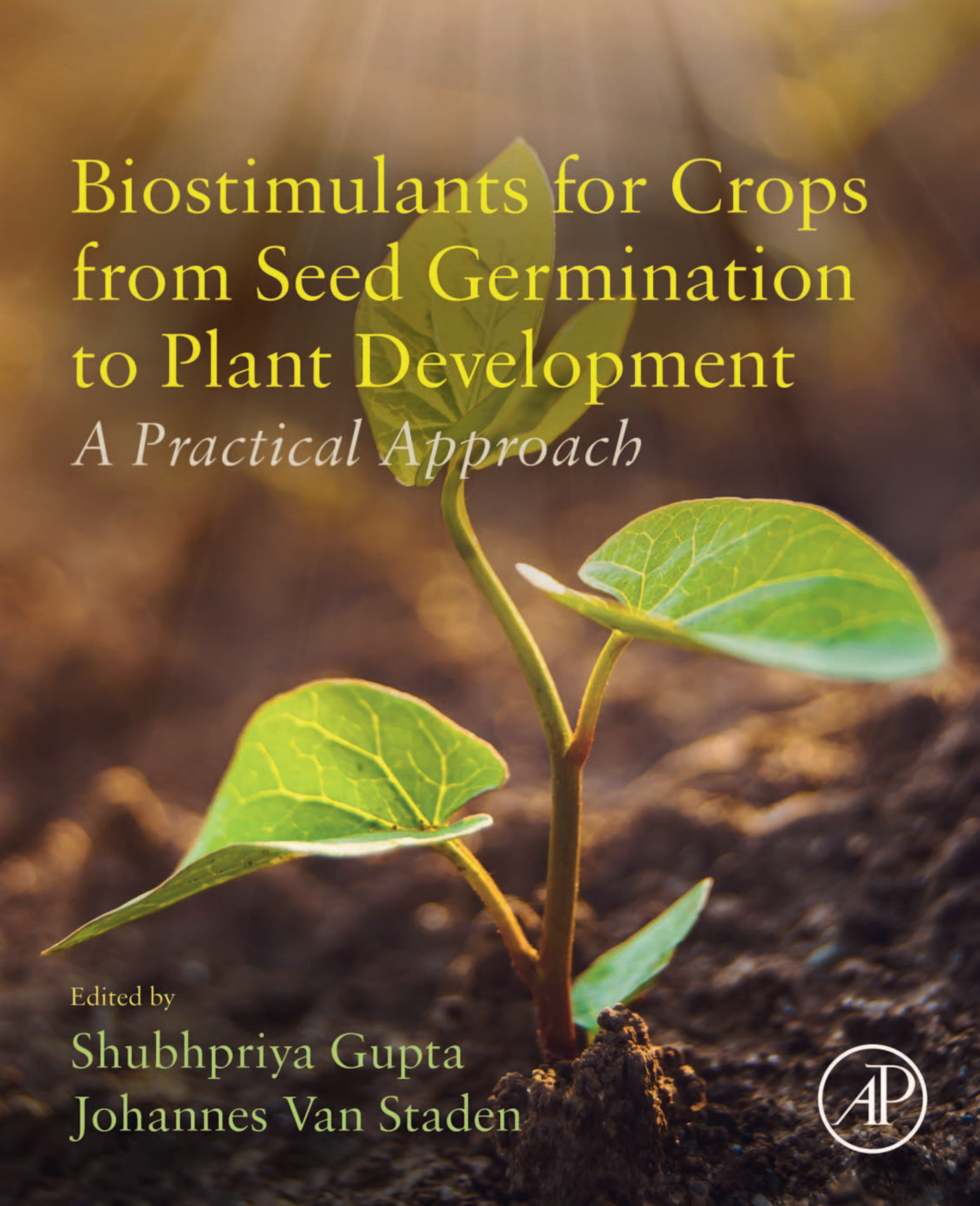

Biostimulants for Crops from Seed Germination to Plant Development

A Practical Approach

Edited by

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ELSEVIER

ACADEMIC PRESS

An imprint of Elsevier

CHAPTER

12

Extracts of seaweeds used as biostimulants on land and sea crops—an efficacious, phyconomic, circular blue economy: with special reference to *Ascophyllum* (brown) and *Kappaphycus* (red) seaweeds

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Introduction

Most of the world's seaweed-derived extracts used for their biostimulatory/bioeffector properties (i.e., abiotic and biotic bioactive properties) are manufactured from brown seaweeds, which are generally harvested from wild populations, or collected as storm-cast off the beach (e.g., *Ascophyllum, Durvillaea, Ecklonia, Laminaria/Saccharina, Sargassum*, etc.).

This chapter, however, largely refers to the red seaweed *Kappaphycus* spp., which are now one the world's largest marine "crops" with significant socio-economic benefits to those countries within a circumtropical distribution of the globe: many of these producing counties have emerging economies and seaweed cultivation is a socio-economic engine for coastal development and sustainability.

Kappaphycus spp. were initially cultivated as a source of kappa carrageenan as used extensively in processed foods and some pharmaceutical applications. Subsequently, several entrepreneurs, notably

Biostimulants for Crops from Seed Germination to Plant Development. https://doi.org/10.1016/B978-0-12-823048-0.00017-4

FIGURE 12.1

The red seaweed *Kappaphycus alvarezii* three color morphotypes from cultivation—photograph courtesy of Miguel Sepulveda, seaweed consultant.

in India, re-looked at the biomass using a proprietary biorefinery approach to sequentially remove valuable bioactive components along the value-chain. In its simplest form, a biostimulatory extract has been made from the red seaweed by pressing, thereby releasing a potassium-rich liquid which has been commercialized as a sap. The “left-over” fiber can then be used for carrageenan extraction. Other methods of producing a biostimulant extract from *Kappaphycus* have been developed. These applications and their benefits for plants are reviewed.

Kappaphycus spp. are largely propagated vegetatively and clonally. They are extensively cultivated, mostly as a monocrop and, as with on land examples in agronomy, there are parallel phyconomic issues associated with abiotic and biotic stresses in mass cultivation of seaweeds including reduced strain vigor, reduced productivity, responses to environmental challenges and associated, increasing issues with pests and diseases.

