

The effect of *Ascophyllum nodosum* extract on the growth, yield and fruit quality of tomato grown under tropical conditions

Nerissa Ali¹ · Aidan Farrell¹ · Adesh Ramsubhag¹ · Jayaraj Jayaraman¹

Received: 7 January 2015 / Revised and accepted: 27 April 2015 / Published online: 12 May 2015
© Springer Science+Business Media Dordrecht 2015

Abstract Tomato plants (*Lycopersicon esculentum* Mill) grown under tropical field conditions were treated with an alkaline seaweed extract made from *Ascophyllum nodosum* (ASWE). Two field experiments and one greenhouse experiment were conducted to evaluate methods of application, dosage of application, and the impact of each on plant growth parameters and on the quality and yield of fruit. Field experiment 1 included 0.2 % ASWE spray, 0.2 % ASWE root drench, fungicide spray and combinations of the above. Plants foliar-sprayed with 0.2 % ASWE had significantly increased plant height (10 %) and plant fruit yield (51 %) when compared to control plants. Similar results were observed for ASWE spray alternated with fungicide or with ASWE root drench. Field experiment 2 included 0.5 % ASWE spray, fungicide spray and ASWE spray alternated with fungicide. The higher concentration of ASWE resulted in a significant increase in plant height (37 %) and plant fruit yield (63 %) compared to control plants. The third experiment under greenhouse conditions also showed that 0.5 % ASWE spray caused a significant increase in plant height (20 %) and plant fruit yield (54 %) compared to control plants. In the greenhouse, ASWE-treated plants had larger root systems and increased concentrations of minerals in the shoots. Fruit from plants treated with ASWE showed significant increases in quality attributes including, size, colour, firmness, total soluble solids, ascorbic acid levels and mineral levels. Overall, the use of ASWE resulted in clear improvements in tomato fruit yield and quality under tropical growing conditions.

Keywords *Ascophyllum nodosum* · Phaeophyceae · Fruit quality · Plant growth · Seaweed extract · Tomato · Tropical agriculture

Introduction

In the tropics, tomato (*Lycopersicon esculentum* Mill.) is cultivated year-round and is recognised as a key vegetable and cash crop for many low-income farmers (Prior et al. 1994). To achieve reasonable yields and high-quality produce, growers use a wide range of fertilisers and pesticides, which accounts for up to 38 and 43 % of total crop production costs, respectively (Lopez et al. 1995, 1996). Abundant use of these synthetic chemicals can result in detrimental effects on non-target species and in chemical contamination of soil, water supplies and harvested products (Barceló and Hennion 1997). Growers continue to search for sustainable strategies that will improve crop yields without adversely impacting on the environment. The use of seaweed extract as bio-fertilisers and bio-stimulants offers a potential solution to this problem (Jayaraj et al. 2008; Khan et al. 2009; Hernandez-Herrera et al. 2014; Satish et al. 2015).

Of the three main groups of macro algae, the brown seaweeds (e.g. *Ascophyllum nodosum*) are the most commonly used in agriculture (Ugarte et al. 2006). Traditionally, growers utilise seaweeds either fresh or dried, as manures or compost (Booth 1969). More recently, growers have begun to use commercial seaweed extract products as foliar sprays (Craigie 2011; Mohanty et al. 2013; Hernandez-Herrera et al. 2014; Mikiciuk and Dobromilska 2014). Seaweed extracts are classified as bio-stimulants because they contain bioactive substances at low concentrations that exhibit growth-stimulating properties (Khan et al. 2009), but they also act as bio-fertilisers (Craigie 2011; Mohanty et al. 2013). Analysis of *A. nodosum* extract revealed a wide range of organic and inorganic

✉ Jayaraj Jayaraman
jaya1965@hotmail.com

¹ Department of Life Sciences, Faculty of Science and Technology, The University of the West Indies, St. Augustine, Trinidad and Tobago

substances including alginic acid, mannitol, laminarin, nutrient minerals, vitamins, phytohormones and antioxidants (Baardseth 1970). Recent studies indicate that brown seaweeds contain 40 to 70 % carbohydrates, such as cellulose, alginate, laminarin, fucoidan and mannitol; 3 to 10 % proteins, such as lectins and free amino acids; 4 to 8 % polyphenols and other pigments; 2 to 4 % phospholipids and glycolipids; trace amounts of vitamins C and A; a wide variety of minerals including phosphorous, potassium, calcium, magnesium, boron, zinc and other trace elements and trace amounts of plant hormones, including auxins (indole acetic acid and indole pyruvic acid), betaines, cytokinins (zetaine) and gibberellins (Sharma et al. 2012; Chojnacka et al. 2012). Plant hormones can alter plant growth by stimulating cell expansion and increasing the photosynthetic rate. Plant hormones from brown seaweeds, such as cytokinins, auxins and gibberellins, have been shown to enhance germination, growth and yield in crop plants (Crouch and van Staden 1993; Rayorath et al. 2008; Craigie 2011). Betaines and betaine-like compounds have been shown to increase tolerance to abiotic stresses such as salinity, drought and frost (Blunden and Gordon 1986; Wang et al. 2003).

Though several reports are available on the use of *Ascophyllum* extract in plant culture, there has been less research on the use of this extract under tropical field conditions. This study assesses, for the first time, the potential of *A. nodosum* extract to improve the yield and quality of tomato growing under tropical field conditions. The study further assesses the efficacy of different concentrations and application methods for use as a phytostimulant in tomato.

