*Biostimulant activity of brown seaweed species from Strangford Lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (*Vigno mungo *L.) and pak choi (*Brassica rapa chinensis *L.)*

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Biostimulant activity of brown seaweed species from Strangford Lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (*Vigno mungo* L.) and pak choi (*Brassica rapa chinensis* L.)

S. H. S. Sharma · G. Lyons · C. McRoberts · D. McCall · E. Carmichael · F. Andrews · R. Swan · R. McCormack · R. Mellon

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Abstract The aims of this study were to characterise the composition of seaweed species and to evaluate the efficacy of aqueous extracts as plant biostimulants. Five species (*Ascophyllum nodosum*, *Fucus serratus*, *Fucus vesiculosus*, *Laminaria hyperborea* and *Sargassum muticum*) of seaweed were harvested from Strangford Lough, Northern Ireland for the evaluation of polysaccharides, indole-3-acetic acid (IAA), carbon, nitrogen, lipid, ash and mineral contents. The compositional analyses of the five species and their freeze-dried extracts were also carried out using thermogravimetric analysis, scanning electron microscopy and X-ray microanalysis. The concentration of IAA in the acid extracts of the five species ranged between 2.74 and 46.8 ng g^{-1}. The carbon, nitrogen, lipid and ash contents ranged between 25.0 and 38.6, 1.37%, and 3.16, 0.83%, and 3.98 and 18.10 and 47.68%, respectively. *L. hyperborea* and *S. muticum* contained the highest amounts of minerals. The biostimulant activities of acidic (pH 3.0), neutral (pH 6.5) and alkaline (pH 9.0) extracts were determined by mung bean bioassay. The alkaline extracts from *F. vesiculosus* and *A. nodosum* stimulated significantly ($P<0.001$) higher dry matter (DM, %) yield of the mung bean plants. The majority of the acidic extracts significantly ($P<0.001$) enhanced root formation on the mung bean stem cuttings compared to alkaline or neutral extracts. The acidic extracts of the five species, water control and a commercial product were evaluated as foliar feeds for pak choi plants using a hydroponic production system. The interaction of species, e.g. *A. nodosum* and *F. vesiculosus* and the two treatment dilutions on DM yield increases of pak choi were significant ($P<0.05$).

Keywords Seaweeds · Phaeophyta · Mung bean · Pak choi

S. H. S. Sharma (✉) · G. Lyons · D. McCall · E. Carmichael · F. Andrews · R. Swan
Plant Health and Environment Protection Branch,
Agri-Food and Biosciences Institute,
Newforge lane, Belfast BT9 5PX, UK
e-mail: shekhar.sharma@afbini.gov.uk

C. McRoberts · R. McCormack
Food Chemistry Branch, Agri-Food and Biosciences Institute,
Newforge lane, Belfast BT9 5PX, UK

R. Mellon
Northern Ireland Environment Agency,
Belfast BT30 6JB, UK

Introduction

Seaweeds have a long tradition of being used in coastal agriculture in the British Isles including Northern Ireland as a soil conditioner to enhance crop growth and productivity (Booth 1969). During the 1950s liquid seaweed extracts were formulated for organic production (Milton 1952) in Europe and North America and later, dry formulations were developed for foliar applications (Stephenson 1974). Research carried out during the 1960s demonstrated the chelating properties of these extracts for improving the utilization of minerals, e.g. phosphorus in soil (Booth 1969; Lyn 1972), the stimula-

tion of plant growth due to auxin-like activities (Blunden 1971) and the priming of seeds for increasing the rate of germination Stephenson 1974. Booth (1969) reported that the full impact of using seaweeds as fertilizer was not only due to nitrogen, phosphorus and potash content but also because of other trace minerals and metabolites. Other researchers have also speculated as to the mechanisms involved as well as the validity of the findings (Beckett and van Staden 1989; Crouch et al. 1990; Hankins and Hockey 1990). During the past two decades, the evidences for the presence of rooting factors in extracts, e.g. indole acetic acid (IAA, Sanderson et al. 1987; Stirk et al. 2004), the growth stimulation and the uptake of minerals under stressful conditions were reported, leading to a better understanding of the mechanisms involved in the control of abiotic and biotic stresses (Crouch and van Staden 1993; Khan et al. 2009; Rayorath et al. 2007). Currently commercial formulations are used widely in tropical and Mediterranean regions to alleviate abiotic stresses (e.g. temperature, water deficit and high salinity) and also to modulate crop plants against biotic stresses (Demir et al. 2006; Spinelli et al. 2010; Zhang and Ervin 2004). Henry (2005) reviewed various studies focused on the performance of alkaline and nonalkaline extracts of seaweeds and concluded that the two extraction protocols may have some value, as crop treatments depending on the conditions of application. Relatively few studies have directly compared different extraction protocols for bioactivity.

Many species of green, brown and red algae are available in and around Strangford Lough, Northern Ireland. However commercially available species are mostly brown algae of the Phaeophyta: *Ascophyllum nodosum*, *Fucus serratus*, *Fucus vesiculosus* and *Laminaria hyperborea* (McLaughlin et al. 2006). Several reports on the introduction of *Sargassum muticum*, a non-native species to the British Isles including Strangford Lough have been published (Gorham and Lewey 1984; McLaughlin et al. 2006). Seaweeds contain a diverse range of organic and mineral fractions (Rioux et al. 2007; Sivasankari et al. 2006). The first four species were studied in many countries for polysaccharide fractions such as laminarin, fucoidan and alginate (Marinho-Sorinano et al. 2006). However the biology and composition of *S. muticum* have not been reported widely (Gorham and Lewey 1984). *S. muticum* is particularly interesting as few studies have been reported on the composition, metabolite content and bioactivity of the polysaccharides and growth hormones. The aims of this study were to characterise the composition of these five seaweed species in terms of their polysaccharide, auxin and mineral content and to evaluate the efficacy of acidic, alkaline and neutral seaweed extracts as plant biostimulants.

