Characterization of 'Ice-Ice' Disease in Kappaphycus alvarezii: Its Morphological Changes, Histochemical Components, and Implications for Management Strategies

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ABSTRACT
Kappaphycus alvarezii, a seaweed known for its carrageenan production, is extensively cultivated in the Philippines, providing livelihoods to fishing communities and generating revenue through exports of dried Kappaphycus and semi-refined carrageenan. Unfortunately, there has been a recent decline in seaweed production attributed to outbreaks of 'ice-ice' disease in seaweed farms. To understand the disease better, this study aimed to document the morphological changes and analyze the pigments and bioactive metabolites of 'ice-ice' disease-infected K. alvarezii. The researchers induced the disease in K. alvarezii thalli under various laboratory conditions involving different salinity (25, 30, 35 ppt) and temperature (25˚C, 27˚C, and 25˚C) levels.
The findings revealed that necrosis at the apical tips of K. alvarezii thalli started on the second day of disease induction, particularly in thalli incubated in 25 ppt salinity, regardless of temperature. By day 8, the infection reached degree 5, where 81-100% of the thallus was affected. The infected thalli showed slimy secretions, bleaching, softening, and fragmentation. As the 'ice-ice' disease progressed, the concentration of pigments (chlorophyll a, total chlorophyll, and carotenoid) decreased, and the number of dead cells increased. The concentration of chlorophyll a, total chlorophyll, and carotenoids dipped significantly, while the percentage of dead cells rose to 52% in thalli with 81-100% infection. Bioactive metabolites such as chitin, pectin, and suberin were detected in both uninfected thalli and those with degree 4 (61-80% of thalli infected) infection, but not in thalli with more than 80% infection. Histochemically, 'ice-ice' disease is characterized by decreased chlorophyll a concentration, cell and tissue necrosis, and loss of bioactive metabolites.
To address the issue, the study recommends developing a management protocol for 'ice-ice' disease in K. alvarezii based on the information gathered. Additionally, the researchers suggest conducting further research on enhancing photosynthetic pigments of seedstocks before planting them in the field.

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INTRODUCTION
Seaweeds are abundant marine macroalgae found in coastal waters and estuaries, offering a wide range of applications in industries such as food, cosmetics, pharmaceuticals, biofuel, aquaculture, and wastewater treatment. They are rich sources of vitamins (A, B, B12, C, D, E), minerals (Calcium, Potassium, Phosphorus, Sodium), and other beneficial compounds. In the Philippines, seaweeds are not only consumed as food but also used to produce agar, align, and carrageenan, which have valuable industrial uses in various products.
However, the country has experienced a decline in seaweed production, with only a fraction of the potential farming area utilized. One of the major factors contributing to this decline is the prevalence of 'Ice-ice' disease, a condition characterized by whitening, softening, and fragmentation of infected seaweed, resulting in reduced biomass and carrageenan yield.
In Northern Samar, the municipalities of Biri, Capul, Gamay, and Lapinig have also been affected by this disease. Despite the challenges, seaweed farmers in the region continue their livelihood activities. The disease tends to occur during specific months of the year, typically the dry season.
Given the diverse perspectives and limited knowledge about the 'Ice-ice' disease, it is crucial to conduct scientific research to understand its physical and chemical components better. This study aims to deepen our understanding of the disease and establish measures to address it effectively.

OBJECTIVES OF THE STUDY

The main objective of this study was to observe and document the changes in morphology and histochemical components of K. alvarezii when infected with 'ice-ice' disease. Specifically, the study aimed to achieve the following goals:

1. Monitor and analyze the alterations in color, texture, and branching patterns of 'ice-ice' disease-infected K. alvarezii.
2. Establish connections between the percentage of dead cells, concentration of Chlorophyll, and pH levels with the different degrees of 'ice-ice' disease infection in K. alvarezii.
3. Identify the bioactive metabolites present in K. alvarezii at various stages of 'ice-ice' disease infection.
4. Investigate the combined effects of salinity and temperature on the development of 'ice-ice' disease in K. alvarezii under controlled laboratory conditions.