To the rescue, perhaps, comes various extracts of brown seaweeds which have been applied within varied phyconomic activities, including micropropagation and cultivation, in particular, for *Kappaphycus alvarezii* (Fig. 12.1) Similar beneficial applications for commercial seaweeds are also reported to include: the green alga *Ulva*, the browns *Laminaria/Saccharina* and the reds *Gracilaria* and *Pyropia*—which will be highlighted.

These applications are increasingly important parts of the global, marine (blue) economy which in addition are part of sustainable marine, circular economies as commercial extracts of seaweeds are being used for the alleviation of abiotic and biotic stress thereby acting as biostimulants/bioeffectors for the benefit of mass cultivated, commercially important seaweeds.

Applications of the red seaweed *Kappaphycus* as a liquid fertilizer/biostimulant

Since the early 1970s, the red seaweed genus *Kappaphycus* has been extensively farmed in southeast Asia, principally as a source of kappa carrageenan. It has also been farmed to a lesser extent in East Africa, India, Pacific Oceana, and Latin America (Hayashi et al., 2017; Neish et al., 2017). The kappa carrageenan extracted from *Kappaphycus* is mainly used in the food industry as a thickener, gelling agent, emulsion stabilizer, and re-crystallization inhibitor (Porse and Rudolph, 2017; Bixler and Porse, 2011; De Ruiter and Rudolph, 1997). Kappa carrageenan is also used in the cosmetics and pharmaceutical industries. Emerging, mechanized cultivation technologies, and biorefinery processing technologies have recently provided opportunistic pathways toward the increased utilization of cultivated *Kappaphycus* spp. biomass. Such technologies enable production of a broad range of value-added agricultural, chemical, and biofuel products that can support future value-chain development with market size and values beyond the present prospects for a scenario dominated solely by carrageenan (Neish and Suryanarayan, 2017).

India has been a leading country in developing commercial applications of *Kappaphycus*-based, biostimulant extracts for agricultural crops, despite limited farmed seaweed biomass available from domestic cultivation; the history of commercial cultivation of *Kappaphycus* in India has been summarized in Neish et al. (2017). Commercial farm development began with ocean plantings in 2000 by the Central Salt and Marine Chemicals Research Institute, Bhavnagar, India, in collaboration with Pepsi Foods Ltd. (a subsidiary of Pepsico India Holdings Ltd.). The economic viability of open-sea cultivation was established in 2001, the technology of commercial cultivation was transferred to PepsiCo, India Holdings Ltd. They then initiated pilot-scale farming along the Palk Bay and Gulf of Mannar coasts of Tamilnadu (Eswaran et al., 2002). The cultivation business rights for *K. alvarezii* were transferred by Pepsi Foods Ltd. to Aquagri Processing Pvt. Ltd. in 2008, and the same company continues to be successful in expanding cultivation activities in Tamil Nadu to this day.

CSMCRI scientists (Eswaran et al., 2005) discovered that fresh *Kappaphycus* biomass could be divided into a liquid "sap" fraction that could be utilized as an agricultural biostimulant, and a solid "pulp" fraction that could be used as raw material for the production of carrageenan. Since then, *Kappaphycus* "K-sap" has been field-tested for its efficacy as a biostimulant for a variety of agricultural applications (Table 12.1). Generally, K-sap is applied as a foliar spray during the early stages of plant growth or is used to soak seeds before sowing for faster germination.

Table 12.1 Summary of various applications of extracts from *Kappaphycus* sp.? on agricultural crops.

Commodity	Type of application	Concentrations	Highlights of results	References
Okra	Foliar	2.5%, 5%, 7.5%, 10%, and 15% K-sap	Yield and nutrition quality of okra fruit got significantly increased (20.47%) at LSF spray 2.50%.	Zodape et al. (2008)
Green gram	Foliar	5%,10%, and 15% K-sap	10% K-sap significantly in yield of pods, weight of pods, and seed yield per plant and 100 seed weight.	Zodape et al. (2010)

Continued

Table 12.1 Summary of various applications of extracts from *Kappaphycus* sp.? on agricultural crops.—cont'd

Commodity	Type of application	Concentrations	Highlights of results	References
Tomato	Foliar	2.5%, 5%, 7.5%, 10%, and 15% K-sap	5% K-sap showed the best yield and yield attributing characters and quality of fruit over rest.	Zodape et al. (2011)
Rice, groundnut, pepper	–	1%, 2%, 5%, and 10% + 100% recommended dose of fertilizer (RDF)	Lower concentrations (2%) promote seed germination, growth, and yield in crop plants.	Babu and Rengasamy (2012)
Rice-potato-green gram	Foliar	2.5%, 5%, 7.5%, 10%, and 15.0% v/v K-sap + RDF	Highest productivity at 15% + RDF in terms of rice equivalent yield (REY) with net monetary returns of RS. 175,608.60 $ha^{-1}yr^{-1}$ and benefit: cost ration of 2.06.	Pramanick et al. (2014a)
Rice	Foliar	2.5%, 5%, 7.5%, 10%, and 15% K-sap + RRF	15% K-sap + RDF resulted to 41.47% increase in transplanted rice with maximum straw yield.	Pramanick et al. (2014b)
Corn	Foliar	2.5%, 5%, 7.5%, 10%, and 15% K-sap plus recommended rate of fertilizers (RRF) (50%, 100%)	Increase in plant height at 7.5% K-sap + 100% RRF compared with 100% RFF only.	Singh et al. (2016)
Red gram	Soaking (powder)	0.03%	Enhanced seed germination.	Karthikeyan and Shanmugam (2016)
	Foliar	0.02%	Enhanced yield of net grain up to 35.93% at vegetative phase, preflowering stage, and pod maturity.	
Rice	Foliar	2.5%, 5%, 7.5%, 10% and 15% K-sap + RRF 80:40: 40 (N: P_2O_5: K_2O kg/ha)	15% K-sap + 50% RRF significantly increased the grain yield in rice at 29% and at least 43% climate change (CC) impact ton^{-1} of rice.	Sharma et al. (2017)

Table 12.1 Summary of various applications of extracts from *Kappaphycus* sp.? on agricultural crops.—cont'd

