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Enhancing Germination and Early Growth of Curly Lettuce Using Fermented Liquid Extract of *Padina australis* Hauck

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Abstract

Fermented seaweed liquid extract serves as an affordable and eco-friendly nutrient supplement, biostimulant, or biofertilizer, effectively promoting crop growth and supporting sustainable agricultural practices. This study evaluates the effects of the fermented liquid extract of *Padina australis* (FLEP) at various concentrations (0, 2, 5, 10, 20, and 100%) on lettuce germination and early growth. Germination parameters were assessed over 14 days under controlled conditions, followed by consecutive greenhouse experiments that examined the impact of foliar FLEP spray on two-week-old seedlings over 21 days, measuring early growth parameters and foliar nutrient concentrations. All data were statistically analyzed using a one-way analysis of variance at a 5% significance level. Results revealed that the FLEP significantly improved the seedling vigor index and length at concentrations ranging from 2 to 20%. The relative growth rate (RGR) for height exhibited significant increases at the 2% and 5% FLEP concentrations, while RGR for leaves, shoot dry biomass, and leaf area demonstrated significant improvements at FLEP concentrations of 2 to 20%. Foliar P content, and not foliar N, was significantly affected by the FLEP treatments, with P levels typically increasing with higher FLEP concentrations. These findings suggest that applying FLEP, particularly at low concentrations (2% and 5%) as a foliar spray significantly enhances lettuce germination and growth. Furthermore, this study highlights the potential of the FLEP as a novel foliar biofertilizer.

Keywords: foliar biofertilizer; macroalgae; *Padina australis*; relative growth rate; seedling vigor index

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INTRODUCTION

Global food security is one of the most pressing challenges facing the agricultural sector, leading to the widespread use of chemical fertilizers, which significantly contributed to about 50% increase in crop yield (Ramya et al., 2015; Chang et al., 2021; Ammar et al., 2022; Begho et al., 2022). Most South Asian countries, including Brunei, largely rely on chemical fertilizers to overcome unfavorable soil conditions and enhance soil fertility (Bijay-Singh et al., 2022; DoAA, 2022). It has been estimated that South Asian nations currently use about 121 kg of chemical fertilizers per hectare, a figure projected to rise to approximately 268 kg ha^{-1} by 2050, thus exacerbating N loss and decreasing N use efficiency (Raghuram et al., 2021; Begho

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et al., 2022; Bijay-Singh et al., 2022). However, the extensive use of chemical fertilizers negatively impacts ecosystem health and poses risks to humans, highlighting their precarious nature and underscoring the need for eco-friendly, bio-based fertilizers like seaweed extracts (Thirumaran et al., 2009; Ramya et al., 2015; Pg Abdul Karim et al., 2021; Dhankar and Kumar, 2023).

Seaweeds are multicellular, macroscopic marine algae found in the intertidal zone of coastlines, featuring multifaceted applications in modern agriculture (Nedumaran and Arulbalachandran, 2015; Pangaribuan et al., 2022). They are generally classified based on their pigments into three groups: Ochrophyta, Phaeophyta, and Rhodophyta. Among these, extracts from Phaeophyta, the brown seaweed group that comprises species such as *Ascophyllum nodosum*, *Ecklonia maxima*, *Macrocystis pyrifera*, and *Sargassum* spp. are widely used in agricultural applications because of their vast spectrum of beneficial compounds and plant growth promoters, including auxins, gibberellins, carotenoids and polysaccharides (Zheng et al., 2016; Arokia rajan et al., 2020; Nasmia et al., 2021; Pangaribuan et al., 2022). Additionally, seaweeds are rich in macromolecules such as proteins, sugars, lipids, polyphenols, fatty acids, and fats; and essential minerals including Fe, Mn, Ca, Cl, Ba, Sr, Al, I, Cu, B, and K. These unique properties make seaweed extracts ideal candidates for promoting agricultural sustainability and serve as eco-friendly alternatives.