Materials and methods

Seed material

Seeds of pak choi (*Brassica rapa chinensis*, green, F1 hybrid) were obtained from Thompsons and Morgan seed company (Ipswich, England). Mung bean (*Vigno mungo* L., KPS1) seeds used in the bioassay were supplied by Moles Seeds (Colchester, England). Seeds were stored in foil bags until required.

Collection

Five species (*A. nodosum*, *L. hyperborea*, *S. muticum*, *F. vesiculosus* and *F. serratus*) of seaweed were harvested from the coastal area of Strangford Lough (54°26′40″ N–5° 35′40″ W) during February and June 2009. The seaweeds were handpicked and washed thoroughly with seawater to remove unwanted debris, grouped according to species and bulked to 20–30-kg lots.

Extraction procedure

The algal materials were subsampled, washed with tap water to remove epiphytes and encrusting materials, followed by careful evaluation for morphological characteristics for species identification. A part of the samples were air-dried (20°C) for compositional analyses, and the remaining fresh samples were cut to 10-cm lengths to ease handling and processed using a blender (Waring Commercial). Fresh material (500 g) was homogenised with 500 mL of deionised and distilled water in a blender for 5 min. The pH of the extract was adjusted to either 3.0 or 9.0 with acetic acid (1 M) or potassium hydroxide (2 N) or left unadjusted (neutral water control) and further homogenised for another 5 min. The final pH of the extract was adjusted if necessary. The materials were stored in the laboratory for 1–2 hours to stabilise and complete the extraction steps. The solid residue was separated first by using a fine muslin cloth, later filtered using Whatman grade 1 paper, and this step required 3–4 h to complete. Subsamples of the acidic, alkaline and neutral extracts were evaluated for pH, electrical conductivity (EC) and freeze-dried for dry matter (%) yield determinations and compositional analyses. Further removal of fine particles present in the extracts were achieved by using a 0.2-μm vacuum filtration unit (Nalgene), and materials stored were frozen at −20°C in aliquots of 200 mL. The sterilised liquid filtrate was taken as 100% concentration of the five seaweed species and samples further diluted as per treatment. The seaweed samples from June 2009 were extracted using the acid protocol only.

Compositional analyses

The air-dried seaweed samples were pulverised using a mill (Tecator, model 1093) equipped with a mesh screen size of 0.5 mm. The milled seaweed samples were stored in plastic jars at room temperature for further analyses. All determinations were performed in triplicate unless specified. Nitrogen and carbon contents were determined by elemental analysis (LECO CN-2000) in duplicate. The ash contents were estimated by heating the samples overnight in a muffle furnace maintained at 600°C. Crude lipids were extracted from the seaweed samples with chloroform to methanol (Sanchez-Machado et al. 2004). The elemental (Ca, Cu, Fe, K, Mg, Na and P) composition of the samples was quantified by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer).

Thermogravimetric analysis of the samples (seaweed and freeze-dried) was carried out by combustion in air using a Mettler Toledo (TGA/DSC1) analyser. A typical sample mass of 3.1 mg was heated from 30 to 780°C at a heating rate of 20°C min^{-1} in a flow rate of 50 mL min^{-1} air (Lyons et al. 2008). The reference standards, alginic acid (A7003), laminarin (L9634), mannitol (M4125) and fucoidan (F5631), were obtained from Sigma-Aldrich (England).

The seaweed samples and extracted polysaccharide fractions were also evaluated using scanning electron microscopy (SEM; Quanta 200, FEI) equipped with an energy-dispersive X-ray microanalysis system (Inca 300, Oxford Instruments). This instrumentation was used to determine visual differences, such as surface topography of the freeze-dried polysaccharides and elemental composition of seaweed species. Internal standards were used for semi-quantitative analysis of the samples under similar microscope parameters. Unextracted seaweed or polysaccharide samples were distributed on adhesive carbon discs on SEM sample support stubs. The samples were imaged at ×200 magnification in the SEM, and ten particles were selected from each image. X-ray spectra of the ten particles of unextracted seaweed samples were collected from five different regions per sample (50 spectra per sample). Results on I and S concentrations presented along with elemental data determined by ICP-OES of the same samples.

IAA was determined by liquid chromatography–mass spectrometry (LC-MS) modified from Hou et al. (2008). The extracts were centrifuged at 769 g for 10 min and purified on 6 mL/500 mg Strata C18-U cartridges (Phenomenex, UK). The cartridges were conditioned with 3 mL methanol followed by 3 mL water. The centrifuged extract was applied followed by washing with 2 mL water then 2 mL 20% methanol. Cartridges were dried under vacuum and IAA eluted with 2 mL 80% methanol. LC-MS was carried out using a Quattro Ultima Pt triple quadrupole MS (Micromass UK Ltd.) coupled with an Agilent 1100 LC-MS system (Agilent UK Ltd.). LC separation was carried out using a Luna phenyl–hexyl column (150×2 mm, 5 μm particle size, Phenomenex UK Ltd.). Chromatography was performed using 95% acetonitrile (channel A) and 5% acetonitrile (channel B) with the following gradient programme: At t=0 min, the mobile phase was 95% channel B, and this was held for 2 min. Between 2 and 6 min the proportion of channel B was reduced to 5%. Mobile phase composition was returned to 95% channel B between 9 and 11 min. Quantification was carried out by comparison against authentic IAA standards.

