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A review of multiple biostimulant and bioeffector benefits of AMPEP, an extract of the brown alga *Ascophyllum nodosum,* as applied to the enhanced cultivation and micropropagation of the commercially important red algal carrageenophyte *Kappaphycus alvarezii* and its selected cultivars

Anicia Q. Hurtado[1] · **Alan T. Critchley**[2]

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Abstract

Commercially manufactured extracts of a variety of seaweeds have been used extensively for several decades for the relief of abiotic and biotic stresses for (terrestrial) agricultural crops and horticulture, to improve yield and quality. However, the use of seaweed extracts for their beneficial properties, as applied to marine macroalgae, only began in the mid to late 2000s. *Kappaphycus alvarezii* is an important red seaweed on the coasts of tropical to sub-tropical waters, mainly because of the various applications of kappa carrageenan, which is the major industrial colloid extracted from this extensively cultivated biomass. Cultivation of this seaweed has brought economic benefits to tens of thousands of seaweed farmers in Southeast Asia and other minor producing countries. Recently, *Ascophyllum* (aka. Acadian) Marine Plant Extract Powder (AMPEP), a commercial seaweed extract from the brown intertidal, macroalga *A. nodosum* was used in steps taken during the micropropagation and field cultivation of *K. alvarezii*. The reasons for utilizing this treatment included addressing the current problems facing the industry, such as decreased productivity, loss of vigor and diminished crop quality. This was brought about by shortages in the availability of good quality propagules (seedlings) and also disease and endo-epiphyte infestations, which affected the ability to grow and harvest saleable biomass, thus decreasing the income, and therefore the interest and participation of the potential seaweed farmers (based on the factor of repetitive, "drudge" labor and their derived income per unit effort). This paper reviews studies on *Kappaphycus* including the use of AMPEP specifically and also some alternative extracts to mitigate both biotic and abiotic stressors, using examples from micropropagation, field cultivation, endophyte mitigation and impacts on the resulting carrageenan qualities. Taken together, this body of evidence provides proof of concept and very promising results which may lead to further studies more specifically to identify the modes of action and the metabolic pathways by which the complex AMPEP extract might improve stress tolerances in *K. alvarezii* in order to obtain higher productivity and enhanced quality characteristics (i.e., exposure to increasing surface seawater temperature, salinity fluctuations and photo-inhibitory irradiance as well as attacks by pathogenic and opportunistic organisms).

Keywords AMPEP · *Ascophyllum nodosum*, · Biostimulant (bioeffector) · *Kappaphycus alvarezii* · Micropropagation · Growth rate · Epiphytes · Endophytes · Carrageenan · Aquatic plant extract

Introduction

Kappaphycus is a red alga grown predominantly in the tropics (Parker 1974; Doty 1985; Doty and Norris 1985; Hurtado et al. 2014a, b, 2015) and to a lesser extent in sub-tropical waters (Hayashi et al. 2014; Msuya et al. 2014). This seaweed is the world's main commercial source of kappa carrageenan (Bixler and Porse 2011; Porse and Rudolph 2017). This type of carrageenan is primarily used in a wide range of processed

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✉ Anicia Q. Hurtado
anicia.hurtado@gmail.com

[1] Integrated Services for the Development of Aquaculture and Fisheries (ISDA) Inc., MacArthur Highway, Tabuc Suba, Jaro, 5000 Iloilo City, Philippines

[2] Verschuren Centre for Sustainability in Energy and the Environment, Cape Breton University, Sydney, Nova Scotia B1P 6L2, Canada

food applications, cosmetics, personal care products and pharmaceutical ingredients (Campbell and Hotchkiss 2017; Loureiro et al. 2017). Most recently, in attempts to diversify and add further value to the cultivated biomass, *Kappaphycus* has been used as a source of biostimulant itself and it has been tested on several agricultural crops (Zodape et al. 2010; Babu and Rengasamy 2012; Pramanick et al. 2014; Singh et al. 2016; Sharma et al. 2017) and also as feedstock for multiple stream processing (biorefinery; Ortiz-Tena et al. 2017) and for biofuel (Neish and Suryanarayan 2017).

Commercial cultivation of *Kappaphycus* began in the Philippines in 1970 (Doty 1973; Parker 1974; Doty and Alvarez 1981; Trono and Valdestamon 1994), from where its practice spread to neighboring Southeast Asian countries (Adnan and Porse 1987; Hurtado et al. 2014a), the western Indian Ocean, particularly Tanzania, Madagascar, India, and Sri Lanka (Msuya et al. 2014), and to Central and South America (Robledo et al. 2013; Hayashi et al. 2014, 2017). It is hard to imagine the availability of many more newly established cultivation sites, especially in view of tightened restrictions on the relocation of marine genetic material from one part of the world to another (Cottier-Cook et al. 2016).

Commercial extracts from different sources of seaweeds (also known as aquatic plants for registration purposes) have been manufactured at an industrial scale for several decades to be used extensively for improved agricultural crop production and harvest quality (see: USDA 2016). These aspects have been comprehensively reported on by Craigie (2011) and Bhattacharyya et al. (2015). Seaweeds extracts are noted to (1) enhance root vigor (Crouch and van Staden 1992), (2) increase leaf chlorophyll content (Blunden et al. 1996), (3) increase the number of leaves (Rayirath et al. 2008); (4) improve fruit yield (Arthur et al. 2003; Kumari et al. 2011; Kumar and Sahoo 2011), (5) enhance the flavonoid content of treated plants (Fan et al. 2011), and (6) enhance vegetative propagation (Leclerc et al. 2006). However, the most substantial improvements and value proposition to the farmers associated with their applications of seaweed extracts are improved tolerances towards abiotic stresses, including drought (Zhang and Ervin 2004; Spann and Little 2011; Martynenko et al. 2016; Santaniello et al. 2017; Shukla et al. 2017; Van Oosten et al. 2017), ion toxicity (Mancuso et al. 2006), freezing (Rayirath et al. 2009), and high temperatures (Zhang and Ervin 2008). Van Oosten et al. (2017) proposed the alternate term: “bioeffector” to encompass the wide range of claims related to biotic benefits of the category of biostimulants (including seaweed extract applications, Shekhar Sharma et al. 2014). Some performance improvements include enhanced tolerance to biotic stresses (i.e., pathogens and disease agents, for. For a number of extracts of seaweeds multiple biotic responses are reported, see: Jayaraman et al. 2011; Coronado and Dionisio Sese 2014; Mireya et al. 2014; Shekhar Sharma et al. 2014; Stadnik and de Freitas 2014; Le Lann et al. 2016; Esserti et al. 2017a, b, 2018), but these fall outside of the normally accepted biostimulant definition for regulatory purposes (du Jardin 2015).

As an aside, it is interesting to note the recent development of commercial, plant biostimulants from the expressed sap of *Kappaphycus alvarezii*. It is not the place of this review to investigate the efficacy of such extracts but Trivedi et al. (2017) should be cited demonstrating parallels in applications of biostimulants derived from red and brown seaweeds and improved salinity tolerance of treated plants. In this case, the role of quaternary ammonium compounds are considered in terms of mode of action for the red seaweed extract.