Commodity	Type of application	Concentrations	Highlights of results	References
Sugarcane	Foliar	0.3% K-sap setts treatment 1% K-sap at growth stages	Higher cane yield obtained in K-sap treated (20.47%–28.79%) over control; natural potash from seaweed may improve yield the quality of the cane and the juice.	Karthikeyan and Shanmugam (2017)
Rice-potato-blackgram	Foliar	2.5%, 5.0%, 10%, and 15.0% V/V + recommended RDF	Highest productivity in terms of REY at 15% K-sap + 75% RDF with annual net monetary returns of 231,686 ha^{-1} and benefit: cost ratio of 2.61.	Pramanick et al. (2018)
Corn	Foliar	2.5%, 5%, 7.5%, 10%, and 15% K-sap	Grain yield increased significantly by 18.54% at 15% K-sap + 50% RDF resulting to increase in the number of rows in cob, cob length, and 100 grain weight.	Basavaraja et al. (2018)
Banana	Foliar	LBS6S (1 mL/L) LBS6S (5 mL/L)	Improved bunch weight significantly by 25.24% at LBS6S1mL/L over control.	Ravi et al. (2018)
Orchid	Foliar	0,6,12,25,50,100 mg/L aqueous extract of K	50 mg/L concentration showed the best results in root stimulation, plantlet development, fresh mass gain, and newly formed shoots.	Amatuzzi et al. (2020)
Tomato	Foliar	20 mL of extracts from shiitake, BFIICaB *(K. alvarezii*), and BKPSGII (*K. alvarezii* + *Sargassum* sp.)	Promoted the highest enzymatic activity, promoted activities of B-1,3-glucanase, peroxidase, and PAL. All three extracts preened activities as potential inducers of resistance in tomato plants against *Fusarium* wilt.	de Melo et al. (2020)

Continued

Table 12.1 Summary of various applications of extracts from *Kappaphycus* sp.? on agricultural crops.—cont'd

Commodity	Type of application	Concentrations	Highlights of results	References
Rice-potato-green gram.	Foliar	7.5% K−sap + 50% RDF, 0.0% K−sap + 50% RDF, 7.5% K−sap + 100% RDF	Best results obtained in 7.5% K−sap + 100% RDF with the highest system productivity.	Pramanick et al. (2020)

PAL, *Phenylalanine Ammonia Lyase;* RDF, *Recommended Dose of Fertilizers;* RRF, *Recommended Rate of Fertilizers.*

Biostimulant impacts on *Kappaphycus* phyconomy and value-chains

A history of *Kappaphycus* and *Eucheuma*, known collectively as "eucheumatoids" seaweed farming development was summarized in Neish et al. (2017) and value-chain issues tied to that history were discussed in Neish and Suryanarayan (2017). Seaweed biostimulant products had already begun to have transformative impacts on seaweed phyconomy and value-chain development in ways that move the industry in new directions. By far, the most significant impact was on value-chains. Early development of *Kappaphycus*-based biostimulants in India led to development of biostimulant production in Indonesia, where seaweed supply constraints did not inhibit market development in ways that were endemic to India. Positive impacts of biostimulant product development transformed value-chains in three fundamental ways, namely:

1. Agricultural biostimulant products were a major new market for *Kappaphycus* biomass beyond its use as a raw material for the manufacture of carrageenan.
2. Biostimulant manufacture necessarily commenced with live seaweed, so value addition commences at, or near, farming communities.
3. Biorefinery technologies used to make biostimulants are designed to utilize the full biomass and to generate minimal waste in a way that is adaptable to production of a wide-range of products, even beyond hydrocolloids and biostimulants.

Dependence on slow-growing markets for marine hydrocolloids resulted in *Kappaphycus* spp. markets becoming virtually a "zero-sum game" during the 21st Century (Neish and Suryanarayan, 2017). Adopting biorefinery processing of live seaweed biomass enabled diversification and expansion of tropical red seaweed production to serve growing markets. In the near-zero-sum market dominated by hydrocolloids, the growth of Indonesian *Kappaphycus* sales occurred at the expense of Filipino producers who suffered impacts from cost-adding factors such as typhoon-risk and peace-and-order impacts that Indonesian producers did not have to contend with. With expanding and growing markets, producing countries can all benefit from expanded farming.

Value-chain impacts of biorefinery products are still in their early days, but trends toward development are firmly in place. However, the direct impacts of seaweed-derived biostimulants on seaweed crop production, as described in the present chapter, are at an even earlier stage of development. Developments described below are leading toward their future commercial applications for biostimulants in an adaptive *Kappaphycus* phyconomy.

Brown seaweed extracts as bioeffector/biostimulants in seaweed cultivation

Seaweed extracts applied as biostimulants and/or bioeffectors are mainly used in agricultural crops, as reviewed extensively by, Khan et al. (2009), Craigie (2011), Dmytryk and Chojnacka (2018), Górka et al. (2018). Reports have shown that extracts of selected seaweeds can improve growth and increase plant resistance to abiotic and biotic stresses (Shukla et al., 2019). Brown seaweeds have been commonly used as a source of biomass for various extraction procedures (hydrolyses) to produce an extract (i.e., powder or liquid) for agricultural crop applications.

Brown seaweeds generally dominate the intertidal and subtidal zones, particularly in northern zones. Hence, several, natural population management studies (Ugarte and Sharp, 2001, 2012) have been reported. Among the brown seaweeds, *Ascophyllum nodosum*, *Fucus* spp., and *Laminaria/Saccharina* spp., flourish in temperate waters, while *Sargassum* spp. and *Turbinaria* spp. (Ang, 1985; Trono and Lluisma, 1990; Hurtado and Ragaza, 1999; Srinivasa Rao and Umamaheswara Rao, 2002; Chan et al., 2013) prosper in tropical waters. These are some of the most sought-after seaweed genera mainly because of their availability. A number of commercial seaweed extracts used in agricultural and horticultural industries are already available in the market as reported by Khan et al. (2009) and Shukla et al. (2019).

This chapter would be remiss if it did not acknowledge that the first report of using an(y) extract of seaweed to benefit another cultivated seaweed was the use of a commercial extract from the South African brown, kelp *Ecklonia maxima* (extract produced by low-pressure cavitation), i.e., Kelpak, used on the algae *Gracilaria* (Rhodophyta) and *Ulva* (Chlorophyta) (Robertsson Anderson et al., 2006). This extract was evaluated in a scenario for enhancing seaweed biomass production as a food for abalone. In spite of promising results, it is not known why this work was not taken further.