Mung bean bioassay

The auxin-like activity (Kollarova et al. 2010) in the seaweed extracts was determined by measuring root formation and dry matter (DM) accumulation in mung bean stem cuttings. Three experiments were carried out during March, April and May 2009. The seeds were soaked in deionised water for 2 h prior to sowing in boxes containing sterilised perlite–vermiculite mix (2:6 litre) to generate 560 seedlings under similar conditions in the glasshouse. Poor-quality seedlings were discarded. The boxes were covered with black polythene and removed when the hook stage was visible after 2–3 days. The seaweed extracts solutions were diluted (10^{-2}, 3×10^{-2}, 10^{-3}; 3×10^{-3}; 10^{-4}; 3×10^{-4}) with distilled deionised water. Cuttings were excised from seedlings 10 days after sowing by removing roots and cotyledons and placed in vials (five plants/vial; four vials/dilution; size 6×2 cm) containing dilute solutions of the extracts (4 mL). An indole-3-butyric acid (IBA, Sigma) dilution series [1 M (inhibiting concentration), 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} (stimulating concentration)] was prepared as standards, and buffer solutions of half-strength Hoagland's nutrient solutions (Hoagland and Arnon 1950) adjusted at pH 6.2–6.5 were used as controls. All plants were incubated for 3 days in the test solutions, and on day 4, test solutions were poured out from each vial and 4-mL buffer solutions were added. The plants were incubated in a growth room maintained at 15°C with 16 h photoperiod (~100 μmol photons m^{-2} s^{-1}) and 8 h dark cycle. The plants were monitored regularly, and vials were topped up with deionised water when necessary. After 10 days, the number of roots was recorded. The fresh and dry matter weights were recorded after drying in an oven at 90°C overnight in triplicate.

Hydroponic production of pak choi

A single pak choi seed was placed in a small hole in a rock wool cube that was presoaked in water to allow rapid germination of the test seeds. The seeded rock wool blocks were covered with a 1-cm layer of milled peat to maintain

Table 1 Comparison of seaweed species (*A. nodosum*, *F. serratus*, *F. vesiculosus*, *L. hyperborea* and *S. muticum*) harvested in February (F) and June (J) 2009 to show changes in nitrogen (% DM), ash (%), lipid (%) and carbon (%), LSD at 5% level (n=3)

	AN	FS	FV	LH	SM
Nitrogen					
F	1.37	3.16	1.79	3.11	2.81
J	1.95	2.20	2.02	1.45	1.84
LSD	0.04*	0.02**	0.05**	0.02***	0.14**
Ash					
F	18.10	23.33	21.91	47.68	41.49
J	20.82	25.39	21.66	34.39	34.03
LSD	0.58**	1.5*	ns	1.17**	0.57**
Lipid					
F	3.98	1.93	3.00	0.83	1.08
J	2.41	2.70	2.23	0.89	1.25
LSD	0.90*	0.66**	0.72*	ns	ns
Carbon					
F	38.6	36.1	36.8	25.0	27.3
J	37.2	34.9	36.9	28.6	29.5
LSD	0.48**	0.22**	ns	0.68**	0.61***

AN A. nodosum, *FS F. serratus*, *FV F. vesiculosus*, *LH L. hyperborea*, *SM S. muticum*, *ns* not significant

*P<0.05; **P<0.01; ***P<0.001

moisture content and placed on trays in a glass house maintained at 25°C. After 14 days the healthy germinated seedlings at the two leaf stage were divided randomly to groups of 12 plants and primed with 20 μL drops of the various acidic seaweed extracts on each of the two leaves or water control (buffered) or a commercial seaweed extract (*A. nodosum*; 1:150, AlgaGreen; Oilean Glas Teo, Ireland). After priming, the plants were transferred to the hydroponic channels along with the rock wool cubes. The two dilutions (1:100 and 1:30) of the extracts (five species) were prepared, using deionised sterile water, and surfactants were not added to the test solutions.

The pak choi hydroponic cropping trials were carried out during May, July and September 2009 in a polytunnel, and each trial was replicated with two randomised blocks. The acidified extracts from the five species were used as foliar sprays. Each block consisted of 12 channels placed on a wooden frame and each housing 12 plants with a total of 144 plants. Treatment details are as follows: five extracts of two dilutions; 1:100 and 1:30, pH adjusted to 5.4–5.6; one control, distilled water, pH adjusted to 5.4–5.6; AlgaGreen diluted at 1:150, pH not adjusted. The beneficial effects of the extracts as foliar feed were tested using a continuous flow solution culture where the nutrient constantly flows past the roots of pak choi seedlings. The temperature in the polytunnel and nutrient containers were monitored daily and recorded. The adjustment for pH, electrical conductivity and concentration of Hoagland's nutrients solutions (Hoagland and Arnon 1950) were performed in a storage tank (250 L capacity) which supplied nutrient to each of the 24 containers set at the bottom of each channel. Each container was equipped with a filter and a pump attached inside to distribute 12 L of nutrient solution around the plant roots over shallow and gently sloping channels. A steady flow of nutrient solution was maintained along the channel, and the roots grew into dense mats, with a thin film of nutrient passing over them. The nutrient solution for each channel was monitored for reduction of volume in the container and topped up, as required by maintaining pH between 6 and 7.5 and conductivity at 2,000 μS cm^{-1} using Hoagland's nutrient solutions. The treatments were applied as foliar sprays using a 30-mL bottle equipped with a hand pump. The volume required for spraying the plants were 0.5 to 2.5 mL per plant depending on maturity, and top shoots were sprayed with even coverage. The total volume of each treatment sprayed (three times) was approximately 4–4.5 mL per plant during the hydroponic trials. The treatments were carried out on day 8, 15 and 20 from the start of the trial. The plants were harvested after 25 to

Table 2 Comparison of elemental composition (%) of the five species (mean of February and June samples) using air-dried samples, LSD significant at 5% level (n=6)