METHODOLOGY

This study took place at the Seaweed Laboratory of the Fisheries Department, located within the College of Agriculture, Fisheries, and Natural Resources (CAFNR) at the University of Eastern Philippines (UEP) in Catarman, Northern Samar. UEP is geographically situated on the eastern boundary of Catarman, Northern Samar (12° 29' 46" North, 124° 38' 19" East). The university houses the CAFNR-Fisheries Department, which maintains a well-equipped seaweed laboratory for conducting research on seaweed. The laboratory is actively involved in the country's seaweed research and development program and possesses a collection of seaweed samples gathered from various locations in the province. Additionally, a seaweed laboratory dedicated to micro-propagation and genetic improvement through biotechnology protocols, such as protoplast fusion, has recently been established.

The seaweed samples used in this study were collected from seaweed farms in Brgy. Dao, Gamay, Northern Samar. Dao is one of the coastal barangays (villages) within the municipality of Gamay, Northern Samar. The area is characterized by fringing coral reefs, volcanic rock formations, mangrove trees, and the marginal marine waters of Brgy. Dao are sheltered within an inland cove. This cove provides protection against the strong currents of the Pacific Ocean, and its sandy substratum with patches of seagrass growth makes it suitable for seaweed farming. Moreover, the inland water in the cove benefits from regular replenishment through the tidal currents of the pristine waters.

RESULTS AND DISCUSSION

Changes in Morphology

Morphological changes in K. alvarezii during the induction of 'ice-ice' disease were observed and documented. Initially, all K. alvarezii samples appeared healthy with pointed tips and a glossy, dark brown to greenish brown color. However, on the second day of the experiment, necrotic tips, indicated by a loss of coloration, were observed in most treatments, except for the treatment with low temperature and high salinity. In this treatment, symptoms of 'ice-ice' disease only appeared on the third day, specifically at the tips of the branchlets.

By the fourth day, necrosis had spread from the branchlet tips to other parts of the thallus, although the infected branchlets remained attached to the main thallus. On the fifth day, slimy secretions and lesions were noticed in thalli incubated at 25 ppt, regardless of temperature levels. The bleached parts of the thallus became flexible and eventually led to fragmentation of the branchlets. Thalli incubated at 30-35 ppt exhibited bleaching and slimy secretions in regions where 40-60% of the thallus was infected, particularly at higher salinity levels.

Between the sixth and eighth days, a significant portion (81-100%) of K. alvarezii thalli were infected in samples incubated at 25 ppt, resulting in the disintegration of most of the thalli. The highest percentage of infection (65.1%) was observed in thalli incubated at 25 ppt, and the percentage of infection increased with higher temperatures. Generally, thalli infections were lower at higher salinity levels (30-35 ppt).

At 30 ppt, the percentage of thalli infection decreased with increasing temperature, while at 35 ppt, thalli infection increased at higher temperatures. The difference in infection rates between salinity levels was statistically significant, indicating that salinity plays a major role in predisposing K. alvarezii to 'ice-ice' disease infection. Therefore, to minimize infection, it is recommended to culture K. alvarezii at salinity levels of 30-35 ppt.

Overall, no interactive effects were observed between temperature and salinity on thallus infection, but significant differences in means were found between salinity levels. This information highlights the importance of salinity as a critical factor in 'ice-ice' disease infection and suggests that proper management practices should consider maintaining the appropriate salinity levels for K. alvarezii cultivation.

Examining the Relationship between Cell Death, Pigment Concentrations (Chlorophyll and Carotenoids), and pH Levels

Analysis of cell staining using Evan's Blue dye revealed distinct staining patterns, indicating the viability of cells. Live cells remained unstained or lightly stained, while dead cells exhibited a deep blue coloration. The percentage of dead cells demonstrated a positive correlation with the degree of infection or the extent of thallus bleaching. In severely infected thalli where more than 81% of the tissue was affected (degree 5), approximately 52% of cells within the tissues were found to be dead. Notably, as the infection progressed to degree 2, with 21-40% thallus infection or bleaching, the percentage of dead cells notably increased, impeding tissue regeneration and contributing to the progressive disintegration of the thalli.

