

Evaluating Three Invasive Algal Species as Local Organic Sources of Potassium for Pak Choi (*Brassica rapa*, *Chinensis* Group) Growth

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Abstract. The application of locally available invasive algae biomass as a fertilizer for crop production in Hawaii is being investigated as a substitute for imported chemical fertilizers. Three closely related greenhouse trials were conducted to determine if the algae served as a source of potassium (K) on growth, yield, and K mineral nutrition in pak choi (*Brassica rapa*, *Chinensis* group). In the first trial, three algal species (*Gracilaria salicornia*, *Kappaphycus alvarezii*, and *Euclidean denticulatum*) were applied at five rates of K, each to evaluate their effects on growth and K nutrition of pak choi plants. The pak choi was direct seeded into 0.0027-m³ pots containing peatmoss-based growth media. In trial 2, pak choi was grown in peat media at six rates of K provided by algae (*E. denticulatum*) or by potassium nitrate (KNO₃). In trial 3, the six rates of K were provided through algae (*K. alvarezii*), KNO₃, and potassium chloride (KCl) and were compared for growth and K nutrition. Results from the first greenhouse trial showed no significant differences among the three algal species in yield or tissue K content of pak choi. However, plant yield and tissue K concentration were increased with application rates. The maximum yield and tissue K were observed when K was provided within the range of 250–300 kg·ha⁻¹. Similarly, in Expts. 2 and 3, there were no significant differences between commercial K fertilizers and algal K species for yield. Only K rates were significant for yields and tissue K concentrations. It was concluded that K in the invasive algae was similarly available as K in commercial synthetic fertilizers for pak choi growth in terms of yield and tissue K content under our experimental conditions.

To meet increasing demand for locally available inputs, the use of seaweed as a source of crop nutrients is a viable option (Pramanick et al., 2014). Many growers in the Pacific region are requesting such an organic fertilizer (Radovich et al., 2012). Marine algae are used in agricultural and horticultural crops, resulting in many beneficial effects on yield and quality (Dhargalkar and Pereira, 2005). In many countries, seaweed and seaweed-based fertilizers are still used in both agriculture and

horticulture (Verkleij, 1992; Zodape, 2001). Not only providing nutrients, these seaweeds improve soil structure and humus content when they are composted and applied to the soil (Haslam and Hopkins, 1996). Seaweed liquid fertilizer (SLF) of *Dictyota dichotoma* was found to increase the yield, growth of roots and shoots,

number of roots, and maturity time of *Abelmoschus esculentus* L. (okra) at low concentrations (Sasikumar et al., 2011). Seaweed concentrate prepared from *Ecklonia maxima* (Osbeck) Papenfuss has improved the growth of tomato seedlings when applied as a soil drench (Crouchand van Staden, 1992). It was observed that the application of SLF of *Ascophyllum nodosum* increased the chlorophyll of cucumber cotyledons and tomato plants (Whaphamem et al., 1993). In Hawaii, the most commonly found macro-sized invasive species are *G. salicornia* (Gorilla Ogo), *K. alvarezii* (*Kappaphycus*), and *E. denticulatum* (*Euclidean*); all are dominant alien algal species in Hawaiian reefs (Smith et al., 2002). They show potential for use as a locally available source of K (Ahmad et al., 2016; Radovich et al., 2012). Several farmers in Hawaii have been using the algal species for some crops, but there is a lack of information about the availability and optimal rates of K for crop growth from these algal species. The overall objective of this research was to evaluate three invasive algal species on yield and K mineral nutrition of pak choi, and then to compare one algal species with KNO₃ and/or KCl.

Materials and Methods

Three greenhouse experiments were conducted at the University of Hawaii's Magoon Research Facility (lat. 21°18'22"N, long. 157°48'37"W). The three algal species were obtained from the Department of Land and Natural Resources on Oahu Island and were dried in the oven at 70 °C for 72 h. The dried algae were ground into a fine powder using a coffee grinder. The required amounts of K provided from each algal species and for each application rate were calculated based on the K analysis report received from the Agricultural Diagnostic Service Center (ADSC) at University of Hawaii, Manoa (Table 1). The amount of K required from algae per plant based on rates was calculated based on pak choi plant density of 71,659 per hectare with a spacing of 0.30 m between plants and 0.45 m between rows. The required amount of K per plant was divided by the % of K present in each species. For trials 1 and 2, we used pak choi (*B. rapa*, *Chinensis* group) cv. Bonsai and for the trial 3, cv. Mei Qing choi

Table 1. Mineral nutrient concentration in *Kappaphycus alvarezii*, *Euclidean denticulatum*, and *Gracilaria salicornia* used in these trials.