Material and methods

Field experiments

Field experiment 1 was conducted in October 2010 at the Orange Grove Estate in North Trinidad (10° 38' 00.6" N, 61° 22' 20.4" W) and consisted of eight treatments applied

over 3 months. Field experiment 2 was conducted in October 2013 at Bernard Road Food Crop Project Estate in Central Trinidad (10° 32' 38.4" N, 61° 26' 40.4" W) and consisted of four treatments applied over 4 months. The treatment details for both field experiments are provided in Table 1. The field preparation, intercultural operations and cultivation methods were adopted as per the recommendations suggested by the Ministry of Food Production, Trinidad and Tobago (<http://agriculture.gov.tt>). The field was prepared by mechanical brush cutting, ploughing and rotovating. Cambered beds were formed 6.10×0.91 m apart. Seven-week-old Hybrid 61 tomato seedlings were transplanted with 0.25 m between plants and 0.9 m between rows. Plants were supported with wooden stakes throughout. Baseline mineral nutrition was provided at the rate of 5 g plant⁻¹ of NPK as a basal dose and 25 g as split doses at monthly intervals. The usual application of foliar fertiliser and plant boosters was omitted. An alkaline seaweed extract prepared from *A. nodosum* (ASWE) by extracting in potassium hydroxide followed by neutralisation was supplied by the Acadian Seaplants Limited, Dartmouth, NS, Canada (see Rayorath et al. 2009 for details). The seaweed extract was applied to tomato plants as foliar spray/soil drench at a concentration of 0.2 or 0.5 %. In the appropriate treatments, a fungicide, Daconex (75 % chlorothalonil), was sprayed at 1.25 g a.i L⁻¹.

Both field experiments were laid out in a completely randomised design with three replicates for each treatment. Treatment sprays were applied 15 days after transplanting and then once every 15 days throughout the experiments. Plant height was measured at the end of the experiment. Fruits were harvested upon maturity at half-ripe stage and fruit yield measured as total marketable harvested fruit weighed after each harvest and accumulated at the end of the experiment.

Greenhouse experiment

For the greenhouse experiment, tomato plants (Hybrid 61) were grown under greenhouse conditions from August to

Table 1 Details of treatments applied in the two field experiments

Field experiment 1	Field experiment 2
Control	Control
Fungicide spray	Fungicide spray
ASWE spray (0.2 %)	ASWE spray (0.5 %)
Fungicide and spray: ASWE spray (0.2 %) alternated with fungicide	Fungicide and spray: ASWE spray (0.5 %) alternated with fungicide
ASWE root drench (0.2 %)	
Spray and drench: ASWE spray (0.2 %) and ASWE root drench (0.2 %)	
Fungicide and drench: ASWE root drench (0.2 %) alternated with fungicide	
All: ASWE spray (0.2 %) and root drench alternated with fungicide	

Treatments include *A. nodosum* seaweed extract (ASWE) applied as a foliar spray or a root drench and fungicide (Daconex; 75 % chlorothalonil) applied as a foliar spray

November 2011 at the University of the West Indies Field Station at Valsayn, Trinidad. Within the greenhouse, treatment plants were laid out in a completely randomised design with each treatment row (ten plants) replicated three times. A growing medium was prepared using a planting mix (Green Formula; Lambert Peat Moss Inc., Canada) and sharp sand in a ratio of 3:1, filled into pots (0.04 m³). Seven-week-old plants were transplanted into the planting mix at one seedling per pot. Plants were fertilised with a total of 25 g of NPK fertiliser applied as split doses at planting and continued at 2-week intervals up to 50 days after planting. No fungicides or phytostimulants were used in this experiment. The treatments included control, 0.2 and 0.5 % ASWE foliar spray. Foliar sprays were applied once every 15 days; control plants were sprayed with water. Plant height was measured at bi-weekly intervals. Chlorophyll content measurements were taken from ten randomly selected plants per treatment, using

mature leaves at the tenth node from the base. Five readings were taken from each leaf using a chlorophyll meter (atLEAF+, FT Green LLC, Detroit, USA) and expressed as a ratio (equivalent to SPAD units; Richardson et al. 2002). At the end of the experiment, the plants were harvested for biomass and mineral content analysed. For biomass estimation, at the end of the experiment, plants were cut at the collar region and separated into shoot (stem and leaf) and root for determination of dry weight. Tissue was placed in paper bags and oven dried at 65 °C for 24 h before weighing. For mineral analysis, 1 kg of dried shoot tissue per treatment was taken from the bulk oven-dried tissue and then milled into a powder from which three subsamples were tested. N was determined using the Kjeldhal distillation method (AOAC 2007); P using spectrophotometry (Murphy and Riley 1962); K and Na by flame photometry (Jackson 1973) and Ca, Cu, Fe and Zn using atomic absorption spectrophotometry (Lindsay and Norvell 1978).

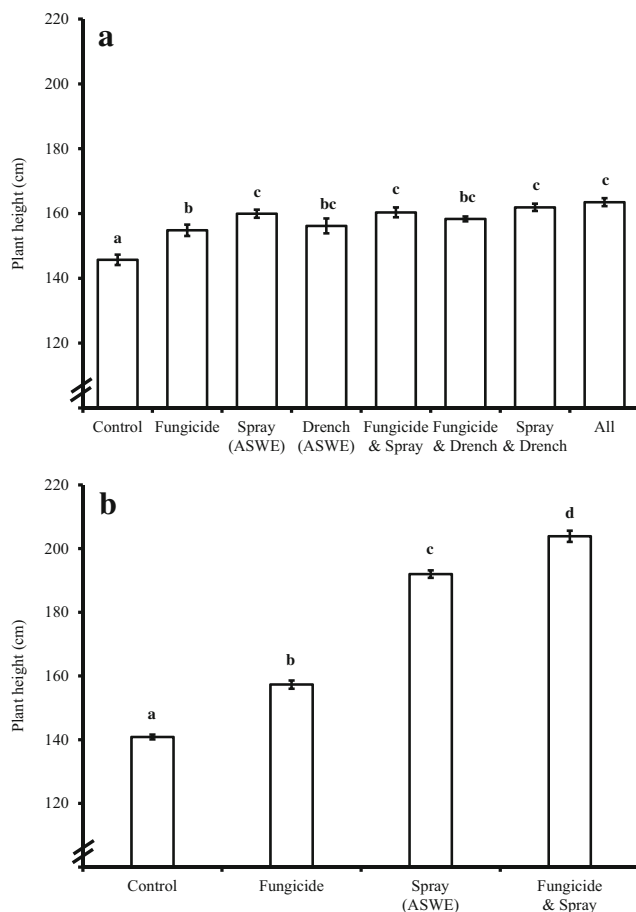


Fig. 1 Plant height of field grown tomato plants from **a** field experiment 1, 90 days after transplantation with eight treatments including seaweed extract made from *A. nodosum* (ASWE) at a concentration of 0.2 % and **b** field experiment 2 120 days after transplantation with four treatments including ASWE at a concentration of 0.5 %. Data are means±SE (n=30 plants); different letters indicate significant differences according to Fisher's Least Significant Difference Test (P=0.05); LSD is 4.2 and 3.6 for **a** and **b**, respectively

