte which was only 13.61%±1.25.

CONCLUSIONS

Mean incidence and degree of infection of ice-ice disease found to be highest in all observations in the seaweed farm in Mampang, Zamboanga City compared in Lapuyan, Zamboanga del Sur. This observation could be related to the unfavourable environmental conditions. Thus, high temperature, low salinity, high pH level and low depth of the water are the suspected factors that play a crucial role in the disease.

This incidence and infection of ice-ice disease are indicative of the varying resistance of *K. alvarezii* to the disease, thus this study may be significant with an implication to management strategies in seaweed farming. This implies that if effective management strategies such as finding a good site, proper cultivation method and good monitoring in the seaweed farm are to be developed, the incidence and infection of this disease in a specific seaweed farm can be minimized.

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