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Effects of Fermented Nori (*Pyropia yezoensis*) Seaweed Liquid Fertilizers on Growth Characteristics, Nutrient Uptake, and Iodine Content of Komatsuna (*Brassica rapa* L.) Cultivated in Soil

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Abstract

We investigated the effects of fermented nori (*Pyropia yezoensis*) liquid fertilizers on plant growth, soil plant analysis development (SPAD) values, and nutrient uptake of komatsuna (*Brassica rapa* L. var. wakana komatsuna) plants. The four types of fermented nori seaweed liquid fertilizers (SLFs) evaluated in this study were prepared by anaerobic fermentation of unwashed nori (SLF1), aerobic fermentation of unwashed nori (SLF2), anaerobic fermentation of washed nori (SLF3), and aerobic fermentation of washed nori (SLF4). In Experiment 1, the highest plant growth, SPAD, and nutrient uptake values were obtained from treatment with SLF2 and SLF4. There were no significant differences between the effects of basal and foliar application of SLFs, except for iodine (I) content; plants treated with SLF1 had the highest I content. In Experiment 2, plant growth and nutrient uptake decreased with higher concentrations of SLFs. Plants treated with 25% SLF2 + 75% chemical fertilizer (CF) or 25% SLF4 + 75% CF exhibited significantly higher plant growth and nutrient uptake. The highest I content resulted from treatment with 75% SLF1 + 25% CF or 100% SLF1. Taken together, our results showed that 25% SLF + 75% CF produced the best plant growth characteristics, nutrient uptake, and I content relative to the controls. Therefore, basal application of these liquid organic fertilizers can be used to increase productivity, nutrient uptake, and I content and to reduce nitrate-nitrogen content in komatsuna production.

Keywords

Nori, Komatsuna, Basal and Foliar Application, Plant Growth, Nutrient Uptake, I Content

1. Introduction

Excessive use of inorganic fertilizers has detrimental effects on the physicochemical properties of soil. In contrast, organic fertilizers contribute to the deposition of crop residues and physicochemical properties of soil [1]. Seaweed extract has been used as a source of organic supplemental fertilizers [2]. The use of seaweed products is known to improve seed germination and seedling development, increase plant tolerance to environmental stress [3], and enhance plant growth and yield [4].

Nori (*Pyropia yezoensis*) is cultivated on the coast of the Ariake Sea in Fukuoka Prefecture, which has become a leading area of nori production in Japan (Ministry of Agriculture, Forestry, and Fishery, 2015) through use of the pole system cultivation method [5]. In recent years, low quality, and faded nori has also been harvested, even in cultivation systems that involve the freezing of seedlings, and this production method is relatively stable. Some of this faded nori is dumped into the sea, which hinders fisheries, or is incinerated without being utilized [6].

Nori is rich in protein, iodine (I), dietary fiber, and several vitamins including folic acid and vitamins B12 and K (Japan Food Standard Ingredient Table 2015). I is an essential micronutrient for human health, as it is required for the synthesis of the thyroid hormones thyroxine and triiodothyronine [7]. I deficiency in the human diet and the need for new ways to fortify foods with this nutrient provide incentive for identifying the most suitable species for biofortification with I [8]. Fruits and vegetables are generally poor sources of I; however, plants can accumulate this element, and increased application of I to the soil or water enhances its accumulation in plants [9].

A vegetable-rich diet provides many health-promoting substances such as minerals, vitamins (A, C, B1, B6, B9, and E), dietary fiber, and phytochemicals. Despite the nutritional benefits of eating vegetables, they also contain substances that adversely affect human health, such as nitrates and nitrites [10]. Vegetables are the major source of the daily intake of nitrate by human beings, accounting for 72% - 94% of the total intake [11]. High nitrate accumulation in plants is harmful to human health [12] as well as to plant growth [13].

In recent years, liquid fertilizers based on seaweed extracts have been commercialized [14]. Natural seaweed fertilizer products can substitute for conventional synthetic fertilizers. In agriculture, seaweeds have been used as soil conditioners, fertilizers, and green manure due to the presence of high amounts of potassium (K) salts, micronutrients, and growth substances. Booth [15] observed that the value of seaweeds as fertilizers was due not only to nitrogen (N), phosphorus (P), and potash content, but also due to the presence of trace elements and metabolites. The higher amounts of water-soluble potash, other minerals, and trace elements in seaweeds are readily absorbed by plants and can be used to control nutrient deficiency diseases [16]. We were interested in the use of organic liquid fertilizers on economically important crops such as komatsuna. The objective of this study was to determine the effects of four types of fermented seaweed liquid fertilizers (SLFs) on the plant growth characteristics, soil plant analysis development (SPAD) values, nutrient uptake, and I and nitrate-nitrogen (NO_3-N) contents of komatsuna (*Brassica rapa* L. var. wakana komatsuna).

2. Materials and Methods

2.1. Nori Fermentation

Experimental materials included faded waste nori, molasses (SPOON SUGAR Co. Ltd., Hyogo, Japan), well water, and the microbial material BASE EIGHT (Sunpowers Co. Ltd., Nagasaki, Japan). Thirty-three liters of well water, 7 kg of nori (unwashed or washed with well water), 2.5 L of molasses, and 2.5 L of BASE EIGHT (total volume 45 L) were added to a 50-L fermentation tank (MH-50; Suico Co. Ltd., Hyogo, Japan) and mixed well. The nori was fermented aerobically and anaerobically. Fermentation was carried out for 3 months from April to July of 2016. Four types of SLF were produced: anaerobically fermented unwashed nori (SLF1), aerobically fermented unwashed nori (SLF2), anaerobically fermented washed nori (SLF3), and aerobically fermented washed nori (SLF4).

