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Seaweed extract as organic bio-stimulant improves productivity and quality of rice in eastern Himalayas

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Abstract Enhancing productivity through integrated nutrient management is pertinent to sustainable intensification of agricultural ecosystems. Field experiments were conducted during 2012 and 2013 in Eastern Himalayas with extracts from *Kappaphycus alvarezii* (K sap) and *Gracilaria edulis* (G sap) to assess growth, productivity and quality enhancement of rice (*Oryza sativa*). A Petri dish experiment was also conducted to assess the efficacy of saps in improving germination percentage and seedling vigour of rice seedlings. Field experiment included foliar spray of K and G saps (2.5, 5, 10 and 15% concentrations) with 100% recommended dose of fertilizers (RDF) along with water spray + 100% RDF (control). The rice seeds soaked with lower concentrations (2.5 and 5%) of K and G saps recorded higher germination percentage, root and shoot length and seedling vigour index as compared to water soaking. Whilst soaking in higher concentrations (7.5% and above) reduced the germination percentage and seedling vigour index. Foliar spray of K or G sap at 5% and above concentrations + 100% RDF significantly increased plant height, dry matter accumulation, chlorophyll index, crop growth rate, yield attributes and yield of rice (5.4 to 18.4% higher) as compared to control. Application of K or G sap at 10% concentrations + 100% RDF also increased micro-nutrient (Fe, Cu, Zn and Mn) and protein concentration in rice grains. Thus, foliar application of 10% K or G sap along with 100% RDF is a recommendable option for improving yield and quality of rice and sustaining soil fertility in eastern Himalayas and similar agroecosystems elsewhere.

Keywords Hill agriculture · Micro-nutrient · Protein content · Rice · Seaweed · Yield

Introduction

Rice (*Oryza sativa* L.) is the principal food crop of the north-eastern region (NER) of India with total geographical area of about 26.2 million ha (M ha). It is cultivated on an area of about 3.5 M ha with an average productivity of 1.78 Mg ha^{-1} which is much below the India's national average (2.2 Mg ha^{-1}) (Das et al. 2013). Rice productivity in the NER of India is low due to inadequate nutrient and water management practices (Das et al. 2014). Soils of about 85% of total geographical area of the NER are moderate to strongly acidic in nature (Manoj Kumar 2011) that limits the nutrient availability to plants (Fageria and Barbosa Filho 2007). Some strategies to improve rice productivity are use of chemical fertilizer (Das et al. 2014), organic manure (Das et al. 2010), high yielding varieties (Patel et al. 2010) and pesticides

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(Bottrell and Schoenly 2012). The organic manures like farm yard manure (FYM) and compost are bulky in nature and very laborious to carry in the hilly terrain of NER. Availability of adequate quantity of organic manure in time and place is another constraint in crop production. Under such situation, seaweed extract is an alternative, being an economic and low-volume organic source of fertilizer (Craigie 2011; Dwivedi et al. 2014). Use of seaweed extracts has gained popularity due to their potential use in organic and sustainable agriculture (Shah et al. 2013; Layek et al. 2015). Seaweeds contain all the trace elements (Devi and Mani 2015; Pal et al. 2015) and some essential plant growth hormones (Zhang and Ervin 2008; Lotze and Hoffman 2016). Natural plant growth regulators (e.g. auxin, gibberellin and cytokinin) present in seaweed extract give a major boost to crop yields by accelerating the plant's metabolic function (Zhang and Ervin 2008; Wang et al. 2016). The use of seaweeds as manure was an ancient and common practice amongst the Romans, and was also practiced in Japan, China, Britain and Spain (Thirumaran et al. 2009). Seaweed cast had been used by the farmers, even in the early 1900s (Wang et al. 2016). In many countries, seaweeds are still used both in agriculture and horticulture (Elansary et al. 2016; Wang et al. 2016). In India, large quantity of macroscopic marine algae is utilised directly as manure or in the form of compost by coastal communities (Nedumaran and Arulbalachandran 2015). About 15 Mt of seaweed products are produced every year across the globe, amongst which a considerable portion is used as nutrient supplements or bio-stimulants to improve plant growth and productivity (FAO 2006). Council of Scientific and Industrial Research (CSIR)-Central Salt and Marine Chemical Research Institute (CSMCRI), Bhavnagar, Gujarat, India, introduced the commercially important seaweeds, *Kappaphycus alvarezii* and *Gracilaria edulis*, in India and developed a practical cultivation technology leading to large-scale farming in shallow coastal waters (Eswaran et al. 2002; Layek et al. 2015).

The recent challenges to enhance food production due to the increasing level of biotic and abiotic stresses are attributed to climate change with a severe impact on agriculture in the twenty-first century (IPCC 2014). Therefore, research for sustainable approaches to alleviate these stresses is a high priority (Bird et al. 2016). Seaweed extract enhances tolerance against environmental stresses and increases plant nutrient uptake from soil (Shah et al. 2013; Yadav et al. 2016). Unlike chemical fertilizers, extracts derived from seaweeds are biodegradable, non-toxic and non-polluting (Pramanick et al. 2013; Pal et al. 2015). The seaweed extract has recently gained much emphasis as foliar spray for inducing faster growth and yield in cereals, vegetables, fruit orchards and horticultural plants (Dwivedi et al. 2014; Elansary et al. 2016). Foliar application of mineral nutrients offers a quicker method of supplying nutrients to plants than methods involving soil application (Shah et al. 2013; Nedumaran and Arulbalachandran 2015). It may be due to active uptake of nutrients through stomatal pores instead of cuticular uptake (Marschner 2011; Fernandez and Brown 2013). Many researchers have reported beneficial effects of seaweed extracts on growth and yield of wheat (*Triticum aestivum* L.) (Shah et al. 2013), tomato (*Solanum lycopersicum*) (Demir et al. 2006), soybean (*Soybean max*) (Rathore et al. 2009), blackgram (*Vigna mungo*) (Murugalakshmikumari et al. 2002) and maize (*Zea mays*) (Layek et al. 2014, 2015). Research of seaweed extracts on rice is very limited in India and mainly done in the plain land rice ecosystems (Satapathy et al. 2014; Patel et al. 2015; Singh et al. 2015). However, the effects of seaweed extract on rice under acid soil and hill ecosystems of India have not been widely studied. Data on effect of seaweed extracts on soil available nutrients and nutrient and protein content in rice grain is also meagre.