Researchers worldwide have documented the exceptional qualities of seaweed extracts in enhancing crop productivity, including increased root growth and development, enhanced nutrient uptake, improved disease resistance, and increased plant tolerance to extreme conditions (Calvo et al., 2014; Di Filippo-Herrera et al., 2019; Waqas et al., 2019; Hines et al., 2021). Seaweed liquid extracts are typically applied as foliar sprays, which facilitate nutrient uptake, induce early flowering, increase crop yield, and confer disease protection (Ali et al., 2016). Additionally, seaweed liquid extracts have been shown to improve seed vigor and health while promoting rapid seed germination, leading to healthy mature plants (Jebasingh et al., 2014; Arokia rajan et al., 2020).

This study aims to preliminarily investigate the potentiality of the brown seaweed species, *Padina australis* Hauck, belonging to the family Dictyotaceae, as a foliar biofertilizer. Selected physicochemical properties of *P. australis* biomass are evaluated before exploring its efficacy in producing a fermented liquid extract fertilizer that enhances seed germination and plant growth. While *P. australis* is recognized for its numerous medicinal and health benefits (Rushdi et al., 2021), its agricultural potential remains largely unexplored. Notably, one of the few studies on *Padina* spp. was conducted by Arokia rajan et al. (2020), who demonstrated that liquid extracts derived from its sister genus, *P. gymnospora*, improved the germination and growth-promoting properties of *Capsicum annuum*. *Padina australis* thrives in the sublittoral regions of tropical and subtropical waters worldwide (Chellapan et al., 2020), making it a readily available resource for agricultural applications. This study contributes to the limited research on foliar biofertilizers that use fresh seaweeds to produce fermented liquid seaweed extract.

To evaluate the potential effects of different concentrations of fermented *P. australis* liquid fertilizer on plants, a fast-growing leafy vegetable, *Lactuca sativa* L. (Asteraceae) or curly lettuce, was chosen as the test species for this study. With a relatively short life cycle of 55 to 70 days, it is a suitable test species for experimentation (Tudela et al., 2017). This versatile and widely cultivated plant is primarily valued for its nutritious leaves, pleasant texture, and taste. Rich in water content (~94.5%) and low in calories, it serves as an excellent source of vitamins, minerals, and bioactive compounds that offer numerous health benefits (Yang et al., 2022). Furthermore, the lettuce market was valued at 3.8 billion USD in 2023 (Precision Business Insights, 2025), highlighting its global economic importance. The use of chemical fertilizers has shown a tremendous increase in lettuce growth, but their impact on the environment, human health, and soil health has raised concerns over their future use in agriculture practices (Hoa et al., 2022). Therefore, this study evaluates the growth-promoting ability of fermented liquid extract of *P. australis* on the germination and early growth of curly lettuce.

MATERIALS AND METHOD

The brown *P. australis* Hauck (Dictyotaceae), found in the intertidal zone of the open ocean in Brunei Darussalam, was identified through macroscopic observations of its morphology and other details, as confirmed by a seaweed expert

from Universiti Brunei Darussalam. It was hand-collected from the rocky shores at Tanjong Batu (115°03'25.2" to 115°03'28.8" E, 5°02'44.4" to 5°02'45.6" N) during low tide. The specimens were thoroughly rinsed with seawater and promptly transported to the laboratory in a plastic bag. In the laboratory, the samples were rinsed multiple times with tap water and subsequently with distilled water to remove any small invertebrates, shells, sand, and debris. The samples were processed for liquid extract preparation on the same day of collection.

Determination of selected physicochemical properties of *P. australis* biomass

Fresh *P. australis* biomass or thallus was analyzed for pH, moisture, organic matter, and ash content following AOAC (2000) and Pérez-Harguindeguy et al. (2016). Distilled water (40 ml) was added to ground fresh thalli of *P. australis* (5 g) at the ratio of 8:1 (v/w), which was mixed using a shaker for 5 minutes. The sample was centrifuged, and the pH of the supernatant was measured using a pH bench meter (Orion Star TM A211, Thermo Scientific, USA). Moisture content based on fresh weight was determined by the oven drying method at 105 °C until constant weight was obtained. Ash content was evaluated by incinerating oven-dried *P. australis* (105 °C) in a muffle furnace at 550 °C for 18 hours until it burned to ash. The ash and organic matter contents were determined using Equation 1 and 2, respectively.