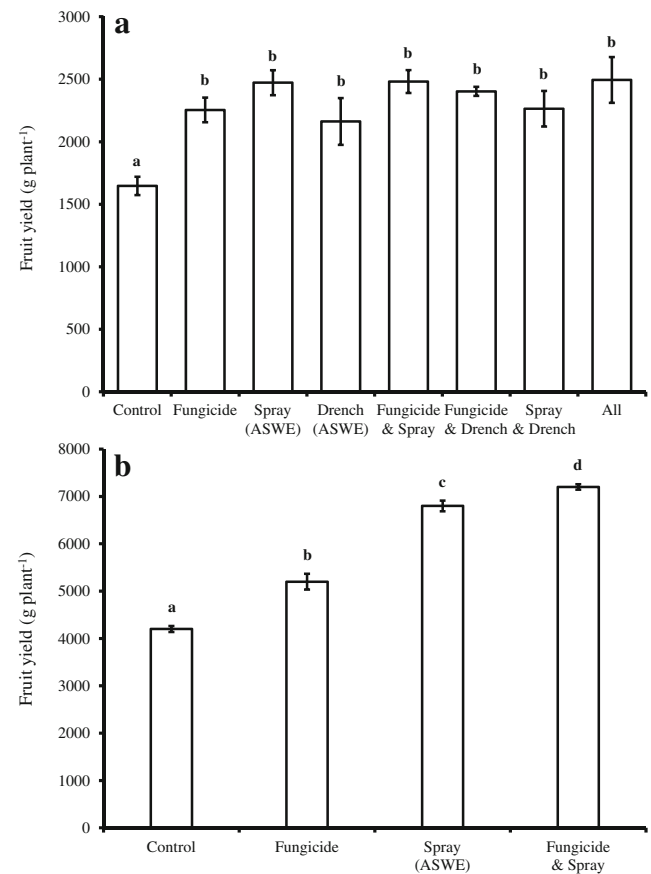


Fig. 2 Fruit yield of field-grown tomato plants from **a** field experiment 1, 90 days after transplantation with eight treatments including seaweed extract made from *A. nodosum* (ASWE) at a concentration of 0.2 % and **b** field experiment 2, 120 days after transplantation with four treatments, including ASWE at a concentration of 0.5 %. Yields are g plant⁻¹ of fresh weight accumulated over several harvests. Data are means±SE (n=30 plants); different letters according to Fisher's Least Significant Difference (LSD) test (P=0.05); LSD is 372.3 and 306.1 for **a** and **b**, respectively

A range of reproductive parameters were monitored throughout the duration of the greenhouse experiment, i.e. number of bearing flower clusters, number of flowers per cluster and number of fruits per cluster. Fruit yield was monitored throughout the greenhouse experiment. Fruits were harvested five times over the 60 days and weighed at each harvest, fruit yield per plant was calculated from the cumulated weights. Harvested fruits were graded based on weight, and the percentage of fruit in each category was calculated: grade A (>70 g), grade B (30–69 g) and grade C (<30 g).

A range of additional fruit quality parameters were measured during the third harvest, i.e. size, flesh and skin thickness, external colour, internal colour, firmness, total soluble solids, total titrable acidity, viscosity, mineral content and ascorbic acid content. For fruit quality analysis, 2 kg of randomly selected fruit per treatment was pooled from the third harvest. Fruits were harvested at the 75 % ripe stage and kept for 5 days until 100 % ripe. All the fruit quality tests were performed in a laboratory at a temperature of 20 °C and a relative humidity of 65 %. Polar fruit length (length from the stem end to the blossom end) and fruit diameter (circumference across the widest part of the fruit) were measured using a caliper, from ten fruit selected at random from each pooled sample. Skin and flesh thickness were also measured in millimetre using a caliper, from ten fruit selected at random from each pooled sample. External and internal fruit colour was measured from ten fruit selected at random from each pooled sample. External colour was measured in L, A, B coordinates using a colorimeter (Konica Minolta Chroma Meter, Sensing Inc., Japan) and placing the portable iristimulus meter at the mid-point between the stem and calyx end of each fruit (Lopez et al. 2004). Internal fruit colour was measured from fruit cut across the equatorial diameter using a colorimeter (Lopez et al. 2004). Fruit and flesh firmness was measured from ten fruit selected at random from each pooled sample using a texture analyser (Penetrom-TA.XT. Plus Texture Analyzer, Stable Micro Systems, UK), with three probes used for each fruit and the calibrated average recorded. Total soluble solids (TSS) were measured from ten random samples from each pooled sample, which were chipped and tested using a refractometer (Digital OPTI Refractometer, USA). Total titrable acidity (TTA) was measured from 25 g of macerated fruit, by titrating centrifuged macerated tomato with sodium hydroxide, using phenolphthalein as an indicator (AOAC 2007). TTA was measured five times for each treatment, and values were used to calculate the grams of citric acid equivalent per 100 mL of tomato juice. Juice viscosity was measured using the Bostwick consistometer (CSC-Scientific, USA), by blending 500 g of seedless tomato fruit per treatment in a Stomacher commercial laboratory blender. The flow rate was measured as distance travelled in centimetre after 20 s (replicated three times per treatment). For mineral analysis, 2 kg of pooled fruit samples from each treatment