Thus, the present investigation was conducted to study the effect of foliar application of seaweed extract [*Kappaphycus alvarezii* (K sap) and *Gracilaria edulis* (G sap)] on germination, growth, yield and quality of rice. The hypothesis tested was that foliar application of K or G sap will increase the yield and quality of rice grain.

Materials and methods

Description of the site

Field experiments were conducted during the rainy season (June–November) of two consecutive years (2012–2013), in the lowland agronomy farm of the Indian Council of Agricultural Research (ICAR) Research Complex for North Eastern Hill (NEH) Region, Umiam, Meghalaya, India. The experimental site situated in a valley land (950 m above sea level, 25° 30′ N latitude and 91° 51′ E longitude) and surrounded by hills. The temperature of the study site is moderate in most of the year except few months of winter. The average annual rainfall of the site is 2450 mm. The maximum temperature (30 °C) was recorded in the month of June and the minimum temperature (4 °C) in January. The maximum relative humidity for most of the year was >75%. The year 2012 and 2013 received less amount of rainfall (2089 and 2022 mm, respectively) than the average annual rainfall (2450 mm) of the site. Data regarding average weekly weather parameters during the crop growth period of year 2012 and 2013 are measured by the automated weather station present in the institute and shown in Figs. 1 and 2, respectively. The soil of the experimental site is a *Typic Paleudalf* (Das et al. 2014), clay loam in nature, acidic in reaction (pH 5.3), low in available nitrogen (N) (253.7 kg ha^{-1}) and phosphorous (P) (11.2 kg ha^{-1}) and medium in available potassium (K) (259.9 kg ha^{-1}).

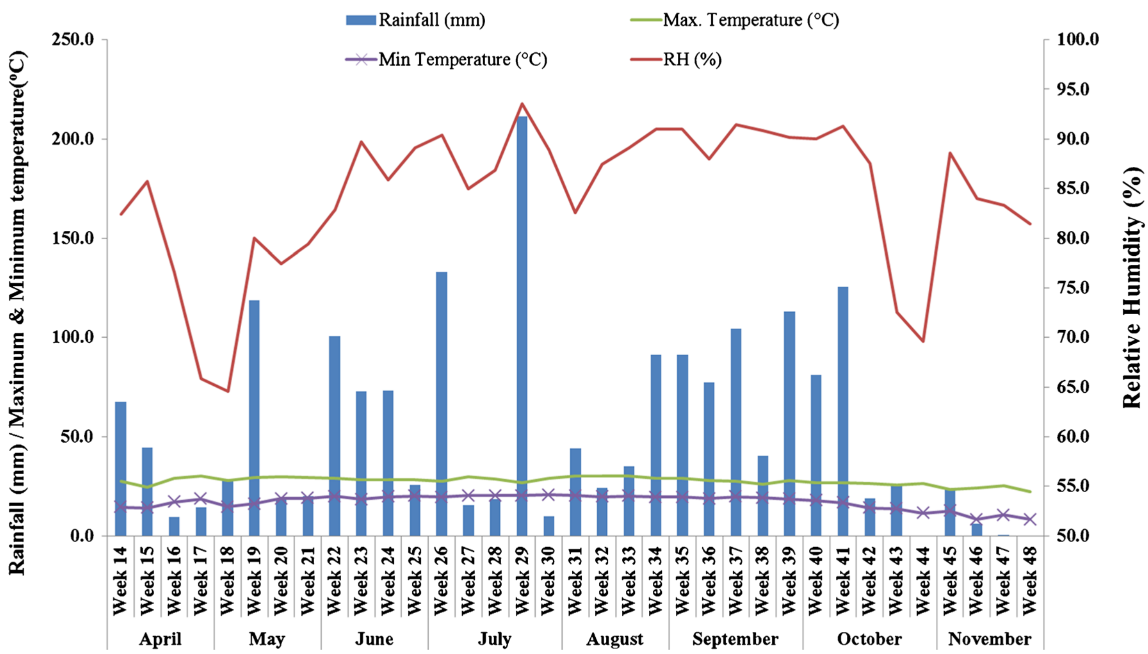


Fig. 1 Weather parameters during rice growing season of year 2012

Treatments and layout

Seaweed extract preparation

Both K and G saps were extracted from fresh *Kappaphycus alvarezii* and *Gracilaria edulis*, respectively, using the methodology of Singh et al. (2016). These seaweeds were cultivated in coastal seawater of Tamil Nadu, India (Layek et al. 2014). The Ksap (commercially available as Aquasap) is being prepared by M/s Aquagri Processing Pvt. Ltd., in collaboration with CSIR-CSMCRI, Gujrat, India. The sap from *K. alvarezii* was expelled mechanically by milling under ambient conditions after washing the seaweed with freshwater. The slurry obtained through milling was centrifuged and preserved (Eswaran et al. 2005). The G sap was prepared by mechanically expelling the sap from *G. edulis* at regional station of CSIR-CSMCRI in Tamil

