SEASONAL OCCURENCES OF EPIPHYTES AND THEIR EFFECTS ON THE QUALITY OF CARRAGEENAN IN COMMERCIALLY CULTIVATED *Kappaphycus alvarezii*.

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ABSTRACT

SEASONAL OCCURENCES OF EPIPHYTES AND THEIR EFFECTS ON THE QUALITY OF CARRAGEENAN IN COMMERCIALLY CULTIVATED *Kappaphycus alvarezii*

Sabah is the only state in Malaysia with a successful seaweed cultivation programme. Malaysia is the third largest producer of *Kappaphycus* and *Eucheuma* in the world with an annual harvest of 6,500 tonnes. The seaweed industry faces various problems in cultivation especially from bacterial causing disease such as "ice-ice" disease and from epiphyte infection. Epiphyte infection of crops has been a long standing problem but very little has been studied on it. This research sets out to look into the characteristics of the epiphytes as well as to study the effects of the epiphytes on the seaweed as well as on the quality of the carrageenan produced. Taxonomical identification has found that the infecting epiphyte to be *Neosiphonia apiculata.* This is concurrent with species found on samples obtained from outbreaks in the Philippines and Tanzania, with all three outbreaks displaying similar symptoms. It was also found that the epiphytes caused massive damage to the infected thalli causing crop loss and degradation; including cortical swellings, pit formation, deformities of the seaweed surface and large growth of epiphytes. The pattern in outbreak showed that the epiphytes are seasonal in Malaysia, reaching peak growth between the months of April to June and August to October, whereby 90% of the *K. alvarezii* were covered with hair like protrusions measuring 0.4 – 5.0 cm. Nutritional testing carried out showed seasonal trend in nutritional properties, farm locations and with the onset of epiphyte infection. Testing was carried out on moisture, ash content, phenolics, lipid, carrageenan yield and fatty acids, showed significant differences. Nutritional values ranged between 90 - 93% for moisture, 40 - 50% for ash, 4 - 7 % for protein, 0.04 - 0.15% lipid, 20 - 120 ng/g total fatty acids, 0.1 - 0.7 mg/g phenolic content and 30 - 75% carrageenan yield. Rheological properties also showed decrease in properties such as carrageenan yield, melting point, viscosity and gel strength for carrageenan extracted from epiphyte infected seaweed. Molecular studies showed that the epiphyte infected carrageenan showed no structural change but the molecular weight was 80 KD as compared to 800 KD of the healthy carageenan.

ABSTRAK

Sabah merupakan negeri tunggal di Malaysia dengan program pengkulturan rumpai laut yang giat berkembang. Malaysia kini merupakan negara ketiga besar dalam pengeluaran Kappaphycus dan Eucheuma di dunia dengan jumlah tahunan sebanyak 6,500 tan. Industri rumpai laut menghadapi pelbagai masalah dalam proses pengeluaran terutamanya daripada penyakit yang disebabkan oleh bakteria seperti penyakit "ice-ice" dan daripada serangan epifit. Serangan epifit ke atas ladang rumpai laut merupakan masalah yang seringkali dihadapi peladang namun tidak banyak yang dikaji ke atasnya. Kajian ini dijalankan bagi meneliti ciri-ciri epifit dan juga kesannya ke atas kualiti karagenan yang dihasilkan. Pengenalan taksonomi telahpun mengenalpasti epifit sebagai Neosiphonia apiculata. Ini adalah selaras dengan keputusan kajian serangan epifit ke atas sampel yang diperolehi daripada serangan di Filipina dan Tanzania, di mana ketiga-tiganya memaparkan ciri-ciri serangan serupa. Kajian juga menunjukkan bahawa epifit ini meninggalkan kerosakan ke atas rumpai laut dan membawa kepada kerugian hasil; termasuk pembentukan lebam kortex, penghasilan pit, perubahan struktur lapisan rumpai laut dan pertumbuhan giat epifit. Serangan epifit di Malaysia menunjukkan terdapat corak serangan dengan pertumbuhan maxima epifit antara bulan April hingga Jun dan Ogos hingga Oktober, di mana sebanyak 90% rumpai laut K. alvarezii diselaputi oleh lapisan berbulu halus berukuran 0.4 – 5.0 cm. Ujian nutrisi yang dijalankan menunjukkan terdapat tren bermusim dalam kandungan nutrisi, antara ladang berbeza dan juga semasa serangan epifit. Ujian termasuk kandungan air, kandungan abu, phenolics, lipid, peratus karagenan dan asid lemak, dengan perbezaan signifikan antara sampel. Nilai nutrisi berada di antara 90-93% untuk kandungan air, 40-50% bagi abu, 4-7% bagi protein, 0.04-0.15% lipid, 20-120 ng/g asid lemak, 0.1-0.7 mg/g kandungan phenolic dan 30-75% peratus karagenan. Ciri-ciri rheologi juga menunjukkan penurunan dalam ciri-ciri seperti peratusan karagenan, takat lebur, viskositi dan kekuatan gel daripada karagenan yang diekstrak daripada rumpai laut yang dijangkiti epifit. Kajian molekular menunjukkan bahawa karageenan daripada ektrak rumpai laut yang dijankiti epifit tidak menunjukkan sebarang perubahaan struktur tetapi jisim molekular menunjukkan penurunan kepada 80 KD berbanding 800 KD sampel karageenan rumpai laut sihat.