Evaluation of chlorophyll and carotenoid pigment concentrations revealed an inverse relationship with the degree of infection. As the infection severity increased, the concentration of pigments decreased, resulting in whitish or colorless regions in severely affected thalli. These findings suggest that tissue fragmentation and disintegration in 'ice-ice' infected K. alvarezii are attributed to critically low pigment concentrations (below 50%) necessary for photosynthesis, as well as tissue necrosis observed as whitening, bleaching, and softening in the infected areas. It is important to note that the results obtained in this study differ from those reported by Pang et al. (2011), where higher chlorophyll a concentrations were found in epiphyte-infected K. alvarezii compared to healthy thalli. This discrepancy can be attributed to the different types of infections (epiphytism by another species of photosynthetic red algae vs. 'ice-ice' disease) and the possibility that the pigment concentration in epiphyte-infected thalli may include contributions from the epiphyte itself, not solely from the host tissue (K. alvarezii).

The pH levels observed in both infected and uninfected K. alvarezii thalli ranged from 6.8 to 7.7, indicating a normal range for the growth and survival of K. alvarezii. Interestingly, the pH level did not demonstrate a correlation with the degree of 'ice-ice' disease infection. Previous studies by Munoz et al. (2004) indicated that the optimal pH range for K. alvarezii growth is between 7.0 and 9.0.

Bioactive Metabolites

The histochemical analysis conducted on the cells of K. alvarezii confirmed the presence of bioactive compounds in both uninfected and 'ice-ice' infected thalli. Using Lugol's iodine and thin layer chromatography, chitin and suberin were detected in decreasing concentrations (based on color intensity) in uninfected thalli until degree 4 of infection (61-80% of thallus infected). Pectin was also found in uninfected thalli and those with 80% infection, but it was not detected in tissues with degree 5 infection. On the other hand, lignin and pectin were not detected at all, suggesting that these compounds are not normal components of K. alvarezii tissues.

The decline in concentration or total loss of these metabolites (chitin, which provides structural support, pectin as a binder of cellulose fibers, and suberin as a waterproofing agent) were the primary factors leading to the softening and disintegration of seaweed tissues, particularly in severe degree 5 'ice-ice' disease infection. These bioactive compounds play essential roles in plant defense against pathogens and herbivores, as well as in plant metabolic processes related to structural support, growth regulation, water transport, and reproduction. They also act as barriers to the movement of water and solutes.

Consequently, the morphological manifestations of 'ice-ice' disease, such as whitening (bleaching), softening, and fragmentation of thalli, primarily result from the reduction in photosynthetic pigment concentration, leading to cell death. The loss of bioactive metabolites further weakens the tissues' capacity for regeneration.

CONCLUSION

Seaweeds, which are macroalgae found in coastal waters and estuaries, have various uses including food consumption and raw material production for agar, alginate, and carrageenan. In the Philippines, Kappaphycus alvarezii, a seaweed species that is extensively farmed, plays a significant role as a major source of carrageenan. Cultivating Kappaphycus provides livelihood opportunities for fishing communities, particularly in Central Visayas and the Sulu Archipelago. However, the country experienced a decline in seaweed production in 2003, with one of the major contributing factors being the prevalence of 'ice-ice' disease in Kappaphycus.

To investigate the effects of 'ice-ice' disease, this study aimed to monitor and document the changes in morphology and histochemical components of K. alvarezii infected with the disease. Specifically, the study focused on observing morphological changes in infected K. alvarezii, such as alterations in color, textures, and branching patterns. It also aimed to examine the correlation between the percentage of dead cells, chlorophyll concentration, pH levels, and the degree of 'ice-ice' disease infection. Additionally, the study aimed to identify the bioactive metabolites present at different degrees of infection and determine the interaction effects of salinity and temperature on the development of 'ice-ice' disease in K. alvarezii.

The researchers monitored the morphological changes in the thallus and the extent of 'ice-ice' infection in K. alvarezii under varying salinity and temperature conditions. Histochemical changes in infected K. alvarezii were assessed by staining tissue sections with specific reagents (Lugol's iodine and Toluidine blue) and confirmed using thin layer chromatography to determine the presence or absence of bioactive compounds such as chitin, lignin, pectin, tannin, and suberin. The number of dead cells, pH levels, and concentrations of photosynthetic pigments (chlorophyll a, total chlorophyll, and carotenoids) were analyzed to establish their correlation with the degree of infection.