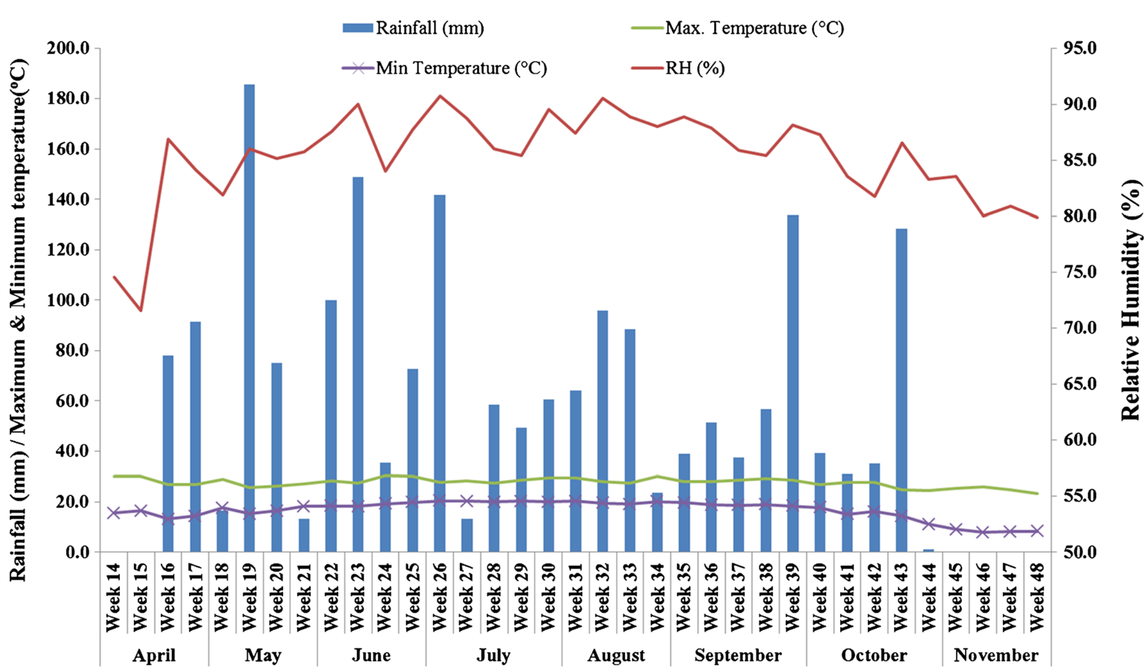


Fig. 2 Weather parameters during rice growing season of year 2013

Nadu followed by filtration. This sap was preserved using a mixture of 0.02% propyl paraben, 0.2% methyl paraben and 0.1% potassium benzoate (Singh et al. 2016). One litre of seaweed extract was considered as 100% concentration of the seaweed extract, and from this, different concentrations (2.5, 5, 7.5, 10 and 15%) were prepared using distilled water (Sivasankari et al. 2006). The composition of both the K and G saps used for this study were from the same lot as that described earlier by us in Layek et al. (2015).

Experiment on germination and seedling vigour

Before initiating field experiment, two seaweeds saps (K sap and G sap) were tested for their efficacy in improving germination percentage and seedling vigour of rice. Rice seeds were soaked with concentrations of 2.5, 5, 7.5, 10 and 15% of K sap and G sap for 24 h, and then, the seeds were placed in Petri plates and watered regularly with distilled water. Water-soaked seeds were used as control. The germination experiment for each of the soaking concentrations was replicated three times. Observations on germination, shoot and root length were recorded after 7 days of soaking. Seedling vigour index was calculated based on germination (%) and seedling length using the following formula (Tanveer et al. 2010):

$$Seedling\ vigour\ index\ I = germination\% \times radical\ length/shoot\ length\ (\mathrm{cm}) \quad (1)$$

Field experiment

Treatments consisted of spray with water (T_1), 2.5% K sap (T_2), 5% K sap (T_3), 10% K sap (T_4), 15% K sap (T_5), 2.5% G sap (T_6), 5% G sap (T_7), 10% G sap (T_8) and 15% G sap (T_9). All the treatments were provided with soil application of 100% recommended dose of fertilizer (RDF), i.e. 80:60:40 kg N, P_2O_5 and K_2O kg ha^{-1} (Das et al. 2013). Three foliar applications of all the sap concentrations (K sap and G sap) were done, first at vegetative stage [25–30 days after transplanting (DAT)], second at tillering stage (50–55 DAT) and third at flowering stage (70–75 DAT) of rice. These nine treatment combinations were tested in a complete randomised block design and replicated three times. The gross plot size was 5 × 4 m. Both K and G saps were used as foliar spray along with surfactants (wetmax plus) for proper adherence. The chemical composition of both K and G saps are given in Table 2.

Cultural practices

Twenty-day-old seedlings of rice variety Shahsarang −1 (high yielding variety recommended for midaltitudes, up to 950 m above sea level) were transplanted manually at 20 × 20 cm spacing in the first week of July using 2 seedlings hill^{-1} (25 hills m^{-2}). Rice was fertilised with 50% N and 100% of P and K (as per the treatments) as basal at the time of transplanting, whilst the remaining 50% of N was applied in two equal splits at tillering (30 DAT) and panicle initiation stages (60 DAT). Urea (46:0:0), single superphosphate (0:16:0) and muriate of potash (0:0:60) were used as source for N, P and K, respectively. Two hand weeding at 25 and 50 DAT and one mechanical weeding with cono-weeder at 40 DAT were done for weed control. No major insect pest and disease problems were observed. A spray of bavistin (methyl benzimidazol-2-ylcarbamate) at 2 g L^{-1} was used as preventive measure against blast disease in the nursery. Rice was harvested manually from net plot area (4 × 3 m) by cutting the aboveground biomass and leaving 20-cm stubble height using a sickle. The harvested produce was sun dried on the concrete floor for 5–7 days before threshing. The grain yield was recorded after cleaning and drying at 14% moisture content.

Plant sampling and analysis

The growth attributes like plant height and dry matter production were recorded at harvest from randomly selected five plants in each plot. The chlorophyll index and crop growth rate (CGR) were recorded at 60 and 90 DAT. The chlorophyll index was recorded using a SPAD meter. The yield parameters (panicles hill^{-1}, effective grains panicle^{-1} and test weight) were measured at maturity from randomly selected five hills in each plot. The post-harvest data on grain and straw yields and harvest index (HI) were recorded from the net plot area of 4 × 3 m. Plant samples were oven dried at 65 °C, ground and sieved through a 0.5-mm sieve and analysed for total N by a micro-Kjeldahl method (Bremner and Mulvaney 1982). The P concentration of plant tissues digested in HNO_3 and $HClO_4$ was determined by the ammonium molybdate method (Olsen and Sommers 1982) and that of K by flame photometry (Jackson 1973). Nutrient uptake (for the aboveground biomass only) was estimated by multiplying the N, P and K concentrations of grains and straw with their respective yield in kilogramme per hectare and summing up the two values. Protein content of the rice grain was obtained by multiplying the total N by 5.95 (Tsukaguchi et al. 2016). The Fe, Mn, Cu and Zn in grain samples were extracted with a DTPA solution (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M triethanolamine, pH 7.3) as outlined by Lindsay and Norvell (1978). The concentration of micro-nutrients in the extract was determined by atomic absorption spectrophotometer (Analyst 200 Atomic Absorption Spectrometer, PerkinElmer).