This chapter largely focuses on the use of an extract from the temperate, intertidal, fucoid brown alga *A. nodosum* (Fig. 12.2) (Pereira et al., 2020) and its applications for benefits to the tropical red alga *Kappaphycus* for micropropagation and field cultivation. Unprocessed *A. nodosum* and its alkaline extracts have been widely used as a biostimulant in terrestrial agricultural production for a wide variety of crop species (MacKinnon et al., 2010; Craigie, 2011; Spann and Little, 2011). Other authors have reported on the direct benefits from applications of various extracts of seaweeds on crop performance such as (a) enhanced root vigor (Crouch and Van Staden, 1992), (b) increased leaf chlorophyll content (Blunden et al., 1996), (c) increased number of leaves (Rayirath et al., 2008), (d) improved fruit yield (Arthur et al., 2003; Kumar and Sahoo, 2011; Kumari et al., 2011), (e) heightened flavonoid content (Fan et al., 2011), and (f) enhanced vegetative propagation (Leclerc et al., 2006). However, more substantial and significant improvements associated with the applications of extracts of seaweeds include improved tolerances toward abiotic stresses, including drought (Shukla et al., 2018; Zhang and Ervin, 2004; Spann and Little, 2011), salinity (Shukla et al., 2019), ion toxicity (Mancuso et al., 2006), freezing stress (Rayirath et al., 2009), and high temperature (Zhang and Ervin, 2008).

Micropropagation

More recently, the varied uses of *A. nodosum* extracts, in various phases of seaweed production, have been published. The first report of such novel applications was made for various species and strains of

FIGURE 12.2

The brown seaweed *Ascophyllum nodosum.*
Ascophyllum nodosum, Spiddal, Co. Galway, Ireland; with mature conceptacles © M.D.Guiry, AlgaeBase.

Reproduced with permission.

the red seaweed *Kappaphycus*. Hurtado et al. (2009) used *Ascophyllum* Marine Plant Extract Powder (AMPEP) as the main ingredient for various culture media, accompanied by specific plant growth regulators (e.g., PGRs: IAA—indole-3-acetic acid and kinetin) to develop new plantlets of various *Kappaphycus* strains, using standard (plant) tissue culture techniques. The research aimed to produce new vegetative propagules for nursery and out-planting purposes and was a technique that could be applied for much-needed steps in the red alga's propagation for the general and successful multiplication and commercial cultivation of eucheumatoid seaweeds. Hurtado et al. (2009) found that strain/species responded differently in the production of new laterals (or direct shoots), at different levels of *Ascophyllum* extract addition, in the presence of PGRs. A 3−5 mg/L AMPEP + PGR showed a significantly reduced duration for emergence of lateral shoots. The report of Yunque et al. (2011) used the same protocol on *K. alvarezii* strains such as vanguard brown and tungawan green. The combination of pH, temperature, and density of explants was studied. The same authors reported that the first shoot emergence was observed on day nine in *K. alvarezii* (tungawan green). Another study used spindle inhibitors (e.g., colchicine and oryzalin) (Hugdahl and Morejohn, 1993), in combination with AMPEP and PGRs to produce more than three new lateral shoots per segment (Neves et al., 2015; Tibubos et al., 2017). However, the former used the normal, red algal culture media such as von Stosch (VS50). The formation of more than three shoots per segment of *Kappaphycus* is a manifestation of polyploid organisms which potentially can grow faster, while being more robust and importantly enhanced resistance to diseases.

The most recent reports on the use of the seaweed extract AMPEP and its variant AMPEP K^+ (potassium boosted) on the micropropagation of several strains of *Kappaphycus* were reported by Ali et al. (2018a, 2020a). In both studies, 3 mg/L of AMPEP, or AMPEP K^+, in combination with 1 mg/L of IAA and kinetin, stimulated the generation of new laterals (i.e., formation of axes, length of the new shoots, and increased lateral shoots after 45 days of incubation under laboratory conditions). The addition of IAA and kinetin with AMPEP K^+ was demonstrated to be more effective than AMPEP alone in the micropropagation of the *Kappaphycu*s tested. In addition, the same authors claimed that new lateral shoots were formed within 9–15 days of incubation. Both studies demonstrated the efficacy of a commercial, soluble extract powder from the brown seaweed *A. nodosum*, normally applied as a land plant biostimulant. It was effective for the generation of microplantlets from several strains of the tropical red seaweeds *K. alvarezii, K.appaphycu*s *striatus*, and *K. melasianus*. This was an important step toward the mass production for seedlings in eucheumatoid farming areas.

Other seaweeds have also been evaluated as candidates for treatment with AMPEP under controlled laboratory conditions. Treatments of seawater with AMPEP at concentrations of 0.1 and 1.0 mg/L stimulated the growth rates of the tropical/subtropical red seaweed *Gracilaria caudata* (Souza et al., 2019), while Dawange and Jaiswar (2020) showed that a 0.1 g/L and 30 min exposure to AMPEP resulted in a higher daily growth rate (DGR) ($7.6 \pm 0.4\%$ day^{-1}) and a number of branches ($40.3 \pm 8.6\%$). The same treatments resulted in the highest total carbohydrates, lipid and phenolic contents at $7.78 \pm 0.08\%$, $0.83 \pm 0.11\%$, and $0.38 \pm 0.01\%$, respectively. Their findings further showed that a combination of von Stosch Enriched Seawater (VS-ES)+AMPEP at 5.0 mg/L stimulated the development of the pigments phycoerythrin, phycocyanin, allophycocyanin, and chlorophyll *a* content in *Laurencia catarinensis* (another tropical red seaweed of potential commercial value). Table 12.2 summarizes reports on the use of soluble, seaweed extract powder from *A. nodosum* for the micropropagation of *Kappaphycus* and *Gracilaria*.

Table 12.2 Summary of the uses of extracts from the brown seaweed *Ascophyllum nodosum* for the micropropagation of *Kappaphycus* and *Gracilaria*.