Species	Ca	Cu	Fe	I	K	Mg	Na	P	S
AN	1.42	0.07	0.07	0.13	3.44	0.87	3.10	0.12	2.64
FV	1.41	0.08	0.15	0.07	3.04	0.86	3.70	0.18	3.13
FS	1.39	0.06	0.13	0.03	4.63	0.88	3.70	0.23	1.54
LH	1.36	0.09	0.30	0.11	8.07	0.86	4.09	0.20	1.01
SM	1.24	0.08	0.29	0.02	7.91	1.88	3.60	0.23	1.28
LSD	ns	ns	0.14*	0.03**	1.7***	0.46**	ns	ns	0.71**

ns not significant; *P<0.05; **P<0.01; ***P<0.001

Table 3 Comparison of seaweed extracts using acidic, neutral and alkaline extraction protocols for pH, electrical conductivity (mS cm^{-1}) and dry matter (%) content for February samples; LSD at 5% level (n=3)

pH	AN	FS	FV	LH	SM
Acidic	3.11	3.20	2.94	3.09	2.98
Water	5.23	6.68	5.14	6.17	6.19
Alkaline	8.98	9.54	8.96	9.00	8.94
LSD	0.04***	0.001***	0.20***	0.05***	0.07***
EC					
Acidic	14.48	17.67	11.07	19.71	20.2
Water	12.49	12.20	17.55	24.8	17.33
Alkaline	14.19	15.87	13.86	20.30	23
LSD	ns	2.08*	2.4*	1.8*	2.6*
DM					
Acidic	2.15	3.24	2.98	1.26	1.15
Water	2.75	2.66	3.03	1.64	1.45
Alkaline	2.85	2.87	3.48	1.75	1.72
LSD	ns	0.32*	0.34*	0.15**	0.24*

ns not significant; *P<0.05; **P<0.01; ***P<0.001

28 days from the start of experiments by individually cutting at the base of each plant followed by weighing and recording the position of plants in the channel. The harvested plants (roots and shoot) were dried for 19 h in an oven maintained at 90°C, and dry matter content was determined for each replicate.

Statistical analysis

The differences between the harvested seaweed samples from the two sampling dates were evaluated by analysis of variance. The results from the mung bean and pak choi experiments were analysed as factorial experiments (GenStat Release 11.1, 2008). Comparisons of biomass yield and root numbers were presented as the measure of the least significance difference (LSD) at 5% level. The calculated mean DM and root numbers for comparison with the extracts were derived from the measured values of the five IBA concentrations.

Results

Composition of seaweed species

The morphological characteristics of the samples were recorded and compared with published descriptions. The nitrogen (%) content (Table 1) varied between 1.45 and 3.11 in *L. hyperborea* to 1.37–1.95 in *A. nodosum*, and the values were within the range reported (Stephenson 1974). The ash (%) content which ranged from 18.1 to 47.68 for all species was higher than the reported values (Marinho-Sorinano et al. 2006; Ruperez 2002; Rioux et al. 2007), and this can be explained by the inorganic salt in sea water absorbed by the seaweeds or by the association of cations

Table 4 Comparison of thermogravimetric analysis of seaweed species harvested in February and June sampling dates (AN, FS, FV, LH and SM—Table 1) in air showing weight losses (WL1–WL4, %) and residue in the four temperature bands (30–190°C, WL1[1], 190–450°C, WL2[2]; 450–660°C, WL3 and 660–780°C WL4), LSD significant at 5% level (n=3)

AN	WL1	WL2	WL3	WL4	Residue (%)	Peak Temp (°C)[1]	Peak Temp (°C)[2]
February	7.59	49.11	19.29	5.27	17.62	267.6	473.2
June	8.02	39.98	21.59	8.30	20.84	269.9	483.2
LSD	0.44*	1.20***	0.98**	0.86**	2.00**	0.56**	6.30**
FS							
February	5.89	39.16	25.25	6.04	22.54	270.0	491.9
June	5.42	39.15	22.54	4.88	26.95	278.5	492.1
LSD	0.12*	ns	0.82**	0.81*	1.23**	0.74***	ns
FV							
February	7.67	43.18	19.64	8.67	19.71	264.4	495.8
June	6.80	40.31	16.22	12.79	22.82	273.5	491.3
LSD	0.79*	2.00***	0.48**	0.92**	1.98**	0.90**	3.90*
LH							
February	4.33	22.16	17.31	1.93	52.97	274.0	485.8
June	4.75	36.29	20.11	3.72	34.07	260.2	488.2
LSD	0.37*	1.10**	0.78**	1.30*	1.50***	0.39***	ns
SM							
February	8.21	25.01	21.23	4.71	39.67	283.2	497.1
June	5.35	32.26	19.42	4.05	37.92	267.0	494.1
LSD	0.17**	0.95**	1.30*	0.64*	1.43*	0.73**	2.90*

ns not significant; *P<0.05; **P<0.01; ***P<0.001

with algal polysaccharides (Marinho-Sorinano et al. 2006). The minimum (0.83) and maximum values (3.98) of lipid were within the range reported (Sanchez-Machado et al. 2004; Marinho-Sorinano et al. 2006). The carbon content (%) of the five species ranged between 25.0 and 38.6. The differences in nitrogen, ash, lipid and carbon contents of the samples were significant (Table 1).