Soil sampling and analysis

Initial as well as post-harvest composite soil samples were collected (500 g composite sample, one sample from each plot) from 0 to 15 cm depth. Three soil samples were collected from each plot and composited. Samples were air dried, processed using 2-mm sieve and analysed for soil pH by Thomas (1996), soil organic carbon (SOC) by Nelson and Sommers (1996), available N by the alkaline permanganate method (Stanford and Smith 1978), available P by Bray method (Kuo 1996) and available K by neutral normal NH_4OAC extraction method (Knudsen et al. 1982).

CGR

It represents dry weight gained by a unit area of crop in a given time. It was computed by using the following formula:

$$\mathrm{CGR} = \left(W_2 - W_2 / T_2 - T_1\right) \text{ and } \textit{expressed} \text{ as g m}^{-2}\text{ day}^{-1} \quad (2)$$

where W_1 and W_2 are the dry weights at times T_1 and T_2, respectively. The CGR was calculated for the duration between 60 and 90 DAT and 90 DAT-harvest.

The HI was determined by the following formula and expressed as percentage (%):

$$\mathrm{HI} = \left(\textit{economic yield} / \textit{biological yield}\right) \times 100 \quad (3)$$

where economic yield is the grain yield and biological yield is the aboveground biomass (grain yield + straw yield).

Statistical analysis

The experimental data pertaining to each parameter studied were subjected to statistical analysis by using the technique of analysis of variance (ANOVA) for single-factor randomised block design (RBD), and their significance was tested by "*F*" test (Gomez and Gomez 1984). Means and standard errors of three replicates were calculated and presented in tables or figures for each level of treatment. Where a significant difference was found with the ANOVA test, the significance of differences between means were compared following Duncan's multiple range test (DMRT) and least significant difference (LSD) at 5% probability ($p = 0.05$). If the difference between two treatment means is greater than the LSD, then the treatment means are significantly different at the particular level of confidence (5%). For each ANOVA, standard error of the mean (SEM) and LSD at 5% probability are reported.

Results

Germination and seedling vigour of rice

The rice seeds soaked with lower concentrations (2.5 and 5%) of both K and G seaweed extracts had higher rates of germination, whilst the higher concentrations (7.5% and above) of the extracts inhibited the germination ($F_{8,16} = 16.1$, $p < 0.05$). Soaking of rice seeds in 2.5% K sap, 2.5% G sap, 5% K sap and 5% G sap in Petri dish increased germination percentage of rice by 5.9, 3.5, 15 and 10.7%, respectively, over water soaking (Table 1). Treatments receiving 5% K and G saps recorded significantly higher seedling vigour index than that for higher concentrations. A significantly higher seedling vigour index was observed even at 2.5% concentration of both seaweed extracts than that for 7.5% and higher concentrations. In comparison with control, soaking seeds in 2.5 and 5% K sap enhanced root length of rice by 16.6 and 36.0% and shoot length by 20.8 and 29.0%, respectively. Shoot length also followed a trend similar to that of root length. The tallest shoots were observed under 5% K sap (3.1 cm) and the shortest under 15% K sap (1.8 cm) treatment. Amongst the two seaweed liquid fertilizers, K exhibited better results than G sap in respect to germination and seedling vigour.

Growth and physiological attributes of rice

Application of K or G sap at 5% and above concentration recorded significantly taller plants than that for the water spray. The tallest plants were observed with 15% G sap ($F_{8,16} = 4.86$, $p < 0.05$ in year 2013) followed by 15% K sap and 10% G sap (Table 2). The shortest plants were recorded under control (water spray). The dry matter production followed similar trend to that of plant height and application of 5% K sap and 10% G sap being equal to corresponding higher levels producing significantly higher dry matter than that of the control. The lowest dry matter production was recorded with control (water spray) ($F_{8,16} = 7.81$, $p < 0.05$ in year 2013) (Table 2). The CGR of rice was significantly influenced by different concentrations of two seaweed extract sprays. At 60–90 DAT, application of 5% K or G sap recorded significantly higher CGR as compared to that of the control in both years of experimentation (Table 2). Significantly higher CGR at 90 DAT-harvest was recorded with 15% K or G sap in 2012 as compared to water spray ($F_{8,16} = 2.78$, $p < 0.05$). However, in 2013, application of 5% K sap and 10% G sap and concentrations more than these recorded significantly higher CGR at 90 DAT-harvest as compared to that for water spray (Table 2). Application of 5% and above concentrations of G or K resulted in significantly higher chlorophyll index than that of the control at 60 and 90 DAT. However, the chlorophyll indices observed for 5, 10 and 15% sap (K or G sap) concentrations were statistically similar (Table 2).

Yield attributes and yield of rice

In comparison with the control, application of saps recorded significantly higher number ($p < 0.05$) of panicles hill^{-1} and number of effective grains panicle^{-1} for 15% K sap, which were at par with that of 10 and 5% K sap treatments (Table 3). In case of G sap, these yield attributes were significantly increased by foliar application of seaweed extracts up to 10%

Table 1 Effect of different concentrations of seaweed sap soaking on germination, root and shoot length and seedling vigour of rice

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling vigour index
Water soaking	80 fg	3.6 d	2.4 bc	192 cd
2.5% K sap	86 cd	4.2 c	2.9 a	249.4 b
5% K sap	92 a	4.9 a	3.1 a	285.2 a
7.5% K sap	82 ef	4.3 bc	2.5 b	205 c
10% K sap	75 h	3.5 de	2.2 cd	165
15% K sap	67 j	3.1 e	1.8	120.6
2.5% G sap	84 de	3.7 d	2.5 b	210 c
5% G sap	89 bc	4.4 b	2.8 a	249.2 b
7.5% G sap	87 c	4.5 b	2.8 a	243.6 b
10% G sap	78 g	4.3 bc	2.2 cd	171.6 de
15% G sap	70 i	3.4 e	2.0 d	140 e
SEM±	2.13	0.13	0.08	9.67
LSD ($p = 0.05$)	6.4	0.40	0.24	29.3