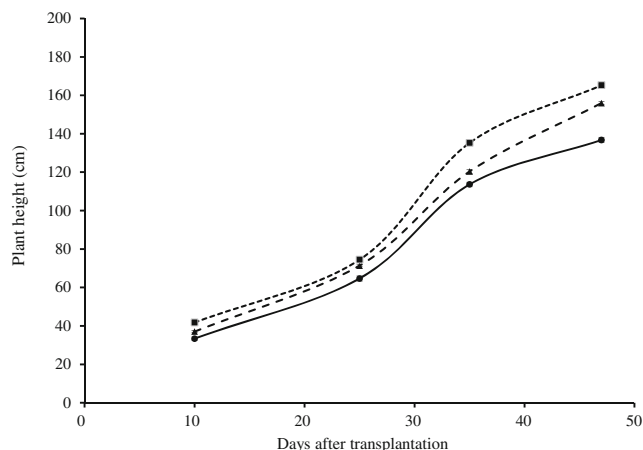


Fig. 3 Plant height of greenhouse-grown tomato plants over 50 days. Plants were sprayed with water (control; circles), seaweed extract made from *A. nodosum* (ASWE) at a concentration of 0.2 % (triangles) or ASWE at a concentration of 0.5 % (squares). Data are means \pm SE ($n=30$); LSD ($P=0.05$) is 3.4 at 50 days after transplantation

were diced and packed into covered foil trays, frozen, freeze dried and milled into a powder and analysed as above for plant tissue. For ascorbic acid content, 20 g of fruit per treatment was homogenised and analysed using the 2,6-dichloroindophenol titrimetric method (AOAC 2007). Ascorbic acid content was measured five times for each treatment, and ascorbic acid per 100 g fruit was calculated using the following formula:

mg ascorbic acid per 100 g fruit

$$= 2/17.2 \times \text{titration value} \times \text{extract volume} \times 4$$

Data analysis

For field experiment 1, factorial ANOVA was performed with ASWE spray, ASWE drench and fungicide as factors. For field experiment 2 and the greenhouse experiment, one-way ANOVA was used with each treatment as a factor (Genstat 8; VSN International Ltd., UK). Post hoc testing used least

Table 2 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on shoot dry weight, root dry weight and root:shoot ratio of greenhouse-grown tomato plants

Treatment	Shoot weight (g)	Root weight (g)	Root:shoot
Control	118.32 \pm 1.427 a	11.08 \pm 0.294 a	0.094 \pm 0.007a
0.2 % ASWE	138.02 \pm 0.790 b	17.38 \pm 0.615 b	0.126 \pm 0.002b
0.5 % ASWE	163.24 \pm 2.828 c	22.13 \pm 0.300 c	0.136 \pm 0.004b

Values are means \pm standard error ($n=30$). Means followed by different letter are significantly different according to Duncan's multiple range test ($P=0.05$)

Table 3 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on number of bearing clusters, flowers per cluster and fruit per cluster of greenhouse-grown tomato plants

Treatment	Number of bearing clusters	Number of flowers per clusters	Number of fruit per clusters
Control	13.70±0.448 a	7.77±0.207 a	4.33±0.188 a
0.2 % ASWE	20.07±0.621 b	8.93±0.191 b	6.07±0.209 b
0.5 % ASWE	24.77±0.471 c	10.43±0.233 c	7.23±0.266 c

Values are means±standard error ($n=30$). Means followed by different letter are significantly different according to Duncan’s multiple range test ($P=0.05$)

significant differences and Duncan’s multiple range test at the 0.05 significance level.

Results

Field experiments

Figure 1 shows plant height as measured in the two field experiments, measured after 90 and 120 days, respectively. Plants treated with ASWE were significantly taller than control plants in both experiments. In field experiment 1, all treatments and all interactions were significant, except ASWE root drench×fungicide spray (Fig. 1a). This shows that ASWE had an additional effect above fungicide treatments when applied as a foliar spray (but not when applied as a root drench). Spray of ASWE at 0.2 % increased the plant height by 10 % (Fig. 1a); but in field experiment 2 with the higher concentration of ASWE spray (0.5 %), plant height increased by 37 % in comparison to the control plants (Fig. 1b).

Figure 2 shows fruit yield as measured in the two field experiments. Plants treated with ASWE had significantly higher yields compared to controls in both experiments. Root drench was again less effective than foliar spray in terms of increased yield (Fig. 2a). In field experiment 2, the 0.5 % ASWE spray resulted in significantly higher yields than both control and fungicide treatments, with a 63 % increase in per plant fruit yield in comparison to the control plants (Fig. 2b).

Greenhouse experiment

After 50 days, the 0.2 and 0.5 % ASWE treatments resulted in a significant increase in plant height in comparison to control plants, by 13 and 20 %, respectively (Fig. 3). These significant increases were also seen in terms of root and shoot dry weight, with the 0.5 % ASWE spray increasing shoot mass by 38 % and root mass by 99 % (Table 2). The greater increase in root mass in ASWE-treated plants also resulted in significantly higher root:shoot ratios at both ASWE concentrations (Table 2).

Both ASWE concentrations produced significant increases in fruit production and fruit yield. The 0.5 % ASWE treatment resulted in a significant increase in the number of bearing

clusters (81 %), number of flowers per cluster (34 %) and number of fruit per cluster (67 %) when compared to the control plants (Table 3). When mature fruit from each treatment was accumulated, the 0.5 % ASWE-treated plants had per plant fruit yields 54 % higher than control plants (Fig. 4). The 0.5 % ASWE-treated plants had achieved the highest fruit weight and fruit size (Table 4). The highest amount of ‘grade A’ fruits was also recorded in the 0.5 % ASWE treatment, while the most ‘grade B’ and ‘grade C’ fruits were found in the control treatment (Table 4). The ASWE-treated fruit had a darker red external (skin) colour, which increased with the higher concentration of ASWE; the fruit internal colour followed the same trend (Table 5). The fruit skin was significantly firmer in ASWE-treated plants with a 40 % increase in the 0.5 % ASWE treatment compared to fruit from control plants. The fruit flesh was also significantly firmer in the ASWE treatments with a 76 % increase in the 0.5 % ASWE treatment compared to fruit from control plants. This trend continued with significantly thicker skin and flesh in the ASWE-treated plants (Table 6). The blended fruit pulp and skin from the ASWE-treated plants showed significant reductions in TTA. The TSS, viscosity and ascorbic acid levels were higher in ASWE-treated plants (Table 7). There was a