Mineral content of seaweed

Two species, *L. hyperborea* and *S. muticum*, contained the highest amounts of minerals (Table 2) compared to the other three species consistent with the ash contents of the five species, confirming high inorganic content. The analyses indicated significant differences in the concentrations of Fe, I, K, Mg and S in the five species. Sulphur content was the highest in *A.*

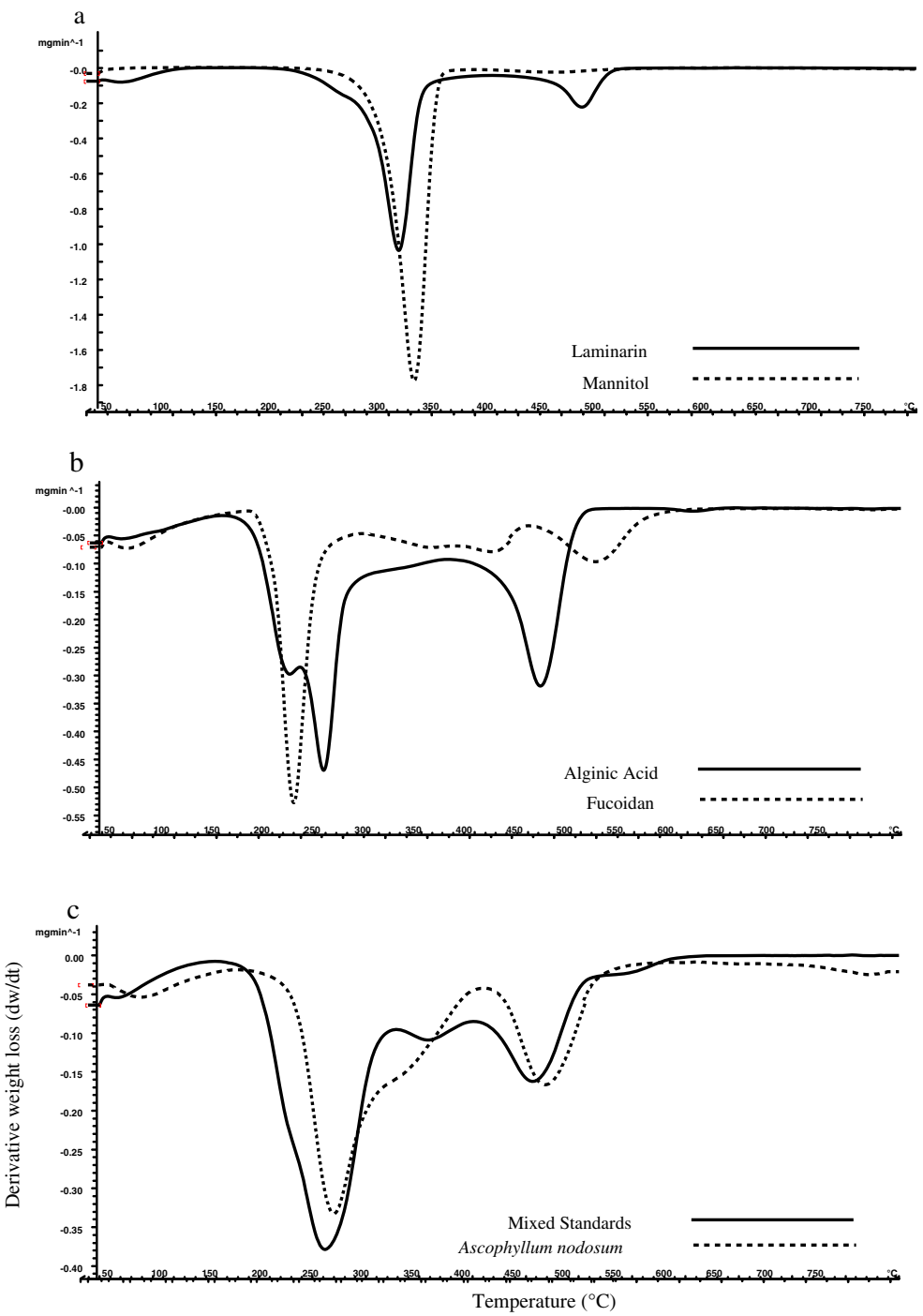

Fig. 1 **a**–**c** Overlays of derivative thermograms: **a** laminarin and mannitol, **b** fucoidan and alginic acid and **c** a mixture of the four components along with unextracted cell wall material of *A. nodosum*, analysed in the presence of air to show differences in the combustion profiles

nodosum and *F. vesiculosus* compared to the other three species, and K content was the highest for *L. hyperborea* and *S. muticum*. The range of mineral concentrations detected agreed with a previous report (Ruperez 2002).

Compositional analyses of seaweed extracts

The differences in pH of the acidic, neutral and alkaline extractions were significant for the five species (Table 3). The EC and DM contents of the three preparations of the five species were quantified, and the differences between the means were significant for all species except for *A. nodosum*. The pH of the aqueous extractions from the June samples extended between 5.21 and 6.30, and mean value of EC was 9.3 mS cm^{-1} and after acidification of the blended materials (pH 4.0), EC increased to 16.64 mS cm^{-1}. The DM (%) contents of the five acid extracts from June (*A. nodosum*, 5.29; *F. vesiculosus*, 5.95; *F. serratus*, 5.36; *L. hyperborea*, 5.15; *S. muticum*, 5.10) were higher than the February samples (Table 3). Similar increase in DM yields from winter to summer sampling periods has been reported by Rioux et al. (2009). The AlgaGreen was also evaluated for pH (5.45), EC (27.9 mS cm^{-1}) and DM content (9.1%). A high concentration of IAA was detected in acid extracts (June samples) of *F. vesiculosus* (46.84 ng g^{-1}) and *A. nodosum* (40.96 ng g^{-1}) compared to lower concentrations detected in extracts of *S. muticum* (2.74 ng g^{-1}), *F. serratus* (10.58 ng g^{-1}) and *L. hyperborea* (5.9 ng g^{-1}). The range of IAA concentration in seaweed samples agreed with previous reports (Sanderson et al. 1987; Stirk et al. 2004).

Thermogravimetric analysis of seaweed species

The weight loss (WL1) in the 30–190°C temperature band was significantly higher for samples collected in February compared to June except for *L. hyperborea* and *A. nodosum*. In both February and June, *L. hyperborea* and *S. muticum* had the lowest weight loss for WL2, the highest residue values compared to other species and the differences in WL2 and residue values were significant except for *F. serratus*. The mean weight losses (WL3) in the 450–660°C decomposition band for all species were significantly higher in June compared to February sampling date, except for *A. nodosum* and *L. hyperborea*. The WL4 values in the 660–780°C were significantly different and a peak at near 700°C could be attributed to the decomposition of stable char (Anastasakis et al. 2011; Ross et al. 2009). The two peak decomposition temperatures (PT1 and PT2) associated with WL2 and WL3 decomposition bands were also significantly different between the two sampling dates (Table 4).