Different letters indicate that the values are significantly different at the $p < 0.05$ level

K sap Kappaphycus alvarezii sap, *G sap Gracilaria edulis* sap

concentrations. The lowest values of all the yield attributes (panicles hill^{-1}, number of effective grains panicle^{-1} and test weight) were recorded for control (water spray). The highest grain and straw yields of rice were recorded for 15% K sap ($F_{8,16} = 3.58$, $p < 0.05$), and it was at par with that for 15% G sap, 10% K sap and 10% G sap in the year 2012 (Table 3). In 2013, application of K or G sap at 5% and above concentrations resulted in significantly higher grain yield as compared to that for the control ($F_{8,16} = 3.58$, $p < 0.05$). In present study, the lowest grain yield was observed for water spray along with soil application of 100% RDF (control), indicating that there is a need for seaweed extract application to obtain the optimum yield. Significantly higher HI ($F_{8,16} = 3.58$, $p < 0.05$) was recorded with application of 10% and higher concentrations of K or G sap as compared to that under water spray in 2012. However, in 2013, there was no significant difference in the HI of rice ($F_{8,16} = 0.75$, $p < 0.05$) amongst the treatments applied along with 100% RDF. The lowest HI was recorded for control (water spray applied with 100% RDF).

Nutrient status in soil after rice harvest

The available N, P and K contents in soil did not vary significantly amongst different concentrations of seaweed extracts (Table 4). The SOC did not vary significantly with the seaweed extract treatments in 2012; however, in 2013, 15% K or G sap recorded significantly higher SOC ($F_{8,16} = 2.71$, $p < 0.05$) compared to that for the control.

N, P and K concentrations in grains and uptake by rice

Concentration of N in rice grain differed significantly amongst treatments (Table 5). Application of 15% K or G saps resulted in significantly ($F_{8,16} = 2.65$, $p < 0.05$) higher N concentration in rice grain (1.49 and 1.50%, respectively) than that for the control (1.40%). However, the N concentration in straw did not vary significantly ($F_{8,16} = 0.43$, $p < 0.05$) amongst seaweed extract concentrations. The significantly highest N uptake ($F_{8,16} = 15.8$, $p < 0.05$) was recorded with 15% K sap spray (129.3 kg ha^{-1}) followed by 15% G sap (127.4 kg ha^{-1}), 10% G sap (126.6 kg ha^{-1}) and 10% K sap (125.5 kg ha^{-1}) (Table 5). Although there was no significant difference between the treatments for P concentration in rice straw ($F_{8,16} = 0.35$, $p < 0.05$), the highest P concentration was recorded with 15% K sap (Table 5). The highest pooled P uptake was recorded with 15% G sap spray (22.4 kg ha^{-1}). The K concentration in rice grain and straw did not differ amongst treatments. However, the highest K uptake was observed for the 15% G sap (117.4 kg ha^{-1}) followed by 15% K sap (115.0 kg ha^{-1}) and these areas statistically higher than water spray (101.5 kg ha^{-1}).

Protein and micro-nutrient content in rice grain

The protein content in rice grain differs significantly amongst treatments (Table 5). Application of 15% K or G saps resulted in significantly higher protein content in rice grain than that in the control. Application of both K and G saps with 100% RDF significantly increased the concentration of Cu in rice grain (5.89 and 5.64 mg kg^{-1}) as compared to that for control (4.04 mg kg^{-1}) (Fig. 3). Whilst the Zn concentration in grain increased significantly up to 10% of the K sap concentration (16.81 mg kg^{-1}), it increased only up to 5% in case of G sap (16.34 mg kg^{-1}). The Fe and Mn concentrations in rice grain also increased with application of K or G sap up to 5% concentrations (Fig. 3). Increasing sap concentrations beyond 10% did not increase the micro-nutrient concentration in rice

Table 2 Effects of different concentrations of seaweed sap sprays on growth and physiological attributes of rice

Treatments	Year 2012						Year 2013					
	Plant height at harvest (cm)	Dry matter at harvest (g hill^{-1})	CGR (g hill^{-1} day^{-1})		Chlorophyll index		Plant height at harvest (cm)	Dry matter at harvest (g hill^{-1})	CGR (g hill^{-1} day^{-1})		Chlorophyll index	
			60–90 DAT	90 DAT-harvest	60 DAT	90 DAT			60–90 DAT	90 DAT-harvest	60 DAT	90 DAT
T1: water spray	90.4 b	48.5 c	0.43 c	0.52 b	34.2 b	36.4 b	84.0 b	45.8 b	0.43 b	0.53 c	36.6 b	29.8 b
T2: 2.5% K sap	93.4 ab	50.5 bc	0.46 c	0.58 ab	36.0 ab	38.2 ab	86.9 a	49.5 a	0.46 ab	0.56 b	38.5 ab	31.2 ab
T3: 5% K sap	94.1 ab	52.1 ab	0.55 abc	0.58 ab	36.7 a	38.9 a	87.7 a	50.9 a	0.47 ab	0.60 a	39.6 a	32.3 ab
T4: 10% K sap	94.9 a	55.6 a	0.57 ab	0.62 ab	36.8 a	39.0 a	88.0 a	51.3 a	0.49 a	0.61 a	40.1 a	32.5 a
T5: 15% K sap	95.1 a	56.8 a	0.56 abc	0.66 a	37.1 a	39.0 a	88.1 a	51.1 a	0.48 a	0.60 a	40.3 a	32.5 a
T6: 2.5% G sap	93.4 ab	50.6 bc	0.48 bc	0.64 a	36.1 ab	38.3 ab	86.1 ab	48.8 ab	0.45 ab	0.56 bc	38.6 ab	31.0 ab
T7: 5% G sap	95.1 a	54.3 ab	0.54 abc	0.58 ab	36.5 a	38.8 a	87.1 ab	50.3 a	0.44 ab	0.59 ab	39.5 a	32.6 a
T8: 10% G sap	95.4 a	55.3 a	0.58 ab	0.61 ab	36.5 a	38.8 a	88.2 a	50.0 a	0.47 ab	0.61 a	39.3 ab	33.1 a
T9: 15% G sap	96.7 a	55.6 a	0.60 a	0.66 a	36.8 a	38.8 a	88.9 a	50.5 a	0.48 a	0.61 a	40.3 a	32.2 ab
SEM±	1.3	1.4	0.03	0.03	0.65	0.65	0.6	0.8	0.01	0.02	0.80	0.72
LSD (p = 0.05)	4.0	4.3	0.09	0.09	1.94	1.94	1.8	2.5	0.03	0.04	2.39	2.15