Nutrient	<i>K. alvarezii</i>	<i>E. denticulatum</i>	<i>G. salicornia</i>
N (%)	0.26	0.57	0.8
C (%)	18.12	18.54	22.05
P (%)	0.04	0.07	0.16
K (%)	20.34	18.02	14.1
Ca (%)	0.21	1.55	4.62
Mg (%)	0.41	0.76	0.78
Na (%)	3.35	3.82	3.88
Fe (µg·L ⁻¹)	173	72	375
Mn (µg·L ⁻¹)	10.5	14	210

Mean values with n = 2. The tissue samples were analyzed by the ADSC laboratory at the University of Hawaii, Manoa.

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was used. All the seeds were obtained from Harris Seeds of Harris Seeds Company (Rochester, NY). Seeds were planted in 0.0027-m³ size pots.

Trial 1. Three algal species (*G. salicornia*, *K. alvarezii*, and *E. denticulatum*) were applied to supply five rates of K at 0, 1.17, 2.35, 3.51, and 4.70 g/plant (which is equivalent to 0, 84, 168, 252, and 336 kg·ha⁻¹), with “0” being the control treatment without K fertilizer. The other major nutrients, nitrogen (N) and phosphorus (P), were provided through tankage (bone meal) (10–3.7–0.8) and triple superphosphate (0–45–0) at constant rates for all the plants at the normal required rates at 168 and 112 kg·ha⁻¹, respectively (Hemphill, 2010). The actual amounts provided were 22.72 g/plant of bone meal and 3.74 g/plant of triple superphosphate. The experiment was set up as a complete randomized design (CRD) with five replicates. Three to four seeds were directly sown to each pot filled with peat-based media (Sunshine Mix No. 4, SunGro, Agawam, MA) on 15 Apr. 2013. A week after seedling emergence in all pots, plants were thinned to one per pot. Overhead irrigation was provided twice a day for 10 min. Plants were harvested on 27 May 2013, 6 weeks after emergence.

Trial 2. This trial was also set up as a CRD with three replications and two fertilizers, *E. denticulatum* and KNO₃. On the basis of the results of trial 1, in which no significant differences were found between the three invasive algal species, *E. denticulatum* was randomly selected and used as a source of K fertilizer and compared with KNO₃ synthetic fertilizer. The two K fertilizers were applied to supply six rates of K at 0, 1.56, 2.35, 3.12, 3.91, and 4.70 g/plant (which is equivalent to 0, 112, 168, 224, 280, and 336 kg·ha⁻¹), with “0” being the control treatment without K fertilizer. The other major nutrients, N and P, were provided through urea (46–0–0) and triple superphosphate (0–45–0) at constant rates for all the plants at the normal required rates at 168 and 112 kg·ha⁻¹, respectively. Urea was provided at 5.10 g/plant to provide N at 2.35 g/plant for all pots, except the pots treated with KNO₃ which received 3.93, 3.37, 2.79, 2.22, and 1.63 g/plant of urea. The triple superphosphate was provided to all the pots at 7.77 g/plant to provide 1.56 g of P per plant. The same procedures were followed as in trial 1 with the following exception. Plants were watered to excess of pot capacity (150 mL) through a drip system once a day. Seeds were sown on 28 Jan. 2014 and were harvested on 11 Mar. 2014, 6 weeks after emergence.