The major organic fractions in the seaweed species are protein, lipid and carbohydrates which can be subdivided into alginic acid, laminarin, mannitol and fucoidan (Anastasakis et al. 2011; Hou et al. 2008; Ross et al. 2009). Reference samples of these carbohydrates were analysed under similar conditions to a sample of *A. nodosum* to allow evaluation of the combustion steps and to identify any overlap in the onset of their decomposition profiles. This included the analysis of a mixture (synthetic sample) of equal proportions of the four carbohydrate fractions listed above. The derivative curves of the reference samples analysed in the presence of air are presented in Fig. 1, showing the characteristic combustion profiles of (a) laminarin and mannitol and (b) fucoidan and alginic acid.

The derivative curve of the synthetic sample was compared with the thermal profile of *A. nodosum* to show similarities and differences in the primary peak due to the presence of lipid, protein and minerals in the unextracted material (Fig. 1c). The freeze-dried polysaccharides resulting from the acidic and alkaline extractions exhibited decomposition peaks between 150–160 and 285–290°C. The peak at 155°C was absent in the neutral fraction but associated with a minor peak at 470°C (Fig. 2). Thermogravimetric analyses have shown significant differences between the five species of seaweeds, mainly in the primary

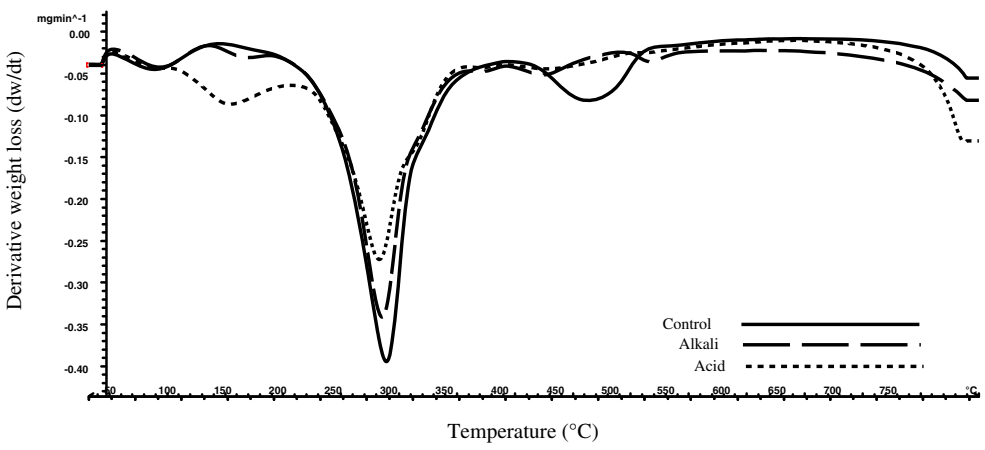

Fig. 2 Overlays of derivative thermograms of *A. nodosum*—polysaccharides extracted in control (neutral), acidic and alkaline pH showing combustion peaks at 150°C, 290°C and 470°C