2.2. Komatsuna Cultivation

Soil pot Experiment 1 was conducted in a greenhouse at Kyushu University from October 10 to November 14, 2017. A randomized complete block (RCB) design was used with three replications and 12 treatments. Treatments were basal and foliar application of chemical fertilizer (CF), 0.4 g of NPK (in distilled water, foliar); 0.3 g of NPK per pot + 0.1 g of N derived from each of the four SLFs, SLF1 (all N content from seaweed), SLF2 (all N content from seaweed), SLF3 (all N content from seaweed), and SLF4 (all N content from seaweed); and a control $(NPK)_0$. Foliar spray applications of 10 mL of 10-fold dilutions of original solutions of SLF1, SLF2, SLF3, and SLF4; CF in distilled H_2O; and distilled H_2O as a control were performed at 5-day intervals beginning 14 days after sowing.

For pot preparation, a/5000 Wagner pot was filled with 3.5 kg (oven-dried basis) of Futsukaichi soil. The physiochemical properties of Futsukaichi soil were analyzed by Myint *et al.* (2011) [17] (**Table 1**). This soil is a sandy loam with a pH of 6.11 (soil:water, 1:2.5) and low total N content (0.68 $g \cdot kg^{-1}$). $CaMg(CO_3)_2$ powder was added to adjust the soil pH to 6.5 before sowing seeds. The CF consisted of 0.4 g of N from $(NH_4)_2SO_4$, 0.4 g of P_2O_5, and 0.4 g of K_2O (from

Table 1. Physical and chemical properties of Futsukaichi soil.

Physicochemical property	Value	Physicochemical property	Value
Soil pH (Soil:H_2O; 1:2.5)	6.11	Available P (mg 100 g^{-1})	5.42
EC (Soil:H_2O; 1:5) (mS/m)	1.77	CEC ($mol_c \cdot kg^{-1}$)	12.55
Total N ($g \cdot ka^{-1}$)	0.68	Exc. Ca ($mol_c \cdot kg^{-1}$)	10.76
Mineralizable N (mg 100 g^{-1})	0.06	Exc. Mg ($mol_c \cdot kg^{-1}$)	0.89
Total P_2O_5 ($g \cdot kg^{-1}$)	0.37	Exc. K ($mol_c \cdot kg^{-1}$)	0.37

Abbreviation: CEC, cation exchangeable capacity: Exc., exchangeable.

KH_2PO_4 + K_2HPO_4). The water holding capacity of the soil was maintained at 60% at the time of sowing.

To study the effects of application of the SLFs in various ratios with CF, soil pot Experiment 2 was conducted in a greenhouse at Kyushu University from April 7 to May 5, 2018. An RCB design was used with three replications and 18 treatments. Basal application treatments included 0.5 g of NPK (100% CF), 0.5 g of N derived from each of the four SLFs (100% SLF, 25% of each SLF + 75% CF, 50% of each SLF + 50% CF, and 75% of each SLF + 25% CF), and a control $(NPK)_0$. All SLF treatments in Experiment 2 were calculated based on the total N content. The pots were prepared as in Experiment 1. The CF contained 0.5 g of N from $(NH_4)_2SO_4$, 0.5 g of P_2O_5, and 0.5 g of K_2O (from KH_2PO_4 + K_2HPO_4). The water holding capacity of the soil was maintained at 60% at the time of sowing.

Komatsuna seeds were sown at 15 seeds per pot and the germinated seedlings were thinned 10 days after sowing to five plants per pot. The pots were watered weekly with tap water according to the water holding capacity of each pot when the pot weight decreased to 200 - 300 g.

2.3. Evaluation of the Effects of SLFs on Komatsuna Growth and SPAD Values

Plant growth characteristics (leaf number and leaf length) were measured and the Soil Plant Analysis Development (SPAD) values were recorded at 3-day intervals beginning 10 days after sowing using a chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc., Osaka, Japan). The final data collection was performed 1 day before harvest. After 35 days, the plants were harvested by cutting at the cotyledonary node and the plants were washed three times with deionized water to remove the nutrients contained in the foliar spray. Plants were freeze-dried for 72 h for I extraction and oven dried at 70˚C for 72 h for nutrient analysis and measurement of shoot dry weight (DW; g).

2.4. Evaluation of the Effects of SLFs on Total Nutrient Uptake of Komatsuna

Oven-dried shoot samples were ground to fine powder using a Cyclotec 1093 sample mill (100 - 120 mesh, Tecator AB, Hoedanaes, Sweden). The fine powders were digested using the salicylic acid-H_2SO_4-hydrogen peroxide (H_2O_2) digestion method [18] as preparation for determining total N and P contents, and by the HNO_3 digestion method [19] as preparation for determining total K, calcium (Ca), magnesium (Mg), and sodium (Na) contents. The total N content was determined using the indophenol method [20]; total P was determined by the ascorbic acid method [21]; and K, Na, Ca, and Mg contents were determined using an atomic absorption spectrophotometer (Z-5300, Hitachi, Tokyo, Japan).

2.5. Evaluation of the Effects of SLFs on I Content of Komatsuna

To determine the I content of plants, samples were extracted with tetramethylammonium hydroxide [$(CH_3)_4NOH$, TMAH] according to the extraction method of Fecher *et al.* [22] and Rädlinger and Heumann [23]. The total I content was determined using an Agilent 7500 ce inductively coupled plasma mass spectrometer ((ICP-MS, Agilent Technologies, Inc., Tokyo, Japan).

2.6. Evaluation of the Effects of SLFs on Nitrate Nitrogen (NO_3-N) Content of Komatsuna

The NO_3-N content of plant samples was determined using a rapid colorimetric method by nitration of salicylic acid according to the method of Cataldo *et al.* [24].

2.7. Data Analysis

Data were statistically analyzed using STATISTIX 8 (Analytical Software, Tallahassee, FL, USA) software and the mean values were compared using Tukey's HSD test at $P < 0.05$.

