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RESEARCH PAPER

Prevalence of Ice-Ice Disease in *Kappaphycus* spp. and *Eucheuma denticulatum* Farms in Sibutu, Tawi-Tawi, Philippines

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Abstract

Kappaphycus spp. and *Eucheuma denticulatum* are commercially farmed in the world, notably in tropical countries such as Indonesia, Philippines, and Malaysia. Diseases and pests, particularly ice-ice disease, are the major hurdles in the sustainability of eucheumatoid seaweed culture. In this study, ice-ice disease prevalence in *Kappaphycus* and *Eucheuma* farms in Sibutu, Tawi-Tawi, Philippines, was assessed and compared according to species, farm depth, and time. Results revealed that in deep water farms, ice-ice disease prevalence was significantly lower in *K. striatus* (4.29±0.97%) than in *K. alvarezii* (10.53±2.64%) in July. In shallow water farms, *E. denticulatum* had the highest ice-ice disease prevalence (21.97±1.73%) significantly among the assessed seaweed species during August, and *K. alvarezii* had the lowest ice-ice disease occurrence (5.43±1.98%) significantly during September. In terms of depth water farm and time comparison, ice-ice disease prevalence (7.41±1.50% - 27.04±4.66%) in deep water (exceeding 2.47±0.16 m during low tide) did not differ significantly from that prevalence (11.35±1.69% - 12.91±1.93%) in shallow water farms (0.61±0.29 m during low tide) across time. This study suggests that ice-ice disease is still a prevalent and persistent problem in eucheumatoid seaweed farming.

Introduction

The genus of *Kappaphycus* and *Eucheuma,* collectively called eucheumatoids, are among the most popular cultivated seaweeds in the world, particularly among coastal communities in tropical regions (Tahiluddin & Terzi, 2021a). Eucheumatoids are commercially farmed for the production of carrageenans –polysaccharides that are extensively utilized in the cosmetic, pharmaceutical, and food industries (Ward et al., 2022). The world eucheumatoid seaweed production in 2018 was nearly 11 million tons, that is, about 34% of the total world production of aquatic plants (FAO, 2020). In the Philippines, the most extensively farmed eucheumatoid seaweeds are *Kappaphycus striatus* and *K. alvarezii* due to the greater demand for kappa-carrageenan extracted from these red seaweeds, whereas iota-carrageenan is less in demand (Dumilag et al., 2022). Hence, the cultivation of *E. denticulatum* is not well-known due to its lower price in the market. The Philippines is one of the top eucheumatoid seaweed-producing countries, ranking fourth in 2018 (FAO, 2020). However, the production of seaweeds (eucheumatoids) in the Philippines has declined from 1.50 million tons in 2019 to 1.47 million tons in 2020 (PSA, 2021). There are multiple factors in

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the production of farmed eucheumatoids; one of these is the prevalence of ice-ice disease (Faisan et al., 2021; Tahiluddin & Terzi, 2021a; Ward et al., 2022; Tahiluddin et al., 2022a).

The ice-ice disease was initially reported by Uyenco (1981) in the Philippines. Now, ice-ice disease is considered a significant problem in eucheumatoid seaweed farming globally (Tahiluddin & Terzi, 2021a). Unfavorable environmental conditions, i.e., high or low temperature, salinity, and light intensity, as well as nutrient deficiency, are the primary factors triggering this disease (Largo et al., 1995a; Largo et al., 2002; Luhan et al., 2015; Tahiluddin & Terzi, 2021a; Tahiluddin & Terzi, 2021b; Tahiluddin et al., 2022a). Secondary factors as causative biotic agents are opportunistic bacteria, taking advantage of the stress seaweeds (Largo et al.,1995b; Largo et al., 1999). Marine-derived fungi also caused ice-ice disease in the *Kappaphycus* species (Solis et al., 2010). This disease is usually showing depigmentation, whitening, or bleaching in the infected thalli of seaweeds (Ward et al., 2022). As the disease progresses, the infected thalli become weak and soft and eventually break off from the cultivation lines; hence losses are expected (Faisan et al., 2021). Ice-ice disease, along with epiphyte infestation, affects the sustainability of eucheumatoid seaweed farming as they have caused numerous problems, including reducing culture stocks and lowering carrageenan quality, lowering income and job opportunities, particularly for marginalized seaweed farmers (Ward et al., 2020). The ice-ice disease is one of the main factors in the 15% production losses of *Kappaphycus* in the Philippines between 2011 and 2013 (Cottier-Cook et al., 2016).