Species	Variety	Optimum conc.	Highlights of results	References
Kappaphycus alvarezii				
Purple	–	3 mg/L AMPEP; 0.1 mg/L AMPEP + PGR	Early emergence of direct shoots at day 21.	Yunque et al. (2011)
Brown	adik-adik	3 mg/L AMPEP; 001 mg/L AMPEP + PGR	Early emergence of direct shoots at day 17.	
	kapilaran	1 mg/L AMPEP + PGR (1 mg/L IAA + Kinetin)	Early emergence of direct shoots at day 49.	
	vanguard	0.1 mg/L AMPEP + PGR (1 mg/L IAA + Kinetin)	Early emergence of direct shoots at day 22.	

Continued

Table 12.2 Summary of the uses of extracts from the brown seaweed *Ascophyllum nodosum* for the micropropagation of *Kappaphycus* and *Gracilaria*.—cont'd

Species	Variety	Optimum conc.	Highlights of results	References
Brown	tambalang	5 mg/L AMPEP K^++ PGR (1 mg/L IAA + Kinetin)	Longest direct shoot formed at 9.6 mm at day 45.	Tibubos et al. (2017)
		0.1 mg/L AMPEP K^++ PGR (1 mg/L IAA + Kinetin) 1 mg/L oryzalin	Longest direct shoot formed at 8.7 mm at day 45.	
		0.5–10 mg/L AMPEP K^++ PGR (1 mg/L IAA + Kinetin)	100% occurrence of direct shoots at day 45.	
Brown	crocodile	3 mg/L AMPEP + PGR (1 mg/L IAA + Kinetin)	Early emergence of direct shoots at day 10. 100% direct axes formed at day 45.	Ali et al. (2018a)
Green	–	3 mg/L AMPEP + PGR (1 mg/L IAA + Kinetin)	Early emergence of direct shoots at day nine. Longest direct shoot at 7 mm at day 45.	
Brown	–	3 mg/L AMPEP K^+ + PGR (1 mg/L IAA + Kinetin)	Longest direct shoot at 7.2 mm at day 45. 93.3% of direct shoots formed at day 45.	Ali et al. (2020a)
Green	tungawan	3 mg/L AMPEP	Early emergence of direct shoots at day 19	Yunque et al. (2011)
Kappaphycus malesianus	–	3 mg/L AMPEP K^++ PGR (1 mg/L IAA + Kinetin)	98% of direct shoots formed at day 45.	Ali et al. (2020a)
Kappaphycus striatus		3 mg/L AMPEP K^++ PGR (1 mg/L IAA + Kinetin)	85.7% of direct shoots formed at day 45.	
Green	–	1 mg/L AMPEP + PGR (1 mg L-1 IAA + Kinetin)	Early emergence of direct shoots at day 25.	Yunque et al. (2011)
	–	3 mg/L AMPEP + PGR (1 mg/L IAA + Kinetin)	Early emergence of direct shoots at day 13	Ali et al. (2018a)
	–	3 mg/L AMPEP K^++ PGR (1 mg/L IAA + Kinetin)	Highest number of direct shoots formed segment^{-1} (3.5) 99.3% of direct shoots formed at day 45	Ali et al. (2020a)
Gracilaria *blodgetti*	–	0.1 mg/L AMPEP	Highest growth rate of 2.7% day^{-1}	Batista de Vega et al. (2020)
		0.1 mg/L AMPEP K^+	Highest growth rate of 1.46% day^{-1}	

Table 12.2 Summary of the uses of extracts from the brown seaweed *Ascophyllum nodosum* for the micropropagation of *Kappaphycus* and *Gracilaria*.—cont'd

Species	Variety	Optimum conc.	Highlights of results	References
Conchocelis *Pyropia yezoensis*	–	Von Stosch with AMPEP or Kelpak at 15, 20 and 25°C	Temperature was the main driver for conchocelis formation in both biostimulants. 15°C at 0.001 ppm Kelpak promoted the early formation of conchocelis 20°C at 1 ppm AMPEP promoted the early formation of conchocelis.	Kim, Pers. comm.

Sea-based nursery

To date, the report of Ali et al. (2020a) is the only study made on the sea-based nursery growth of micropropagated *Kappaphycus* strains. About 45-day-old microplantlets bearing > 7 mm long shoots were generated in the laboratory and then placed in cylindrical net cages (25 cm×40 cm); 500 microplantlets were grown in the sea for 45 days which were to be later used as vegetative propagules for sea-based, field cultivation purposes. The use of vegetative cultivars of *Kappaphycus* which were dipped in AMPEP before out-planting for nursery purposes, over a 12-month study resulted in healthy and robust propagules (sometimes referred to as "seedlings") suitable for commercial cultivation (Hurtado et al., 2012). Table 12.3 summarizes reports on the use of a soluble, seaweed extract powder from *A. nodosum* as used in tank-sea-based culture (nursery) of *Kappaphycus* spp.

Field cultivation

Vegetative strains

Borlongan et al. (2011) used two strains of *K. alvarezii* which were grown at different depths (i.e., surface and 150 cm below surface) with and without immersion (dipping) into an AMPEP solution. Results showed that propagules dipped in AMPEP solution, before out-planting, had significantly higher growth rates than the control. Likewise, the growth rates of the red alga decreased with increasing water depth. The highest growth rates obtained were strains grown at 0–50 cm, with prior AMPEP treatment. Each strain responded differently to AMPEP in growth rate, similar to responses into land plant applications as reviewed extensively by Khan et al. (2009) and Craigie (2011). The concentration of the AMPEP solution and duration of dipping was found to be important. Hurtado et al. (2012) reported that a 30-min dip using 0.1 g/L AMPEP was optimal to obtain satisfactory growth rates of three color morphotypes of *K. alvarezii* (i.e., reddish-brown, yellowish-brown, and purple).

Brazilian phyconomists also reported on the use of AMPEP as a growth stimulant for *K. alvarezii* grown in tubular nets which were hung on commercial floating rafts (Loureiro et al., 2014a).

Table 12.3 Summary of the use of a soluble, seaweed extract powder from *Ascophyllum nodosum* as used in tank-sea-based culture (nursery) of *Kappaphycus* spp.