3. Results

3.1. Physiochemical Properties of SLFs

The nutrient contents of the four SLFs evaluated in this study are shown in **Table 2**. The SLFs contained macro- and micronutrients including N, K, P, Ca, Mg, and Na. SLF2 had significantly higher N content (107.68 mM) than SLF1 and SLF3, followed by SLF4 (76.90 mM). The K, Mg, and Na contents were higher in SLF1 and SLF2 produced from unwashed nori, followed by SLF3 and SLF4, which were produced from washed nori with reduced salt content. The SLFs also contained inorganic ions such as ammonia nitrogen (NH_4-N), NO_3-N, phosphate ($H_2PO_4^+$), chlorine (Cl^-), and sulfate (SO_4^{2-}) with different pH and electrical conductivity values (**Table 3**). SLF1 and SLF3 had significantly lower NH_4-N and NO_3-N contents than SLF2 and SLF4. SLF1 and SLF2 obtained from unwashed nori had higher Cl^- and SO_4^{2-} contents than SLF3 and SLF4 obtained from washed nori. The color of the SLFs was assessed visually. SLF1 and SLF3 were reddish brown and SLF2 and SLF4 were dark brown in color.

Table 2. Macronutrient and micronutrients content of fermented seaweed liquid fertilizers (SLFs).

SLFs	N	P	K	Ca	Mg	Na
	Concentration (mM)					
SLF1	49.64 ± 0.10	4.13 ± 0.08	55.12 ± 0.13	13.10 ± 0.25	24.48 ± 0.21	59.37 ± 0.87
SLF2	107.68 ± 0.93	3.93 ± 0.11	59.34 ± 1.53	11.52 ± 0.36	23.04 ± 0.41	61.88 ± 0.33
SLF3	42.18 ± 0.52	3.71 ± 0.11	40.03 ± 0.13	10.09 ± 0.39	15.22 ± 0.41	14.35 ± 0.87
SLF4	76.90 ± 0.83	4.17 ± 0.00	38.36 ± 1.28	12.71 ± 0.04	18.10 ± 0.00	22.51 ± 0.76

Data show mean values ± standard deviation (SD).

Table 3. Inorganic ions concentration, Cl^-, $H_2PO_4^-$, pH and EC of (SLFs).

SLFs	NH_4^+	NO_3^-	$H_2PO_4^-$	CL^-	SO_4^{2-}	pH	EC
	Concentration (mM)						(s/m)
SLF1	0.12 ± 0.04	2.68 ± 0.00	0.84 ± 0.00	2.09 ± 0.00	0.26 ± 0.00	3.48 ± 0.01	1.51 ± 0.01
SLF2	63.56 ± 0.56	3.03 ± 0.10	1.65 ± 0.03	2.15 ± 0.00	0.17 ± 0.00	7.16 ± 0.01	2.06 ± 0.01
SLF3	0.40 ± 0.00	1.94 ± 0.02	0.85 ± 0.01	1.43 ± 0.00	0.23 ± 0.00	3.85 ± 0.01	1.01 ± 0.00
SLF4	50.22 ± 0.60	2.08 ± 0.06	2.13 ± 0.02	2.01 ± 0.00	0.26 ± 0.00	6.38 ± 0.01	1.51 ± 0.00

Data show mean values ± standard deviation (SD).

3.2. Effects of SLFs on Plant Growth Characteristics and SPAD Values of Komatsuna (Experiment 1)

Plant growth characteristics and SPAD values differed significantly among plants treated with basal and basal-plus-foliar applications of SLFs or CF and control $(NPK)_0$ plants (Table 4). Plants treated with SLF4 exhibited significant differences in leaf number and shoot dry weight compared with plants treated with SLF1 or SLF3 and with the control plants. The leaf lengths of SLF2-treated plants differed significantly from those of SLF1-treated and control plants. CF treatment produced highly significant differences in SPAD values compared with treatment with the four SLFs and the control treatment. The plant growth characteristics and SPAD values of komatsuna did not differ significantly between basal and foliar applications of the fertilizers.

3.3. Effects of SLFs on Nutrient Uptake of Komatsuna (Experiment 1)

Uptake of N, P, K, Ca, Mg, and Na by plants treated with basal and basal-plus-foliar applications of the four SLFs or CF differed significantly from that of the control plants (Table 5). Treatment with CF, SLF2, and SLF4 produced significant differences in N, Ca, and Mg uptake relative to SLF1-treated and control plants. Treatment with CF, SLF3, and SLF4 yielded significant differences in P uptake compared to SLF1-treated and control plants. The difference in K uptake between SLF3- and SLF2-treated plants and CF- and SLF1-treated plants and control plants was highly significant. Mg uptake of SLF2- and SLF4-treated plants differed significantly from that of SLF1-, SLF3-, and CF-treated plants and control plants. There was a highly significant difference in Na uptake between CF-treated plants and SLF-treated and control plants. The N, P, K, Ca, Mg, and Na uptake of komatsuna plants did not differ significantly between basal and foliar applications of the fertilizers.

3.4. Effects of SLFs on I Content of Komatsuna (Experiment 1)

Basal and basal-plus-foliar applications of SLF1 resulted in significantly higher I content (38.30 and 39.30 μg·g^{-1}, respectively) than the control treatment (**Figure 1**).

3.5. Effects of SLFs on NO_3-N Content of Komatsuna (Experiment 1)

Basal and basal-plus-foliar application of CF produced significantly higher NO_3-N (1.3 and 1.1 mg·g^{-1}, respectively) content than all basal and basal-plus-foliar applications of SLF treatments (**Figure 2**).

Table 4. Effects of SLFs on leaf number, leaf length, SPAD value and shoot DW of komatsuna (Soil Expt. 1).