Different letters indicate that the values are significantly different at the $p < 0.05$ level. All the treatments were provided with soil application of 100% recommended dose of fertilizer (RDF)

K sap Kappaphycus alvarezii sap, *G sap Gracilaria edulis* sap, *CGR* crop growth rate, *DAT* days after transplanting, *SEM±* standard error of mean, *LSD* least significance difference

Table 3 Effects of different concentrations of seaweed sap sprays on yield attributes and yield of rice

Treatments	Year 2012						Year 2013					
	Panicles hill^{-1}	Effective grains panicle^{-1}	Test weight (g 1000 seed^{-1})	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Harvest index	Panicles hill^{-1}	Effective grains panicle^{-1}	Test weight (g 1000 seed^{-1})	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Harvest index
T1: water spray	10.0 c	124.1 b	22.1 a	4.79 b	7.17 b	39.26 b	11.3 b	131.9 b	22.6 a	4.28 b	5.96 b	41.8 a
T2: 2.5% K sap	10.9 bc	130.8 ab	22.7 a	5.05 ab	7.23 ab	41.20 ab	11.8 ab	138.0 ab	22.8 a	4.63 ab	6.31 ab	42.3 a
T3: 5% K sap	11.6 ab	134.1 a	22.5 a	5.11 ab	7.31 ab	41.19 ab	12.3 ab	140.7 ab	22.8 a	4.97 ab	6.64 ab	42.8 a
T4: 10% K sap	12.0 a	134.1 a	23.0 a	5.28 a	7.33 a	41.88 a	12.3 ab	150.3 a	23.3 a	5.05 a	6.97 a	42.0 a
T5: 15% K sap	12.9 a	134.8 a	23.0 a	5.36 a	7.34 a	42.24 a	12.9 a	153.0 a	23.3 a	5.07 a	7.26 a	41.1 a
T6: 2.5% G sap	10.4 c	129.8 ab	22.3 a	4.97 ab	7.30 ab	40.58 ab	11.6 ab	143.7 ab	22.7 a	4.55 ab	6.15 ab	42.5 a
T7: 5% G sap	11.8 ab	133.0 a	22.9 a	5.11 ab	7.32 ab	41.13 ab	11.8 ab	147.5 ab	23.0 a	4.88 ab	6.39 ab	43.3 a
T8: 10% G sap	11.9 ab	134.3 a	23.2 a	5.26 a	7.46 a	41.37 a	12.3 ab	155.3 a	23.2 a	4.97 ab	6.69 ab	42.7 a
T9: 15% G sap	11.7 ab	133.4 a	22.8 a	5.28 a	7.48 a	41.55 a	12.4 a	158.5 a	22.9 a	5.01 a	6.77 a	42.5 a
SEM±	0.4	2.5	0.5	0.10	0.21	0.70	0.2	4.8	0.5	0.13	0.14	0.7
LSD (p = 0.05)	1.2	7.6	NS	0.31	0.62	2.07	0.6	14.3	NS	0.38	0.41	2.2

Different letters indicate that the values are significantly different at the $p < 0.05$ level. All the treatments were provided with soil application of 100% recommended dose of fertilizer (RDF)

K sap Kappaphycus alvarezii sap, *G sap Gracilaria edulis* sap, *CGR* crop growth rate, *DAT* days after transplanting, *SEM±* standard error of mean, *LSD* least significance difference

Table 4 Soil pH; soil organic carbon (SOC); and available N, P and K status in soil after rice harvest as influenced by different concentration of seaweed sap sprays

Treatments	Year 2012				Year 2013			
	SOC (g kg^{-1})	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})	SOC (g kg^{-1})	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available (kg ha^{-1})
T1: water spray	24.2 a	243.8 a	10.4 a	247.3 a	24.0 a	236.6 a	13.0 a	249.7 a
T2: 2.5% K sap	24.9 a	246.2 a	10.7 a	248.4 a	24.6 ba	238.4 a	12.5 a	250.9 a
T3: 5% K sap	25.2 a	243.9 a	11.0 a	245.0 a	24.9 b	238.9 a	12.8 a	249.6 a
T4: 10% K sap	25.3 a	244.4 a	10.5 a	241.0 a	24.7 ab	235.2 a	13.2 a	248.3 a
T5: 15% K sap	25.2 a	243.7 a	9.9 a	242.9 a	25.1 b	236.5 a	12.1 a	247.3 a
T6: 2.5% G sap	25.0 a	241.8 a	10.6 a	257.3 a	24.5 ab	236.0 a	12.7 a	250.0 a
T7: 5% G sap	25.3 a	242.8 a	10.7 a	258.0 a	24.7 ab	233.1 a	12.3 a	249.0 a
T8: 10% G sap	25.3 a	239.9 a	10.5 a	255.5 a	24.8 b	234.6 a	12.6 a	248.3 a
T9: 15% G sap	25.4 a	239.2 a	10.0 a	255.9 a	24.8 b	235.4 a	12.5 a	247.3 a
SEM±	0.5	3.2	0.6	4.5	0.3	2.1	0.3	1.8
LSD (p = 0.05)	NS	NS	NS	NS	0.8	NS	NS	NS

Different letters indicate that the values are significantly different at the $p < 0.05$ level. All the treatments were provided with soil application of 100% recommended dose of fertilizer (RDF)

K sap Kappaphycus alvarezii sap, *G sap Gracilaria edulis* sap, *CGR* crop growth rate, *DAT* days after transplanting, *SEM±* standard error of mean, *LSD* least significance difference

grain significantly, and in some cases, concentration higher than 10% decreased the micro-nutrient content.