A considerable amount of iterative research has been reported for the improved cultivation of many eucheumatoids of commerce (e.g., *Kappaphycus* and *Eucheuma*), not only in the Philippines but in all countries to which their seedstock has been dispersed for commercial reasons. The activity of seaweed farming has transformed the lives of tens of thousands of seaweed farmers as a result of their economic (cash) gains derived from farming (Valderrama et al. 2013). Benefits provided to carrageenan processors have been substantial and demand for carrageenan has increased on average 3% year^{-1} and is currently worth US$ 758 M with predictions to reach US$ 1047 M by 2020 (Markets and Markets 2016; see also Campbell and Hotchkiss 2017; Neish and Suryanarayan 2017).

However, after four decades of good economic returns, the marine agronomy of eucheumatoids faces a number of new challenges (not unlike the history of terrestrial farming), see Buschmann et al. (2017) for a review of the bottlenecks and “vital needs” of the industry to support its perceived potential for growth. The reduced quality and yields of carrageenophytes per unit area of cultivated raw materials are of considerable concern, the primary causes of which have been due to the apparent loss of strain vigor and repeated disease and pest infestations of unprotected crops. These issues may be considered to be entirely predictable, yet the carrageenan industry, as a whole, paid little attention until crop shortages became evident in 2008, particularly noted first in the Philippines then spreading to other areas of SE Asia (Baricuatro, pers. comm.; Hurtado et al. 2014). Reasons ascribed to declining seaweed crop production included the intensive nature of the farming activities, poor farm management, introduction of non-indigenous species to new farm sites, and climate change (Cottier-Cook et al. 2016). The fact that the total reported volumes of cultivated eucheumatoid seaweeds continued to increase was due to yet more carrageenophyte farms being established around the world (Hayashi et al. 2017).

There remains a limited, yet important group of studies undertaken on *Kappaphycus* cultivation and the use of commercial seaweed extract from the brown seaweed *Ascophyllum nodosum* in its micropropagation, field cultivation, mitigation of various epibionts and enhancement of final carrageenan qualities (yield and physical properties) (Hurtado et al. 2009, 2012; Loureiro et al. 2010, 2012, 2014a, b; Borlongan et al. 2011; Yunque et al. 2011; Marroig et al. 2016; Ali et al. 2017a, b; Tibubos et al. 2017).

The present authors would like to highlight reports made for academia and industry in an effort to inspire further scientific endeavor by researchers and new entrants to the industry involved in open-water, extensive *Kappaphycus* and *Eucheuma* seaweed production. It is important to continue the quest and improve the sustainability of the carrageenophyte industry. This paper reviews the uses of *Ascophyllum* (Acadian) Marine Plant Extract Powder (AMPEP) as manufactured from *A. nodosum* particularly for the benefits of the genus *Kappaphycus* (and its variants) based on their present (colloid) and future potential (biological activity) uses of high economic value (Bixler and Porse 2011; Campbell and Hotchkiss 2017; Porse and Rudolph 2017). New publications of Garcia-Vaquero et al. (2016); Guan et al. (2017); and Pereira et al. (2017) make an encouraging departure including examinations of new methods of extraction and in particular production of seaweed polysaccharides for their, as yet untapped, value-added potential as biologically active molecules (i.e., not just fibers for their rheology).

The uses of seaweed extracts of various seaweed outside of their traditional range of commercial (biostimulant) applications in terrestrial agriculture/horticulture, such as marine agronomy are in their infancy. The reviewers are only aware of one other example where a commercial seaweed extract (i.e., Kelpak® manufactured from the subtidal kelp *Ecklonia maxima)* has been applied to benefit enhance the growth of another cultivated seaweed (i.e., *Ulva* sp.; Robertson-Andersson et al. 2006). Very recently, Shi et al. (2017) used published a first use of an *A. nodosum* extract to enhance the antioxidant and defense systems of two freshwater microalgae. Whilst the reviewed studies here pioneer the applications of AMPEP for the enhanced cultivation of eucheumatoids, the authors of this review would also like to encourage the applied phycological research community to examine applications of other biostimulant/bioeffector sources as inputs to a well-managed, marine agronomy program (see also Buschmann et al. 2017). The principles for AMPEP treatments could also be applied to other groups of commercially important, cultivated seaweeds, and, at present, some studies are underway for the use of AMPEP in various stages of nori and kelp cultivation (no data available); a case of "watch this space."

Various effects of AMPEP on *Kappaphycus*

Micropropagation

The first report on the use of an *A. nodosum* extract, coined AMPEP (Acadian Marine Plant Extract Powder), was made by Hurtado et al. (2009). Table 1 shows a summary of the *Kappaphycus* varieties (and their abbreviations) which were used in micropropagation (Hurtado et al. 2009). The authors claimed that a shorter duration was observed for shoot formation when the explants were treated with AMPEP+Plant Growth Regulator (PGR = PAA + zeatin at 1 mg L^{-1}; PAA = phenylacetic acid), as compared to AMPEP when used as a single treatment. However, the four explants responded differently when it came to the number of days required for shoot formation. Hence, there were varietal differences in responses. The authors further observed that amongst the four explants used, the purple and adik-adik cultivars initiated shoot formation with the use of AMPEP only at the higher concentrations tested (i.e., 3–5 mg L^{-1}, Table 1) after a shorter period based on the findings of the authors, the use of AMPEP alone and/or in combination with a PGR as a cost-effective and reliable culture medium was highly encouraging for this tissue culture technique.

The results of Hurtado et al. (2009) led to further investigations by Yunque et al. (2011) on the use of AMPEP in the micropropagation of different varieties of *Kappaphycus.* The authors optimized the AMPEP concentrations required, and determined the effects of pH-temperature combinations and explant density. Positive results were obtained using a number of the commonly available cultivars.

Table 2 summarizes results from the addition of AMPEP and various plant growth regulators (PGRs). Performance was evaluated based on the shortest period required for new shoot emergence. The study showed that an addition of PGR to low concentrations of AMPEP was the most effective treatment to hasten shoot formation. When brown and purple color morphotypes of *K. alvarezii* var. tambalang and a green morphotype of *K. striatum* var. sacol were tested for pH-temperature combinations with 1.0 mg L^{-1} of AMPEP + PGR (PAA and zeatin at 1 mg L^{-1}), the brown morphotype produced the greatest number of shoots at pH 7.7, at 20 °C, after as little as 20 days. Data presented in Table 3 clearly demonstrate that each color morphotype of *K. alvarezii* responded differently with regard to the number of explants and volume of culture media combinations. This study again reinforced the findings that AMPEP +PGRs were effective in the enhancement of shoot formation, since it took only 9 days for both the green and purple color morphotypes of *Kappaphycus,* and 21 days for the brown variety, to initiate shoot formation, in all the densities investigated. However, within the ranges tested, the density of explants did not have a significant effect on the rate of shoot formation, but it did influence the average number of shoots generated from the culture. The reasons for these different observations still remain unknown, further. Future studies are required on the physiology and metabolic pathways of the different strains and morphotypes and when available will undoubtedly provide useful information for enhanced cultivation practices.