Treatments	Application (AVG)	Leaf no plant^{-1}	Leaf length (cm)	Shoot dry weight g·pot^{-1}	SPAD
Control	B	5.10 ± 0.03 d	12.07 ± 0.60 c	2.71 ± 0.14 e	32.50 ± 0.9 e
	B + F				
CF	B	8.45 ± 0.24 ab	23.34 ± 1.22 ab	10.85 ± 1.10 ab	50.65 ± 1.3 a
	B + F				
SLF1	B	7.51 ± 0.24 c	22.31 ± 0.71 b	8.02 ± 0.83 d	45.22 ± 0.9 d
	B + F				
SLF2	B	8.39 ± 0.26 ab	24.14 ± 0.53 a	10.27 ± 0.97 bc	47.22 ± 1.1 bc
	B + F				
SLF3	B	8.04 ± 0.35 b	22.82 ± 0.71 ab	9.49 ± 0.44 c	46.18 ± 1.0 cd
	B + F				
SLF4	B	8.77 ± 0.24 a	22.87 ± 0.77 ab	11.65 ± 0.48 a	48.92 ± 1.1 b
	B + F				
Source of variance					
Treatments		0.49**	1.43**	1.53**	1.89**
Application method		ns	ns	ns	0.73**
Interaction (T × A)		ns	ns	ns	ns
CV%		3.57	3.75	9.17	2.34

Data show mean values ± standard deviation (SD) in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). T: treatment; A: application; CF: chemical fertilizer; B: basal, B + F: basal plus foliar. *$P < 0.05$; **$P < 0.01$.

Table 5. Effects of SLFs on N, P, K, Ca, Mg and Na uptake (mg·pot^{-1}) of komatsuna (Soil Expt. 1).

Treatments	Application (AVG)	N	P	K	Ca	Mg	Na
Control	B	30.89 ± 1.89 c	24.71 ± 1.99 c	46.50 ± 6.77 e	51.79 ± 3.54 d	4.45 ± 0.46 d	5.90 ± 0.86 e
	B + F						
CF	B	325.52 ± 12.42 a	138.58 ± 11.73 a	321.02 ± 15.61 d	227.00 ± 27.20 b	14.56 ± 1.38 ab	74.51 ± 10.35 a
	B + F						
SLF1	B	248.85 ± 13.35 b	108.82 ± 8.99 b	394.52 ± 28.72 c	180.83 ± 15.55 c	10.87 ± 1.36 c	23.68 ± 4.66 d
	B + F						
SLF2	B	317.84 ± 12.25 a	123.11 ± 7.83 ab	425.96 ± 10.13 ab	233.93 ± 23.79 ab	15.73 ± 2.31 a	59.03 ± 7.95 b
	B + F						
SLF3	B	279.14 ± 28.31 b	126.99 ± 8.80 a	437.28 ± 9.21 a	207.98 ± 13.52 bc	12.40 ± 0.66 bc	20.20 ± 5.98 d
	B + F						
SLF4	B	325.78 ± 22.03 a	132.83 ± 9.44 a	401.55 ± 14.49 bc	265.45 ± 17.47 a	17.17 ± 1.01 a	39.97 ± 7.67 c
	B + F						
Source of variance							
Treatments		26.66**	17.53**	33.24**	35.85**	2.46**	12.88**
Application method		ns	ns	ns	ns	0.95*	ns
Interaction (T × A)		ns	ns	ns	ns	ns	ns
CV%		7.3	8.93	5.47	10.25	10.92	19.64

Data show mean values ± standard deviation (SD) in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). T: treatment; CF: chemical fertilizer; A: application; B: basal, B + F: basal plus foliar. *$P < 0.05$; **$P < 0.01$.

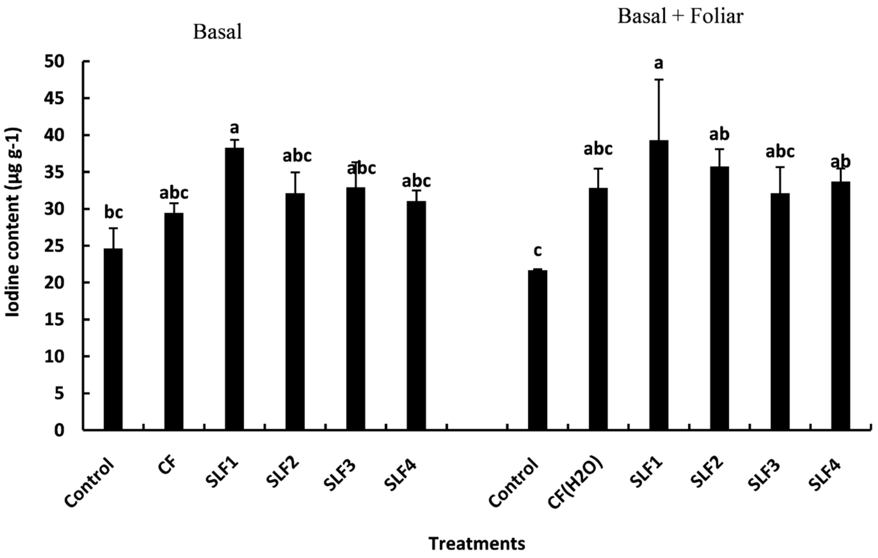

Figure 1. Effects of SLFs between the basal and foliar application on iodine content (µg·g^{-1}) of komatsuna Soil Expt. 1. Mean values followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). The bar on each histogram indicates standard deviation (SD).

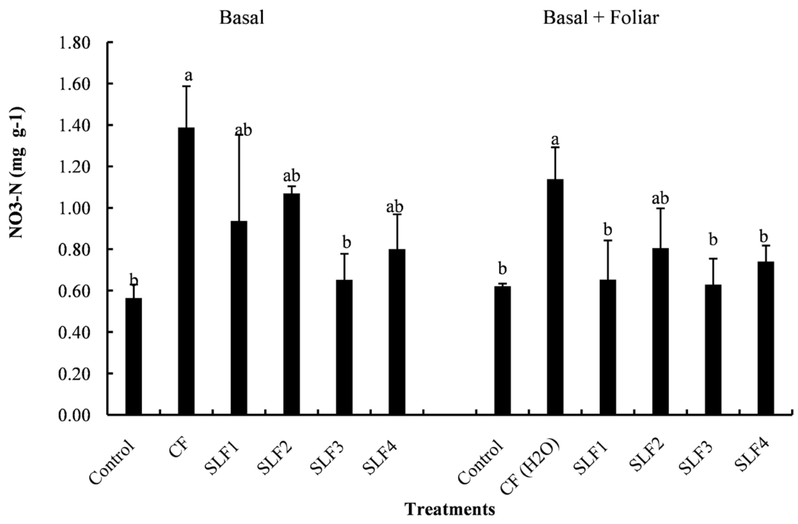

Figure 2. Effects of SLFs on Nitrate Nitrogen (NO_3-N) content ($mg \cdot g^{-1}$) of komatsuna in Soil Expt. 1. Mean values followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). The bar on each histogram indicates standard deviation (SD).