Discussion

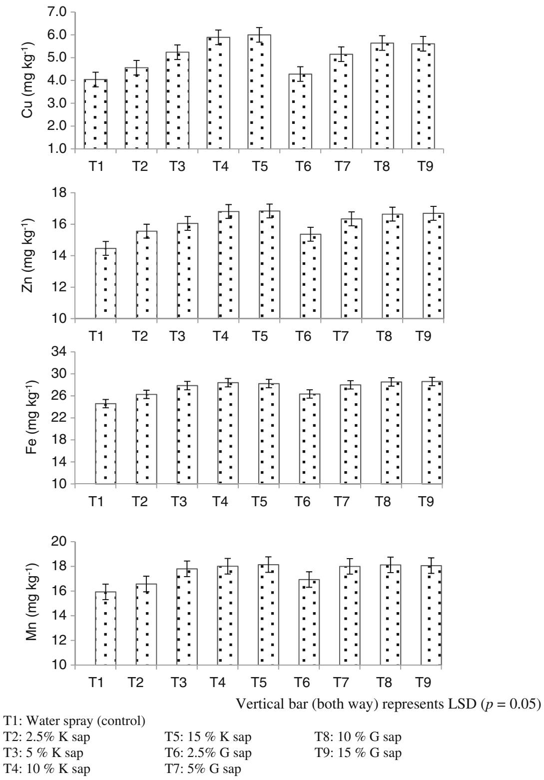
Germination and seedling vigour Soaking of seeds with lower concentrations of seaweed extracts recorded higher rates of germination, shoot and root length and seedling vigour index as compared to those for the control. Higher concentrations of the extracts inhibited the germination and produced shorter root and shoot lengths in rice. Increase in germination and seedling vigour by soaking with seaweed extracts was reported for pigeon pea (*Cajanus cajan*) (Mohan et al. 1994), maize (*Zea mays*) (Rajkumar and Subramanian 1999) and lablab (*Dolichos biflorus*) (Anantharaj and Venkatesalu 2001, 2002), green gramme (*Vigna radiata*) (Venkataraman and Mohan 1997) and cowpea (*Vigna sinensis*) (Sivasankari et al. 2006). The increase in germination and seedling vigour at low concentrations of seaweed extracts may be due to the presence of growth-promoting substances like auxin and gibberellins, phenyl acetic acid (Sivasankari et al. 2006) and micro-nutrients (Layek et al. 2014). Amongst the two seaweed liquid

Table 5 N, P and K contents in rice grain and straw and their total uptake as well as protein content in grain as influenced by seaweed sap sprays (2-year pooled data)

Treatments	N in grain (%)	N in straw (%)	N uptake (kg ha^{-1})	P in grain (%)	P in straw (%)	P uptake (kg ha^{-1})	K in grain (%)	K in straw (%)	K uptake (kg ha^{-1})	Protein content (%)
T1: water spray	1.40 b	0.69 a	110.8 d	0.25 b	0.09 a	17.3 c	0.85 a	0.97 a	101.5 a	8.33 b
T2: 2.5% K sap	1.45 ab	0.72 a	118.5 d	0.26 ab	0.09 a	18.8 bc	0.87 a	0.98 a	107.7 a	8.63 ab
T3: 5% K sap	1.47 a	0.71 a	122.8 bcd	0.27 ab	0.10 a	20.4 ab	0.87 a	1.00 a	113.2 a	8.75 a
T4: 10% K sap	1.48 a	0.69 a	125.5 bc	0.28 ab	0.10 a	21.6 ab	0.90 a	0.98 a	116.2 a	8.81 a
T5: 15% K sap	1.49 a	0.71 a	129.3 ab	0.29 ab	0.11 a	21.5 ab	0.87 a	0.99 a	117.4 a	8.87 a
T6: 2.5% G sap	1.47 a	0.70 a	117.0 d	0.26 ab	0.09 a	18.8 bc	0.85 a	0.98 a	105.9 a	8.75 a
T7: 5% G sap	1.47 a	0.70 a	121.3 cd	0.26 ab	0.10 a	19.3 bc	0.86 a	0.98 a	110.2 a	8.75 a
T8: 10% G sap	1.49 a	0.71 a	126.6 abc	0.28 ab	0.10 a	20.9 ab	0.87 a	1.00 a	115.0 a	8.87 a
T9: 15% G sap	1.50 a	0.70 a	127.4 abc	0.31 a	0.10 a	22.4 a	0.86 a	1.01 a	115.0 a	8.93 a
SEM±	0.02	0.02	2.0	0.02	0.01	0.7	0.02	0.02	2.1	0.12
LSD (p = 0.05)	0.06	NS	6.0	0.04	NS	2.1	NS	NS	6.2	0.35

Different letters indicate that the values are significantly different at the $p < 0.05$ level. All the treatments were provided with soil application of 100% recommended dose of fertilizer (RDF)

K sap Kappaphycus alvarezii sap, *G sap Gracilaria edulis* sap, *CGR* crop growth rate, *DAT* days after transplanting, *SEM±* standard error of mean, *LSD* least significance difference



All the treatments were provided with soil application of 100% recommended dose of fertilizer (RDF).

Fig. 3 Micro-nutrient content in rice grain as influenced by different concentration of seaweed sap sprays (the *vertical bars* indicate LSD at $p = 0.05$)

fertilizers, K sap produced more favourable results than those for G sap in respect to germination and seedling vigour. This may be due to presence of higher amount of growth hormones like auxin, gibberellin and kinetin in K sap as compared to G sap (Layek et al. 2014; Singh et al. 2016).