In the Philippines, the occurrence of ice-ice disease is widespread in cultured eucheumatoid seaweeds, notably in Mindanao (Faisan et al., 2021). Previous studies also reported that ice-ice disease is predominant in eucheumatoid seaweed farming in different parts of the country (Uyenco et al., 1981; Largo et al., 1995; Hurtado et al., 2006; Tisera & Naguit, 2009; Solis et al. 2010; Alibon et al., 2019; Tahiluddin et al., 2022a; Sarri et al., 2022; Tahiluddin et al., 2022b; Bermil et al., 2022; Tahiluddin et al., 2022c) and worldwide (Tahiluddin & Terzi, 2021a). In Sibutu, Tawi-Tawi, Philippines, where one of the largest eucheumatoid seaweed farms in the country is situated, ice-ice disease is a long issue in farmed eucheumatoid seaweeds (Tahiluddin et al., 2022a) that cease the farmers from cultivating sustainably. Usually, the farmers simply cut off the ice-ice-diseased thalli and let the seemingly unaffected branches continue to regenerate and regrow (Hurtado et al., 2019). However, this process seemed not to wholly eradicate the disease. As a consequence, despite being discouraged by the Philippine National Standard in 2021, farmers were urged to incorporate inorganic nutrient enrichment using inorganic fertilizers, mainly ammonium phosphate, which seemed to be an effective way of decreasing the occurrence of ice-ice disease and improving the growth of farmed eucheumatoid seaweeds, thereby increasing the production (Tahiluddin et al., 2022a). However, despite the assistance of inorganic nutrient enrichment, ice-ice disease is still a common problem in eucheumatoid seaweed farming (Tahiluddin et al., 2022a). Hence, this study assessed the prevalence of ice-ice disease in different farms of eucheumatoid seaweeds

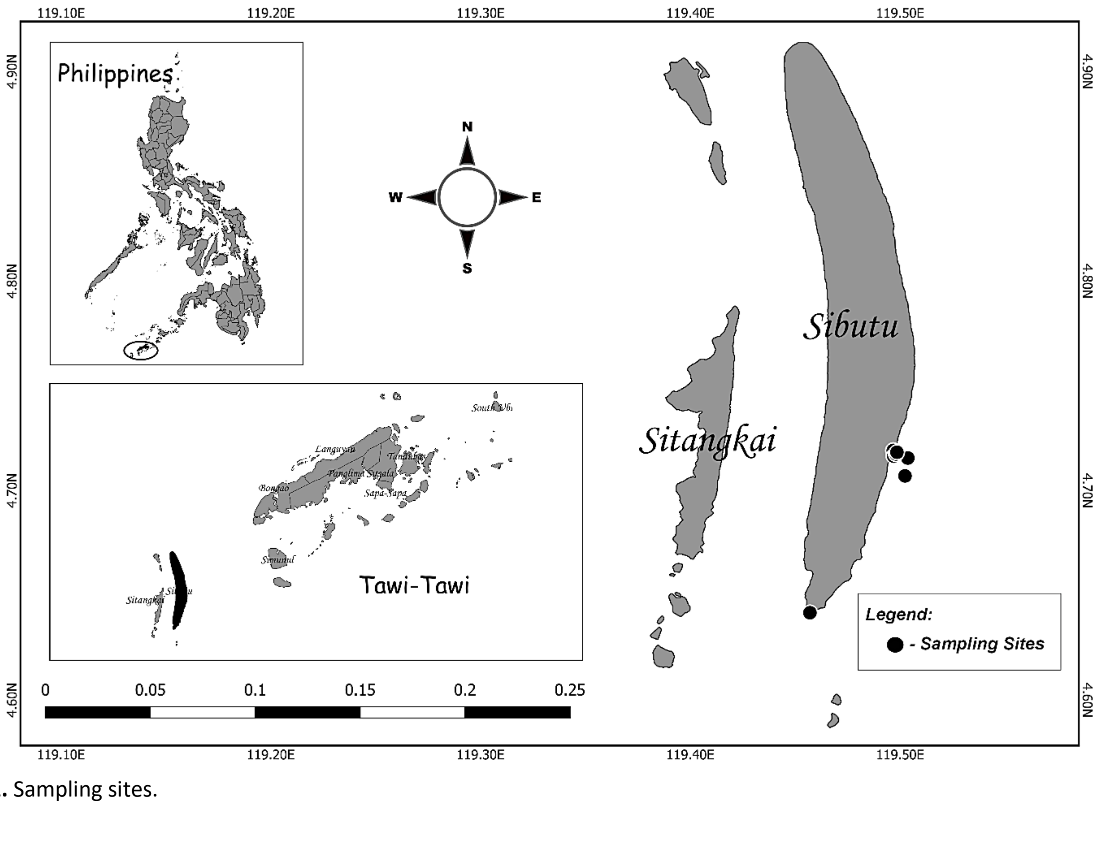

Figure 1. Sampling sites.