Trial 3. This was also a CRD with four replications of three fertilizers, *K. alvarezii*, KNO₃, and KCl, to supply six rates of K at 0, 1.56, 2.35, 3.12, 3.91, and 4.70 g/plant (which is equivalent to 0, 112, 168, 224, 280, and 336 kg·ha⁻¹). Three to four pak choi seeds were sown into each pot filled with peatmoss on 6 Mar. 2015. The

same amount of N and P were provided from urea and triple superphosphate as used in trial 2 with adjustments made in KNO₃-treated plants. The same procedures were followed as in trials 1 and 2 with the following exceptions. A deionized (DI) water system (US Water system, Indianapolis, IN) filter was fitted in the greenhouse connecting to the regular greenhouse irrigation system producing DI water at 0.0075 m³ per minute. The collected DI water was used to irrigate the pots using a watering can, 4 times per week. Plants were harvested on 17 Apr. 2015, 6 weeks after emergence.

Plant harvest and measurement. For trials 1 and 2, aboveground fresh weights (tops) were immediately measured after harvest and data were recorded. The plants were then placed in an oven (Precision Mechanical Convection Oven; Thermo Scientific, Waltham, MA) at 70 °C and were dried for 72 h to constant weight, and the dry weights were recorded. The dried tissue samples were analyzed for K and other nutrients at the ADSC laboratory, University of Hawaii at Manoa, using an inductively coupled plasma spectrometer (Optima 7000DV, Waltham, MA). The fresh petioles harvested from trials 1 and 2 were also analyzed for leaf sap K concentrations using the portable ion-specific electrode (B-731LAQUAtwin Cardy meter; Horiba Scientific, Irvine, CA). The fourth leaf plus midrib were collected from each plant immediately after the harvest and pressed in a garlic presser to extract plant sap. Each 1 mL of sap was diluted with DI water to a volume of 5 mL and the diluted 1-mL sap was analyzed for K concentrations in the Cardy ion meter which was calibrated after every 30 measurements using a KCl standard calibrating solution. For trial 3, the same procedures were followed as in trials 1 and 2 with the exception that sap K was not measured.

Statistical analysis. Analyses of variance on data from plant tissue K content, and fresh

and dry weights were performed using general linear model (GLM) on main treatment effects and their interactions. Data were analyzed using PROC GLM in SAS v.9.2 statistical software (SAS Institute Inc., 2003). Regression analysis was conducted using SigmaPlot v.13 (San Jose, CA) to examine the relationship between the rates of K fertilizers and yield or tissue K.

Results

Trial 1. There was no significant difference in fresh or dry weights of tops or tissue K concentration due to algal species (Table 2). A significant effect due to K fertilizer rate was found in which increasing rates resulted in increased fresh or dry weight and tissue K. The highest yields (dry weight mean 8.34 g/plant) and tissue K (mean 8.2%) were recorded when K was provided in the range of 250 to 300 kg·ha⁻¹ (Figs. 1A and 2A). The interactions of species and rates were not significant.

Trial 2. The results showed that main treatment effects of K rates from both fertilizers had significant, positive effects on fresh or dry weights of tops ($P < 0.05$) and tissue K ($P < 0.01$) (Figs. 1B and 2B; Table 3). There were no significant differences due to the two fertilizer types, *E. denticulatum* and KNO₃, with regard to fresh or dry weights of tops. The two fertilizer types differed significantly ($P < 0.022$) in tissue K concentrations, with algae-treated plants (8.38%) having greater tissue K than KNO₃-treated plants (7.38%). The interaction of fertilizer type and rates were not significant with regard to yield or tissue K content. The highest tissue K (8.9%) was recorded when K was provided at 284 kg·ha⁻¹. On the basis of the quadratic equation formula, the highest dry weight (5.22 g) was observed when was provided at 395 kg·ha⁻¹.

Trial 3. Fertilizer rates significantly increased fresh ($P < 0.0001$) or dry weights of tops ($P < 0.001$), and tissue K concentrations ($P < 0.001$; Figs. 1C and 2C). These results

Table 2. Effect of three invasive algae species on plant growth and tissue K from trial 1.

