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Seasonal occurrences of epiphytic algae on the commercially cultivated red alga *Kappaphycus alvarezii* (Solieriaceae, Gigartinales, Rhodophyta)

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Abstract

Common problems faced in farming of the red algal genus *Kappaphycus/Eucheuma* are "ice-ice disease" and the occurrence of epiphytes. Considerable work has been documented on "ice-ice disease" and it's mode of infection but limited information is available on the emergence of epiphytes. The present study addresses the phenomenon of epiphyte infection, its prevalence in commercially cultivated red alga, *Kappaphycus alvarezii,* and their variability associated with seasonality. Cultured seaweed became susceptible to epiphytes in the dry seasons (1) between March – June and (2) September – November. Findings revealed *Neosiphonia savatieri* (Hariot) M. S. Kim *et* I. K. Lee, as the dominant infecting epiphyte, representing up to 80–85% of the epiphyte present during peak seasons. Besides *N. savatieri*, *Neosiphonia apiculata*, *Ceramium* sp., *Acanthophora* sp. and *Centroceras* sp. were observed in smaller quantities. SEM (Scanning Electron Microscope) micrographs revealed the epiphyte's attachment to the host. Further histological study showed the extent of penetration of epiphytes into the host's cortex tissues and condition of its surrounding tissues. The outbreak of epiphytic filamentous red algae also correlated with drastic changes in seawater temperature and salinity during March – June and September – November.

Introduction

In Malaysia, seaweed farming was introduced in the late 1970s and commercial cultivation started few years after, with the culture of *Gracilaria* in Peninsula Malaysia and *Eucheuma* in North Borneo. Today, after almost 35 years, the red algal genera *Kappaphycus* and *Eucheuma* are intensively cultivated in two locations; Semporna (east coast of Borneo), and Kudat (north Borneo). Concomitant with the increase in farm size and intensification of culture practices is the rise in seaweed diseases, particularly; "ice-ice disease" and invasion of epiphytes (filamentous red algae). Epiphytic invasion is not a new phenomenon and has been known to exist since the dawn of farming practices (Doty & Alvarez, 1975, 1981). However, little is known of their causative agents, seasonality, mode of action and factors causing outbreaks (Parker, 1974; Collen et al., 1995; Fletcher, 1995; Ask, 1999, Ask & Azanza, 2002; Critchley et al., 2004). Knowledge of these aspects is vital, since epiphytic algae invade regularly, at times affecting the marketability of the harvested seaweeds. Epiphyte outbreaks have also shown to weaken the seaweed, making it susceptible to bacterial attack (unpublished data).

Hence, the present study was initiated to gather substantial information and in-depth understanding of the causative agent and some indication of the factors affecting outbreaks. Dynamics of epiphyte outbreaks were monitored in relation to abiotic seawater factors such as temperature and salinity. In addition, histology and Scanning electron microscopy (SEM) investigations were carried out to determine the epiphyte's attachment and intrusion into the host.

Materials and methods

Sampling location

Infected seaweed specimens were collected from a seaweed farm in Teluk Lung, Pulau Balambangan, Kudat, Sabah (04°32′43″N, 116°58′30″E). Sampling was conducted fortnightly during the epiphyte outbreak. Specimens were transported in a cold ice-chest (4 ∼ 6 °C) and fixed in 10% formalin in seawater at the laboratory. Sections and epiphytes were removed from the host by hand under stereo microscope (Stemi-2000 CS, Carl Zeiss, Germany) using a razor blade and pith stick and stained with 0.5% (w/v) cotton blue in lactic acid/phenol/glycerol/water [1:1:1:1 (v/v)] solution and mounted in 50% glycerol/seawater microscope slides. Some of the isolated epiphytes were immediately mounted in seawater on microscope slides and observed. Epiphytes were counted randomly in an area of 1 cm × 1 cm under a stereo microscope at 2 ∼ 5 times magnification. A total of 20 individuals were observed during each observation and data presented in Figure 1 represent average numbers of epiphytes for 12 months observation.