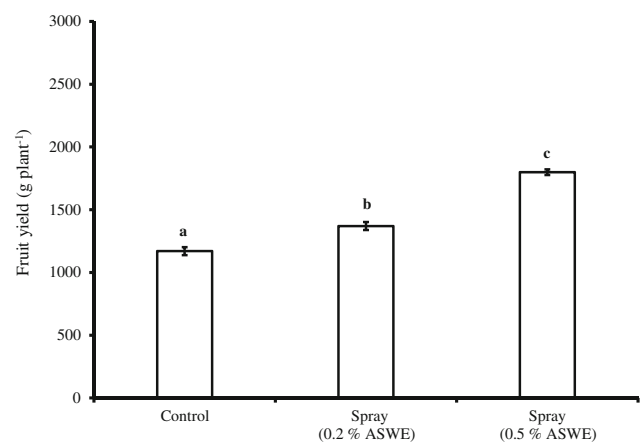


Fig. 4 Fruit yield of greenhouse-grown tomato plants after 50 days. Yields are g plant⁻¹ of fresh weight accumulated over several harvests. Data are means±SE ($n=30$); different letters indicate significant differences according to Fisher’s Least Significant Difference (LSD) test ($P=0.05$); LSD is 87

Table 4 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on fruit weight, fruit size and fruit grades of greenhouse-grown tomato plants

Treatment	Fruit weight (g)	Polar length (mm)	Equatorial diameter (mm)	A (>70 g)	Grade B (30–69 g)	A (<30 g)
Control	46.29±2.053 a	43.00±1.217 a	39.41±1.039 a	18	46	36
0.2 % ASWE	58.42±2.302 b	52.80±1.127 b	43.90±0.875 b	38	43	19
0.5 % ASWE	74.33±1.563 c	64.39±1.063 c	49.06±1.494 c	55	30	15

Values are means±standard error ($n=30$). Means followed by different letter are significantly different according to Duncan's multiple range test ($P=0.05$)

Table 5 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on fruit external and internal colour of greenhouse-grown tomato plants

Treatment	L	External A	B	L	Internal A	B
Control	35.26±1.33 a	18.25±0.61 a	11.62±0.44 a	41.46±0.99 a	9.48±0.96 a	6.69±0.66 a
0.2 % ASWE	29.91±0.80 b	20.32±0.65 b	12.74±0.40 b	29.76±1.36 b	10.05±1.05 b	6.11±0.86 a
0.5 % ASWE	21.37±0.47 c	29.83±0.79 c	12.93±0.27 c	28.88±0.70 b	12.8±0.60 b	6.31±0.55 a

Values are means±standard error ($n=10$). Means followed by different letter are significantly different according to Duncan's multiple range test ($P=0.05$)

Table 6 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on tomato fruit skin and flesh firmness and fruit skin and flesh thickness of greenhouse-grown tomato plants

Treatment	Skin firmness (N)	Flesh firmness (N)	Skin and flesh thickness (mm)
Control	328.41±15.68 a	59.01±2.07 a	5.69±0.065 a
0.2 % ASWE	418.53±26.25 b	76.19±7.03 b	8.14±0.253 b
0.5 % ASWE	461.07±26.90 c	104.28±8.52 c	8.69±0.205 c

Values are means±standard error ($n=10$). Means followed by different letter are significantly different according to Duncan's multiple range test ($P=0.05$)

Table 7 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on fruit skin and flesh viscosity, total titratable acidity (TTA), total soluble solid (TSS) and ascorbic acid levels of greenhouse-grown tomato plants

Treatment	Skin and pulp viscosity (cm s^{-1})	TTA (g citric acid equivalent 100 mL^{-1})	TSS (Brix)	Ascorbic acid ($\text{mg } 100^{-1}$)
Control	23.33±0.718 a	0.057±0.000 a	3.33±0.118 a	17.97±0.359 a
0.2 % ASWE	20.00±0.610 b	0.050±0.001 b	4.61±0.111 b	19.41±0.311 b
0.5 % ASWE	18.38±0.161 c	0.043±0.001 c	4.83±0.083 c	21.57±0.273 c

Values are means±standard error ($n=10$). Means followed by different letter are significantly different according to Duncan's multiple range test ($P=0.05$)

significant increase in the chlorophyll content of ASWE-treated plant leaves, with an 18 % increase in the 0.5 % ASWE-treated plants compared to the control plant leaves (Table 8). This corresponded with a 14 % increase in nitrogen content in the shoots of 0.5 % ASWE-treated plants. ASWE spray resulted in significant increases in the mineral content of shoots for all of the minerals tested except sodium (Table 8). Fruit from the 0.2 and 0.5 % ASWE-treated plants exhibited significant increases in N, P, K, Fe and Zn content (Table 9). The 0.5 % ASWE treatment had a significant increase in Cu levels in comparison to the control and 0.2 % ASWE treatments. The fruit from the control plants had higher levels of Na, while there was no significant difference in Ca levels among the treatments.

Discussion

Application of *Ascophyllum* seaweed extract resulted in significant increases in tomato plant height and in root and shoot biomass. Effects of *A. nodosum* on growth parameters have been reported in other tropical crops (Abdel-Mawgoud et al. 2010; Danesh et al. 2012). There is limited information on the response of tomato plants to *A. nodosum* extract under tropical conditions. Koyama et al. (2012) reported increased fruit yield that was associated with increased plant size (stem diameter). Koyama et al. (2012) found that *A. nodosum* stimulated vegetative growth in the early stages and promoted reproductive growth in the later stages of crop development and suggested that the increased fruit yield was partly due to increased translocation of nutrients in the large stems. Here, we observed clear increases in the biomass of both shoot and root (Table 2), evidencing stimulation of vegetative growth. The greenhouse experiment also showed there was a significantly higher root:shoot ratio in plants treated with ASWE compared to control plants, which suggests that there would have been a change in the partitioning of carbon in favour of the root system. Similar results were found by Rayorath et al. (2008)

Table 9 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on nutrient and mineral content of greenhouse-grown tomato fruits

Mineral content (%)	Control	ASWE (0.2 %)	ASWE (0.5 %)
N	0.016±0.000 a	0.021±0.000 b	0.029±0.000 c
P	0.639±0.000 a	0.689±0.000 b	0.689±0.000 b
K	3.020±0.001 a	3.784±0.003 b	4.538±0.019 c
Ca	0.010±0.000 a	0.009±0.000 a	0.067±0.029 b
Cu	0.001±0.000 a	0.001±0.000 a	0.001±0.000 a
Fe	0.004±0.000 a	0.004±0.000 a	0.014±0.000 c
Na	0.161±0.000 a	0.104±0.000 b	0.157±0.000 c
Zn	0.003±0.000 a	0.003±0.000 a	0.004±0.000 c

Values are means±standard error (n=3). Means followed by different letter are significantly different according to Duncan’s multiple range test (P=0.05)

using *Arabidopsis thaliana*, who found a 58 % increase in root growth with *A. nodosum* extract. Rayorath et al. (2008) attributed this growth promotion to increases in the concentration and decentralisation of auxins and abscisic acid.