Species of algae	K rates (g/plant) ²	Fresh wt (aboveground) (g/plant)	Dry wt (aboveground) (g/plant)	Tissue K (%)
<i>Kappaphycus alvarezii</i>	4.70	144.7 (10.5)	7.7 (1.2)	8.1 (0.4)
	3.51	179.8 (14.1)	9.4 (0.8)	8.3 (0.4)
	2.35	138.6 (15.8)	6.3 (0.6)	6.5 (0.6)
	1.17	125.78 (10.4)	6.1 (0.4)	5.4 (0.4)
<i>Euclima denticulatum</i>	4.70	133 (15.4)	5.9 (0.6)	8.3 (0.4)
	3.51	169.2 (27.8)	10 (2.8)	7.8 (0.4)
	2.35	136.9 (11.9)	6.7 (0.5)	6.8 (0.4)
	1.17	127.8 (4.9)	6.5 (0.2)	5.2 (0.6)
<i>Gracilaria salicornia</i>	4.70	144.8 (8.6)	7.3 (0.2)	8.3 (0.7)
	3.51	123.6 (16.1)	6.2 (0.3)	8.2 (0.4)
	2.35	135.5 (10)	6.1 (0.3)	6.2 (0.3)
	1.17	103.2 (10.6)	5.1 (0.6)	5.4 (0.5)
Control	0	95.5 (1.7)	4.6 (0.4)	2.1 (0.5)
Rates		$P < 0.0111$	$P < 0.0131$	$P < 0.0001$
Species		NS	NS	NS
Rates × species		NS	NS	NS

Mean values represent five replicates from each treatment with SE in parentheses.

NS = not significant at $\alpha = 0.05$.

²The amounts of K rates (g/plant) shown in the table are calculated from the fertilizer application rates of 84, 168, 252, and 336 kg of K/ha.

Discussion

These three very similar trials all address whether these invasive algae serve as a K source to a *Brassica* model crop under a range of experimental conditions. In all three trials, increasing rates of K application significantly increased fresh or dry weights of plant tops and tissue K. In the first greenhouse trial, no significant differences were found among three algae species with regard to fresh or dry weights of tops or tissue K. No significant interactions were found. The second trial was modified to compare the organic source of K (algae species) with synthetic source (KNO_3) of K. However, since the first trial showed no significant difference between the three algae species, one species was used. Both algae and synthetic sources of K were applied at the same application rates. In the second trial, we compared one algal species *E. denticulatum* with KNO_3 and found no significant differences between these two sources of K with regards to fresh or dry weights of tops. Fertilization with algae (8.38%) resulted in significantly higher tissue K than plants fertilized with KNO_3 (7.38%). No significant interactions were found. The results from first and second trials showed that pak choi yield was declining at some point (application rate 336 kg K/ha). We suspected the decline might be related to K toxicity or salt effects that may occur with high application of algae. To address both possibilities, a different species of algae (*K. alvarezii*) was used and an additional synthetic K source (KCl) was included in the third trial to ensure that the chloride content may indicate possible toxic effects. In trial 3, no significant differences were found between these three sources of K with regards to fresh or dry weights of tops (Table 4). Source of K fertilizer significantly affected tissue K with KCl, resulting in higher tissue K (3.7%) than KNO_3 (3.59%) and algae *K. alvarezii* (3.16%). No significant interactions were found. No significant differences among three algae species were detected in total fresh or dry weights of tops or tissue K (Table 2). Our results confirm those previously reported by Crouch et al. (1990) who noted increased uptake of K in lettuce with seaweed concentrate application. A possible reason for lower yields in trial 2 can be attributed to the change in the method of irrigation switched from overhead sprinkler to drip system. The change of pak choi cultivar from Bonsai to Mei Qing choi in the trial 3 may have contributed to some extent for lower tissue K concentrations. Chemical analysis of algae and their extracts made by other researchers showed the presence of a whole range of various plant growth regulators such as auxins, cytokinins, gibberellins, and abscisic acid (Prasad et al., 2010; Yokoya et al., 2010), which at high concentrations may either inhibit or stimulate plant growth depending on the concentration (Crouch and van Staden, 1993). Seaweed constituents including