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Micropropagated	*Gracilaria changii*	–	25% PES supplemented with 5 mg/L AMPEP	High growth rate at 4.7% day^{-1} under tank culture conditions.	Jong et al. (2015)
Micropropagated	*G. caudata*	–	0.1 and 1 mg/L AMPEP; VSES/ 2 + 5 mg/L AMPEP	Stimulated the growth rates; evident of primary branches formation higher concentrations of chlorophyll-a and phycoerythrin.	Souza et al. (2019)
Micropropagated	*L. catarinensis*	–	VSES/ 2 + 5 mg/L AMPEP	Evident of primary branches formation; highest concentrations of phycoerythin, phycocyanin, allophycocyanin and chlorophyll-a.	
Micropropagated	*K. alvarezii* *K. striatus* *K. malesianus*	Brown green green	1 g:10 L seawater: 1 kg seaweed (AMPEP K^{+})	~100% growth rate of each strain/species in cylindrical net cages for 45 days.	Ali et al. (2020a)
Vegetative	*K. alvarezii*	yellowish-brown	0.1 g/L AMPEP at 30 min dipping	Seasonal DGR with highest DGR (5.7%–6.7%) from Jan–Feb over a period of 12-month growth period in the field.	Hurtado et al. (2012)

Table 12.3 Summary of the use of a soluble, seaweed extract powder from *Ascophyllum nodosum* as used in tank-sea-based culture (nursery) of *Kappaphycus* spp.—cont'd

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Sporophytes	*Sarcostemma angustissima* Saccharina latissima	none	30 and 60 min dipping in Kelpak at 0.001, 0.005, 0.05, 1 and 5 mL/L	Both species showed highest SGRs at day five posttreatment with Kelpak. Differences in SGR is temperature-dependent with no clear effect of Kelpak concentrations nor dipping time. Bleaching and blade degradation of sporophytes at 19°C while 100% mortality at 23°C and 25°C during the first 7 days of incubation.	Umansor et al. (2020)

No significant differences were found in growth rates between AMPEP-treated *Kappaphycus* (i.e., 4.1% DGR) and the untreated control (i.e., 3.6% DGR). The same authors found that the treatment of propagules (seedlings) of *K. alvarezii* with an AMPEP solution could be used as an alternative strategy to enhance resistance to lower temperature effects on crops in tanks and in the sea during periods of low-surface seawater temperatures. This result is economically important, especially during the transition period from laboratory to out-planting in the sea.

Marroig et al. (2016) reported on AMPEP-pretreated *K. alvarezii* propagules (seedlings) before being grown in tubular nets, which were hung from floating rafts. These plants showed low growth rates of 0.2%−1.9% DGR during the three periods of field study, which were much lower in comparison to results obtained by Loureiro et al. (2014b) using the same culture technique and concentration of AMPEP (20 g/L) which is probably because of time of cultivation.

Strains of *K. alvarezii* (crocodile and giant) and *K. striatus* responded differently in growth performance when dipped with AMPEP (1 g AMPEP:10 L seawater:1 kg seaweed, 30 min dipping) before out-planting. Positive growth rates were observed in all strains tested during each of the three grow-out cycles, with the exception of the September−October period for both *K. alvarezii* strains. However, significant differences ($P < .01$) in daily growth rate were only determined between the strains dipped in AMPEP versus control during the September−October periods (Ali et al., 2018b).

Micropropagated strains

Ali et al. (2020b) first reported on the use of micropropagated *Kappaphycus* for field cultivation. They determined that AMPEP, enhanced with potassium (i.e., AMPEP K^+) increased mean DGRs (i.e., 3.8%–5.1%) for four strains of *Kappaphycus*, when dipped (i.e., DGR = 2.5%–4.1%). The highest mean DGR (i.e., 5.1%) was recorded between October and January for K. *alvarezii* (green), followed by *K. striatus* (5.03%), *K. alvarezii* (brown, 4.7%), and *Kappaphycus malesianus* (4.1%) when dipped in AMPEP K^+. The lowest mean DGRs (i.e., 2.5%–2.8%) were recorded in control plants in the August–October period. This study demonstrated that the application of AMPEP K^+ was an effective tool in phyconomic crop management to increase the productivity and quality of selected *Kappaphycus* species and strains. Table 12.4 summarizes reports on the use of seaweed extract powder from *A. nodosum* in the field cultivation of *Kappaphycus* spp.

Table 12.4 Summary of the use of soluble seaweed extract powder from *Ascophyllum nodosum* on the field cultivation of *Kappaphycus* spp.

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Vegetative	*Kappaphycus alvarezii*	giant tungawan	0.1 g/L AMPEP	Higher DGRs at the surface than at 150 cm in both varieties with AMPEP treated than untreated two varieties, 3.1% DGR at water surface (0 cm), 1.3% DGR at 150 cm below water surface. 4.1% DGR at water surface, 1.4% DGR at 150 cm below water surface	Borlongan et al. (2011)
Vegetative	*K. alvarezii*	reddish-brown yellowish-brown purple	0.01–0.1 g/L at 30 min dipping	Lower concentrations of AMPEP and shorter time of dipping, DGR is species-specific and seasonal. Highest DGR at 0.01 g/L at 30 min dipping, Highest DGR at 0.01 g/L at 30 min dipping, Highest DGR at 0.01 g/L at 60 min dipping.	Hurtado et al. (2012)

Table 12.4 Summary of the use of soluble seaweed extract powder from *Ascophyllum nodosum* on the field cultivation of *Kappaphycus* spp.—cont'd

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Vegetative	*K. alvarezii*	purple	0.1 g/L AMPEP at 30 min dipping	Better growth performances with AMPEP dippings than untreated ones positive growth rates with AMPEP dipping, Higher phenolic content and ferrous chelating activity with AMPEP dipping than those untreated ones.	Hurtado et al. (2012)
Vegetative	*Kappaphycus striatus*	green	0.1 g/L AMPEP at 30 min dipping	Negative growth rates without AMPEP dipping Higher phenolic content and ferrous chelating activity with AMPEP dipping than those untreated ones.	Hurtado et al. (2012)
Micropropagated	*K. alvarezii* *K. malesianus* *K. striatus*	brown green	1g:10L:1 kg (AMPEP K^+: seawater: Seaweed)	Higher DGRs in all three species than those untreated ones for three growth cycles at 45 days per growth cycle. Highest DGR of 4.7% in Nov–Jan. Highest DGR of 5.1% in Nov–Jan. Highest DGR of 4.1% in Nov–Jan. Highest DGR of 5.0% in Nov–Jan.	Ali et al. (2020b)

Mitigating properties

Epi-endophytes are normally filamentous algae like *Ulva* spp. which are attached either loosely to the surface of the cultured seaweed species or attached by means of penetrating rhizoids which grow into the cortical and or the medullary layers of the host plant. Leonardi et al. (2006) described various host-epiphyte associations.