Total P and N contents of *P. australis* were measured using the method described by Allen et al. (1989). Approximately, 0.2 g of oven-dried (60 °C for 2 days) and ground *P. australis* samples were digested with 5 ml of concentrated H_2SO_4 and Cu-Kjeldahl tablets in a preheated digestive block (Block Digestor BD-46, LACHAT Instruments, USA) at 360 °C for 90 minutes. After cooling, the filtrates were transferred to a 50 ml volumetric flask and diluted with distilled water. The filtrates were analyzed for the total N and P contents using a Flow Injector Analyzer (FIAstar 5000, Hoganas, Sweden). All analyses were done in triplicates.

Preparation of fermented liquid extract of *P. australis* (FLEP)

P. australis liquid extract was prepared following the protocol described by Pise and Sabale (2010) with some modifications. About 1 kg of fresh thalli of *P. australis* was cut into smaller pieces and blended with 3 l of distilled water, attaining a ratio of 1:3 (w/v). The blended mixture was kept in a 5 l conical flask at room temperature (22 °C) and the mouth of the flask was covered with an aluminum foil. After 2 days, the mixture was filtered using a muslin cloth to obtain a concentrated extract, referred to as a crude or 100% FLEP. Different working concentrations were prepared from this crude extract by diluting it with distilled water. This study examined five different concentrations of the extract treatments: 0 (control), 2, 5, 10, 20, and 100%. The 100% FLEP treatment was included to evaluate the effects of crude extract on *L. sativa*. The FLEP solutions were stored at 4 °C until further use.

Seed germination bioassay

The seed germination bioassay methodology adopted by Zheng et al. (2016) was followed in the study to examine and ascertain the seed germination abilities of different concentrations of FLEP on *L. sativa*. The curly green *L. sativa* (lettuce hereafter) (Echo) seeds used in the study were sourced from a local agricultural company (Rimba Garden Central) in Brunei to ensure their relevance and adaptability. The lettuce seeds were first surface sterilized with 4.0% sodium hypochlorite solution for 15 minutes at room temperature (22 °C). Subsequently, the seeds were rinsed thrice with distilled water.

The seed viability test was conducted by immersing them in a beaker of water for 15 minutes and only viable seeds that sank to the bottom of the beaker were used. Each FLEP treatment was investigated with 3 replicates containing 20 seeds each. The seeds were placed on sterilized petri dishes, which were double layered with sterile Whatman filter papers No. 1 (9.0 cm in diameter) that was moistened with 5 ml of each FLEP concentration, i.e., 0, 2, 5, 10, 20, and 100%, separately. In total, this bioassay utilized 360 lettuce seeds. The petri dishes with seeds were incubated in a seed germination chamber set at 25±1 °C, 16-hours light/8-hours dark regime, and a light intensity of 37.7±5.8 μmol m^{-2} s^{-1}. Germination was observed and recorded daily for 14 days.

In the study, a seed was considered to germinate when its radicle length was longer than 2 mm (Hernández-Herrera et al., 2014). Germination parameters measured include final germination percentage (GP) (Equation 3), seedling length, germination index (GI) (Equation 4), and seedling vigor index (SVI) (Equation 5).

$$\text{Ash content (\%)} = \frac{\text{Mass of seaweed after furnace (g)}}{\text{Oven-dried weight (g)}} \text{ x } 100\% \quad (1)$$

$$\text{Organic matter content (\%)} = 100\% - \text{Ash content (\%)} \quad (2)$$

$$\text{GP} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \text{ x } 100\% \quad (3)$$

(Hernández-Herrera et al., 2014)

$$\text{GI} = \sum \frac{G_t}{T_t} \quad (4)$$

(AOSA, 1983)

$$\text{SVI} = \text{Seedling length (cm) x GP} \quad (5)$$

Where G_t is the number of seeds germinated on day t and T_t is the number of days (Orchard, 1977).

$$\text{RGR} = \frac{\ln G_2 - \ln G_1}{t_2 - t_1} \quad (6)$$

Where G_2 and G_1 are the final and initial shoot heights for RGR height (mm mm^{-1} day^{-1}) or final and initial leaf counts for RGR leaves, and $t_2 - t_1$ is the total number of days, i.e., 21 days.

The seedling length was measured from the plumule tip to the radicle tip using a vernier caliper.