Histological study

Sections (0.5 cm in length) of the infected algal thallus were fixed for 24 h in 4% glutaraldehyde in 0.1 M cacodylate buffer (salinity; 30 ppt). Fixed specimens were then dehydrated in the following series of ethanol; 70, 80, 90, 95 and 99%, each for 1 h. Dehydrated specimens were then cleared twice with xylene: each step lasted for 1 h. Finally, specimens were impregnated with paraffin (twice), each for 80 min, before blocking. Sections were cut at 15 μm thickness and stained in haematoxilin/eosin or pirodic acid Schiff's reagent prior to viewing.

Scanning electron microscope study

Algal thalli with epiphytes were cut ca. 1.0 cm in length and fixed for 24 h in 4% glutaraldehyde in 0.1 M cacodylate buffer before post-fixation in 1% OsO_4 at 4 °C for 2 h, followed by dehydration with a graded acetone series and finally, critical point drying. Dehydrated specimens were mounted on stubs and coated with a 10–30 nm layer of gold before observation with a Leica Cambridge S360 electron microscope.

Physical water parameters

Seawater temperature and salinity was recorded on a multi-probe water quality checker (YSI meter, Model 85–25) three times daily at 6 am, 12 pm and 6 pm. Measurements were taken at a maximum depth of 0.5 m below the seawater surface. Data are presented as averages of those measured, for each two-week interval.

Results and discussion

Seasonal variation of epiphytes

Occurrence of epiphytes was monitored from January to December 2003. The presence of epiphytes was first observed at the end of February and vegetative plants emerged 3–4 weeks later and persisted until the end of June. A second invasion was shorter: the first sign was seen in early September and the epiphyte disappeared by late November. A total of 5 epiphyte species were

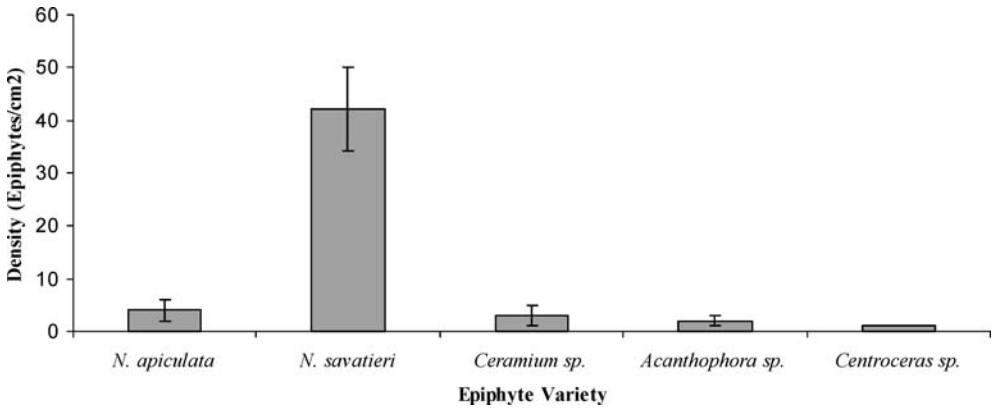

Figure 1. Average species composition of epiphytes isolated from the surface of the cultivated red alga, *Kappaphycus alvarezii*, during outbreaks.

isolated in both outbreaks, identified and their average distribution during the outbreak is shown in Figure 1. Species abundance was: *Neosiphonia savatieri* > *Neosiphonia apiculata* > *Ceramium* sp. > *Acanthophora* sp. > *Centroceras* sp. This trend was consistent at the study site for almost two years, from 2003 ∼ 2004. Detailed observations of the dominant epiphyte were made and the findings are illustrated below.