Table 1 Number of days to shoot emergence in three varieties of *K. alvarezii* (kapilaran (KAP), tambalang purple (PUR), adik-adik (AA) and *K. striatus* (green sacol) (after Hurtado et al. 2009)

	Conc.	Shoot primordia (days post-culture initiation)			
	g L^{-1}	Kapilaran (KAP)	Tambalang (PUR)	Adik-adik(AA)	Green sacol(GS)
AMPEP	0.001	–	–	25	–
	0.01	–	–	39	–
	1.0	–	–	25	–
	2.0	–	–	19	–
	3.0	–	21	17	–
	4.0	60	21	17	–
	5.0	60	21	17	–
AMPEP + PGR	0.001	–	41	17	–
	0.01	–	22	–	–
	0.1	–	22	17	–
	1.0	49	22	21	25
	2.0	49	21	19	–
	3.0	46	21	17	–
	4.0	46	31	17	–
	5.0	–	78	17	–

Hayashi et al. (2008) and Neves et al. (2015) reported on the use of spindle inhibitors such as colchicine and oryzalin, in combination with inorganic culture media such as von Stosch's solution (VS 50) or 50% Guillard & Ryther solution (F/2 50), and synthetic ASP 12-NTA medium and PGRs on callus induction and direct shoot formation in *K. alvarezii.* The authors demonstrated that they were successful in regenerating microplantlets from their procedures; however, the report of Tibubos et al. (2017) using the same spindle inhibitors, with AMPEP K^+ (a potassium fortified extract of *A. nodosum)* as the main culture medium, plus the addition of PGRs (i.e., IAA and kinetin at 1 mg L^{-1} each) was also highly encouraging. It should be noted that the former authors used inorganic culture media, while Tibubos et al. (2017) used an organic culture medium. The length of the direct axes formed, within 45 days of incubation, was the key indicator taken in determining the success rate of different combinations of AMPEP K^+ with and without + PGRs and AMPEP K^+ + PGRs + colchicine or oryzalin (Table 4). Table 4 summarizes the longest and shortest direct axes shoots formed with the corresponding combinations applied. Furthermore, significant differences were observed in the length of the newly formed shoots amongst the different concentrations of AMPEP K^+, as used in combination with colchicine ($P < 0.1$) or oryzalin ($P < 0.05$). The use of either of the two spindle inhibitors resulted in more than 4–5 direct axis shoots per explant, as compared to control (those not treated with AMPEP K^+ and PGRs only, which was 1–2 direct shoots). The efficacy of AMPEP was further reported by the recent findings of Ali et al. (2017a) on three strains of *K. alvarezii* and one strain of *K. striatus* in Sabah, Malaysia wherein the highest percentage of direct axes formed, *K. alvarezii* (tambalang brown*)*, *K. alvarezii* (tambalang green), and *K. striatus* (sacol green) were recorded as follows: 100 ± 0.00, 99 ± 1.34, and 98 ± 2.66%, respectively. The authors further claimed that the shortest duration taken for the emergence of direct axes was observed in *K. alvarezii* (tambalang green) followed by tambalang brown and *K. striatus* (sacol green) on days 9, 10, and 15, respectively. All four studies reported on the use of AMPEP for the micropropagation of different color morphotypes *K. alvarezii* and *K. striatus* and concluded that the application of this brown seaweed extract could be used as

Table 2 Number of days to shoot emergence in four *Kappaphycus* varieties, with each corresponding AMPEP concentration (after Yunque et al. 2011)

	Variety	# of days (first occurrence of shoot (bold)/ Concentration of AMPEP
K. alvarezii	Tambalang purple	**21** (3.0 mg L^{-1}; 0.1 mg L^{-1} + PGR
	Kapilaran brown	**49** (1.0 mg L^{-1} + PGR)
	Vanguard brown	**22** (0.1 mg L^{-1} + PGR)
	Adik-adik brown	**17** (3.0 mg L^{-1}; 0.001 mg L^{-1} + PGR)
	Tungawan green	**19** (3.0 mg L^{-1}; 0.001 mg L^{-1} + PGR)
K. striatus	Sacol green	**25** ((1.0 mg L^{-1} + PGR)

Table 3 Effects of pH-temperature combinations on shoot emergence of *K. alvarezii* (tambalang purple and tambalang reddish brown) and *K. striatus* (sacol green) ($n = 24$) (after Yunque et al. 2011)

Species	Variety	Temperature	pH			
		(°C)	6.7	7.7	8.7	9.7
K. alvarezii	Tambalang purple	20	0	0	0	0
		23	5	2	4	3
		25	10	8	11	11
	Tambalang reddish brown	20	6	4	6	4
		23	8	6	10	5
		25	15	13	11	4
K. striatus	Sacol green	20	5	5	4	3
		23	2	2	0	0
		25	3	5	0	3

a possible management protocol to mass produce robust and healthy plantlets for land-sea-based nursery cultivation operations for commercial purposes. Yong et al. (2014) and Luhan and Mateo (2017) also reviewed usage of inorganic and organic media (some including *Ascophyllum* seaweed extract) and additional PGRs with favorable responses for the clonal propagation of *Kappaphycus* for enhanced seedling production. Though the economics of using seaweed extracts for commercial purposes was beyond the scope of the earlier proof of concept studies, it would be worth determining that value in the near future. It is encouraging that other reports of using natural seaweed extracts (with and without additional PGRs) as media for micropropagation appears to be gaining interest, e.g., Sivanandhan et al. (2014) used simple water extracts of a red and a brown seaweed, *Sargassum wightii* and *Gracilaria edulis*, respectively, for both the micropropagation and metabolite enhancement of an important medicinal plant (*Withania somnifera*) producing steroidal lactones collectively called “withanolides.” In this evaluation, the red algal extract provided the most desired effects. Subsequently, Sharma et al. (2015) reported on using an extract from *K. alvarezii* for the mass production of *Picrorhiza kurroa*, which also enhanced production of bioactives in the treated medicinal herb. This extract was a commercial biostimulant (a pressed “sap” and not a chemically modified hydrolysate) provided by Sea6Energy Pvt. Ltd. (Bangalore, India). Esserti et al. (2017a, 2018) proposed growth media derived from extracts of *Cystoseira myriophylloides* and *Fucus spiralis* for in vitro plant tissue culture. Hence, it would seem seaweed extracts may play important multiple current and future, multiple roles in micropropagation of both land plants and seaweeds.