Enhanced plant growth following application of seaweed extract can be due to bio-fertilisation (Jensen 2004; Kumari et al. 2011). We found a significant increase in several minerals in the shoot and fruit of plants treated with ASWE. With the higher ASWE concentration (0.5 %), mineral content in the fruit was greatly increased (N: 81 %, P: 8 %, K: 50 %, Ca: 570 %, Fe: 250 %, Zn: 33 %), while Na levels decreased by 2 %. The increase in mineral content of the fruit was considerably greater than that reported by Dobromilska et al. (2008) who applied Bio-algeen at 0.3 % under temperate conditions and found increases in fruit mineral content (N: 12 %, P: 17 %, K: 8 %, Ca: 6 %, Fe: 13 %, Zn: 29 %). In general, the larger increases in mineral content seen here are due to higher levels of minerals in the plants treated with ASWE (rather than low values in the control plants). Increased mineral content in the shoot and fruit may be explained, at least in part, by the

Table 8 Effect of *A. nodosum* seaweed extract (ASWE) at two concentrations on chlorophyll content and mineral content of greenhouse-grown tomato shoots

Mineral content (%)	Control	ASWE (0.2 %)	ASWE (0.5 %)
Chlorophyll	44.504±1.285 a	49.722±1.070 b	52.552±0.879 c
N	1.400±0.058 a	1.500±0.000 b	1.600±0.004 c
P	0.667±0.033 a	0.800±0.000 b	0.833±0.033 c
K	0.200±0.000 a	0.233±0.033 b	0.500±0.000 c
Ca	1.257±0.003 a	1.277±0.009 b	1.837±0.003 c
Cu	0.047±0.001 a	0.105±0.001 b	0.134±0.000 c
Fe	0.131±0.000 a	0.171±0.001 b	0.197±0.000 b
Na	0.002±0.000 a	0.002±0.000 a	0.002±0.000 a
Zn	0.120±0.000 a	0.127±0.000 b	0.148±0.000 c

Values are means±standard error (n=3). Means followed by different letter are significantly different according to Duncan’s multiple range test (P=0.05)

increase seen in root biomass, which is expected to increase the foraging capacity of roots and uptake of soil minerals. Also, seaweed extract contains small quantities of available minerals which can be incorporated into the plants. This is especially true when seaweed extract is applied by root drench but also occurs when it is applied as a foliar spray (Jayaraj et al. 2011). Indeed, our results suggest that application by foliar spray is as effective as application by root drench for tomato crops. Similar increases in nutrient minerals have been found in tomato sprayed with other seaweed extracts (Kumari et al. 2011; Zodape et al. 2011), and in other crops sprayed with *A. nodosum* extract including cucumber watermelon and pepper (Abdel-Mawgoud et al. 2010). In addition to minerals, *A. nodosum* seaweed extract contains alginic acid and polyuronides, which improve soil water-holding capacity, crumb structure, aeration and capillary action, all of which can stimulate plant root systems, boost soil microbial activity and enhance mineral availability and absorption (Moore 2004), as well as increasing mobility of carbohydrates and other organic compounds within the plant (Mohanty et al. 2013).

The beneficial effects of ASWE on growth can also be due to a bio-stimulation effect, due to the hormones and related compounds found in *Ascophyllum*. Rayorath et al. (2008) who investigated vegetative growth promotion using *A. thaliana* treated with *A. nodosum* extract concluded that growth promotion was due to auxins and gibberellins and similar compounds that elicited endogenous cytokinin-like activity. In addition to these plant hormones, polysaccharides compounds, such as laminaran and fucoidan, found in *Ascophyllum* extracts, have been shown to exhibit a wide range of elicitor and growth regulatory activities (Rioux et al. 2007).

The increased biomass seen here suggests stimulation of the photosynthetic capacity of the plants. Previous studies have observed higher chlorophyll content in tomato following application of seaweed extract, including *A. nodosum* (Dobromilska et al. 2008; Blunden et al. 1997; Whapham et al. 1993). Here, we found that chlorophyll levels were 18 % higher in ASWE-treated plants compared to control plants. It is likely that the higher chlorophyll content is partly due to a bio-fertilisation effect as nitrogen content, a limiting factor in chlorophyll formation, was also higher in ASWE-treated plants. Betaines present in *A. nodosum* extract have also been shown to enhance leaf chlorophyll content and to reduce its degeneration rate. Blunden and Gordon (1986) found that betaines and betaine-like compounds present in the form of glycinebetaine, γ -aminobutyric acid betaine and 6-aminovaleric acid betaine accounted for enhanced leaf chlorophyll, photosynthetic rates and subsequently increased tomato production.

Alternating fungicide spray with ASWE produced significantly increased plant and fruit biomass in field experiment 2. However, the magnitude of the increase was less than that

seen when fungicide spray was compared to untreated control plants. In other words, the yield gain from applying fungicide was less in ASWE-treated plants. This suggests that some of the benefits of ASWE are due to a reduction in fungal pathogens. These effects are discussed in detail in an accompanying paper.