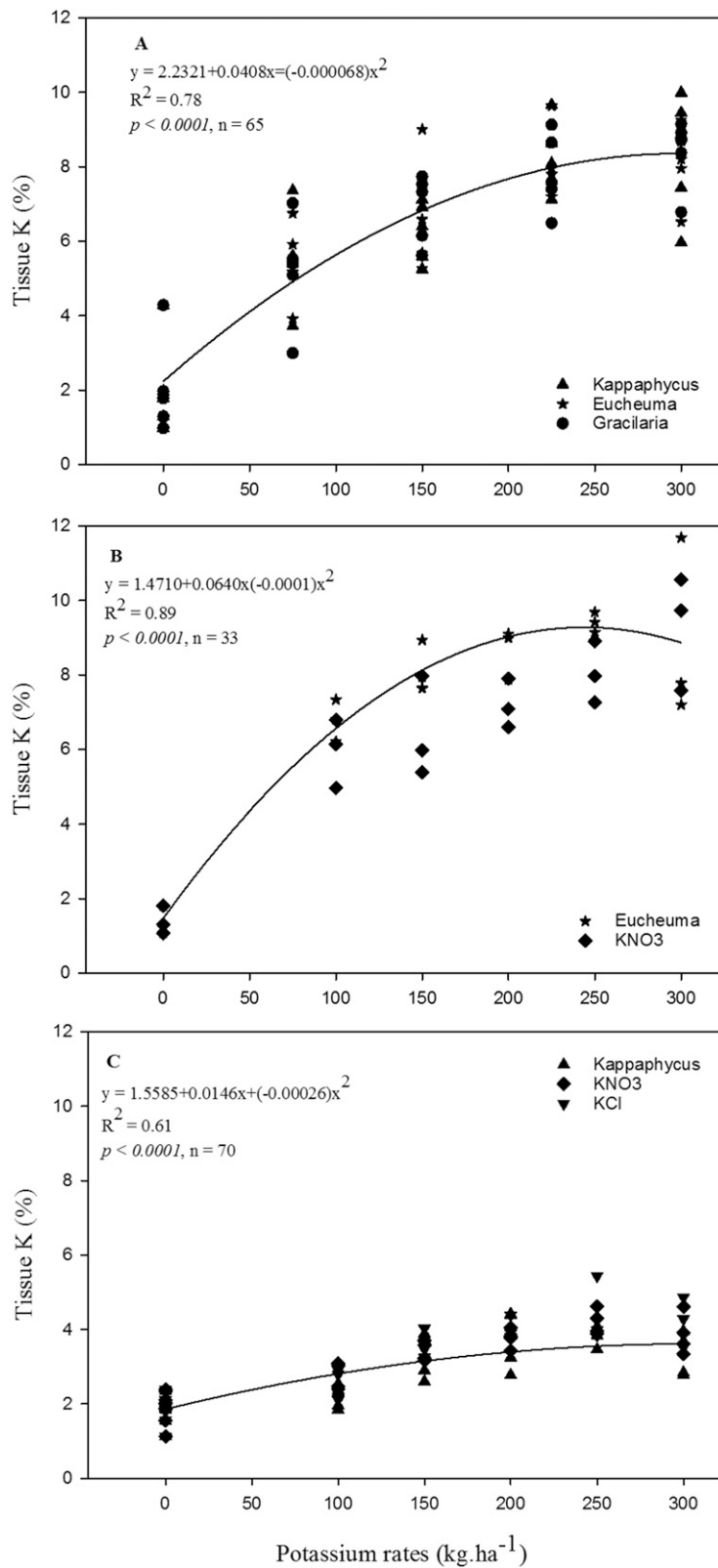


Fig. 1. Effect of K fertilizer rates on tissue K levels (%) of pak choi from (A) trial 1, (B) trial 2, and (C) trial 3.

were consistent with those of trials 1 and 2. No significant differences in fresh or dry weights of tops were found among the three fertilizer types; however, the source of fertilizer significantly ($P < 0.018$) affected tissue K with KCl resulting in higher

tissue K than KNO_3 and algae *K. alvarezii* (Fig. 1C). No significant interactions were found among the main treatments. The highest dry weight (7.61 g) and tissue K (3.87%) were recorded when K was provided at $350 \text{ kg} \cdot \text{ha}^{-1}$.