Field cultivation

The efficacy of AMPEP at different concentrations (i.e., 0.01, 0.1, and 1.0 g L^{-1}) and dipping duration (i.e., 30 and 60 min) was tested under field cultivation conditions, for optimization purposes, for three different color morphotypes of *K. alvarezii*, for a period of 3 months, which represented the wet season in the Philippines (Hurtado et al. 2012). During the period, from August to November, the three color morphotypes showed a distinct pattern of growth in response to the AMPEP treatments, i.e., the lower concentrations, irrespective of dip time, performed better. Significant differences in growth rate amongst the three color morphotypes were observed.

Table 4 Average (±SE, $n = 20$) longest and shortest, direct axis shoot formed in *K. alvarezii* amongst the treatments tested (after Tibubos et al. 2017)

	Direct axes shoot (mm)	
	Longest	Shortest
AMPEP K^+ only	5.3 ± 1.84	2.7 ± 0.33
	0.5 mg L^{-1}	0.1 mg L^{-1}
AMPEP K^+ with PGR	9.6 ± 0.33	8.3 ± 0.33
	0.5 mg L^{-1}	0.1, 1, 10 mg L^{-1}
AMPEP K^+ colchicine with PGR	8.6 ± 1.20	5.3 ± 0.89
(A + C)	A 10 + C 0.1 mg L^{-1}	A 0.5 + C 0.1 mg L^{-1}
AMPEP K^+ oryzaline with PGR	8.7 ± 0.70	6.3 ± 0.60
(A + O)	A 0.1 + O 1.0 mg L^{-1}	A 5 + O 0.5 mg L^{-1}

The efficacy of AMPEP was further evaluated in a commercial nursery using the yellowish-brown morphotype of *K. alvarezii* for a period of 12 months (Hurtado et al. 2012). Significant differences in growth rates were observed over the entire growth period. Expectedly, growth rates were much lower in July and August (the rainy months; lower SST and salinity) and they slowly increased thereafter. The proliferation of young, multiple shoots of the yellowish-brown *K. alvarezii*, grown in a commercial nursery, after 10–14 days of field growth was noteworthy to mention. This could be attributed to the presence of growth priming, promoting/signaling compounds within the *Ascophyllum* extract which stimulated auxin-like activity, which further promoted shoot growth (analogous to land plant, rooting responses, see Wally et al. 2013). The results of this study could be correlated with auxin-like, gibberellin-like (Rayorath et al. 2008a, b), cytokinin-like, precursors of ethylene and betaine stimulation (MacKinnon et al. 2010) which are individually and synergistically involved in growth and performance responses when land plants are treated with various seaweed extracts (Crouch and Van Staden 1993). Likewise, the treated eucheumatoids in this experiment were observed to be darkly pigmented and epiphyte-free, indicating the benefits of AMPEP in improving thallus pigmentation and enhancing resistance to epiphytism.

One further interesting result of the study of Hurtado et al. (2012) was that *K. alvarezii* (purple) and *K. striatus* (green) were analyzed for their phenolic content, and Fe^+ chelating ability. Though no significant effects of AMPEP dipping were found, in regard to total phenolics in either *K. alvarezii* or *K. striatum*, the AMPEP-treated sacol green showed a significant increase ($P < 0.05$) in total antioxidant capacity during the October–November trial period. The tambalang purple strain with AMPEP treatment produced a sustained, high Fe^{2+} chelating ability from September to October through to January/February, which coincided with the enhanced growth of *K. alvarezii* in situ during that time frame. The increased free radical scavenging ability and transition metal chelating ability might be of interest from the point of view of resistance to pathogens and abiotic stresses (Dring 2006). The benefits ascribed to AMPEP are not necessarily exclusive to the processing of *A. nodosum*, as Catarino et al. (2017) reviewed the Fucaceae as being a resource for bioactive phlorotannins. Belda et al. (2016) provided evidence that polyphenols from the brown alga *Himanthalia elongata* provided protection from oxidative stresses. Further work is called for in this most important area of crop husbandry, and it will undoubtedly provide important insights to effective seedling production.

In the study of Loureiro et al. 2014a, Brazilian *K. alvarezii* was grown in tubular netting. However, there were no significant differences in the growth rates of treated seaweeds between the varied durations of dipping ($p = 0.44$). In another study (Marroig et al. 2016), also using *K. alvarezii* in tubular nets, the daily growth rates of treated seedlings were not significantly different between the cultivation period treatments applied ($t = 0.92$; $p = 0.36$, $n = 15$), with a total mean growth rate of $1.48 \pm 0.84\%$ day^{-1}. However, the results did show a significant difference, when compared between sampling periods ($F = 60.78$, $p < 0.001$, $n = 10$).

The latest study to date, reporting on the use of AMPEP in the field cultivation of *Kappaphycus* seaweeds, was made by Ali et al. (2017b). The authors reported findings that the growth rate was species/variety specific, when grown for three consecutive, 45-day growth cycles (August–November) in Semporna, Sabah. Significant differences ($P < 0.01$) in growth rates between the species/variety and growth periods in relation to the treatment of AMPEP were observed.

Table 5 shows a summary of the daily growth rates of the different species/varieties grown in the field using AMPEP at different concentrations. The table illustrates that increased growth rates were obtained when *Kappaphycus* was dipped in AMPEP prior to out-planting in the sea.

Epiphyte mitigation

Kappaphycus cultivation in tropical and sub-tropical waters has seen marked increases (Hayashi et al. 2017) due to growing demand for its applications in the global market. It is utilized as an important structural ingredient in processed food applications, pharmaceuticals, cosmetics, and personal care products, and more recently as a source of raw biomass for biostimulant and biofuel production. However, the mass production of biomass may need to consider perhaps hitherto unforeseen ecological and societal consequences such disease outbreaks. The eucheumatoid seaweeds are typically produced extensively by vegetatively propagated monocrop systems. With the benefit of hindsight, it can be predicted that these types of farming methods are sensitive to the introduction of pests and pathogens; the reality is that there is a need to urgently change farm management practices (Cottier-Cook et al. 2016). The authors of this paper believe that greater societal benefits will be derived when the biological activity of multiple components of the cultivated biomass are fully appreciated and made available in a multi-use, zero effluent MUZE; biorefinery approach (Kapilkumar et al. 2017; Neish and Suryanarayan 2017; Ortiz-Tena et al. 2017).