In the present study, *A. nodosum* extract consistently produced significant increases in fruit yields, with 0.5 % ASWE sprayed at 15-day intervals in the field increasing fruit yield by 63 % compared with control plants and 31 % compared to fungicide only treatment plants (Fig. 2b). Increases in fruit yields have been seen in other crops sprayed with *A. nodosum* seaweed extract (Abetz and Young 1983; Jeannin et al. 1991; Norrie and Keathley 2006; Danesh et al. 2012; Bozorgi 2012) or with extracts from other seaweed species (Khan et al. 2009; Briceno-Dominguez et al. 2014; Satish et al. 2015). These increases in fruit yield are thought to be due to the bio-stimulant and bio-fertilisation effects discussed above. The increases seen in this study were greater than those reported by Koyama et al. (2012) in a subtropical environment, where no consistent increase in fruit yield was found with fortnightly sprays of 0.3 % *A. nodosum* extract over 100 days. They are also greater than those seen for tomato grown in a temperate environment, where yields increased by 22 % using up to four applications of 0.3 % Bio-algeen (Dobromilska et al. 2008) or for tomato treated with other seaweed extracts (Crouch and van Staden 1992; Kumari et al. 2011). The greater increase in fruit yield seen here is likely due to a combination of factors including an overall increase in vegetative and reproductive growth, an increase in chlorophyll content and enhanced uptake and accumulation of nutrients. The results from the greenhouse experiment also show greater improvements in fruit weight, fruit size and fruit set (Tables 3 and 4), in comparison with the results of Koyama et al. (2012). In particular, the number of bearing clusters and number of fruit per cluster were increased by >60 % in the plants sprayed with 0.5 % ASWE. The greater increases seen here could be as a result of the precise concentration and frequency of application of the ASWE or to the tomato variety used. However, they more likely reflect the greater potential for yield improvement under tropical growing conditions than other climatic conditions.

Tomatoes provide a major source of important minerals, especially K and Zn, and *A. nodosum* extract increased both of these elements as well as others mentioned above, thereby increasing the nutritional value of the fruit (Dobromilska et al. 2008). Dobromilska et al. (2008) also found that Bio-algeen sprays significantly increased K and had a positive influence on tomato fruit colour. Our results show that the quality of the fruit juice and whole fruit significantly improved with ASWE treatment. Fruits from ASWE-treated plants had firmer flesh and skin than fruits from control plants, and they were darker red internally and externally (as expected with increased K;

Hartz et al. 2005). Increased ascorbic acid and total soluble solids also contribute to the nutritional value of the fruit. Increases in fruit quality in response to *A. nodosum* extract have been observed in other crops (Abdel-Mawgoud et al. 2010), but this is the first time such improvements have been reported in tomato.

Overall, the use of ASWE to promote growth and crop yields was seen to be more effective in the tropics than in reports from subtropical and temperate growing environments. Our results suggest that under tropical conditions *A. nodosum* extract at 0.5 % applied as a foliar spray at fortnightly intervals results in considerable improvements in plant growth performance and in fruit yield. Greater performance was seen when ASWE was incorporated into an integrated tomato cropping programme alternated with reduced crop inputs (fungicides). Under current conditions, this represents a significant cost saving in comparison to the existing practice which relies on intensive use of synthetic crop inputs. The observed improvement in fruit quality following application of *A. nodosum* extract further supports its usefulness in enhancing the quality of the produce. Being an organic input, it also invites the attraction of both farmers and consumers and also from the government for encouragement of good agricultural practices in crop production.

Acknowledgments This research project was supported by the research grants awarded to J.J by Acadian Seaplants Limited, Dartmouth, NS, Canada and Conservation, Food and Health (CFH) Foundation, Boston, MA, USA.