3.6. Effects of SLFs on Komatsuna Growth and SPAD Values (Experiment 2)

Plant growth and SPAD values of plants treated with combinations of SLFs and CF in various proportions differed significantly from those of control plants (Table 6). The plant growth, SPAD, and shoot DW values of plants treated with 100% CF, 25% SLF2 + 75% CF, or 25% SLF4 + 75% CF differed significantly from those of plants treated with SLF2, SLF1, or SLF3 and control plants. The plant growth and shoot DW decreased with increasing proportions of SLFs (50% SLF + 50% CF, 75% SLF + 25% CF, and 100% SLF). Increasing proportions of SLF1 and SLF3 markedly depressed plant growth and shoot DW. In contrast, increasing proportions of SLF2 and SLF4 had no detrimental effects. Treatment with 100% CF, 25% SLF2 + 75% CF, or 25% SLF4 + 75% CF resulted in better plant growth, shoot DW, and nutrient uptake than the other treatments.

3.7. Effects of SLFs on Nutrient Uptake of Komatsuna (Experiment 2)

N, P, K, Ca, Mg, and Na uptake differed significantly between the plants treated with various proportions of SLFs and the control plants (Table 7). Treatment with 100% CF, 25% SLF2 + 75% CF, or 25% SLF4 + 75% CF produced highly significant differences in N, P, K, and Ca uptake relative to treatment with SLF2, SLF4, SLF1, or SLF3 or the control treatment. Plants treated with 25% SLF2 + 75% CF or 25% SLF4 + 75% CF exhibited significantly higher Mg uptake than plants subjected to the other treatments, and treatment with 100% CF produced significantly higher Na uptake than the other treatments.

3.8. Effects of SLFs on I Content of Komatsuna (Experiment 2)

The I content of plants treated with 25% SLF4 + 75% CF or 75% SLF4 + 25% CF and the control plants differed significantly from those of plants treated with any of the other proportions of SLFs and CF (**Figure 3**). The plants treated with 75% SLF1 + 25% CF or 100% SLF1 exhibited the highest I contents of 118.18 and 120.83 μg·g^{-1}, respectively.

3.9. Effects of SLFs on NO_3-N Content of Komatsuna (Experiment 2)

Basal and basal-plus-foliar applications of CF, 100% SLF1, and 75% SLF4 + 25% CF produced significantly higher NO_3-N contents of 0.43, 0.38, and 0.38 mg·g^{-1}, respectively, than all of the other SLF treatments (**Figure 4**).

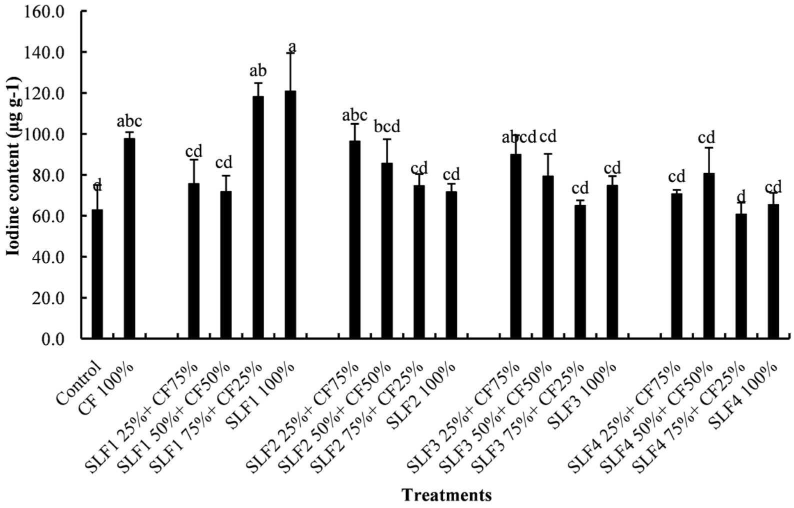

Figure 3. Effects of different ratio of SLFs and CF on Iodine content (μg·g^{-1}) of komatsuna in Soil Expt. 2. Mean values followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). The bar on each histogram indicates standard deviation (SD).

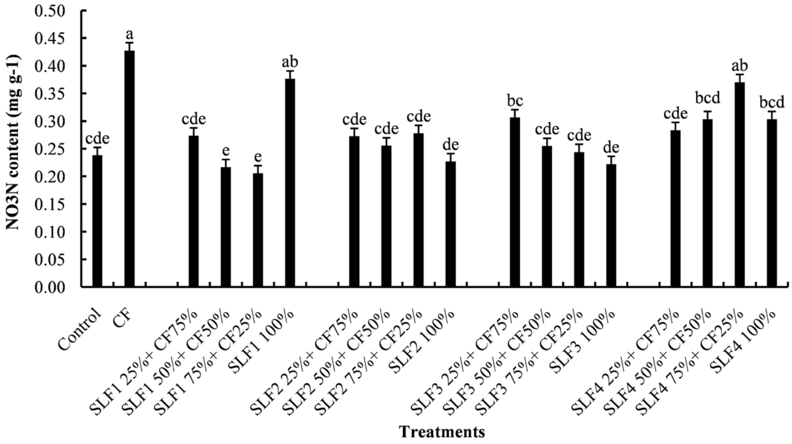

Figure 4. Effects of different ratio of SLFs and CF on Nitrate Nitrogen (NO_3-N) content (mg·g^{-1}) of komatsuna in Soil Expt. 2. Mean values followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). The bar on each histogram indicates standard deviation (SD).