“Ice-ice” malaise, epiphytes, and structurally damaging endophytes are detrimental to the health and growth of *Kappaphycus*. These problems are the primary causes of biomass loss in field cultivation which then had a “knock on” effect leading to shortages of dried seaweed for carrageenan processing, with an accompanying, considerable decrease in carrageenan yield and quality (i.e., average molecular weight of extract and almost elimination of both iota- and methylated-carrageenan content—see Mendoza et al. 2002). In addition, reduced biomass production also dramatically reduced the availability of good quality propagules (vegetative seedlings)

Table 5 A summary of Daily Growth Rate (DGR) amongst *Kappaphycus* varieties treated with AMPEP

Species	Type of cultivation	Cultivation period	AMPEP conc./Duration of dipping g L^{-1}/min	DGR (% increase da^{1})	Reference
K. alvarezii					Loureiro et al. 2010
Brown	In vitro		5, 10, 15, 20, 25, 30	3.5,4.1,6.2, 6.1,5.2,4.2	
Green	In vitro		5, 10, 15, 20, 25, 30	3.1,3.8,6.0,6.2,5.0,4.0	
Red	In vitro		5, 10, 15, 20, 25, 30	3.0,2.7,5.0,4.7,4.8,4.6	
K. alvarezii (tambalang)	HLL		0.1/45	1.3 ± 0.8–3.1 ± 0.8	Borlongan et al. 2011
K. akvarezii (tungawan)	HLL		0.1/45	1.4 ± 1.0–4.1 ± 1.0	
K. alvarezii					Hurtado et al. 2012
Reddish brown	HLL	1	0.01, 0.1, 1.0 L^{-1}/30	7.0, 7.0, 5.9	
			0.01, 0.1, 1.0 L^{-1}/60	6.1, 6,2,7.0	
		2	0.01, 0.1, 1.0 L^{-1}/30	5.5, 6.6, 4.6	
			0.01, 0.1, 1.0 L^{-1}/60	5.1,5.6,4.6	
		3	0.01, 0.1, 1.0 L^{-1}/30	5.1, 5.5, 5.1	
			0.01, 0.1, 1.0 L^{-1}/60	6.0,5.1, 4.3	
Yellowish brown	HLL	1	0.01, 0.1, 1.0 L^{-1}/30	7.7, 7.0, 5.1	
			0.01, 0.1, 1.0 L^{-1}/60	6.9, 6.5, 6.7	
		2	0.01, 0.1, 1.0 L^{-1}/30	5.6, 6.9, 4.5	
			0.01, 0.1, 1.0 L^{-1}/60	6.3, 5.3, 4.2	
		3	0.01, 0.1, 1.0 L^{-1}/30	5.2, 4.6, 3.5	
			0.01, 0.1, 1.0 L^{-1}/60	2.8, 3.8, 3.3	
Purple	HLL	1	0.01, 0.1, 1.0 L^{-1}/30	6.8, 6.5, 6.2	
			0.01, 0.1, 1.0 L^{-1}/60	7.3, 7.2, 6.5	
		2	0.01, 0.1, 1.0 L^{-1}/30	6.8, 7.2, 4.6	
			0.01, 0.1, 1.0 L^{-1}/60	7.2, 6.2, 4.6	
		3	0.01, 0.1, 1.0 L^{-1}/30	6.9, 6.8. 6.5	
			0.01, 0.1, 1.0 L^{-1}/60	5.5, 5.8, 4.9	
K. alvarezii (Yellowish brown)	HLL		0.1/30	0.8–6.7	Hurtado et al. 2012
K. alvarezii			20	6.2 ± 1.0	Loureiro et al. 2014
			40	7.3 ± 1.7	
K. alvarezii	TN		20/60	0.7 ± 0.5–2.2 ± 0.4	Marroig et al. 2016
K. alvarezii					
Crocodile	HLL	1	0.1/45	5.4 ± 0.1	Ali et al. 2017b
		2		5.6 ± 0.1	
		3		5.4 ± 0.2	
Giant	HLL	1	0.1/45	4.1 ± 0.3	
		2		3.7 ± 0.2	
		3		4.6 ± 0.4	
K. striatus	HLL	1	0.1/45	5.4 ± 0.2	
		2		5.5 ± 0.1	
		3		5.5 ± 0.7	

HLL hanging long line, *TN* tubular net

for the next growth cycle and most importantly, represented an important loss of income and motivation for seaweed farmers, who may then be tempted to revert to less sustainable activities and alternative income generating activities out of sheer, economic necessity (Solante, per comm).

Though the ice-ice (whitening of the thallus) problem was first reported by Uyenco (1977) and Uyenco et al. (1981) in the Philippines, it was the epiphytic filamentous algae (EFA) (Ask 1999) infestation in Calatagan Is. Camarines Norte, Philippines, which came to be the most damaging (Largo 2002; Critchley et al. 2004; Hurtado et al. 2006). These reports were the earliest to provide an account of the incidence of EFA in *Kappaphycus* farms. EFA infestations (in terms of very large epiphytic loads on cultivated biomass) were later reported in other places such as Indonesia, Malaysia and Tanzania (Vairappan et al. 2008), China (Pang et al. 2012, 2015), and Madagascar (Ateweberhan et al. 2015; Tsiresy et al. 2016). Further studies showed that the culprits were species of *Neosiphonia* (i.e., *N. apiculata* and *N. savatieri*) which produced internal filaments within the eucheumatoid tissues and resulted in decay due to secondary bacterial infections and significant fragmentation of crops and losses from cultivation lines (Vairappan 2006; Vairappan et al. 2008). Tsiresy et al. (2016) stipulated their identification of the endophyte as a *Polysiphonia* sp. and suggested that *Polysiphonia-Neosiphonia* species exhibit different symptoms upon infecting various eucheumatoid species. However, the impacts of epiphyte/endophyte infestations are deleterious to crop production and the quality of the yield. In all cases, investigated so far, infestations have led to tissue disintegration and secondary bacterial infections of the carrageenophyte crops. Clearly, this threat is spreading within existing cultivation sites over an extremely wide geographical range, the likely remote vector being the international relocation of infected seedlings, and the marginal vectors for localized infections being the numerous reproductive spores of the polysiphonous epi/endophytes. There is an urgent need for dedicated efforts on these issues and it is fortunate that the GlobalSeaweedSTAR Initiative has projects to undertake further detailed research and provide well-founded interventions and best-practice management recommendations (Cottier-Cook et al. 2016).