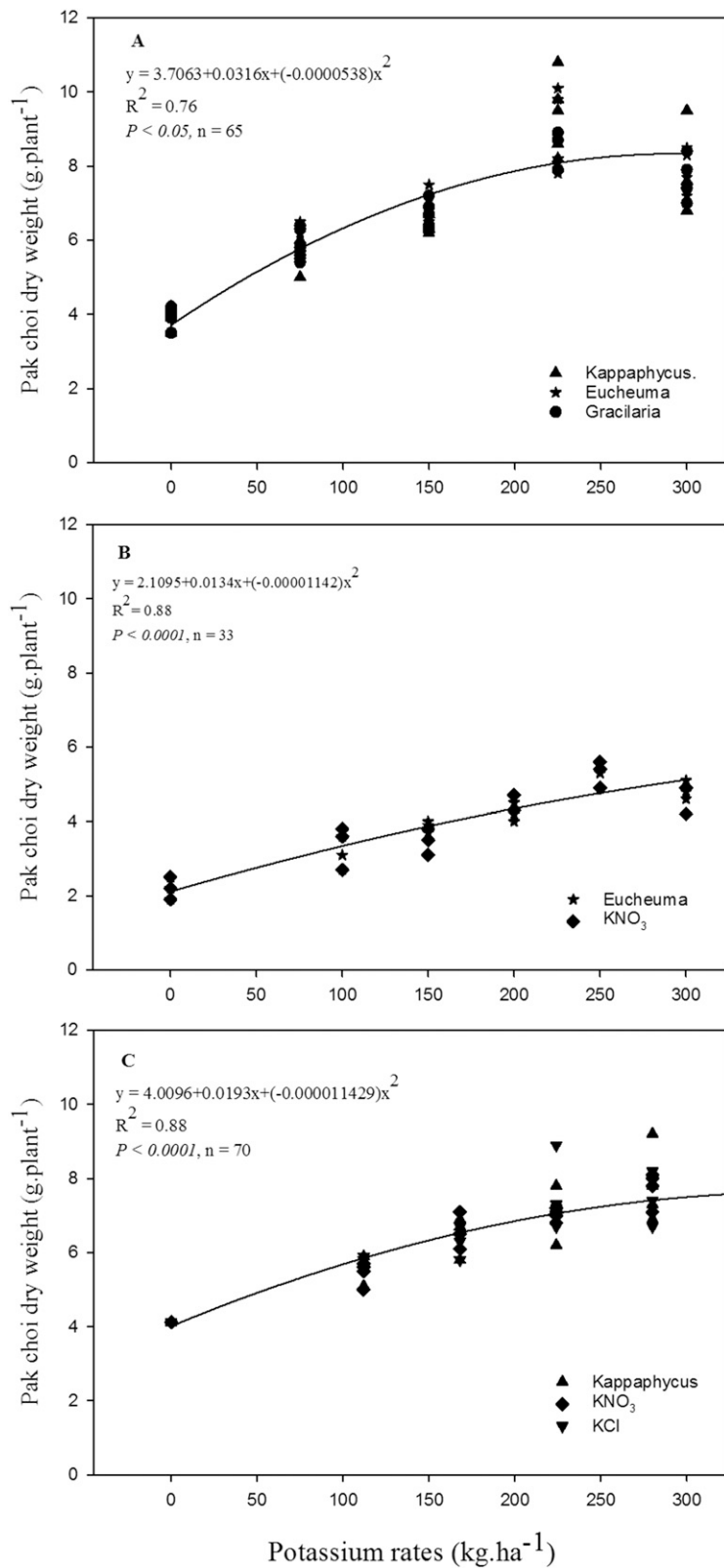


Fig. 2. Effect of K fertilizer rates on dry weight (g) of pak choi from (A) trial 1, (B) trial 2, and (C) trial 3.

macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid that affect cellular metabolism in treated plants may have enhanced pak choi growth and yield. Another possible contribution to increased yield is the presence of polysaccharides in some of the red

algae as sugars that are known to improve plant growth in a similar way to hormones (Rolland et al., 2002).

Conclusion

The invasive algae used in these studies positively influenced the growth and K

nutrition of pak choi. The consistent results from these studies suggest that the these invasive algae species of Hawaiian Islands have potential to be used as a replacement for synthetic K fertilizer in vegetable crop production and are particularly beneficial when used for crops with high K requirements. The algae waste biomass could be mixed with commercially available fertilizers to enhance the plant growth. These seaweeds may provide an effective approach to nutrient management in places where inorganic fertilizers are expensive and limited. Further studies on specific mechanisms attributed to plant growth including K availability over the time are required to determine the potential of these algae species as a commercially viable product.