(*Kappaphycus* species and *Eucheuma denticulatum*) in Sibutu, Tawi-Tawi, Philippines, between July to September 2022. The prevalence of ice-ice disease was compared according to farm depth (shallow and deep waters), species (*Kappaphycus striatus, K. alvarezii,* and *Eucheuma denticulatum*), and time (July, August, and September).

Materials and Methods

Sampling Sites

This study was conducted in the municipality of Sibutu, province of Tawi-Tawi, Philippines (Figure 1) since this area has extensive eucheumatoid seaweed farms and is considered as one of the major suppliers of dried eucheumatoid seaweeds in the Philippines. The study was carried out in July, August, and September 2022. The characteristics of the assessed farms can be seen in Table 1. Different farms according to depth (shallow and deep-water farms) and species (*Kappaphycus alvarezii* and *K. striatus)* were assessed, done in triplicate farms with five random lines in each farm for each species. However, for *E. denticulatum*, due to limited farm availability as a result of its lower price, only one farm (shallow area) and five random lines were assessed. As practiced by the farmers in the area, *K. alvarezii and K. striatus*, both farmed in shallow and deep-water farms, were nutrient-enriched with ammonium phosphate at a concentration ranging between 2.26 and 25 g L^{-1} by shortly dipping the seaweeds in the fertilizer solution for 5-60 seconds, gathered on the bamboo drying platform, covered with canvas overnight, and planted the following day. Re-application was done after 7-10 days of the initial planting (Tahiluddin et al., 2022a). On the other hand, *E. denticulatum* is untreated due to its lower market price. Shallow water farms (0.61±0.29 m during low tide) used the modified fixed-off bottom method, while deep water farms (exceeding 2.47±0.16 m during low tide) employed the floating method. A modified fixed-off bottom method utilized stakes on both sides installed on the seabed with floaters (mainly empty plastic bottles), while the floating method utilized floaters supported with anchored sinkers (mainly rocks or sacks filled with sand) on both ends.

Ice-Ice Disease Assessment

Ice-ice disease was assessed on each farm through visual observation once a month. Any signs or symptoms, such as showing white and soft thalli, are considered an ice-ice disease (Tahiluddin et al., 2022a). Diseased thalli were counted per line (31-56 bundles per line). The prevalence of ice-ice disease was calculated by obtaining the quotient from counted ice-ice disease-infected thalli and the total number of seaweed bundles per line (Faisan et al., 2021).

Monitoring of Water Parameters

Water parameters, such as temperature and salinity, were respectively determined using a thermometer and refractometer during the assessment once a month. The surface water (7-12 cm depth) of the farms was measured (in triplicates) just beside the cultivated lines by submerging the thermometer and obtaining the water sample for salinity measurement.

Statistical Analysis

The obtained data were expressed as mean±SE. Statistical analysis was performed using the IBM SPSS version 20. Non-parametric one-way analysis of variance (ANOVA) Kruskal-Wallis was utilized to determine significant differences among species at each time. This was done after the data were found not to be normally distributed using Shapiro-Wilk's test, and the variances were not homogeneous. In addition, to compare the significant differences in ice-ice disease prevalence between the depth (deep water and shallow water farms) and time (July, August, and September), a non-parametric Friedman's two-way analysis of variance (ANOVA) was used. The prevalence of ice-ice disease and water parameters, i. e., temperature and salinity, were also correlated using Pearson Correlation analysis. The level of significance was set at $p<0.05$.

Results

The prevalence of ice-ice disease of *Kappaphycus* species in deep water (floating method) can be seen in Table 2. In July, the ice-ice disease percentage of *K.*

Table 1. Characteristics of the assessed farms.