References

- Abdel-Mawgoud AMR, Tantaway A, Hafez MM, Habib HAM (2010) Seaweed extract improves growth, yield and quality of different watermelon hybrids. *J Agric Biol Sci* 6:161–168
- Abetz P, Young CL (1983) The effect of seaweed extract sprays derived from *Ascophyllum nodosum* on lettuce and cauliflower crops. *Bot Mar* 26:487–492
- AOAC (2007) Official methods of analysis, 18th edn. AOAC International, Gaithersburg
- Baardseth E (1970) Synopsis of biological data on knobbed wrack, *Ascophyllum nodosum*. *FAO Fish Synop* 1:41
- Barceló D, Hennion MC (1997) Trace determination of pesticides and their degradation products in water. *Techniques and Instrumentation in Analytical Chemistry*. Elsevier, Amsterdam 19:542
- Blunden G, Gordon SM (1986) Betaines and their sulphonyl analogues in marine algae. *Prog Phycol Res* 4:39–80
- Blunden G, Jenkins T, Liu Y (1997) Enhanced leaf chlorophyll levels in plants treated with seaweed extract. *J Appl Phycol* 8:535–543
- Booth E (1969) The manufacture and properties of liquid seaweed extracts. *Proc Int Seaweed Symp* 6:655–662
- Bozorgi HR (2012) Effects of foliar spraying with marine plant *Ascophyllum nodosum* extract and nano iron chelate fertiliser on fruit yield and several attributes of eggplant (*Solanum melongena* L.). *ARPN J Agric Biol Sci* 7:357–362
- Briceno-Dominguez D, Hernandez-Carmona G, Moyo M, Stirk W, Van Staden J (2014) Plant growth promoting activity of seaweed liquid extracts produced from *Macrocystis pyrifera* under different pH and temperature conditions. *J Appl Phycol* 26:2203–2210
- Chojnacka K, Saeid A, Witkowska Z, Tuhy L (2012) Biologically active compounds in seaweed extracts—the prospects for the application. *Open Conf Proc J* 3:20–28
- Craigie JS (2011) Seaweed extract stimuli in plant science and agriculture. *J Appl Phycol* 23:371–393
- Crouch IJ, van Staden J (1992) Effect of seaweed concentrate on the establishment and yield of greenhouse tomato plants. *J Appl Phycol* 4:291–296
- Crouch IJ, van Staden J (1993) Evidence for the presence of plant growth regulators in commercial seaweed products. *J Plant Growth Regul* 13:21–29
- Danesh RK, Bidarigh S, Azarpour E, Moraditochae M, Bozorgi HR (2012) Study effects of nitrogen fertiliser management and foliar spraying of marine plant *Ascophyllum nodosum* extract on yield of cucumber (*Cucumis sativus* L.). *Int J Agric Crop Sci* 4:1492–1495
- Dobromilska R, Mikiciuk M, Gubarewicz K (2008) Evaluation of cherry tomato yielding and fruit mineral composition after using of Bioalgeen S-90 preparation. *J Elementol* 13:491–499
- Hartz TK, Johnstone PR, Francis DM, Miyao EM (2005) Processing tomato yield and fruit quality improved with potassium fertigation. *Hort Sci* 40:1862–1867
- Hernandez-Herrera RA, Santacruz-Ruvalcaba F, Ruiz-Lopez MA, Norrie J, Hernández-Carmona G (2014) Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *J Appl Phycol* 26:619–628
- Jackson ML (1973) Soil chemical analysis, 2nd edn. Prentice Hall, New Delhi
- Jayaraj J, Wan A, Rahman M, Punja ZK (2008) Seaweed extract reduces foliar fungal diseases on carrot. *Crop Protect* 27:1360–1366
- Jayaraj J, Norrie J, Punja ZK (2011) Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber. *J Appl Phycol* 23:353–361
- Jeannin I, Lescure JC, Morot-Gaudry JF (1991) The effects of aqueous seaweed sprays on the growth of maize. *Bot Mar* 34:469–473
- Jensen E (2004) Seaweed; fact or fancy. *Broadcaster* 12:164–170
- Khan W, Rayirath UP, Jithesh MNSS, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2009) Seaweed extracts as biostimulants of plant growth and development. *J Plant Growth Regul* 28:386–399
- Koyama R, Bettoni MM, Roder C, de Assis AM, Roberto SR, Mogor AF (2012) Seaweed extract of *Ascophyllum nodosum* (L.) on tomato yield and vegetable development. *Amazon J Agric Environ Sci* 55:282–287
- Kumari R, Kaur I, Bhatnagar AK (2011) Effect of aqueous extract of *Sargassum johnstonii* Setchell & Gardner on growth, yield and quality of *Lycopersicon esculentum* Mill. *J Appl Phycol* 23:623–633
- Lindsay WL, Norvell WA (1978) Development DTPA soil test for zinc, iron, manganese and copper. *Soil Sci Soc Am J* 42:421–428
- Lopez VF, Morais M, Cock MJW (1995) Preliminary results of a socioeconomic survey of farmer's pest control strategies in four vegetable crops in Trinidad In: report of the Regional Workshop on Integrated Production and Pest Management Practices for Vegetable Crops, FAO, p 21–22
- Lopez VF, Morais M, Dowlat P, Lessee M, Lewis C, Dowlath N, Mohammed H, Ramsamooj D, Ramjit MS, Ali N (1996) A socioeconomic survey of farmers' practices in four vegetable crops in South Trinidad. *International Institute of Biological Control Caribbean and Latin America Station, Trinidad*, p 30
- Lopez C, Andres F, Perla AG (2004) Comparison of color indices for tomato ripening. *Hortic Bras* 22:534–537
- Mikiciuk M, Dobromilska R (2014) Assessment of yield and physiological indices of small-sized tomato cv Bianca F1 under the influence of biostimulators of marine algae origin. *Acta Sci Pol Hortorum Cultus* 13:31–41

- Mohanty D, Adhikary SP, Chattopadhyay N (2013) Seaweed liquid fertiliser (SLF) and its role in agricultural productivity. *Ecosan* 3: 147–155
- Moore KK (2004) Using seaweed compost to grow bedding plants. *BioCycle* 45:43–44
- Murphy J, Riley J (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27: 31–36
- Norrie J, Keathley JP (2006) Benefits of *Ascophyllum nodosum* marine-plant extract applications to Thompson seedless' grape production. *Acta Hort* 727:243–248
- Prior P, Grimault V, Schmith J (1994) Resistance to bacterial wilt in tomato: present status and prospects: In: Hayward AC, Hartman GL (ed) *Bacterial Wilt. The diseases and its causative agent*. CAB International. pp 209–223
- Rayorath P, Jithesh MN, Farid A, Khan W, Palanisamy R, Hankins SD, Critchley AT, Prithiviraj B (2008) Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L.) Le Jol. using a model plant, *Arabidopsis thaliana* (L.) Heynth. *J Appl Phycol* 20:423–429
- Rayorath P, Benkel B, Hodges DM, Allan-Wojtas P, Mackinnon S, Critchley AT (2009) Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in *Arabidopsis thaliana*. *Planta* 230:135–147
- Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of non-invasive methods to estimate foliar chlorophyll content. *New Phytol* 153:185–194
- Rioux LE, Turgeon SL, Beaulieu M (2007) Characterization of polysaccharides extracted from brown seaweeds. *Carbohydr Polym* 69:530–537
- Satish L, Rameshkumar R, Rathinapriya P, Pandian S, Rency AS, Sunitha T, Ramesh M (2015) Effect of seaweed liquid extracts and plant growth regulators on in vitro mass propagation of brinjal (*Solanum melongena* L.) through hypocotyl and leaf disc explants. *J Appl Phycol* 27:993–1002
- Sharma HSS, Lyons G, McRoberts C, McCall D, Carmichael E, Andrews F, Swan R (2012) Biostimulant activity of brown seaweed species from Strangford Lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (*Vigna mungo* L.) and pak choi (*Brassica rapa chinensis* L.). *J Appl Phycol* 24:1081–1091
- Ugarte RA, Sharp G, Moore B (2006) Changes in the brown seaweed *Ascophyllum nodosum* (L.) Le Jol. plant morphology and biomass produced by cutter rake harvests in Southern New Brunswick, Canada. *J Appl Phycol* 18:351–359
- Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218:1–14
- Whapham CA, Blunden G, Jenkins T, Hankins HD (1993) Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *J Appl Phycol* 5:231–234
- Zodape ST, Gupta A, Bhandari SC, Rawat US, Chaudhary DR, Eswaran K, Chikara J (2011) Foliar application of seaweed sap as biostimulant for enhancement of yield and quality of tomato (*Lycopersicon esculentum* Mill). *J Sci Ind Res* 70:215–219