The use of AMPEP was tested on *Kappaphycus* (both laboratory and field) in order to evaluate if dipping a red algal tissue in a brown seaweed extract could increase tolerances to biotic stressors. If so, this would be effect a response similar to that of a "bioeffector" as described for some land plants (Fan et. 2011; Rayirath et al. 2009; Van Oosten et al. 2017). Treatments evaluated if AMPEP had any consequences for associated pathogen and/or epi-endophytes. The presence of epiphytes such *Cladophora* sp. and *Ulva* sp. and *Polysiphonia subtilissima* on the apical tips of green, red, and brown variants of *K. alvarezii* (when dipping in to AMPEP solutions at 5, 10, 15, 20, 25, and 30 g L^{-1} for 1 h), was tested in Brazil (Loureiro et al. 2010. Of the associated epiphytes tested, *Polysiphonia* proved to be the most tolerant to AMPEP treatment since this red alga survived at the higher seaweed extract concentrations (i.e., 25–30 g L^{-1}). The findings led to a more in-depth study on the in vitro relationships between red algal defense mechanisms and AMPEP treatments. It would be interesting to investigate if AMPEP treatments could be successful against the *Polysiphonia* sp. currently hindering eucheumatoid cultivation in Madagascar (Tsiresy et al. 2016); this may be tested in a future study. Loureiro et al. (2012) reported that the administration of the extract reduced the effects of a defensive, oxidative burst (i.e., production of hydrogen peroxide) by *Kappaphycus* which may be extremely aggressive both for both the host carrageenophyte and for its polysiphonous epiphytes. Bleaching of the non-corticated portions of *P. subtilissima* thalli which were cultivated as simulated epiphytes, confirmed that AMPEP protected *K. alvarezii* from the effects of its own superficial bursts of hydrogen peroxide. The same authors proposed that the use of AMPEP acted as a potential "vaccine," eliciting the activation of the treated host's natural defenses against pathogens and ameliorating the negative effects of self-harm due to long-term exposure to oxidative bursts. The *modus operandi* here seems to be not unlike the findings supporting Vacciplant®, a commercial biopesticide which was manufactured from *Ascophyllum nodosum* by Goëmar in France (Klarzynski et al. 2000). Further information on its applications for terrestrial crops affected by apple scab and fire blight are presented in Bernardon-Méy et al. (2013); mode of action including a patent were established based on the presence of laminarin in the extract and β-1,3 glucan activity (see also Klarzynski et al. 2000; Kadam et al. 2015; Raimundo et al. 2017). This vaccine-like effect also described for AMPEP-treated, Brazilian *Kappaphycus* was suggested to comprise a biochemically activated cascade of reactions initiated with the β-1,3 glucan (AMPEP)-β-1,3 glucanase (*K. alvarezii*) interaction (Fig. 1), thereby stimulating the production of volatile halogenated compounds which culminated in an oxidative pulse of self-cleansing peroxide (Loureiro et al. 2017). Esserti et al. (2017b, 2018) detailed further studies on the priming of antioxidant defense mechanisms of *Lycopersicon* (tomato, see also Stadnik and de Freitas 2014; Sangha et al. 2015) and *Nicotiana* (tobacco) using experimental extracts of *Cystoseira myriophylloides*, *Laminaria digitata* and *Fucus spiralis* which may provide new insights regarding modes of action of brown and red seaweed extracts, and new sources of raw materials and extracts for applications on plants and potentially, other seaweeds for the amelioration of biotic and abiotic stresses.

AMPEP was also tested in the field for its ability to provide treated *Kappaphycus* with some level of tolerance to epiphytic infestations. Two varieties of *Kappaphycus alvarezii* from Sibugay, Zamboanga, Philippines, were tested using AMPEP dips, first as a source of nutrients for growth, and secondly to determine if such applications had any effect on the percentage occurrence of an epiphytic infestation of *Neosiphonia* sp., at four different depths in open-water cultivation. Borlongan et al. (2011) reported that an increased growth rate was significantly ($P < 0.05$) influenced by the pre-treatment of seedlings by dipping with AMPEP prior to out-planting, and likewise, a significantly reduced percentage occurrence of *Neosiphonia* sp. was found. A lower percentage occurrence (6–50%) of *Neosiphonia* sp. was assessed when *K. alvarezii* was dipped in AMPEP, as compared to control (10–75%). Their study further demonstrated that *K. alvarezii* had to be grown between 50 and 100 cm below the water surface in order to reduce the incidence of the damaging endophyte. Traditionally, *K. alvarezii* and *K. striatus* (although both normally subtidal in their natural habitat) are grown at, or near, to the surface of the water, exposing them to intense sunlight, this was considered a crucial factor in the huge *Polysiphonia* sp. incidence in Calaguas Is. Camarines Norte, Philippines (Hurtado et al. 2006) and possibly Madagascar (Tsiresy et al. 2016). The findings of Borlongan et al. (2011) were further supported by Ali et al. (2017b) where *K. alvarezii* (both Crocodile and Giant variants) and *K. striatus* were dipped in AMPEP at 0.1 g L^{-1} concentration for a duration of 45 min which resulted in a lower incidence of *Neosiphonia* sp., as compared to control, this study was carried out in the waters of Semporna, Sabah, Malaysia.

The work of Marroig et al. (2016) showed that when *K. alvarezii* was not dip-treated with AMPEP (i.e., the control), a higher epibiont biomass (i.e., 0.76 ± 0.52 g dwt tubular net^{-1}) was recorded, whilst the AMPEP-treated samples had significantly ($P < 0.002$) less epibiont biomass (i.e., 0.26 ± 0.27 g dwt tubular net^{-1}). Their findings demonstrated the prompt response of carrageenophyte tissues to AMPEP treatment and provided notable benefits against the early settlement of epibionts, especially during the first two growth periods. The rapid response of the seedlings with reduced, early epibiont spore settlement, and provision of ongoing protection, until the second sampling period can be explained by the defense-elicitation which AMPEP initiates in *K. alvarezii* (and a wide variety of its cultivars) which reverts to the possible vaccine-like, mode of action explanation as proposed by Loureiro et al. (2012). Though the epibionts measured and reported by Marroig et al. (2016) were not fully identified taxonomically, their results clearly demonstrated the positive and powerful effects of AMPEP in the mitigation of epi-endophytes, consequently, improving the growth and industrial quality of the treated host plants.

In the study of Ali et al. (2017b), the percentage occurrence of *Neosiphonia apiculata* on *K. alvarezii* (Crocodile and Giant) and *K. striatus* ranged from 35.6 ± 1.1–41.6 ± 2.4% which were treated with AMPEP prior to out-planting. Those in the control groups ranged from 44.4 ± 0.9–86.3 ± 2.2% over the same three, consecutive 45-day growth cycles and these were found to be significantly different from one another ($P < 0.01$). In general, a higher percentage occurrence of *N. apiculata* was observed in the controls. Amongst the three *Kappaphycus* tested, *K. striatus* seemed to be more tolerant to *N. apiculata,* especially during the Sept.–Oct. growth period. A positive growth rate (2 ± 0.4% day^{-1}) and a lower percentage incidence of *N. apiculata* (53.8 ± 2.1%) were obtained, as compared to *K. alvarezii* (Crocodile and Giant, viz. growth of −2.1 ± 0.35% and −3.3 ± 0.91%, respectively) with concomitantly higher incidences of *N. apiculata* (75 ± 3.9% and 86.3 ± 2.2%, respectively).

Carrageenan quality

The global market demand for kappa carrageenan, as extracted from the cultivated biomass of *Kappaphycus* and its commercial varieties, has many valuable applications as a food texturizing ingredient. Growth in demand for these end-use markets has been steady over the ten-year period 2006–2015, with an estimated growth of 1.5–3% $year^{-1}$, in each sector (Hotchkiss et al. 2016). This growth is reflective of the global trend and increasing demands for processed/convenience foods and was in response to both population growth and changes in regional economies and food aspirations/preferences. Hence, strong demand for the dried carrageenophytes is expected to continue (Campbell and Hotchkiss 2017).