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LIST OF SYMBOLS AND ABBREVATIONS

o	-	Degree
°C	-	Degrees Celsius
%	-	Percentage
κ-	-	Kappa
λ-	-	Lambda
ι-	-	Iota
α	-	Alpha
β	-	Beta
µm	-	Micrometers
µs	-	Microsecond
cm	-	Centimeter
cm^2	-	Centimeter squared
cP	-	Centipose
DW	-	Dry weight
EFA	-	Epiphytic Filamentous Algae
g	-	Grams
g/cm^2	-	Gram per centimeter squared
ISDA	-	Integrated Services for the Development of Aquaculture and Fisheries
kDa	-	Kilo Dalton
kg	-	Kilograms
L	-	Liters
LKIM	-	Lembaga Kemajuan Ikan Malaysia
m	-	Meter
M	-	Molarity
MARDI	-	Malaysia Agriculture Research and Development Institute
mg/cm^2	-	Milligram per centimeter squared
mg/cm^3	-	Milligram per centimeter cubed
mg/g	-	Milligram per gram
MHz	-	Megahertz
min	-	Minutes
ml	-	Milliliters
mL/min	-	Milliliters per minute
mm	-	Millimeters
mm^3	-	Millimeter cubed
mt	-	Metric tons
mt/yr	-	Metric tons per year
MUFA	-	Mono Unsaturated Fatty Acids
N	-	Normality

ng/g	-	Nanogram over gram
nm	-	Nanometers
PNG	-	Philippine Natural Grade
ppm	-	Parts per million
ppt	-	Parts per thousand
PUFA	-	Poly Unsaturated Fatty Acids
RPM	-	Rounds per minute
s	-	Seconds
SD	-	Standard Deviation
SEM	-	Scanning Electron Microscope
SFA	-	Saturated Fatty Acids
UMS	-	Universiti Malaysia Sabah
USD	-	United States Dollar
v/v	-	Volume over volume
w/w	-	Weight over weight

CHAPTER 1

INTRODUCTION

1.1 Seaweeds and Their Uses

Seaweed has been utilized by humans for centuries for a wide variety of purposes. Seaweeds are generally categorized into three main divisions; Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) (Renn, 1997). Total worldwide seaweed production, both from natural sources and cultured seaweed totaled 2,005,459 tons (DW) in 1995 (Lindsey and Ohno, 1999). The breakdown of the different classes produced is listed in Figure 1.1 below.