	Characteristics					
	Depth (m)	*Farming method*	*No. of assessed farms*	*No of assessed line per farm*	*No. of bunches per line*	*Inorganic nutrient enriched*
Deep water						
K. alvarezii	3.10±0.08	Floating	3	5	31-47	Yes
K. striatus	2.85±0.17	Floating	3	5	34-44	Yes
Shallow water						
K. alvarezii	0.56±0.05	Modified fixed- off bottom	3	5	40-53	Yes
K. striatus	0.59±0.07	Modified fixed-off bottom	3	5	38-49	Yes
E.denticulatum	1.60±0.28	Modified fixed-off bottom	1	5	45-56	No

striatus (4.29±0.97%) was significantly lower ($p<0.05$) than *K. alvarezii* (10.53±2.64%). However, in August, albeit the ice-ice disease occurrence was lower in *K. alvarezii* (5.45±0.67%) than in *K. striatus* (10.33±2.00), it was not significantly different ($p>0.05$). Also, in September, there was no significant difference ($p>0.05$) between the ice-ice disease prevalence of *K. striatus* (21.69±6.54%) and *K. alvarezii* (32.39±6.58%).

In shallow water (modified fixed-off bottom method), the ice-ice disease prevalence is also shown in Table 2. During July, ice-ice disease prevalence of *E. denticulatum, K. striatus,* and *K. alvarezii* were not statistically significant ($p>0.05$), with a prevalence of 15±4.33%, 12.59±1.97%, and 8.9±3.12%, respectively. In August, ice-ice disease prevalence of *E. denticulatum* (21.97±1.73%) was statistically higher ($p<0.05$) when compared with *K. striatus* (10.33±2.42%) and *K. alvarezii* (6.83±1.40%). In September, the percentage of ice-ice disease of *K. alvarezii* (5.43±1.98%) was significantly lower ($p<0.05$) than *K. striatus* (20.14±3.05%) but not significant ($p>0.05$) with *E. denticulatum* (7.73±2.17%).

According to water depth, the prevalence of ice-ice disease of eucheumatoid seaweed species in July and August was lower in deep water farms (7.41±1.50 and 7.89±1.13%, respectively) than in shallow water farms (11.35±1.69% and 10.50±1.47%, respectively). During the month of September, deep water farms (27.04±4.66%) were greater than shallow water farms (12.91±1.93%) (Table 3). However, Friedman's two-way ANOVA revealed that no significant differences ($p>0.05$) were detected in the ice-ice disease prevalence between depths and time.

The physico-chemical parameters (temperature and salinity) of the farms are shown in Table 4. In July, an extreme temperature of nearly 33°C in *Kappaphycus* farms was recorded. For the months of August and September, the temperature varied according to farm depth and species but generally ranged between 29.67±0.33 (*E. denticulatum*) to 32.78±0.49 (*K. alvarezii*, deep water). For salinity, all farms were within the favorable salinity throughout the samplings, which ranged from 31.00±0.24 (*K. striatus*, deep water) to 34.00±0.00 (*E. denticulatum*). Pearson Correlation analysis revealed that temperature and salinity were not correlated with the prevalence of ice-ice disease (Table 5).

Table 2. Ice-ice disease prevalence (%) of *Kappaphycus* and *Eucheuma* species farmed in deep and shallow waters.

Farm	Ice-ice disease prevalence (%)		
	July	*Aug*	*Sept*
Deep water			
K. alvarezii	10.53±2.64^{a}	5.45±0.67^{a}	32.39±6.58^{a}
K. striatus	4.29±0.97^{b}	10.33±2.00^{a}	21.69±6.54^{a}
Shallow water			
K. alvarezii	8.9±3.12^{a}	6.83±1.40^{b}	5.43±1.98^{c}
K. striatus	12.59±1.97^{a}	10.33±2.42^{b}	20.14±3.05^{ab}
E. denticulatum	15±4.33^{a}	21.97±1.73^{a}	7.73±2.17^{bc}

Note: Superscriptions of different letters in each time across farm depth were significant at 0.05.

Table 3. Ice-ice disease prevalence (%) of eucheumatoid seaweeds in different farm depths.

Farm depth	Ice-ice disease prevalence (%)		
	July	*Aug*	*Sept*
Deep water	7.41±1.50^{a}	7.89±1.13^{a}	27.04±4.66^{a}
Shallow water	11.35±1.69^{a}	10.50±1.47^{a}	12.91±1.93^{a}

Note: Superscriptions with the same letter across farm depth and time are not significant at 0.05.

Table 4. Temperature and salinity of eucheumatoid seaweed farm waters.