Seedling growth bioassay

The growth assay was conducted in a greenhouse located at Universiti Brunei Darussalam, Brunei-Muara for 21 days. The average daily temperature, relative humidity, and maximum photosynthetically active radiation (PAR, Quantum MQ-200 PAR Meter, Apogee Instruments, UK) in the plant shade were measured at noon and were observed to be 29±3 °C (n = 3), ~80% and ~300 µmol m^{-2} s^{-1}, respectively. New sets of lettuce seeds were initially grown in polytrays filled with autoclaved commercial sandy potting soil (BVB Substrates, De Lier, the Netherlands) purchased from Asia Enterprise Trading Company, Tanjung Bunut, Brunei with physicochemical properties as presented in Table 1.

Once the seedlings in the polytrays developed 2 to 3 true leaves after 14 days, they were transplanted into plastic pots (18 cm diameter x 20 cm height) filled with 400 g of sandy potting soil (Table 1). The seedlings were allowed to acclimatize for a day before being exposed to the different FLEP concentrations. The experiment was conducted using a randomized complete block design with three blocks, with each block or replicate comprising six FLEP concentrations, namely 0 (control), 2, 5, 10, 20, and 100%. Each FLEP concentration was represented by six pots, each containing one seedling, resulting in a total of 108 seedlings.

After acclimatization, 3 ml of FLEP was sprayed without any surfactant onto the leaves in the early morning daily for 7 days, followed by applications every 5 days until day 21, which was the final harvest stage. The pot was also irrigated

Table 1. Selected chemical properties of sandy potting soil (BVB substrates) used in the seedling growth bioassay

Properties	Potting soil
Texture	Sandy
Clay (%)	2.4±1
Silt (%)	14.0±4
Sand (%)	83.6±4
pH	4.75±0.02
Moisture (%)	35.8±0.33
Organic matter (%)	91.7±0.17
Total N (mg g^{-1})	47.9±1.70
Total P (mg g^{-1})	11.4±0.17

Note: Values mean±standard error, SE (n = 3)

with 50 ml of distilled water once a day. Shoot height, dry biomass, leaf count, and leaf area were measured periodically. The initial and final estimates of shoot height, which were measured from the top of the newly formed leaf to the base of the shoot where the first root hairs appear, and leaf count were taken on day 1 (the first day of FLEP application) and day 21, respectively. The relative growth rate (RGR) was calculated based on the shoot height (RGR height) and leaf count (RGR leaves) using the formula given by Hunt et al. (2002) shown in Equation 6.

Shoot dry biomass was obtained after oven-drying the shoots for 2 days at 60 °C. The leaf area of three randomly selected leaves from each seedling was measured on day 21 using Leafbyte 1.3.0 to calculate the mean leaf area for each treatment (Getman-Pickering et al., 2020). Three leaves of three randomly selected seedlings in each treatment and experimental block were used for total N and P concentrations. Approximately 0.2 g of oven-dried (60 °C for 2 days) and ground leaf samples were acid-digested. The total N and P analysis methods in leaves were similar to the one described for seaweed samples above.

Statistical analysis

All statistical analyses were performed using R 4.2.3 software (R Core Team, 2023). The data were checked for normality of residuals and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. A one-way analysis of variance (ANOVA) was used to assess all germination and growth parameters as well as total N and P contents in lettuce leaves. Any significant differences at a 5% significance level for each parameter were further analyzed using the Tukey HSD to determine the effects of FLEP concentrations on the seed germination and plant growth parameters.

RESULTS AND DISCUSSION

Table 2 describes the properties of the wild brown seaweed *P. australis* and the study emphasizes the utilization and exploitation of these nutrients available in *P. australis* for agriculture application as fertilizers, as it is the most abundantly and easily available seaweed in the coastal regions of Brunei.