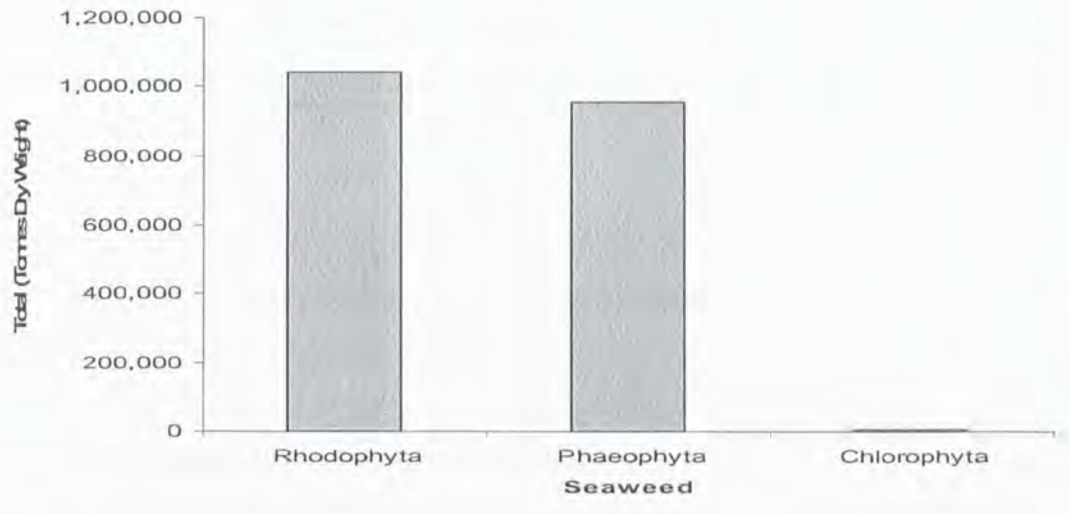

Figure 1.1: Worldwide seaweed production (1995)

Various Asian and Pacific nation cultures have produced foodstuff from seaweeds, often as salads or as ingredients used in cooking (Doty, 1987; Renn, 1997; Sulu *et al.* 2004). *Caulerpa, Sargassum, Laminaria, Porphyra, Undaria, Gracilaria* and *Ulva* are some examples of common seaweeds eaten as food (Phang, 1998; Lindsey and Ohno, 1999). The Japanese and the Republic of Korea are especially adapt in making use of their coastal resources with seaweeds featured as an important staple in their culinary palate, used for such dishes as sushi, noodle making, soup stock, jellies and as fresh salads (Norziah and Ching, 2000; McHugh, 2003). In Japan at present, seaweed farming has become an important economic activity in the country with annual harvests of 6 million tons of wet seaweed annually, valued at USD 5 billion (2002).

On a smaller scale, seaweeds have also been used as fertilizer and animal feed with little or no processing needed (Legasto, 1988; Phang, 1998). Brown seaweed with its high fiber and mineral content, such as *Laminaria, Hypnea* and *Ascophyllum,* acts as excellent soil amendment and fertilizers (Wong and Cheung, 2000). *Ascophyllum* can also be processed by grinding into a fine powder and added as additives to animal feed. Other uses for seaweed include industrial processing to yield various important substances and compounds.

Red and brown algae have been an important source of commercially valuable polysaccharides. Algins, carrageenans and agar are some of the main products derived from seaweed and contribute as a main income earner in the food and processing industry (Renn, 1997; Trono, 1998). Annual production (1995) is estimated at 108,229 tons (DW) for agarophytes, 81,858 tons (DW) for carrageenophytes and 826,178 tons (DW) for alginophytes. (Lindsey and Ohno, 1999) Each different type of seaweed produces polysaccharides with differing and unique properties that can be applied to a variety of purposes.

1.2 The *Kappaphycus* and *Eucheuma* Industry

Kappaphycus alvarezii is grown commercially around the world as a source of carrageenophytes and marketed as '*cottonii*'(Critchley *et al.*, 2004). *Eucheuma denticulatum* (*Eucheuma spinosum*) is another widely grown crop but is less popular compared to the former (Ask and Azanza, 2002). Naturally, *K. alvarezii* is found growing in the sub-littoral zone, just below the tide line with the reef area consisting of mainly sandy-corally to rocky substrate and water flow being slow to moderate (McHugh, 2004).

Past practices involved harvesting of wild stocks but during the 70's large scale commercial farms started to gain popularity in the Philippines and Indonesia, later spreading to Tanzania, Vietnam, Malaysia and several Pacific islands (Legsato, 1988; McHugh, 2004). In the Philippines alone, *K. alvarezii* (known locally as tambalang) make up 80% of total seaweed production in the country with an estimated 10,000 hectares of seaweed farms centered in areas such as south west Mindanao, Sulu and Tawi-tawi, and Southern Pahlawan (Trono, 1998). Two main grades of crops are currently sold; refined and semi-refined. *K. alvarezii* produces *kappa* carrageenan while *E. denticulatum* produces *iota* carrageenan (Sulu *et al.*, 2004; Vairappan *et al.*, 2003).

Favored method of cultivation includes; long line, semi raft, raft, cages, off bottom and staking. Cultivation requires favorable environmental conditions such as salinity, water temperature, water movement, sea bottom conditions and sunlight (Doty, 1987). The culture site is placed a distance from freshwater sources such as rivers and not too near land runoff to avoid low salinity conditions of below 30 ppt. Water temperature is best between 25 – 30 °C while water movement is essential to provide a constant stream of nutrients to the plants. The sea bottom condition may also affect growth with white sandy bottom being the best while a silt disturbed bottom may cause sediment to settle on the plant as well as reduce lighting. A growing depth of 0.5 – 1.0 m has been found to be ideal to allow adequate sunlight penetration (Gerung and Ohno, 1997; Luxton, 1999).