Table 6. Effects of different ratio of SLFs and CF on leaf number, leaf length, SPAD and shoot DW of komatsuna (Soil Expt. 2).

Treatments	Leaf no plant^{-1}	Leaf length (cm)	Shoot DW g·pot^{-1}	SPAD
Control	6.19 ± 0.11 j	12.20 ± 0.65 h	4.09 ± 0.13 g	34.87 ± 0.25 i
CF 100%	10.83 ± 0.03 a	24.05 ± 0.54 a	17.19 ± 0.32 ab	49.30 ± 0.16 ab
SLF1 25% + CF 75%	9.99 ± 0.06 de	22.97 ± 0.40 ab	14.81 ± 0.79 cde	46.27 ± 0.61 ef
SLF1 50% + CF 50%	8.89 ± 0.25 g	19.39 ± 0.37 ef	9.74 ± 0.27 f	45.50 ± 0.41f
SLF1 75% + CF 25%	6.91 ± 0.14 i	15.82 ± 0.43 g	3.83 ± 0.29 g	40.77 ± 0.19 i
SLF1 100%	5.11 ± 0.84 k	10.68 ± 0.62 i	1.33 ± 0.35 h	39.57 ± 0.37 hi
SLF2 25% + CF 75%	10.47 ± 0.22 ab	24.03 ± 0.12 a	18.08 ± 0.74 a	48.80 ± 0.28 abc
SLF2 50% + CF 50%	10.00 ± 0.04 cde	21.37 ± 0.09 bcd	15.55 ± 0.70 bcd	47.07 ± 0.24 de
SLF2 75% + CF 25%	9.96 ± 0.06 de	20.37 ± 0.27 def	14.13 ± 0.64 de	47.80 ± 0.14 bcd
SLF2 100%	9.28 ± 0.20 fg	19.62 ± 0.10 ef	12.92 ± 0.99 de	46.07 ± 0.12 ef
SLF3 25% + CF 75%	9.92 ± 0.10 de	22.62 ± 0.27 b	15.86 ± 0.23 bcd	49.57 ± 0.33 abcd
SLF3 50% + CF 50%	9.65 ± 0.09 ef	20.71 ± 0.51 de	12.85 ± 0.29 e	43.97 ± 0.17 g
SLF3 75% + CF 25%	8.99 ± 0.02 g	18.98 ± 0.10 f	10.27 ± 1.45 f	43.53 ± 0.50 g
SLF3 100%	7.67 ± 0.55 h	16.51 ± 0.45 g	5.47 ± 0.78 g	42.67 ± 0.48 gh
SLF4 25% + CF 75%	10.57 ± 0.38 ab	24.09 ± 0.05 a	18.37 ± 0.38 a	48.97 ± 0.95 ab
SLF4 50% + CF 50%	10.51 ± 0.34 abc	22.46 ± 0.41 bc	17.01 ± 0.56 abc	47.43 ± 0.42 cde
SLF4 75% + CF 25%	10.23 ± 0.29 bcd	21.07 ± 0.11 cd	16.74 ± 0.61 abc	47.90 ± 0.16 abcd
SLF4 100%	10.31 ± 0.35 bcd	21.11 ± 0.13 cd	15.37 ± 0.28 bcd	49.63 ± 0.40 abcd

Data show mean values ± standard deviation (SD) in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test).

Table 7. Effects of different ratio of SLFs and CF on the nutrients uptake (mg·pot^{-1}) of komatsuna (Soil Expt. 2).

Treatments	N	P	K	Ca	Mg	Na
Control	46.43 ± 1.65 i	42.62 ± 0.09 ij	44.92 ± 1.51 i	113.6 ± 8.27 g	8.90 ± 0.53 h	11.84 ± 3.02 f
CF	356.14 ± 14.44 a	219.57 ± 0.30 a	362.13 ± 1.95 a	464 ± 19.36 ab	37.22 ± 1.32 bc	127.08 ± 3.77 a
SLF1 25% + CF 75%	252.76 ± 14.30 cde	150.41 ± 0.19 cd	252.76 ± 0.80 cde	337.20 ± 10.46 de	21.60 ± 0.40 ef	64.22 ± 5.83 bcd
SLF1 50% + CF 50%	191.27 ± 4.28 fg	112.23 ± 0.38 fg	191.27 ± 0.34fg	243.56 ± 17.94 f	17.61 ± 0.92 fg	51.89 ± 3.92 d
SLF1 75% + CF 25%	161.06 ± 7.23 gh	57.46 ± 0.01 hi	161.06 ± 0.69 gh	107.96 ± 14.62 gh	10.26 ± 0.46 h	50.98 ± 3.33 d
SLF1 100%	59.35 ± 15.32 i	20.36 ± 0.01 j	59.35 ± 0.11 i	32.53 ± 8.49 h	3.15 ± 0.55 i	18.14 ± 4.49 f
SLF2 25% + CF 75%	361.73 ± 13.81 a	199.49 ± 0.39 ab	361.73 ± 2.36 a	474.55 ± 48.69 ab	39.61 ± 2.01 ab	84.35 ± 10.60 b
SLF2 50% + CF 50%	279.46 ± 12.46	146.07 ± 0.19 cde	279.46 ± 0.82 cd	417.83 ± 21.53 bc	32.80 ± 0.23 c	76.76 ± 5.80 bc
SLF2 75% + CF 25%	246.10 ± 9.79de	131.34 ± 0.18 def	246.10 ± 0.86 de	370.93 ± 6.53 cde	27.82 ± 0.47 d	69.07 ± 3.08 bcd
SLF2 100%	238.90 ± 17.16 def	144.45 ± 0.16 ef	238.90 ± 1.11 def	327.83 ± 2.44 e	24.93 ± 2.99 de	67.95 ± 4.83 bcd
SLF3 25% + CF 75%	274.42 ± 3.81 cd	154.57 ± 0.20 cd	274.42 ± 0.69 cd	368.07 ± 6.20 cde	27.72 ± 0.40 d	54.54 ± 3.97 cd
SLF3 50% + CF 50%	245.66 ± 6.47 de	150.01 ± 0.48 cd	245.66 ± 2.15 de	327.22 ± 11.67 e	25.66 ± 0.75 de	52.02 ± 2.33 d
SLF3 75% + CF 25%	221.03 ± 30.56 ef	107.62 ± 0.00 fg	221.03 ± 1.07 ef	242.58 ± 25.31 f	16.38 ± 0.81 g	46.49 ± 6.49 de
SLF3 100%	135.26 ± 19.35 h	79.20 ± 0.08 gh	135.26 ± 0.30 h	114.32 ± 19.81 g	10.73 ± 2.71 h	25.57 ± 5.73 ef
SLF4 25% + CF 75%	365.49 ± 11.49 a	199.88 ± 0.01 ab	365.49 ± 1.92 a	497.41 ± 36.57 a	44.00 ± 0.70 a	76.27 ± 2.23 bc
SLF4 50% + CF 50%	339.82 ± 10.34 ab	171.21 ± 2.33 bc	339.82 ± 1.61 ab	472.75 ± 25.21 ab	43.64 ± 0.82 a	60.47 ± 4.96 cd
SLF4 75% + CF 25%	300.45 ± 10.41 bc	147.79 ± 2.06 cd	300.45 ± 1.35 bc	460.11 ± 5.93 ab	25.45 ± 0.36 de	58.16 ± 18.14 cd
SLF4 100%	269.00 ± 0.47 cde	126.02 ± 1.41 def	269.00 ± 0.95 cde	414.82 ± 25.88 bcd	24.56 ± 0.45 de	55.27 ± 2.35 cd