Table 2. Selected physicochemical properties of the thalli of *P. australis* Hauck

Properties	*P. australis*
pH	7.56±0.11
Moisture (%)	84.74±0.35
Organic matter (%)	58.70±6.57
Total N (mg g^{-1})	10.60±0.31
Total P (mg g^{-1})	0.77±0.05

Note: Values mean±standard error, SE (n = 3)

Several studies worldwide have reported the abundant macronutrients and minerals in marine seaweed compared to those found in terrestrial plants (Calvo et al., 2014; Nasmia et al., 2021). Likewise, *P. australis* available in the Brunei waters was rich in beneficial minerals (N and P). Both seaweed and seaweed liquid extract significantly promote seed germination and crop growth and yield, thereby enhancing agricultural economic viability and ensuring food security (Khan et al., 2009; Hernández-Herrera et al., 2014; Arokia rajan et al., 2020). Hernández-Herrera et al. (2014) and Khan et al. (2009) have stated that P in seaweed extracts facilitates root proliferation, thus increasing the root/shoot ratio, while N in seaweed promotes plant growth, yield, and overall plant vigor.

However, it is important to acknowledge that the mineral contents of seaweed species are greatly influenced by several factors such as growth season, environmental conditions, geographical locations, physiological variations, and the analytical methods used in assessing and processing seaweeds (Xu et al., 2023). Additionally, certain minerals in seaweed species might be associated with polysaccharides, which could limit the absorption of these minerals. For example, monovalent cations (e.g., Na) and divalent cations (e.g., Ca and Mg) have a strong affinity for carboxylic polysaccharides (e.g., alginates in brown seaweed and carrageenan in red seaweed), potentially restricting the availability of associated minerals in seaweeds (Xu et al., 2023). Therefore, it is apparent that both intrinsic (or inherent) and extrinsic factors play a pivotal role in determining the macronutrient and mineral composition of seaweeds, which can vary with geographical locations and climatic conditions.

Table 3 describes that final GPs of > 90% were observed in all the concentrations of FLEP, with the control seeds (0% FLEP concentration) and seeds treated with 10% FLEP showing the highest final GP of about 97%. The GI of seeds ranged between 17.5 and 19.0, with the control and 100% FLEP treatments recording the highest GI (19.0) and the lowest GI (17.5), respectively. However, neither the GP nor GI of lettuce seeds was significantly affected by FLEP concentrations (p > 0.05).

In contrast, both the SVI and seedling length of lettuce seeds were significantly affected by the foliar application of FLEP concentrations (Table 3, $p < 0.001$). Apart from the 100% FLEP treatment, the SVI values of lettuce seeds (2 to 20% FLEP) showed a significant increase ranging from 1.8- to 2.3-fold compared to the control seeds. The most and least vigorous seeds were observed at 20% FLEP treatment (SVI of 1229) and 100% FLEP treatment (SVI of 822), respectively. Notably, even at 100% FLEP concentration, the seed vigor remained significantly higher (SVI of 822) than that was observed with the control seeds (SVI of 528). Moreover, seeds showing greater SVI were associated with longer seedling lengths (Table 3). Additionally, all FLEP treatments significantly stimulated the mean seedling length compared to the control seedlings ($p < 0.001$). The increase in seedling length ranged from 1.6- to 2.5-fold for the different FLEP concentrations used. Notably, the 20% FLEP treatment resulted in the longest mean seedling length of 13.7 cm, whereas the shortest mean seedling length of 8.81 cm was recorded in the 100% FLEP treatment ($p < 0.001$). Nevertheless, 100% FLEP treatment still demonstrated a significant promotion of seedling growth compared to the control seedlings.

Figure 1 represents the role of different concentrations of FLEP used in the study on the early growth of lettuce seedlings. The FLEP treatments significantly influenced the growth traits of lettuce seedlings ($p < 0.01$). RGR based on shoot height (RGR height) was relatively greater, i.e., 2.4- and 2.3-fold increase, respectively, was observed in the 2% and 5% FLEP concentrations compared to the control seedlings ($p < 0.001$, Figure 1a). RGR based on leaf count (RGR leaves), shoot dry biomass, and leaf area were also found to have remarkable improvement, i.e., ranging from 3.3- to 3.7-fold ($p < 0.01$, Figure 1b), 6.0- to 8.3-fold ($p < 0.001$, Figure 1c) and 4.7- to 6.3-fold ($p < 0.001$, Figure 1d), respectively in the 2 to 20% FLEP concentrations compared to the control seedlings. From this, it is inferred that the FLEP concentrations of 2 to 20% exhibited better growth performance compared to the 100% FLEP treatment, confirming the growth-promoting abilities of lower concentrations of FLEP obtained from *P. australis* on lettuce seedlings ($p < 0.01$, Figure 1a-d). However, FLEP treatments did not contribute to the total foliar N and P in the 21-day-old lettuce seedlings (Table 4). From Table 4, it is conspicuous that the effect of FLEP was found to be statistically significant on the mean total P concentration ($p < 0.01$) but not the total N concentration in the lettuce leaves ($p > 0.05$, Table 4). The control treatment significantly exhibited the highest total P concentration compared to most FLEP treatments, except for the 100% FLEP treatment.