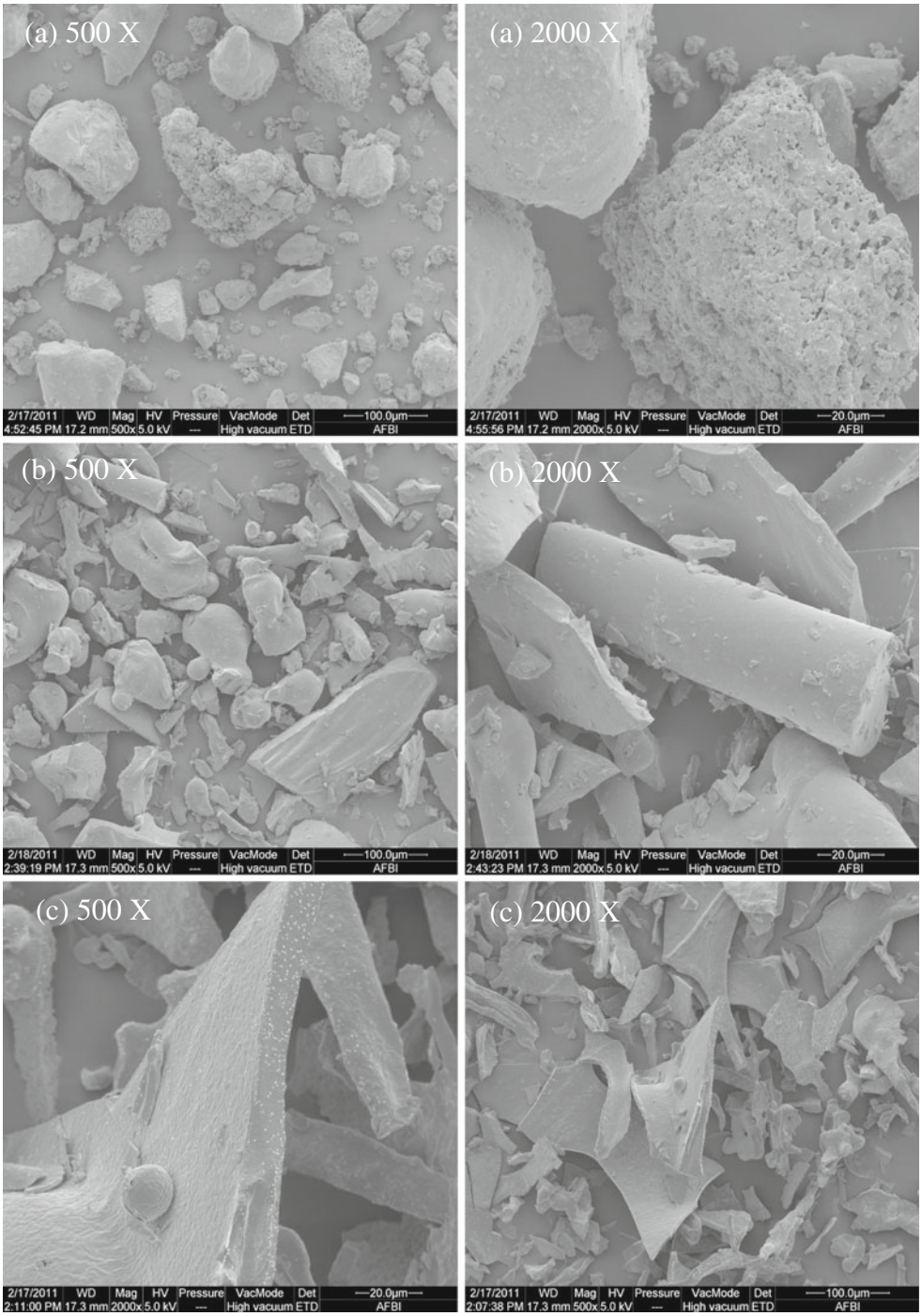

Fig. 3 **a**–**c** Typical scanning electron microscope images of freeze-dried polysaccharides of *A. nodosum* after **a** acidic, **b** neutral and **c** alkaline extractions showing differences in particle forms (×500 and ×2,000)

and secondary decomposition peaks (WL1–WL4), indicating compositional variations in both organic and inorganic fractions (Ross et al. 2009).

Evaluation of extracted polysaccharide

The freeze-dried polysaccharide fractions extracted by acid, neutral and alkaline protocols were evaluated for surface features and structural forms by SEM (Fig. 3a–c). The materials from the alkaline extractions were well degraded (fine particle size), indicating depolymerisation during extraction but formed a highly regular structure and pores on the materials were not visible. The polysaccharides extracted in acid pH exhibited granular structures with open and porous form compared to materials derived from the alkaline or neutral extraction protocols. The polysaccharides extracted from *F. serratus* in acidic, neutral and alkaline pH were very sticky and non-granular compared to the other four species. Several species including *F. serratus* can release phenol–alginate compounds that exhibit adhesive

Table 5 A comparison of dry matter (%) content of mung bean plants treated with five extracts prepared as acidic, neutral, alkaline and control (IBA), LSD significant at 5% level (270 replicates for seaweed extracts and 15 replicates for control); control IBA value mean of five IBA test concentrations

Preparation	AN	FS	FV	LH	SM	Mean
Acidic	9.58	9.72	8.30	9.77	9.71	9.42
Neutral	10.13	9.97	10.10	9.80	9.94	9.98
Alkaline	10.46	10.09	10.41	10.35	10.07	10.28
Control (IBA)	8.99					

LSD preparation=0.36; LSD species=0.46; LSD preparation×species =0.79

properties to both hydrophobic and hydrophilic surfaces in aqueous conditions (Berglin et al. 2004).

Mung bean bioassay

Mung bean stem cuttings were incubated in the test concentrations of the five seaweed extracts and IBA. This resulted in a significant ($P<0.001$) increase of DM content of all treatments except for the acidic extract of *F. vesiculosus* compared to the control (Table 5). The alkaline extracts showed the highest DM yields of the treated plants (Table 5). A comparison of DM content of the plants incubated in different dilutions (10^{-2} to 3×10^{-5}) of the extracts revealed a gradual increase of DM (9.67 to 10.56%) yield, indicating higher nutrient uptake with alkaline compared to acidic. The dry matter contents of mung bean plants, treated with IBA in 1 M, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} solutions, were 8.06, 8.89, 8.83, 9.53 and 9.66%. The treatment also resulted in a significant ($P<0.001$) increase in root numbers for all seaweed species when prepared in acidic pH, and *F. vesiculosus* showed significantly ($P<0.001$) higher root numbers compared to the other four species (Table 6). The root numbers of mung bean plants, treated with IBA in 1 M, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} solutions, were 21.27, 24.73, 29.93, 35.43 and 44.63%. The mean root numbers were higher for IBA-treated cuttings compared with plants treated with seaweed extracts (Table 6).

Table 6 A comparison of root numbers of mung bean plants treated with acidic, neutral and alkaline seaweed extracts and control, LSD significant at 5% level (270 replicates for seaweed extracts and 15 replicates for control); control IBA value mean of five IBA test concentrations

Preparation	AN	FS	FV	LH	SM
Acidic	11.09	11.21	14.04	10.40	9.95
Neutral	9.43	9.78	12.37	9.29	9.59
Alkaline	10.76	9.72	10.20	9.12	9.28
Control (IBA)	31.20				

LSD species=1.13; LSD preparation=0.88

Pak choi production trial

The test solutions of seaweed extracts (e.g. acidic extracts of the five species, water control and AlgaGreen) were adjusted to pH 5.43–5.62 before application and consequently EC increased after pH adjustments to 27.94 mS cm^{-1}. Mean DM content of the acidic extracts and AlgaGreen preparations were 2.17 and 9.12%, respectively, indicating significant differences in the availability of potential nutrients for plants. Furthermore the DM contents of the June samples were higher at a mean value of 5.37%. During the pak choi trials, the plants treated with acidic extracts (100×dilution) would have received a lower dose of nutrients compared to AlgaGreen (150×dilution) due to differences in the DM content. Two weeks after the transplantation in the channels, plant leaves from some of the treated (e.g. dark green colour) and control plants were easily distinguishable by visual evaluation. The foliar application of the extracts positively influenced the vegetative growth of pak choi plants (Table 7). The seaweed extracts, e.g. *A. nodosum* and *F. vesiculosus* showed significant ($P<0.05$) differences in DM yield for the two dilutions.