Description of Neosiphonia savatieri

The first emergence of an epiphytic infection on *K. alvarezii* was observed in late February with the appearance of tiny black spots on surface cuticle cells layer (Figure 2A). These black spots then became rough and the vegetative epiphyte surfaced after 3–4 weeks (Figure 2B). Tissue cross-sections of the black spots revealed the presence of tetrasporelings embedded between the outer cortex cells (Figure 2C). By the end of March, the vegetative state of the epiphytes could be seen as shown in Figure 2D. Epiphytes were observed as solitary plants growing on the algal surface with rhizoids penetrating into the tissue of the cortical cell layers. In the peak season, the dominant epiphytes, *N. savatieri*, were seen to grow close to each other at a maximum density of 40–48 epiphytes/cm^{-2}.

N. savatieri is a plant with vertical axes and a height of 4–20 mm. Its basal attachment system is composed of a primary rhizoid with one or several secondary rhizoids. The latter were cut off from their pericentral cells at lower segments of the axis. These rhizoids penetrate into tissue of the basiphyte *via* cortical cells and at times are seen to penetrate down to the inner cortical

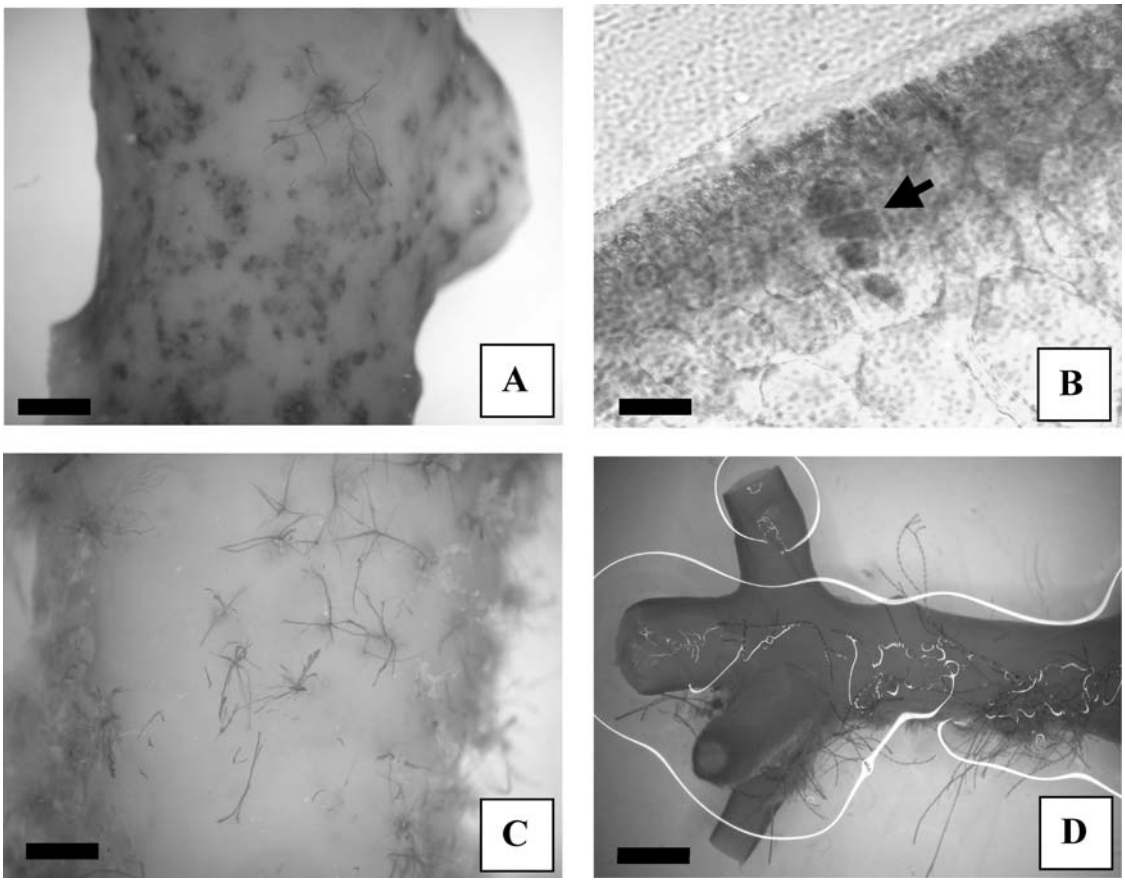

Figure 2. *Kappaphycus alvarezii* infected with *Neosiphonia savatieri*. (A) Host plant with early stage of epiphytes imbedded as tiny black spots in outer and inner cortex cell layers in late February (scale bar = 300 μm); (B) Tetrasporeling implanted between the outer and inner cortex cell layers (scale bar = 50 μm); (C) Host plant with epiphyte germlings by end of March (scale bar = 300 μm); (D) Host plant with mature epiphyte (scale bar = 300 μm).