Assessment of the growth improvement properties of the FLEP prepared in the study has inferred that the FLEP concentrations of 2 to 20% were found to improve the SVI and seedling length significantly without affecting the final seed GP and GI of the lettuce seeds. This could be due to the physicochemical properties, such as pH and electrical conductivity (EC), of FLEP, as many plants are sensitive to changes in growth medium during germination and seedling growth phases (Hernández-Herrera et al., 2014). Arthur et al. (2013) and Arokia rajan et al. (2020) noted the enhanced benefits of 20% liquid extracts of *E. maxima* at an almost neutral pH (~pH 6.5). However, the crude or 100% FLEP in this study had a pH of 7.81 and an EC of 24.4 mS cm^{-1}, indicating high pH but low salt concentrations that may affect seed germination. Generally, excessive

Table 3. Mean final GP, GI, SVI, and seedling length (cm) of *L. sativa* seeds in response to different concentrations of FLEP

Treatment (%)	Final GP (%)	GI	SVI	Seedling length
Control (0)	96.7±1.7^{a}	19.0±0.8^{a}	528±44.3^{c}	5.5±0.4^{d}
2	95.0±2.9^{a}	18.7±0.4^{a}	1,037±82.6^{ab}	10.9±0.6^{b}
5	93.3±4.4^{a}	18.1±0.8^{a}	968±42.1^{ab}	10.4±0.04^{bc}
10	96.7±3.3^{a}	18.2±0.9^{a}	1,093±70.3^{ab}	11.3±0.4^{b}
20	90.0±5.0^{a}	17.8±0.9^{a}	1,229±106.7^{a}	13.7±0.6^{a}
100	93.3±4.4^{a}	17.5±1.8^{a}	822±51.2^{bc}	8.8±0.2^{c}
F-value	0.445^{ns}	0.486^{ns}	12.3***	47.5***

Note: GP = Germination percentage, GI = Germination index, SVI = Seedling vigor index. Values mean±standard error, SE ($n = 3$). *F*-value is obtained from one-way ANOVA. *, ** or *** denotes significant difference at $p < 0.05$, $p < 0.01$ or $p < 0.001$, respectively. Different letters within a column indicate significant differences in mean values after the Tukey HSD test at a 5% significance level

Table 4. Mean total foliar N and P concentrations of 21-day-old *L. sativa* seedlings in response to different concentrations of FLEP

Treatment (%)	Foliar N (mg g^{-1})	Foliar P (mg g^{-1})
Control (0)	20.5±0.3^{a}	5.3±0.01^{a}
2	10.3±2.1^{a}	1.9±0.4^{c}
5	15.4±3.9^{a}	3.5±1.1^{bc}
10	9.6±2.0^{a}	2.2±0.4^{bc}
20	11.6±1.0^{a}	2.4±0.2^{bc}
100	15.5±5.5^{a}	4.8±0.5^{ab}
F-value	1.04^{ns}	5.79**

Note: Values mean±standard error, SE (n = 3). *F*-value is obtained from one-way ANOVA. *, ** or *** denotes significant difference at $p < 0.05$, $p < 0.01$ or $p < 0.001$, respectively. Different letters within a column indicate significant differences in mean values after the Tukey HSD test at a 5% significance level

salt accumulation can be toxic to the embryo and developing seedlings, leading to non-uniform germination and hindered seedling growth (Tarchoun et al., 2022). Nonetheless, maintaining a low salt concentration (i.e., low EC) in the growth medium promotes efficient seed imbibition, metabolic activities, and production of hydrolytic enzymes that are necessary for seed germination and subsequent seedling growth (Hernández-Herrera et al., 2014; Pompelli et al., 2023). However, high concentrations of FLEP, specifically the 100% FLEP treatment, were found to have detrimental effects on the seeds, such as browning of the radicles and inhibition of both radicle and shoot growth, possibly linked to the elevated pH observed in the growth medium. Similar observations have been reported by Hernández-Herrera et al. (2014).