The efficacy of the seaweed extract AMPEP to reduce the occurrence of *Neosiphonia* sp. showed promising results when tested with two strains of *K. alvarezii* (giant tambalang and tungawan) (Borlongan et al., 2011). The authors claimed that the percentage occurrence of *Neosiphonia* sp. infection (6%−50% at all depths) of both *Kappaphycus* varieties with AMPEP treatment was significantly lower than the controls (i.e., 10%−75% at all depths). Furthermore, the authors observed a higher percentage occurrence of *Neosiphonia* sp. infection when the strains were grown at the water surface (0 cm) as compared to 50, 100, and 150 cm below the water surface.

Loureiro et al. (2012) confirmed results from Borlongan et al. (2011) when the former tested the efficacy of AMPEP on *Polysiphonia subtilissima*−infected *K. alvarezii* under *in vitro* conditions. The bleaching of the noncorticated portions of *Polysiphonia subtilissima* thalli that were cultivated as simulated epiphytes with AMPEP samples suggested that AMPEP protected *K. alvarezii* from hydrogen peroxide effects. They claimed that the AMPEP powder acted as a potential "vaccine" which elicited the activation of *K. alvarezii* natural defenses against pathogens and consequently ameliorated the negative effects of long-term exposure to oxidative bursts.

In another field study, Marroig et al. (2016) found that the epi-biont settlement of *K. alvarezii* grown in tubular nets hung from rafts was reduced following treatment with AMPEP. Control samples displayed higher biomass of epi-bionts (0.76 ± 0.52 g [dry mass]/tubular net) when compared to the AMPEP-treated samples (0.26 ± 0.27 g [dry mass]/tubular net).

Both vegetative (Ali et al., 2018b) and micropropagated (Ali et al., 2020b) strains of *Kappaphycus*, when pre-treated with AMPEP or AMPEP K^+ before out-planting to the sea, demonstrated lesser degrees of *Melanothamnus apiculatus* (formerly *Neosiphonia apiculata*) incidence, as compared with the untreated control. However, the response of each *Kappaphycus* strain to AMPEP was different. The lower percentage incidence of *M. apiculatus* in AMPEP-treated *Kappaphycus* strains was possibly because of effects of oxidative bursts (i.e., extracellular production of hydrogen peroxide), which can be extremely aggressive or even harmful to individual thalli or attaching spores and epiphytes. Molecular studies are currently exploring this effect.

Gracilaria fisheri grown under controlled conditions was also tested for the efficacy of AMPEP. A significant reduction of epiphytes (>90%) was observed after one week of treatment of AMPEP at 1 g/L concentration, as compared to those treated with 0.1 g/L PES (Provasoli's Enriched Seawater) (Chirapart et al., 2019).

Umanzor et al. (2019) reported that A*scophyllum* extract and Kelpak (an extract of *Ecklonia maxima*) enhanced the growth capacity of the temperate brown kelp *Saccharina* in the northwestern Atlantic when exposed to sub-optimal water temperatures (18°C), allowing them to overcome thermal stress more effectively, while maintaining their growth rate. The above information on the use of AMPEP seaweed extract providing both abiotic and biotic stress relief is promising and if widely integrated can provide economic gains for farmers of many types of seaweed. Earlier reports on the use of an extract of *A. nodosum* to cope with biotic and abiotic stresses, e.g., mitigation of the occurrence of ice-ice, epi-endophytes, epi-bionts, and thermal tolerance in treated seaweeds (Table 12.5).

Carrageenan quality

An account of the varying benefits of AMPEP-treated *Kappaphycus* would be incomplete without describing the effects on the carrageenan quality of field cultivated *Kappaphycus* strains. To date, there is increasing evidence on the positive effects of AMPEP on yield, viscosity, and gel strength of

Table 12.5 Summary of the use of soluble seaweed extract powder from *Ascophyllum nodosum* to mitigate the occurrence of ice-ice, epi-endophytes, epi-bionts and thermal tolerance in treated seaweeds.

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Vegetative	*Kappaphycus alvarezii*	–	15–20g/L AMPEP	Reduced presence of *Cladophora* and *Ulva* after 45 days of in vitro growth.	Loureiro et al. (2010)
Vegetative	*K. alvarezii*	giant tambalang	0.1g/L AMPEP	Lower occurrence of *Neosiphonia* sp. at all depth levels with AMPEP treated than those untreated ones. Tungawan variety showed lower % occurrence of *Neosiphonia* sp. than variety tambalang in all depth levels.	Borlongan et al. (2011)
Vegetative	*K. alvarezii*	–	20 g/L AMPEP	AMPEP acted as a potential vaccine, eliciting the activation of *K. alvarezii* natural defenses against pathogens and ameliorated the negative effects of long-term exposure to oxidative bursts.	Loureiro et al. (2012)
Vegetative	*K. alvarezii*	–	20 g/L AMPEP	*K. alvarezii* treated with AMPEP were more resilient to lethal temperatures than the untreated ones.	Loureiro et al. (2014)

Continued

Table 12.5 Summary of the use of soluble seaweed extract powder from *Ascophyllum nodosum* to mitigate the occurrence of ice-ice, epi-endophytes, epi-bionts and thermal tolerance in treated seaweeds.—cont'd