Discussion

Major components of the five species changed significantly during the winter and summer months (Rioux et al. 2009). The range of carbon, nitrogen, lipid, ash, minerals and IAA concentrations in the samples agreed with previous reports (Rioux et al. 2007; Ruperez 2002; Sanchez-Machado et al. 2004; Sanderson et al. 1987; Stephenson 1974). The changes in dry matter content, pH and EC of acidic,

Table 7 Comparison of dry matter (%) yield of pak choi plants (g) treated with foliar applications of five acidic extracts at two concentrations 1/100 and 1/30, water control and AlgaGreen (1:150 concentration), LSD significant at 5% level; seaweed treatments, $n=900$; water control, $n=90$; AlgaGreen, $n=90$

Treatment	Concentration 1/100	Concentration 1/30
AN	5.06	4.54
FS	4.72	4.95
FV	4.60	4.97
LH	4.66	4.65
SM	4.93	4.72
Water control	4.72	
AlgaGreen (1/150)	4.69	

LSD species×concentrations=0.34

alkaline and neutral extractions indicated affects on the bioactivity of the extracts (Booth 1969; Henry 2005).

The analysis of reference standards (e.g. alginic acid, fucoidan, laminarin and mannitol) and the synthetic sample demonstrated the changes in the rate of decomposition of some components of the mixture due to a catalytic effect of alginic acid. Since alginic acid is composed of two types of uronic acids, D-mannuronic (*M*) acid and L-guluronic (*G*) acid, the *M*/*G* ratio will determine solubility and viscosity (Mizuno et al. 1983). The *M*/*G* ratio is likely to determine thermal profile and the shoulder at 225°C may be linked to compositional differences. Peak combustion of the synthetic sample occurred between 160 and 320°C with a shoulder near 220°C indicating decomposition of fucoidan and/or alginic acid followed by the primary peak at 250°C representing laminarin. At 275°C a shoulder to the main peak was observed which could be linked to combustion of mannitol and the earlier onset of combustion for all fractions indicated a catalytic effect caused by the acid group in alginic acid (Anastasakis et al. 2011; Roman and Winter 2004). The next combustion step was a two-stage char formation at 350°C and 450°C followed by a minor shoulder at 520°C possibly linked to decomposition of residual carbon/inorganic fractions (Anastasakis et al. 2011).

The pyrolysis profile of polysaccharides present in different seaweed species has been reported (Anastasakis et al. 2011). Thermogravimetry can be used as a semi-quantitative protocol for comparing compositional differences between mannitol, alginic acid, fucoidan and laminarin fractions of seaweeds including the catalytic effect of alginic acid. In this study similar results were observed for both the synthetic sample and *A. nodosum*, indicating a reduction in temperature of the primary peaks from laminarin and mannitol by nearly 100°C (Fig. 1c). The thermal profiles of the extracted polysaccharides showed the influence of the extraction process demonstrated by the higher weight loss below 200°C in the acidic and alkaline extracts (Fig. 2).

The acidic preparations showed significantly increased root numbers in four of the five species and a previous study demonstrated that auxin-like compounds were present in higher concentration compared to alkaline extracts (Crouch and van Staden 1993; Stirk and van Staden 1977). The acidic extracts from *A. nodosum* and *F. vesiculosus* showed high IAA content compared to the other three species (Sanderson et al. 1987; Stirk et al. 2004). The presence of bioactive substances can enhance efficiency of stomatal uptake of nutrients and minerals compared to the untreated plants (Mancuso et al. 2006).

Many researchers (Demir et al. 2006; Rathore et al. 2009; Spinelli et al. 2010) have reported positive effects that seaweed extracts have on seed vigour as a result of priming and the productivity, and alleviation of biotic and abiotic stresses in field crops. Key examples are the role of plant growth regulators and polyols (e.g. mannitol) for alleviating abiotic stresses, the development of protocols for determining growth hormones and the identification of specific genes in *Arabidopsis thaliana* linked to the signalling factors present in *A. nodosum* extracts (Rayorath et al. 2008; Zhang and Ervin 2004). Furthermore the results suggested that *A. thaliana* could be used effectively as a rapid tool for testing the bioactivity of seaweed extracts (Rayorath et al. 2008). Zhang and Ervin (2004) were one of the first to report that the treatment of cool season grasses with seaweed extracts and humic acids improved resistance to moisture deficit conditions possibly by upregulation of plant defence systems against oxidative stress. During our trials, the temperature inside the plastic house reached 25–30°C during at least 7 days and the evening temperatures averaged 4–8°C. The abiotic conditions (e.g. high and low temperature) may not have been stressful enough to the pak choi plants. Other studies have shown that crop plants, e.g. potato, cucumbers and onions are more likely to respond to foliar treatments under stressful growing conditions (Kuisma 1989; McGeary and Birkenhead 1984; Nelson and van Staden 1984).

In conclusion, this study has confirmed that carbon, nitrogen, lipid, polysaccharides and mineral contents of the species can change between winter and summer months. The results from this study have provided supporting evidence for improved uptake of nutrients leading to greater DM yields of both mung bean and pak choi plants. The alkaline extracts from *F. vesiculosus* and *A. nodosum* stimulated higher DM yield of the mung bean plants. The majority of the acidic extracts enhanced root formation on the mung bean stem cuttings compared to alkaline or neutral extracts. The interaction of species (*A. nodosum* and *F. vesiculosus*) and the two treatment dilutions on DM yield differences of pak choi plants were significant. The use of plant biostimulants should be combined with precision agriculture and innovative decision-making systems, for maximizing yield and quality of crop plants.

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