The FLEP at lower concentrations enhanced the SVI and seedling growth, promoting the relative growth rate (RGR height and leaves), shoot dry biomass, and leaf area of lettuce. This growth promotion could be attributed to the presence of essential bioactive compounds and broad-spectrum growth-promoting substances in the FLEP. Similar evidence has been accorded by Khan et al. (2009) and Small and Degenhardt (2018) regarding the presence of various bioactive compounds, plant growth regulators (PGRs) (e.g., auxins, cytokinins, gibberellins, betaines), polysaccharides (e.g., fucoidan, agar, algin, laminarin), macro- and micronutrients, trace elements, vitamins, amino acids, lipids, natural pigments, and antioxidants in seaweeds that protect crops from drought, salinity, and diseases (Mannan et al., 2023). Benítez García et al. (2020) have identified the presence of 10 different PGRs or phytohormones in aqueous *Padina durvillaei* (brown seaweed) extract which includes abscisic acid (ABA), indoleacetic acids (IAA), cytokinins (transzeatin, isopentyladenine, dihydrozeatin), gibberellins (GA_1, GA_4), jasmonic acid (JA), and salicylic acid (SA) that are known to promote seed germination, plant growth, and protect the crops from unfavorable conditions. Unfortunately, none of these bioactive compounds and phytohormones were analyzed in *P. australis*. This study suggests that further work for elucidating the bioactive compounds and determining complex mechanisms of FLEP on lettuce growth and development is necessary.

Additionally, seaweed polysaccharides are well-recognized globally for their ability to improve plant growth (Blunden et al., 2010). Consequently, Monteiro et al. (2021) have worked on fermented seaweed that had a rich content of polysaccharides capable of promoting nutrient release and availability in the soil through effective decomposition of the biomass available. Karthik and Jayasri (2023) have stated that the utilization of 20% liquid extract obtained from *Turbinaria ornata* (a brown seaweed) as foliar spray enhanced the seed germination growth traits of mung beans with improved yield. Thirumaran et al. (2009), Kumar and Sahoo (2011), Arthur et al. (2013), Jebasingh et al. (2014), Zheng et al. (2016), Arokia rajan et al. (2020), Atteya et al. (2022), Huda et al. (2023) and Karthik and Jayasri (2023) have well documented the potentiality of seaweed liquid extract in promoting the seed germination, seedling vigor, disease protection, crop growth, and yield at lower concentrations as a foliar spray and other modes of applications. The primary benefit of using foliar spray methods with seaweed liquid extract is the prevention of nutrient leaching from the soil matrix, which simultaneously promotes crop growth (Waraich et al., 2015; Youssef et al., 2022).

Foliar P content, and not foliar N content, of lettuce seedlings was affected by FLEP treatments, with P levels typically increasing with higher FLEP concentrations. This may be