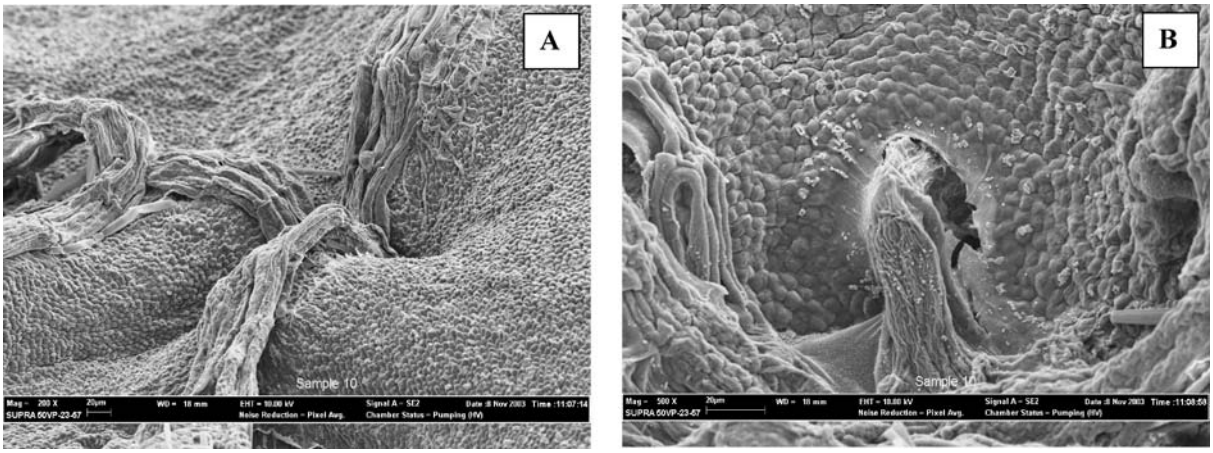

Figure 3. Scanning Electron Microscopy micrographs showing epiphyte's attachment to the host plant; (A) Emergence of epiphyte branching from a common location (200 ×); (B) Areas around the point of attachment have lesions/openings, providing ideal opportunity for grazers and microbial attack (500×).

cell layers as described by Kim and Lee (1999), Masuda et al. (2001), and Hollenberg (1968).

Epiphytic attachment phases

N. savatieri attached itself to the host *via* a basal attachment using its primary rhizoid or/and secondary rhizoids. Close examination of its attachment location revealed the emergence of 2–4 branches from one location as shown in Figure 3A. Horizontal branches that exceeded 8–10 mm were seen attached to the host again at a different locations *via* secondary rhizoids, but this was not seen for the axial branches. SEM micrographs in Figure 3B also showed slight lesions or cracks at the point where epiphytes entered the host plant**.** Presence of such lesions and cracks weakens the host plants, making them vulnerable to thallus breakage and bacterial attack. Histological study was carried out on tissue cross-sections at locations where the epiphyte was seen to penetrate the host, and the findings are shown in Figures 4A and 4B. Figures 4A shows an epiphyte with its main axis horizontally aligned to the host and with two of its rhizoids penetrating the host. In Figure 4B, two rhizoids are shown to penetrate into the cortex cell layers of the host; (1) first rhizoid **(I)** can be seen to penetrate deeply, to the inner cortex cells, where it is surrounded by outer cortex cells and inner cortex cells, (2) the second rhizoid only penetrates to the outer cortex cell layers **(II).**