Kappaphycus and *Eucheuma* are sensitive to environmental changes which could lead to slower growth rate or failure of the crops (Iain, 2004). Changes in environmental parameters such as reduction of salinity, increase in water surface temperature and decrease in light intensity have been cited to induce effects like seaweed bleaching, stress and "ice-ice" disease (Largo *et al.*, 1995).

1.3 Problems in Seaweed Cultivation

Kappaphycus and *Eucheuma* farming often face various problems that may cause loss or the complete collapse of seaweed stocks. Grazing fishes and turtles, disease, epiphyte outbreak and environmental conditions can all lead to depreciation in crop production (Doty, 1987; Sulu *et al.*, 2004). *Siganids* (rabbit fishes), sea urchins and puffer fishes have been identified as pests. Turtles are often seen feeding on farms and may eat large amounts of seaweed, at times leading to the disappearances of seedlings in an entire plot (Ramlan, 2006). "Ice-ice" disease occurs during severe environmental conditions such as low salinity and high temperature (Doty, 1987). Symptoms include a loss of pigmentation turning the plant surface translucent followed by a weakening of the affected part and finally breakage from the parent plant. Largo *et al.* (1995) also found that bacteria were responsible for the cause of "ice-ice" disease, isolating two different pathogenic strains from diseased seaweed. Recently, epiphytes have been found growing on the seaweed itself leading to excessive growths during seasonal periods of the year whereby the entire surface of the seaweed is overgrown with epiphytic protrusions rendering a hair like appearance to the seaweed (Ask and Azanza, 2002).

1.4 Epiphyte Infection of Seaweeds

Of all the problems, epiphytic outbreak is the least studied. Currently very little is understood about the cause and effect of epiphytic growth on *K. alvarezii* farms and no management measures are in place to cope when extensive outbreaks are evident. The only solution thus far practiced is to isolate the afflicted crops into smaller plots and wait until the outbreaks peters out (Ramlan, 2006). If growth of the epiphyte is too dense, affected crops are taken out and dried, later to be sold along with healthy crops as batches. The effect of the presence of epiphytes is unknown and how it affects carrageenan production using epiphyte infected seaweed has not been looked into. It is believed that by using mixed batches of dried seaweed in carrageenan production, the impact of any effects from the epiphyte is minimal or nonexistent at all.

Past studies have identified *Neosiphonia* and *Polysiphonia* as the main epiphytic species inhabiting *K. alvarezii* found in the Philippines (Ask and Azanza, 2002). Cortical swelling was apparent where epiphyte thallus emerged from the plant's surface resulting in pit formation. *Neosiphonia savatieri* and *Neosiphonia apiculata* were also characterized in outbreaks from farm crops in Kudat, Malaysia (Vairappan, 2006). Yet not much else has been studied on the characteristics of these epiphytes while other countries have stated problems with epiphytes without identifying the causation agent.

It would be an important interest to note if the identical epiphyte is prevalent and responsible for outbreaks in different countries or if different epiphytes are present. It has been noted that outbreaks are seasonal in nature and may be affected by changes in weather and environmental conditions. Another avenue of interests may be to see if the presence of epiphytes on crops will affect industry standards of carrageenan production as mixed raw materials; both epiphytic infected seaweed as well as healthy seaweed, are used. Present investigations attempts to record the epiphyte outbreak in major carrageenophyte producing countries and the epiphyte's impact on the quality of the carrageenan. This study does not cover measures to address the epiphyte infection as understanding of the epiphyte and their mode of infection has yet to be established, of which this research aims to address.

1.5 Objectives

The research focused on seven main objectives;

1) To evaluate (quantitatively and qualitatively) the symptoms and extent of epiphyte outbreak in farms found in Banggi, Kudat within an annual cycle;
2) To identify and characterize epiphytes isolated from *K. alvarezii* from major carrageenan producing countries such as the Philippines and Tanzania;
3) To determine if nutritional values, phenolics content, fatty acids profile and carrageenan yield differs depending on location of farms in Kudat;
4) To determine if there exists an annual seasonal variation in nutritional values, phenolics content, fatty acids profile and carrageenan yield for *K. alvarezii* cultured in Kudat;
5) To determine if nutritional values, phenolics content, fatty acids profile and carrageenan yield for healthy and epiphyte infected *K. alvarezii* differ between both samples;
6) To carry out rheological analysis on carrageenan extracted from healthy and epiphyte infected *K. alvarezii*; and
7) To study the differences in functional groups, molecular size and chemical structure in healthy and epiphyte infected carrageenan.