Both AMPEP-treated and control *Kappaphycus alvarezii* were subject to lethal, low temperatures (e.g., 16–18 °C) under laboratory conditions, in order to determine their response, growth rate, and carrageenan quality. Though lower carrageenan yield and viscosity were measured when *K. alvarezii* was treated with AMPEP, at the three temperatures tested, the yield values obtained for the treated material (ca. 30%) was within industry standards (Hayashi et al. 2007; Hung et al. 2009; Góes and Reis Goes and Reis 2011, Góes and Reis 2012; Loureiro et al. 2014a). However, in addition, an increased gel strength was measured in the AMPEP treatments. These authors concluded that the use of AMPEP as a treatment for *K. alvarezii* seedlings in Brazil could be used as a farmer-mediated response to the effects of the seasonally lower SST, which is a factor delaying out-planting of field material due to the detrimental effects of "colder water" on the production of eucheumatoid crops.

Table 6 shows a summary of carrageenan quality characteristics of the different *Kappaphycus* species/varieties when dipped with AMPEP. When AMPEP-treated and control *K. alvarezii* were grown on commercial floating rafts in Brazil, the carrageenan yield was highest in the treated samples ($p < 0.001$) after 20 days of growth. Likewise, after 40 days, the carrageenan yield in the treated samples was significantly higher ($p = 0.009$) than the control. However, the extracted carrageenan gel strength was higher ($p = 0.03$) and the viscosity was highest in the samples which were not treated with AMPEP (i.e., control; $p < 0.001$; Loureiro et al. 2014b). The same authors postulated that the higher carrageenan yields obtained in the AMPEP-treated *K. alvarezii* may be explained in the light of the fact that the biostimulant/bioeffector can act as both elicitor or primer of responses to abiotic stress conditions, thereby prompting the seaweed's defenses resulting in greater production of carrageenan, which is a structural component of the thalli to provide strength and flexibility in its natural habitat (Loureiro et al. 2012). This might explain the higher carrageenan yield results of *K. alvarezii* samples exposed to AMPEP as early as 20 days after the initiation of cultivation, when compared to the control samples.

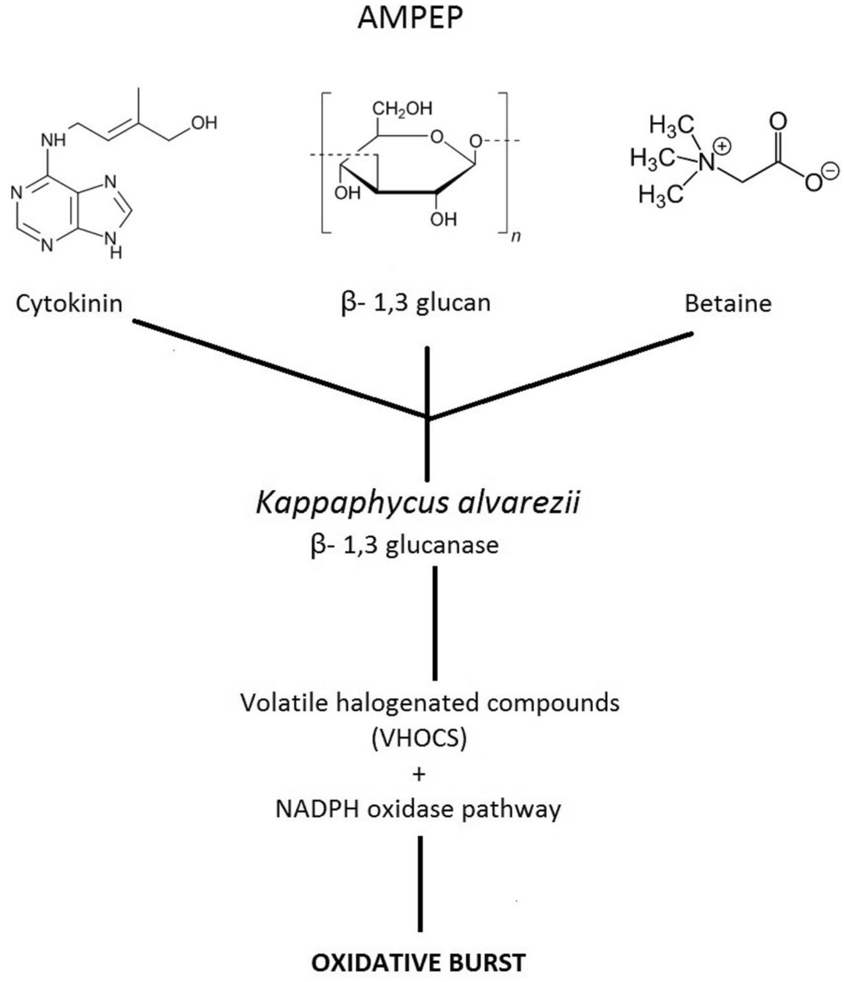

Fig. 1 Suggested biochemical pathway for the beneficial effects of AMPEP on *Kappaphycus alvarezii* (after Loureiro et al. 2017)

However, the findings of Marroig et al. (2016) using AMPEP, also on *K. alvarezii* on commercial floating rafts was the opposite to those findings of Loureiro et al. (2014a), since the former authors obtained higher gel strengths than those of the control (no AMPEP treatment), but a lower gel viscosity. However, higher carrageenan yields were obtained in AMPEP-treated *K. alvarezii*. The carrageenan yield (44.65 ± 7.09%) showed no significant differences between the treatments ($t = 0.46$; $p = 0.64$), with larger percentages by the end of the third sampling period ($F = 130.96$, $p < 0.001$). The findings of Loureiro et al. (2014b) and Marroig et al. (2016) agreed with the findings of Ali et al. (2017b) in that, relative to control, there were higher carrageenan yields for both refined carrageenan (RC) and semi-refined carrageenan (SRC) when two varieties of *K. alvarezii* (Crocodile and Giant) and *K. striatus* were pre-dipped in AMPEP and out-planted on long-lines in Semporna, Sabah, Malaysia. However, between the RC and SRC formats, in the treated samples, the carrageenan yield and viscosity were higher in the SRC with the gel strength of RC being much higher.