Farm	Temperature (°C)			Salinity (‰)		
	July	*Aug*	*Sept*	*July*	*Aug*	*Sept*
Deep water						
K. alvarezii	33.11±0.11	31.89±0.11	32.44±0.18	33.44±0.29	33.67±0.29	33.67±0.17
K. striatus	33.11±0.11	31.89±0.11	32.78±0.32	31.00±0.24	33.56±0.34	34.22±0.15
Shallow water						
K. alvarezii	33.00±0.24	32.00±0.00	32.78±0.49	33.56±0.24	33.44±0.24	33.89±0.26
K. striatus	32.78±0.15	31.44±0.18	32.56±0.38	32.89±0.42	32.33±0.55	33.56±0.18
E. denticulatum	31.33±0.33	29.67±0.33	30.00±0.00	34.00±0.00	33.67±0.33	33.33±0.33

Table 5. Pearson Correlation coefficients (r) and p values. Significant level was set at 0.05.

	Temperature	Salinity
Deep water		
K. alvarezii	0.121 (0.923)	0.33 (0.780)
K. striatus	-0.90 (0.943)	0.876 (0.320)
Shallow water		
K. alvarezii	0.316 (0.795)	0.969 (0.159)
K. striatus	0.558 (0.623)	-0.625 (0.570)
E. denticulatum	-0.176 (0.887)	0.518 (0.653)

Discussion

The present study revealed that the prevalence of ice-ice disease varied significantly according to farmed species, ranging between 4 and 32% from July to September. However, farm depth (deep and shallow waters) and time had no significant effect on the ice-ice disease occurrence. According to Tahiluddin & Terzi (2021a), ice-ice disease occurrence may vary according to places, cultivation period, and farmed species. Ice-ice disease is common in *K. striatus* in other parts of Tawi-Tawi, Philippines, with a percent occurrence ranging between 24 and 64% farmed in Bongao municipality (Sarri et al., 2022) and 5 and 65% in Panglima Sugala municipality (Tahiluddin et al., 2022b). A similar study also reported that the farm of *K. alvarezii* in Zamboanga City and Zamboanga del Sur, Philippines, was infected with an ice-ice disease with a prevalence ranging from 22 to 30% sampled during the month of July to September (Alibon et al., 2019), which is comparable to the results in the present study. In Bais Bay, Zamboanga del Norte, and Negros Oriental, Philippines, the highest prevalence of ice-ice disease (52-56%) was noticed during April, October, and December in the farms of *K. alvarezii* and *E. denticulatum* (Tisera & Naguit, 2009). A massive occurrence of ice-ice disease (70-87%) was recorded on the *Kappahycus* farm in Calaguas Is., Camarines Norte, Philippines (Hurtado et al., 2006). In other countries, like in the study of Pang et al. (2015) in Lian Bay, Hainan Province, China, an ice-ice disease outbreak of *Kappaphycus* species was notably observed from May to August. In Mannar Gulf and Palk Bay, southern India, the ice-ice disease was noticed in *K. alvarezii* farm during March and April (Arasamuthu & Edward, 2018). A prominent ice-ice disease of up to 99% prevalence was observed in eucheumatoid seaweed farms during the hot and dry season, typically from February to March in Zanzibar, Tanzania (Largo et al., 2020). In South Sulawesi, Indonesia, ice-ice disease occurred between September and October (Badraeni et al., 2020).

Although there was no correlation between temperature and salinity and ice-ice disease prevalence, the temperature of the assessed farms was generally high (nearly 33°C) in the present study, notably during July, which may have caused stress to the seaweeds. Changes in the environmental factors, such as a temperature of 33-35°C, a salinity of less than 20 ppt, a low light intensity of less than 50 mol photon m^{-2} s^{-1} (Largo et al., 1995a), and lack of nutrients (Luhan et al., 2015; Tahiluddin et al., 2022a) have been linked to ice-ice disease occurrence in eucheumatoid seaweeds (Tahiluddin & Terzi, 2021a). The synergistic effect of these stressful environmental conditions, together with the presence of opportunistic marine bacteria (*Vibrio* sp. P11 and *Cytophaga* sp. P25), have been shown to cause ice-ice disease in eucheumatoid seaweed species (Largo et al., 199.5b). Marine-derived fungi have also triggered ice-ice disease development (Solis et al., 2010), and their abundance was significantly higher in ice-ice diseased thalli of *Kappaphycus* species (Bermil et al., 2021). However, the exact nature of the complex interactions between biotic and environmental factors leading to ice-ice disease remains mysterious (Faisan et al., 2021).