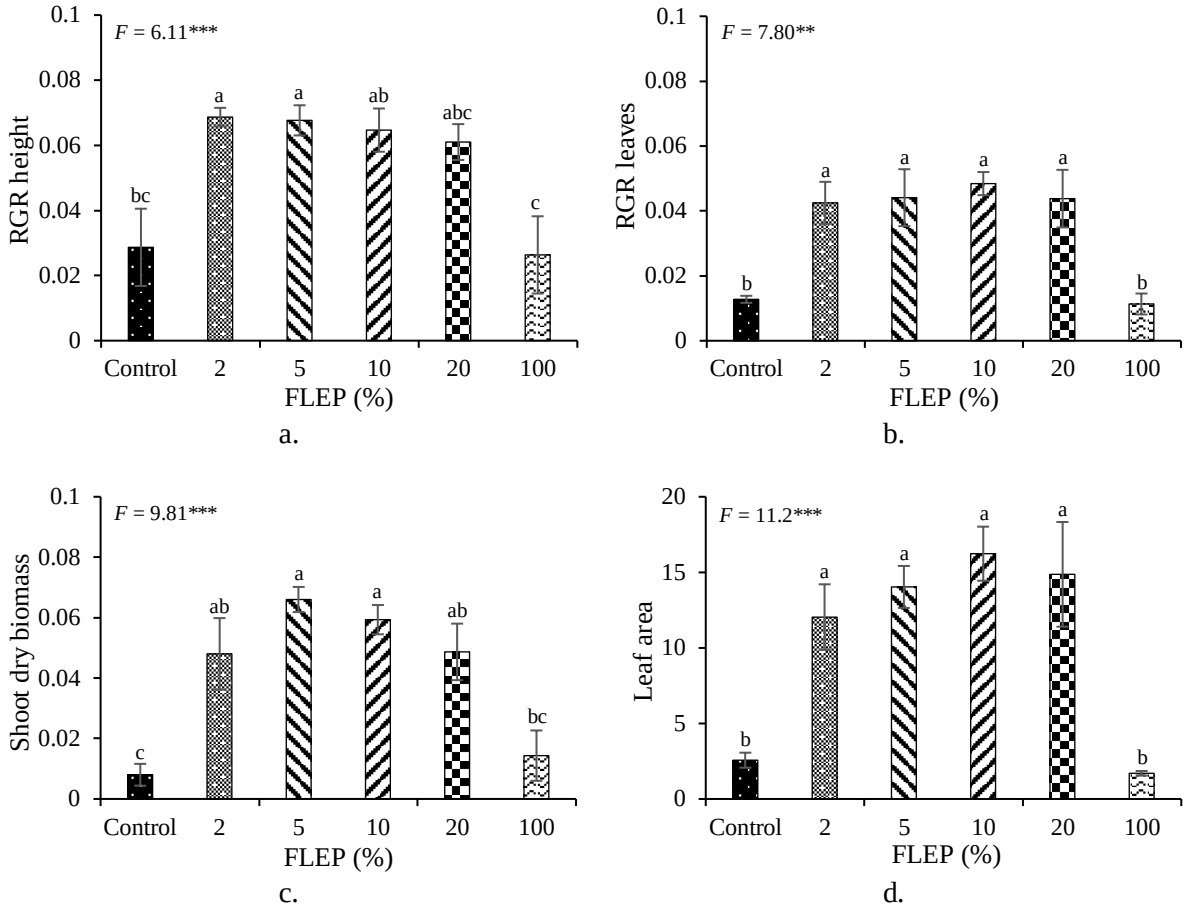

Figure 1. Effect of different concentrations of FLEP on the growth parameters of 21-day-old *L. sativa* seedlings: (a) Mean relative growth rate based on shoot height (RGR height; mm mm^{-1} day^{-1}), (b) Mean relative growth rate based on leaf count (RGR leaves; unit $unit^{-1}$ day^{-1}), (c) Mean shoot dry biomass (g), and (d) Mean leaf area (cm^2)

Note: Values mean±standard error, SE (n = 3). The F-values and P-values are obtained from one-way ANOVA at a 5% significance level. Different letters within a panel represent significant differences between FLEP concentrations as obtained from Tukey HSD test at a 5% significance level. *, ** or *** denotes significant difference at $p < 0.05$, $p < 0.01$ or $p < 0.001$, respectively

attributed to the ability of various seaweed extracts to influence the regulation of genes involved in nutrient uptake (Battacharyya et al., 2015). Furthermore, the control seedlings exhibited higher concentrations of total foliar N and P compared to seedlings treated with FLEP, which aligns with the observations made by Tougaard et al. (2023), which states that the high levels of remobilization of P observed in young and fully expanded leaves results in a decreased level of P concentrations to 25 to 33% within 7 days.

CONCLUSIONS

The current findings suggest that the FLEP effectively promotes seed germination and growth of lettuce at lower concentrations, specifically at 2% and 5%, thus corroborating the significant efficacy of this seaweed-derived fertilizer in enhancing crop and plant growth. Moreover, the presence of inorganic minerals in the FLEP makes it a suitable organic fertilizer for sustainable agriculture. This study demands the need for further research to identify bioactive compounds in FLEP and elucidate the complex mechanisms influencing lettuce germination and growth. Future research should also examine the long-term impacts of foliar applications of seaweed biofertilizers on crop yield and health. Harnessing the full potential of foliar biofertilizers could significantly advance sustainable agriculture practices worldwide.

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