Seasonality of epiphytic invasion

Seasonal variation in epiphyte species composition was consistent for two consecutive year of crop cultivation (2003–2004). Correlation between the changes in epiphyte composition and physical abiotic factors at the culture site showed an interesting trend. Figure 5 shows the fluctuation in abiotic factors for 12 months at the study site. Emergence of epiphytes in late February/March coincides with drastic increases in salinity and temperature, e.g. seawater temperature increased from 27 to 31 °C, and salinity increased from 28 to 34 ppt. The opposite occurred in September (second epiphyte invasion), when both salinity and temperature were decreasing, e.g. temperature dropped from 30 to 25 °C, and salinity decreased from 29 to 27 ppt, between September to late November. Hence, there could be a possible correlation between the fluctuations in the abiotic factors and the emergence of epiphytes. Drastic changes in the abiotic factors could act as a triggering mechanism or cue for the epiphytes to infect *K. alvarezii*, as described by Mtolera et al. (1996) for *Eucheuma denticulatum* (N.L. Burman) F.S. Collins *et* Hervey. A similar situation has been reported in various bacterial diseases in Japanese kelp and *K. alvarezii* (Glenn & Doty, 1990; Vairappan, 2001; Largo et al., 1995a,b). Besides seawater temperature and salinity fluctuations, other physical factors such as seawater nutrient levels and photoperiod could also play an important role in epiphyte germination and outbreak. However, both