Growth and physiological attributes Application of K or G sap at 5% and higher concentration produced significantly higher plant height and dry matter production in rice than that for the control. Such increment in plant height and dry matter production might be due to the presence of macro- and micro-nutrients, cytokinins, auxins and betaines in seaweed extracts, which boosted the photosynthetic process, thereby stimulating vegetative growth (Devi and Mani 2015), as has also been reported for gramme (Pramanick et al. 2013) and wheat (Shah et al. 2013). Crop production is determined by CGR which has a linear relationship with intercepted irradiance (Singh et al. 2016). Increase in growth rate (CGR) is also recorded in the experiment with application of 5% K or G sap and higher concentrations recorded at 60–90 DAT (Table 3). Increase in the growth rate may be due to the presence of macro- and micro-nutrients (Sridhar and Rengasamy 2011) and presence of growth-promoting substances in the seaweed extract (Devi and Mani 2015; Pal et al. 2015). The presence of bioactive substances in seaweed extract can improve stomatal uptake efficiency (Mancuso et al. 2006; Rathore et al. 2009) and improve the growth rate of plants. Increase in growth of plant due to application of seaweed extract has been reported in rice (Satapathy et al. 2014; Patel et al. 2015; Singh et al. 2015), blackgram (*Vigna mungo*) (Murugalakshmikumari et al. 2002) and okra (*Abelmoschus esculentus*) (Zodape et al. 2008). Increase in chlorophyll content with application of sap concentration of up to 5% and above may be attributed to the reduction in chlorophyll degradation due to the presence of betaines in the seaweed extract (Whapham et al. 1993).

Yield attributes and yield Yield attributes of rice (e.g. panicles hill^{-1} and number of effective grains panicle^{-1}) increased with increase in seaweed extract concentration, and the highest value was obtained for 15% K sap. However, it was being statistically at par with that for 10 and 5% K sap concentrations (Table 4). Further, these yield attributes were significantly increased only up to 10% G sap concentrations. These improvements in yield parameters can be attributed to the increase in movement of photosynthates from vegetative part to the developing grains (Shah et al. 2013). The rice grain yield increased by 16.1 and 14% by application of 5% K or G sap, and by 18.4 and 17.0% by application of 15% K or G sap compared to that of the control, respectively, in 2013 (Table 4). Similar results of increase in yield attributes and yield by application of K and G saps have also been reported for rice (Patel et al. 2015; Singh et al. 2015), wheat (Zodape et al. 2009; Shah et al. 2013), green gramme (*V. radiata*) (Pramanick et al. 2013), tomato (Zodape et al. 2011), okra (Sylvia et al. 2005) and soybean (Rathore et al. 2009). Presence of micro-elements and plant growth regulators, especially cytokinins in K and G saps, increased the yield of diverse crops fertilised with recommended RDF (Zhang and Ervin 2008; Zodape et al. 2009). Significantly higher HI was recorded with application of 10% and higher concentrations of K or G sap as compared to that for the control in 2012.

Available N, P, K and SOCs concentration in soil Assessing the available nutrient concentration in soil after any treatment is important to the sustainability issue. The different concentrations of seaweed extracts did not make any significant change in concentrations of soil available N, P and K. After 2 years, significantly higher SOC following the harvest of rice was recorded under 15% K or G sap application. This increase in SOC may be due to better crop growth under higher concentrations of seaweed extract sprays (Sharma et al. 2003) and possible enhancement in microbial activity as a result of biostimulation (Kadian et al. 2012) as compared to that for the control. More biomass production can lead to greater amount of root exudation and increase the SOC concentration (Banerjee et al. 2006).

Nutritional quality of grains The N and P concentrations in rice grains and K concentration in rice straw as well as their uptake increased with increase in concentrations of seaweed extracts (Table 5). However, there was no significant difference amongst treatments in N and P concentrations in rice straw and K concentration in rice grain. Similar results of increased uptake of N, P, K and Mg in grapevines (*Vitis vinifera*) and cucumber (*Cucumis sativus*) with application of seaweed extract has been reported (Turan and Kose 2004; Mancuso et al. 2006). Seaweed extracts may enhance the effectiveness of fertilizers as well as nutrient utilisation from soil (Frankenberger and Arshad 1995; Rathore et al. 2009) and thus increase the nutrient concentration and uptake by rice. Foliar spray of seaweed extract can enhance the effectiveness of fertilizers as well as of nutrient utilisation from soil (Frankenberger and Arshad 1995; Sharma et al. 2014). The total nutrient uptake being a product of nutrient concentration in grain, straw and their biomass (Layek et al. 2013); higher nutrient concentration in grain and straw; and higher yield of rice due to seaweed extract application led to higher uptake of N, P and K. Application of 15% K or G saps also resulted in significantly higher protein content in rice grain than that in the control. Protein content in wheat was also found to increase by 15.64 and 13.09% with 7.5 and 5.0% concentrations of K and G saps, respectively (Shah et al. 2013). Increase in protein content with the application of seaweed extracts has also been reported in *Vigna catajung* (Anantharaj and Venkatesalu 2001). This might be because of promotive effects on root proliferation and higher uptake of N, P and sulphur needed for protein synthesis (Shah et al. 2013). Seaweed extract application also increased the micro-nutrient content in rice grains like Cu and Zn up to 10% concentrations and Fe and Mn up to 5% concentrations (Fig. 3). These results are in accordance with those of earlier studies in okra and wheat (Zodape et al. 2009; Shah et al. 2013). Seaweed extract contains chelating compounds (i.e. mannitol) that can increase availability of some micro-nutrients to plants (Shah et al. 2013).

Conclusion Soaking of rice seeds in lower concentration (2.5 and 5%) of saps extracted from K sap and G sap improved the germination and seedling vigour of rice. Application of either K or G sap up to 10% concentrations along with RDF resulted in significant improvement in yield and quality of grain over water spray. However, application of higher concentration (15%) of K or G sap along with 100% RDF did not cause any significant enhancement of yield and nutritional quality of grains compared to 10% seaweed extract sprays. Thus, application of 10% concentration of K or G sap spray along with 100% recommended dose of nutrients is a feasible option to obtain high yield and grain quality of rice in NER region of India.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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