Data show mean value ± SD (n = 3). The same letters not significantly different at $P < 0.05$ (Tukey's test) in each column.

4. Discussion

In our study, treatment with SLFs resulted in significant increases in yield. In Experiment 1, the growth characteristics of plants treated with SLFs or CF differed significantly from those of the control plants. Moreover, SLF2 and SLF4, which were produced from aerobic fermentation, caused significantly different increases in plant growth compared with SLF1 and SLF3, which were produced from anaerobic fermentation, but their effects did not differ significantly from those of CF treatment. In the aerobic fermentation process, sufficient oxygen is present and many low molecular weight organic substances are decomposed into carbon dioxide and inorganic matter. Therefore, SLF2 and SLF4 were easily decomposed into an available form in the soil and thus easily absorbed by the plants. Kannan *et al.* [25] observed that soil application of SLF produced from *Enteromorpha clathrata* and *Hypnea musciformis* improved the growth characteristics of green gram, black gram, and rice.

Application of SLFs and CF produced SPAD values that differed significantly from those of the control $(NPK)_0$. Fan *et al.* [26] reported that chlorophyll content increased in a wide variety of crops treated with seaweed extract, including grapevine and strawberry. This increase in chlorophyll content at lower SLF concentrations may be due to the presence of high amounts of Mg [27]. Our study shows similar results in that the SPAD values and chlorophyll contents of komatsuna plants treated with CF or any of the nori SLFs differed significantly from those of the control plants.

In Experiment 1, treatment with SLFs and CF resulted in significantly different N, P, K, Ca, Mg, and Na uptake from that of the control treatment. Plants treated with SLF2, SLF3, or SLF4 exhibited increased K uptake compared with SLF1- and CF-treated plants and control plants. Kingman and Moore [28] reported that seaweed manure is rich in K, but poorer in N and P than farm manure. As SLF is a very good source of K, it helps regulate the water status of plants by controlling the opening and closing of the stomata, thereby affecting photosynthesis to a large extent [29]. The addition of the foliar applications of the SLFs produced no significant effects on plant growth characteristics, SPAD values, or nutrient uptake of komatsuna over the basal application treatments alone. In Experiment 1, application of CF, SLF2, SLF3, or SLF4 increased N, P, K, Ca, Mg, and Na uptake compared with SLF1 and the control treatment. This result may be due to the presence of micro- and macronutrients such as N, P, K, Ca, Mg, and Na in the SLFs (**Table 2**).

Experiment 2 showed that higher concentrations of SLFs resulted in decreased plant growth, shoot DW, and nutrient uptake. Plant growth and shoot DW were markedly depressed by increased concentrations of SLF1 and SLF3, but increased concentrations of SLF2 and SLF4 had no detrimental effects. The plants treated with 25% SLF + 75% CF exhibited better plant growth, shoot DW, and nutrient uptake than the plants subjected to higher concentration treatments (50% SLF + 50% CF, 75% SLF + 25% CF, and 100% SLF). Similar results were obtained in okra by Sasikumar *et al.* [30], who showed that the highest shoot length, root length, fresh shoot weight, dry root weight, lateral root number, leaf area index, leaf number, and flower number resulted from treatment with a 25% concentration of *Dictyota dichotoma*.

Experiment 2 also showed that plants treated with 100% CF, 25% SLF2 + 75% CF, or 25% SLF4 + 75% CF exhibited highly significantly differences in N, P, K, and Ca uptake relative to plants treated with SLF2, SLF4, SLF1, or SLF3 and the control plants. Mg uptake was significantly greater in plants treated with 25% SLF2 + 75% CF or 25% SLF4 + 75% CF, and Na uptake was significantly higher with 100% CF treatment than with the other treatments. These results confirm the findings previously reported by [31], who noted increased uptake of Mg, K, and Ca in lettuce treated with seaweed concentrate. Mancuso *et al.* [date] also observed increased N, P, K, and Mg uptake in grapevine and cucumber with the application of seaweed extract.