Conclusions

AMPEP, a commercial extract from the brown seaweed *Ascophyllum nodosum,* was used singly or in combination with PGRs (e.g., IAA and Kinetin) and spindle inhibitors (e.g., colchicine and oryzalin). It was demonstrated to be an effective culture medium for the mass generation of microplantlets which are increasingly, desperately required for nursery purposes and which ultimately enhance field cultivation through biomass production and colloid quality. The use of AMPEP has been demonstrated as a suitable biostimulant for the benefit of early production of these red seaweed seedlings, providing levels of protection against abiotic stresses. The ability of AMPEP to act as a potential vaccine, eliciting/priming activation of eucheumatoid seaweeds' endogenous, natural defenses against pathogens, and ameliorating the negative effects of self-imposed, long-term exposure to oxidative bursts is another noteworthy finding for the multiple modes of action of AMPEP as a bioeffector. Taken together, these findings suggest the AMPEP treatment of seedlings prior to out-planting can play an important, economic role in reducing superficial epiphytes and effects of damaging

Table 6 Summary of carrageenan quality characteristics of *K. alvarezii* and *K. striatus* pre-treated with AMPEP (values ave. ±SD)

Species	Concentration/period of AMPEP dipping g L^{-1}/min	Cultivation period	Refined carrageenan(Cg)			Semi-refined carrageenan (Cg)			Reference
			Cg yield %	Viscosity cPs	Gel strength g cm^{-2}	Cg yield %	viscosity cPs	Gel strength g cm^{-2}	
K. alvarezii	20/60		–	–	–	38.0 ± 6.7	–	–	Loureiro et al. 2014b
	40/60		–	–	–	42.0 ± 5.9	527.2 ± 133.6	369.0 ± 133.6	
K. alvarezii	20/60	1	–	–	–	40.0 ± 1.5	62.6 ± 9.9	250.8 ± 110.4	Marroig et al. 2016
	20/60	2	–	–	–	38.8 ± 4.4	74.3 ± 13.8	340.5 ± 82.9	
	20/60	3	–	–	–	53.4 ± 1.6	128.1 ± 8.2	281.4 ± 35.7	
K. alvarezii	0.1/45	1	45.1 ± 1.4	61.3 ± 4.4	1881 ± 23.6	67.4 ± 1.7	67.4 ± 1.7	394 ± 5.1	Ali et al. 2017a, b
Crocodile		2	40.7 ± 0.8	54.5 ± 1.7	1690 ± 130.4	67.2 ± 1.1	67.2 ± 1.1	314 ± 7.4	
		3	41.2 ± 1.3	59.9 ± 2.2	1732 ± 67.5	67.2 ± 1.1	67.2 ± 1.1	330 ± 9.5	
Giant	0.1/45	1	39.7 ± 0.3	50.5 ± 1.4	1589 ± 42.4	68.9 ± 0.9	68.9 ± 0.9	281 ± 7.2	
		2	32.7 ± 0.8	44.2 ± 2.7	1255 ± 95.7	64.3 ± 1.6	64.3 ± 1.6	261 ± 5.0	
		3	35.7 ± 0.3	49.9 ± 1.1	1308 ± 49.3	64.3 ± 1.6	64.3 ± 1.6	265 ± 1.5	
K. striatus	0.1/45	1	39.6 ± 0.4	41.3 ± 0.9	1383 ± 43.9	60.2 ± 1.2	60.2 ± 1.2	266 ± 3.6	
		2	39.8 ± 0.9	44.7 ± 2.7	1402 ± 53.3	57.7 ± 0.8	57.7 ± 0.8	221 ± 7.8	
		3	40.3 ± 0.9	44.3 ± 1.5	1384 ± 43.7	58.8 ± 0.9	58.8 ± 0.9	237 ± 15.3	

endophyte settlement and development to the point of dramatic crop losses.

There are many parallels to be drawn between land plant and selected red seaweed responses. One other account of AMPEP benefits can be found using the green alga, *Ulva* (Kumar et al. 2013). The authors showed that supplementation of AMPEP (150 μg mL^{-1}) to their culture medium significantly reduced the accumulation of ROS and lipid peroxidation together with the inhibition of lipoxygenase (LOX) activity specially LOX-2 and LOX-3 isoforms. Likewise, the positive results of AMPEP treatment on economically important carrageenan quality characteristics such as yield, viscosity, and gel strength are not to be under-estimated.

Clearly, further work is required on concentrations and application timings of AMPEP and treatments for multiple groups of other cultivated seaweeds (e.g., agarophytes and alginophytes) should be investigated. The protocols required would be very similar to the ongoing work where the product is used for its biostimulant/bioeffector properties, with multiple benefits for terrestrial crops. AMPEP when constituted as a solution is a complex mixture of hydrolysed components of the *Ascophyllum nodosum* thalli and does not provide a "single chemistry" (i.e., one compound/one defined response), as with many agrochemicals or pharmaceuticals. To date, there has been very little work undertaken on the role extracts of seaweed may play in the future of marine agronomy. It is quite plain to see that there will be many needs for inputs such as fertilizers, biostimulants, and bio-pesticides for the future, sustainable practices of seaweed husbandry on a massive scale. The time has come for this situation to change if seaweed agronomy is to reach its fullest global potential (Cottier-Cook et al. 2016; Buschmann et al. 2017). It is patently evident that further work is called for to examine which components of a commercial extract of the brown seaweed such as *Ascophyllum* effects the priming and signaling physiological pathways of a red seaweed such as *Kappaphycus*.

The fundamental question remains, why would a brown seaweed extract have such significant impacts on the growth and productivity of land plants and some selected seaweeds? The extract is produced by hydrolysis (in the case of AMPEP, open atmosphere and KOH). AMPEP is only one commercial extract, and there are others from a number of brown seaweeds (Shekhar Sharma et al. 2014), different methods of hydrolysis are employed by different manufacturers, as in atmospheric pressure and various pH; see United States Department of Agriculture (USDA) regulations and definitions related to the organic uses of aquatic plant extracts (USDA 2016). The interactions of different biomass and different extraction procedures results in pools of different hydrolysates; Shekhar Sharma et al. (2014) reviewed a range of plant biostimulants and extraction methods in relation to abiotic and biotic properties of the extracts. Although *Ascophyllum* processed over different seasons and from different geographies had "remarkably similar extract profiles" (Craigie et al.

2007), the same species of different macroalgae processed by different techniques resulted in different biostimulant characteristics (Craigie 2011; Sangha et al. 2015; Goñi et al. 2016; Lötze and Hoffman 2016; Olivares-Molina and Fernández 2016; Urbani et al. 2016; Moreira et al. 2017) therefore expressing different efficacies and properties when applied in bioassays and field trials. Whilst the extracts are described as being "crude," they indeed contain many products of hydrolysis not normally found in nature. The processing is not merely liberation of active compounds within the seaweed biomass, bioactivity is created as a result of the chemical reactions taking place during hydrolysis, yet even to date, these chemical reactions remain to be characterized. In essence, such extracts comprise new compounds resulting from the interaction of algal thalli and the hydrolytic process applied. Most seaweed (aquatic plant) extracts are sold for applications in agriculture/horticulture on the basis of their beneficial effects on plants and their alleviation of abiotic and biotic stresses (Craigie 2011; Bhattacharyya et al. 2015), yet very few modes of action have been elucidated. It would be extremely germane if the nature of the signaling/priming compounds within a seaweed extract could be ascertained and then to understand how these might have conserved/analogous functions across terrestrial plants and seaweed physiological pathways in general.

From this review, it would seem that there is an urgent need to elucidate the biochemical and physiological pathways behind the observations of positive benefits and current applications and learn to use extracts of *Ascophyllum* (and potentially other seaweeds) strategically and surgically (i.e., effective rates and timings of applications) for its most cost-effective benefits. In particular, it is anticipated that AMPEP can make further significant contributions to the successful and sustainable production of global seaweed raw material requirements.

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