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Vegetative	*K. alvarezii*	–	20 g/L AMPEP	AMPEP was efficient against epi-biont settlement on *K. alvarezii* grown on tubular nets tied to floating rafts.	Marroig et al. (2016)
Vegetative	*K. alvarezii* *K. striatus*	crocodile giant	1 g:10 L seawater: 1 kg seaweed	*K. striatus* was the most resistant among the seaweeds tested for the incidence of *N. apiculata* especially when treated with AMPEP.	Ali et al. (2018b)
Vegetative	*G. fisheri*	–	1g/L AMPEP	Significant reduction (>90%) of epiphytes in *G. fisheri* after 1 week of treatment.	Chirapart et al. (2019)
Micropropagated	*K. alvarezii* *K. striatus* *K. malesianus*	Brown green green	1 g:10 L seawater: 1 kg seaweed (AMPEP K^+)	Significant differences in % incidence of *N. apiculata* between treated and untreated of the 4 strains tested *K. alvarezii* (green) had the highest 82.4%) incidence of *N. apiculata* in Nov–Jan while the lowest (37.9%) was recorded in *K. malesianus* in Oct–Jan during the entire study period.	Ali et al. (2020b)

Kappaphycus carrageenan. Loureiro et al. (2014b) demonstrated a higher carrageenan yield in AMPEP-treated *K. alvarezii* both with 20 and 40 min dipping periods. However, Marroig et al. (2016) reported using the same concentration (i.e., 20 g/L) of AMPEP without similar effects. Ali et al. (2018b, 2020b) using vegetative and micropropagated strains of *Kappaphycus*, respectively, demonstrated higher carrageenan yield, viscosity, and gel strength, as compared with control. Furthermore, each species/strain performed differently in terms of the variables tested. Among the three species of *Kappaphycus* tested (*K. alvarezii, K. striatus*, and *K. malesianus*), *K. malesianus* showed the poorest performance in terms of carrageenan quality (Ali et al., 2020b).

There are several unpublished reports from the seaweed cultivation industry that fertilizer (i.e., N, P, and K) treatments of *Kappaphycus* during field cultivation resulted in poorer carrageenan qualities, in particular, the percentage recovery rate of carrageenan. Personal observations including interviews with some seaweed farmers (Hurtado pers. comm.) revealed claims that soaking propagules of *Kappaphycus* spp. with inorganic fertilizer, i.e., urea, on a weekly basis, resulted in robust thalli. In some cases, treated thalli were harvested, dried, and sold to processors in as little as 21 days. However, propagules of that age do not contain fully developed kappa carrageenan characteristics. It takes 45–60 days of the seaweed culture for kappa carrageenan to fully develop within the cell wall matrix (Villaneuva et al., 2011; Periyasamy et al., 2019). This information suggests that the biostimulatory effects of *Ascophyllum* extract on carrageenan development can provide benefits to the seaweed farmers. Table 12.6 summarizes earlier reports on the quality of carrageenan from *Kappaphycus* treated with an extract from *Ascophyllum nodosum*.

Table 12.6 Summary of the quality of carrageenan from *Kappaphycus* sp. treated with an extract from *Ascophyllum nodosum*.

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Vegetative	*Kappaphycus alvarezii*	–	20 g/L AMPEP	Higher carrageenan yield in AMPEP treated than those untreated ones but lower in viscosity and gel strength.	Loureiro et al. (2012)
Vegetative	*K. alvarezii*	–	20 g/L AMPEP	Significant differences in carrageenan yield between AMPEP treated and untreated *K. alvarezii*. Higher viscosity and gel strength were recorded to AMPEP untreated *K. alvarezii* than treated ones.	Loureiro et al. (2014)

Continued

Table 12.6 Summary of the quality of carrageenan from *Kappaphycus* sp. treated with an extract from *Ascophyllum nodosum*.—cont'd

Type of cultivars	Species	Variety	Optimum conc.	Highlights of results	References
Vegetative	*K. alvarezii*	crocodile giant	1 g:10 L seawater: 1 kg seaweed	Both semirefined and refined carrageenan were tested in the three samples treated and untreated with AMPEP.	Ali et al. (2018b)
Vegetative	*K. striatus*	–	–	Carrageenan yield, viscosity and gel showed higher values in AMPEP treated than those untreated ones.	Ali et al. (2018b)
Micropropagated	*K. alvarezii* *K. striatus* *K. malesianus*	brown green green	1 g:10 L seawater: 1 kg seaweed (AMPEP K^+)	Carrageenan yield and viscosity carrageenan yield (%) and viscosity (cPs) did not differ greatly among the four strains tested across the three growth cycles with either AMPEP K^+ dipping or control treatments.	Ali et al. (2020b)

Conclusions

Various extracts of several types of seaweeds have a positive role to play in improving the cultivation of a number of economically important seaweeds. Indubitably more research is required to explore the fullest potential for applications of the various and varied extracts of seaweeds in marine phyconomy.

This review does not suggest that the beneficial properties described herein are exclusive to any one seaweed, nor indeed process applied in the extraction procedure. The chapter discusses novel technical improvements to several red seaweeds in cultivation, specifically *Gracilaria* and *Kappaphycus*, and a representative brown kelp *Saccharina* and applications of brown seaweed extracts as biostimulants/ bioeffectors/biofortifiers in red seaweed micro- and macropropagation. Whereas growth and development benefits associated with marine plant extract treatments in land agriculture have been extensively documented over the past 20 years, applications in field (phyconomic) production are novel and much more recent. Regardless, a growing body of evidence suggests that through the application of extracts of brown seaweeds such as those derived from *A. nodosum* and *Ecklonia maxima* that the mitigation of biotic and abiotic stresses which have proven advantageous to horticultural and agronomic crops, including biotic and/or abiotic stress relief, can also be extended to *Kappaphycus* production (as well as other commercially important species; research is on-going). It may be reasonable to assume that biochemical and physiological effects from these marine, macroalgal extracts would impart similar benefits to other algae, as they do to land plants. However, this field of discovery has only been explored and discussed very recently.

In short, re-focusing of the science of extracts of seaweeds from land plants to marine macroalgae can contribute to significant regional and international resource development, constitute a key role in sustainable marine agriculture (phyconomy) and benefit the circular, blue economy. This review suggests that involvement of extracts of various seaweeds in Integrated Crop Management programs may provide equal value to Integrated "Marine" Crop Management programs, including red, brown, and green macroalgae and perhaps other aquatic algal species (i.e., freshwater and microalgae), leading to the sustainable development of many economically important aquatic resources.

Acknowledgments

We are most grateful to Professor M.D. Guiry for use of the photograph of *Ascophyllum nodosum* from www.algaebase.org. We thank Miguel Sepulveda—Seaweed Consultant for use of the picture of *Kappaphycus alvarezii*.

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