CHAPTER 2

LITERATURE REVIEW

2.1 Carrageenan Industry

The ASEAN region comprising the Philippines, Indonesia and Malaysia mainly cultivates *Kappaphycus* and on a smaller extent *Eucheuma* for the production of carrageenan for the world market. Production of both *Kappaphycus* and *Eucheuma* is estimated to be 114,300 mt/yr and 22,400 mt/yr with an average selling price of USD 550-650/mt in the year 2002 (http://www.seaplant.net). The main markets for carrageenan derived from the seaweeds are mostly in Europe (35%), North America (25%) and South East Asia (25%).

2.2 *Kappaphycus* and *Eucheuma*

Kappaphycus and *Eucheuma* (Figure 2.1) are the main species of red seaweed (Rhodophyta) cultivated commercially at present around the world. The evolution of this trade can be traced back to the early 70s when farming of these two seaweeds were beginning to expand and soon found ready interests in various coastal nations until finally evolving into its current state.

Figure 2.1: *Eucheuma spinosum* (A) and *Kappaphycus alvarezii* (B)

2.2.1 *Kappaphycus* and *Eucheuma* Biology and Life Cycle

The life cycle of *Kappaphycus* and *Eucheuma* seaweed are not well understood in entire and the one suggested is only an ideal life cycle. It is believed that the seaweed employs a triphasic life cycle with gametophyte (N), tetrasporophyte (2N) and carposporophyte (2N) phases (Sulu *et al.*, 2004). The diploid tetrasporophytic phase produces haploid, non-motile meiospores called tetraspores. These produce separate haploid male and female gametophytes. A diploid carposporophyte develops *in-situ* on the female gametophyte after fertilization and the carposporophyte releases diploid carpospores which initiates the tetrasporophytic stage again (Ask and Azanza, 2002). Growth is not dependent on settling on any substrate but also grow well drifting in the water column (Lundsor, 2002). Sexual reproductive organs might be absent from the seaweed on occasions and growth is wholly dependent on vegetative growth. Vegetative growth is achieved from sectioned thallus in which all parts are able to grow into new plants and this is the propagation method most favoured for aquaculture of the species (Doty, 1987; Lundsor, 2002). Figure 2.2 below displays a summary of the *K. alvarezii* life cycle.

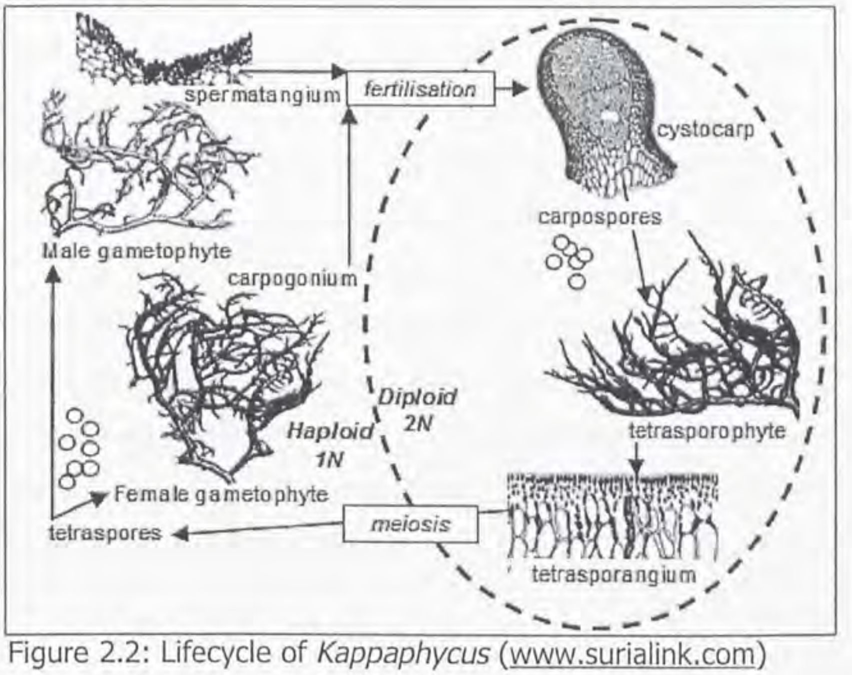

Figure 2.2: Lifecycle of *Kappaphycus* (www.surialink.com)

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