Every eucheumatoid seaweed species has different responses to ice-ice disease. As revealed in the present study, *K. striatus* had a lesser prevalence of ice-ice disease, particularly in deep water farms. This is similar to the previous reports. For example, *K. alvarezii* has shown greater sensitivity to ice-ice disease when compared to *K. striatus* varieties (Tahiluddin and Toring, 2013). Also, Hurtado et al. (2008) emphasized that *K. striatus* var. sacol is more resistant to ice-ice disease. In terms of the number of marine-derived fungi – one of the causative biotic agents of ice-ice disease, *K. striatus* had 10-100 times lower than *K. alvarezii,* indicating that *K. striatus* has a higher resistance to potential ice-ice-causing marine-derived fungi (Bermil et al., 2022). Although *E. denticulatum* is known to be resistant to ice-ice disease due to the production of volatile halocarbons, which provide a defense mechanism for diseases (Tahiluddin & Terzi, 2021a), the present study showed the opposite finding. *Kappaphycus* species in the present study were nutrient-enriched with inorganic fertilizers, while *E. denticulatum*, due to its low market price, inorganic nutrient enrichment is not being applied. A previous study revealed that inorganic nutrient enrichment reduced ice-ice disease prevalence significantly (Tahiluddin et al., 2022a). In terms of depth, i.e., deep and shallow waters, the prevalence of ice-ice disease in the present study did not differ significantly, albeit shallow water was higher (11.35±1.69% and 10.50±1.47%) than deep water (7.41±1.50 and 7.89±1.13%) during July and August, and deep water (27.04±4.66%) was much higher than shallow water (12.91±1.93%) in September. This result was in agreement with the study of Faisan et al. (2021), where depth (shallow and deep-water farms) did not affect the prevalence of ice-ice disease, although it was higher (14.8%) in deep-water farms compared to shallow-water farms (5.0%). It has been suggested that prolonged low or high rainfall volume, high near-surface temperature, and low wind speed in deep water farm sites may contribute to the stress of seaweeds leading to the development of ice-ice disease (Faisan et al., 2021).

The assessed cultivated eucheumatoid seaweeds (*K. alvarezii* and *K. striatus*) in the present study were all inorganic nutrient-enriched as a control measure and growth booster for seaweeds. Despite this, the prevalence of ice-ice still occurred. Tahiluddin et al. (2022a) reported that inorganic nutrient enrichment did not totally eliminate the disease but rather significantly reduced the prevalence of ice-ice disease and improved the growth of cultivated *Kappaphycus.* This is maybe due to the higher nitrogen content in those exposed to luxury nutrients during enrichment (Tahiluddin et al., 2021b). Apart from incorporating inorganic nutrient enrichment, a practice being discouraged by the Philippine National Standard, translocating the ice-ice diseased seaweeds to nearshore – serves as a recovery area for the infected seaweeds is also done by the seaweed farmers in the study site; hence recovery can be expected after few weeks of transferring (Pers. Com.). The science behind the recovery is still unknown. However, several authors have stressed the importance of biosecurity measures, such as quarantine procedures recommended to be implemented during translocating cultivars, notably in those major eucheumatoid seaweed farms with greater prevalence of pests and diseases, including ice-ice disease in order to hinder or reduce the disease and pest transmission among farms in a wider geographical range (Mateo et al., 2020; Kambey et al., 2020; Rusekwa et al., 2020; Ward et al., 2020; Faisan et al., 2021; Kambey et al., 2021; Mateo et al., 2021; Tahiluddin & Terzi, 2021b; Ward et al., 2022).

Conclusion

Ice-ice disease is indeed one of the lingering problems in farmed eucheumatoid seaweeds. The present study showed that the prevalence of ice-ice disease varied according to farmed species, ranging from 4-32%. *Kappaphycus striatus* showed less vulnerability to ice-ice disease, notably in deep water farms. Both farm depths, i. e., deep and shallow waters, were infected with the ice-ice disease from July to September. Inorganic nutrient enrichment seemed to influence the ice-ice disease since untreated *E. denticulatum* had the highest prevalence of the disease. Hence, *K. striatus* is recommended to be used in eucheumatoid seaweed farming as it has a high tolerance to ice-ice disease when cultivated in deep water. This study serves as baseline information for further investigating and understanding the ice-ice disease in eucheumatoid seaweed cultivation.

Ethical Statement

Not applicable.

Funding Information

Not applicable.

Author Contribution

ABT conceptualization, data analysis, original draft preparation, writing - review and editing, SUD data collection, data analysis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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