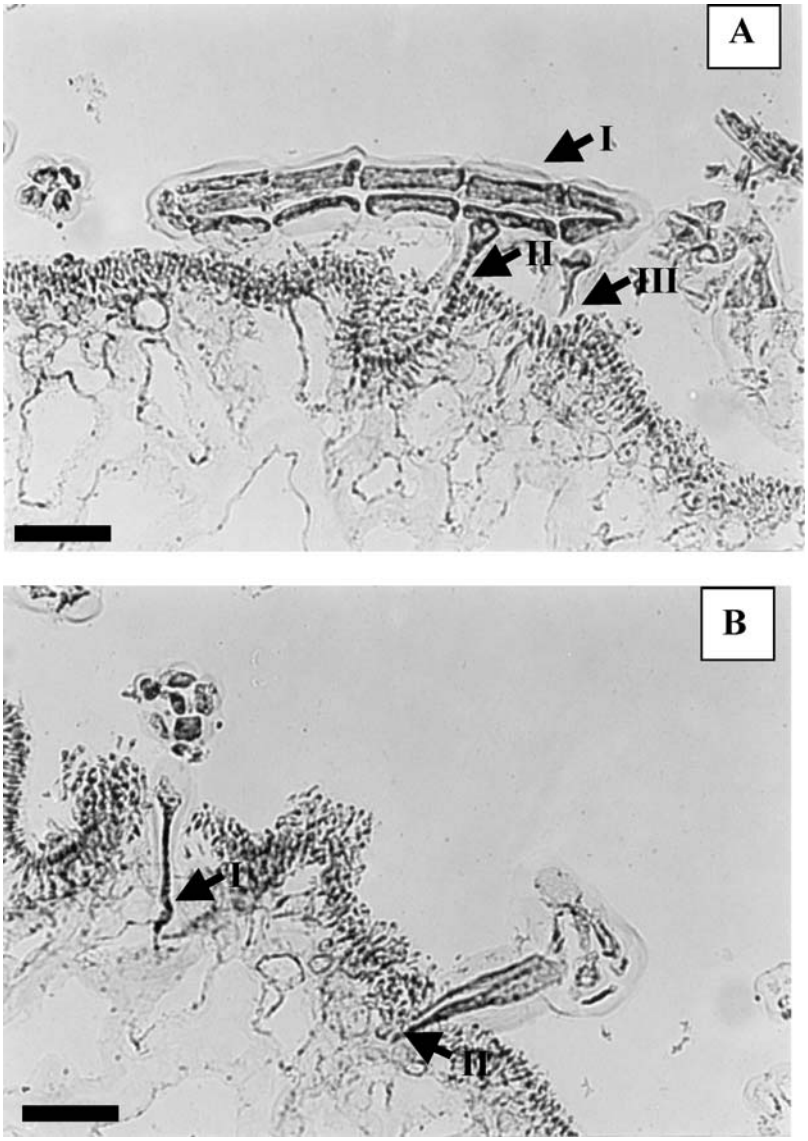

Figure 4. Histological section showing attachment of the epiphyte to the host plant; (A) Cross section of epiphyte's first-order branch (I) and two rhizoids attached to the host (II & III); (B) Slides showing penetration of rhizoids to the inner cortex cell layers (I) and outer cortex cell layers (II).

these factors could not possibly play any important role in this case since the culture site was far from anthropogenic sources of nutrients and there were no significant difference in photoperiod at the study site.

Conclusion

In the South East Asian region, where most commercial red algae are cultivated for carrageenan, occurrence of epiphytes is not given due importance. This could be due to lack of understanding or because cultivation is often a practice in remote areas, by poor farmers. However, the fact remains that epiphytic growth is on the rise and it has always been seen as a serious constraint to commercial seaweed cultivation, particularly in the tropics (Wheeler et al., 1981; Zemke-White & Ohno, 1999). Emergence of an epiphytic outbreak is a complex problem and the extent of the outbreak often depends on the quality of the cultivated strain, abiotic parameters of the culture site and seasonal weather fluctuations. However, considerable effort is warranted to better understand this problem due to its importance in

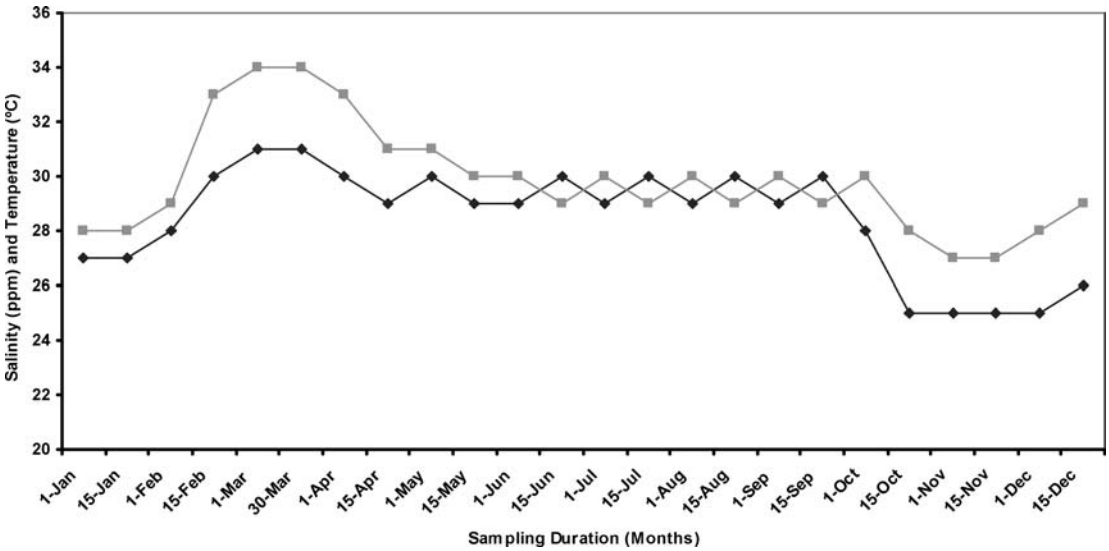

Figure 5. Fluctuations of abiotic factors, seawater temperature (♦) and salinity (■), at seaweed culture location in Teluk Lung, Pulau Banggi, Kudat, during the study period – 2003 (Data shown are average of 3 readings, standard deviation is not shown to avoid data congestion).

the marketability of the harvested seaweed and possible impacts on the quality of its phycocolloid. Hence, the present study could be regarded as a forerunner in our attempt to fully understand the impact of epiphyte on the growth, production and quality of carrageenan produced by *K. alvarezii*.

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