Linear regression analysis showed that the plants treated with CF or the SLFs exhibited decreased shoot DW, whereas the nutrient (N, P, K, Na, N/P, Mg/K) content of the plants increased. However, the shoot DW increased in plants treated with CF or the SLFs when the nutrient content of the plants decreased. These results suggest that treatment with higher concentration of nutrients depressed the shoot DW of komatsuna. Based on linear regression analysis, the plants treated with the higher concentrations of CF and SLFs exhibited decreased shoot DW (**Figure 5**). Plants treated with a low concentration (25%) of *Dictyota dichotoma* exhibited enhanced growth and yield compared to those treated with a higher concentration (100%) [32]. Treatment with 25% *Dictyota dichotoma* increased the yield parameters, maximum fruit number, fruit length, fruit dry weight, and yield of okra. In this study, linear regression analysis showed strong relationships between shoot DW and N (0.67), P (0.52), K (0.71), Na (0.48), N/P (0.35), and Mg/K (0.39) (**Figure 5** and **Table 8**); however, there was no relationship between shoot DW and Ca (0.07), Mg (0.05), or Ca/Mg (0.13) (**Table 8**).

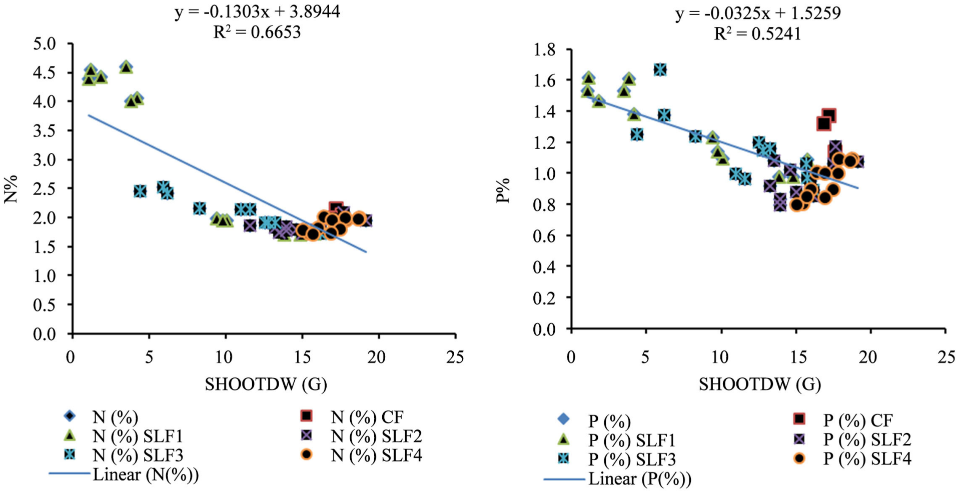

Figure 5. Linear regression between shoot DW (g) and N and P content affected by CF, SLF1, SLF2, SLF3 and SLF4 (Soil Expt. 2).

Table 8. Regression analysis between DM and Nutrients content (%) in Soil Expt. 2.

Nutrients (%)	Regression analysis between DM and Nutrients content (%)
N	$y = -0.1303x + 3.8944$
	$R^2 = 0.6653$
P	$y = -0.0325x + 1.5259$
	$R^2 = 0.5241$
N/P	$y = -0.0448x + 2.558$
	$R^2 = 0.3544$
K	$y = -0.1576x + 5.1263$
	$R^2 = 0.7055$
Ca	$y = 0.0132x + 2.3878$
	$R^2 = 0.0747$
Mg	$y = -0.0019x + 0.2244$
	$R^2 = 0.0467$
Na	$y = -0.0415x + 1.0671$
	$R^2 = 0.4762$
Ca/Mg	$y = 0.1836x + 10.882$
	$R^2 = 0.1279$
Mg/K	$y = 0.0024x + 0.0379$
	$R^2 = 0.385$

Total samples numbers were n = 54.

In Experiment 1 of this study, both basal and basal-plus-foliar applications of SLF1 resulted in the highest I contents, 38.30 and 39.30 $\mu g \cdot g^{-1}$, respectively. In Experiment 2, the plants treated with 75% SLF1 + CF 25% and 100% SLF1 had the highest I contents, 118.18 and 120.83 $\mu g \cdot g^{-1}$, respectively. These results reflect that nori is rich in protein, I, dietary fiber, and several vitamins, including folic acid and vitamins B12 and K (Japan Food Standard Ingredient Table 2015). The I content surveyed for nori was 29.3 - 45.8 $mg \cdot kg^{-1}$ [33].

The plants treated with basal and basal-plus-foliar applications of CF had significantly higher nitrate N (1.3 and 1.1 $mg \cdot g^{-1}$) content than plants treated with basal and basal-plus-foliar applications of the SLFs. In Experiment 2, the plants treated with 100% CF, 100% SLF1, or 75% SLF4 + 25% CF had higher nitrate N contents of 0.42, 0.38, and 0.37 $mg \cdot g^{-1}$, respectively. In our study, the nitrate contents of plants subjected to all of the SLF and CF treatments in Experiments 1 and 2 were lower than the level considered harmful for human health. Markiewicz *et al.* [34] found that the maximum limits for NO_3-N and NO_2-N content in fresh vegetables that were safe for the human consumption were 167 and 0.67 $mg \cdot kg^{-1}$, respectively.

5. Conclusion

Our results show that fermented nori SLFs enhanced plant growth, SPAD values, and nutrient uptake of komatsuna compared with controls. Treatment of komatsuna with 25% SLF + 75% CF produced the best plant growth, shoot DW, nutrient uptake, highest I content, and lowest NO_3-N content relative to control plants. Although basal application of SLFs was effective on komatsuna, foliar application was not effective, except for its effects on I content. The I content of komatsuna was increased by SLF treatment. Taken together, we conclude that basal application of fermented SLFs can enhance plant growth, nutrient uptake, and I content while decreasing NO_3-N content in komatsuna. SLFs could be used as an organic fertilizer to reduce chemical fertilizer usage, leading to more sustainable agriculture.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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