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Table 3. Effect of *Eucheuma denticulatum* algae and potassium nitrate on plant growth and tissue K from trial 2.

Fertilizer types	Fertilizer K rates (g/plant) ^z	Fresh wt (aboveground) (g/plant)	Dry wt (aboveground) (g/plant)	Tissue K (%)
<i>E. denticulatum</i>	4.70	61.4 (1.8)	3.8 (0.03)	8.9 (1.4)
	3.91	70.2 (4.5)	4 (0.08)	9.4 (0.1)
	3.12	74.6 (5.2)	3.7 (0.3)	8.6 (0.3)
	2.35	66.5 (2.3)	3.8 (0.08)	8.1 (0.3)
	1.56	58.7 (3.0)	3.1 (0.2)	6.7 (0.3)
Potassium nitrate	4.70	71.2 (3.9)	3.8 (0.2)	9.2 (0.8)
	3.91	67.6 (1.3)	3.8 (0.1)	8.0 (0.4)
	3.12	75.5 (2.1)	3.6 (0.1)	7.1 (0.3)
	2.35	63.3 (4.9)	3.0 (0.06)	6.4 (0.7)
	1.56	68.3 (1.6)	3.3 (0.3)	5.9 (0.5)
Control	0	45.3 (0.8)	2.2 (0.1)	1.4 (0.2)
Rates		$P < 0.0156$	$P < 0.0198$	$P < 0.0024$
Fertilizer types		NS	NS	$P < 0.0022$
Rates × fertilizer types		NS	NS	NS

Mean values represent three replicates from each treatment with SE in parentheses.

NS = not significant at $\alpha = 0.05$.

^zThe amounts of K rates (g/plant) shown in the table are calculated from the fertilizer application rates of 112, 168, 224, 280, and 336 kg of K/ha.

Table 4. Effect of *Kappaphycus alvarezii*, potassium nitrate (KNO₃), and potassium chloride (KCl) on plant yield and tissue K from trial 3.

Fertilizer types	Fertilizer K rates (g/plant) ^z	Fresh wt (aboveground) (g/plant)	Dry wt (aboveground) (g/plant)	Tissue K (%)
<i>K. alvarezii</i>	4.70	90 (0.9)	5.4 (0.2)	3.3 (0.2)
	3.91	131 (9.9)	7.8 (0.5)	3.4 (0.4)
	3.12	106 (8.6)	5.9 (0.9)	3.6 (0.4)
	2.35	100 (10.7)	6.4 (0.8)	3.2 (0.3)
	1.56	89.7 (7.3)	5.6 (0.7)	2.1 (0.1)
Potassium nitrate (KNO ₃)	4.70	94.3 (11.6)	5.9 (0.5)	3.7 (0.3)
	3.91	133 (7.8)	7.5 (0.4)	3.9 (0.3)
	3.12	110 (13.4)	6.1 (0.5)	3.8 (0.1)
	2.35	104 (16.4)	6.1 (0.9)	3.4 (0.1)
	1.56	97.4 (6.3)	5.6 (0.2)	3.1 (0.2)
Potassium chloride (KCl)	4.70	108 (5.1)	6.1 (0.08)	4.0 (0.3)
	3.91	121 (19.1)	6.8 (0.6)	4.0 (0.5)
	3.12	126 (12.6)	6.7 (0.8)	3.6 (0.3)
	2.35	86.5 (11.2)	4.8 (0.4)	3.4 (0.1)
	1.56	89.6 (7.1)	5.1 (0.3)	3.2 (0.3)
Control	0	66.8 (2.4)	4.1 (0.1)	1.9 (0.1)
Rates		$P < 0.0001$	$P < 0.0015$	$P < 0.0026$
Fertilizer types		NS	NS	$P < 0.0185$
Rates × fertilizer types		NS	NS	NS

Mean values represent four replicates from each treatment with SE in parentheses.

NS = not significant at $\alpha = 0.05$.

^zThe amounts of K⁺ rates (g/plant) shown in the table are calculated from the fertilizer application rates of 112, 168, 224, 280, and 336 kg of K/ha.