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Efficiency of bio-foliar fertilizer extracted from seaweed and water hyacinth on lettuce *(Lactuca sativa)* vegetable in Central Vietnam

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Foliar fertilizer is important for lettuce vegetable development and yield. The present investigation studied different types and ratios of bio-foliar fertilizer to water seaweed extract and water hyacinth (0:1, 1:10, 1:20 and 1:30) in comparison to different two commercially available foliar fertilizers on growth, and quality attributes of lettuce. Four pot and field experiments were undertaken in spring seasons of 2020 and 2021 in randomized complete block design using three replications. The findings revealed that lettuce yield and quality were dependent on the type and spraying ratio of bio-foliar fertilizer from seaweed and water hyacinth. Improved yield and quality of lettuce was found when bio-foliar fertilizers from seaweed and water hyacinth were sprayed at ratio 1:10 (increasing compared to control in 68–81% with seaweed; 61–70% with water hyacinth in pot experiment and 19% with seaweed; 14–17% with water hyacinth in field experiment). Nitrate content was within standard range <1000 mg kg^{-1} and brix content from 2.20-2.65% as compared to commercial foliar fertilizer. A close relationship was found between biomass yield (R^2 = 0.46 to 0.66) and economic yield (R^2 = 0.50 to 0.64) with type and ratio of bio-fertilizer. In conclusion, a bio-foliar fertilizer extracts from seaweed at a spraying ratio of 1:10 can be used to achieve better yield and quality for lettuce crop.

Keywords: Aquatic plant, bio-foliar fertilizer, lettuce, quality, yield.

INTRODUCTION

The rapidly growing world population demand an increase in food production and product quality at the expense of decreasing land, increasing water scarcity and climate change phenomenon. Tam Giang - Cau Hai lagoon is located in Thua Thien Hue province, Central Vietnam which covers roughly 22,000 hectares has many areas of rivers, lakes, and ponds with many types of water bodies rich in many common aquatic weeds about a total of 16 species (Ancion *et al.*, 2009; Ton That Phap *et al.,* 2001; 2009). Aquatic weeds, including seaweed, are widely used to produce nutritious food for humans, raw materials and have recently been used for fertilizer production by leaf and root. Protein, amino acids, and essential plant growth stimulants can be found in aquatic weeds and their hydrolysate solutions (Blunden *et al.,* 1997; Rosalba *et al.,* 2014). The seaweed extract contains many biologically active compounds including phytohormones and mineral compounds. As a result, seaweed extract has been used as a growth stimulant in several studies (Malmoud *et al.,* 2019). Seaweed extract application has also been found to increase the vigor of the pea (Carvalho *et al.*, 2013). Aquatic plants have been used to assess diversity, use as animal feed and soil mulch. However, studies on aquatic weeds to provide nutrients for crops are not much studied. Chemical fertilizers, growth stimulants, and pesticides play a vital part in modern agricultural production, with the ability to prevent disease, boost crop productivity, and provide amazing cost efficiency. However, excessive synthetic fertilizers use, growth stimulants, and pesticides has resulted in unfavorable outcomes in agricultural production (Hoang *et al.*, 2018; Makawita *et al*., 2021). The initial survey results showed that people used a variety of growth stimulants for crops derived from pesticides. These pesticides are very cheap, highly toxic, kill insects, stimulate more growth and make vegetables greener (Nguyen *et al.*, 2018). And growers harvest vegetables before the time, to fetch high market price. Therefore, the use of biological fertilizers and natural nutrients, especially biological active substances, is increasingly in agricultural production in Vietnam. Developing foliar bio-fertilizers extracted from aquatic weeds can provide alternative natural source, will reduce the

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environmental impact of chemical fertilizers and pesticides application, increase productivity and quality of crops, for sustainable agricultural development. Therefore, this study determined the best ratio and type of bio-foliar fertilizer from seaweed extract and water hyacinth in improving growth, yield and quality for lettuce for two growing seasons.

MATERIALS AND METHODS

***Experimental sites and soil physio-chemical properties*:** The pot experiments were conducted under wire house condition at Faculty of Agronomy, University of Agriculture and Forestry, Hue University from February to April, 2020. The field experiments were carried out in Huong Long ward, Hue city, Thua Thien Hue province, Central Vietnam from April to May, 2020 and January to February, 2021. The soils used in these experiments were alluvial (Fluvisols). This region has a humid tropical climate with two seasons as dry from April to September and other rainy from October to March with an average rainfall of 2,300 mm year^{-1} concentrated over a short period from October to December and average temperature of 26 and 35ºC. Soil of these experiments were rather acidic (5.2 pH_{KCl}), low in total organic carbon of1.35%, total N, P and K of 0.06%, 0.05% and 0.25%.

***Bio-foliar fertilizer preparation*:** Bio-foliar fertilizer extraction**:** Aquatic plant samples including seaweed (*Vallisneria spiralis)* in lagoon and water hyacinth (*Eichhornia crassipes*) were collected from ponds, lakes in Thua Thien Hue province. They were then drained and taken to the laboratory and washed three or four times with clean water to eliminate all sand particles, especially fungal pathogens. A plastic container with capacity of 100 L consisted of the 50 kg of each aquatic plant type and added with 0.5 kg of *Trichoderma* sp., 2 L of molasses, after which stirred, covered tightly, and stored until sampling. Foliar fertilizer was extracted after composting for 2 months by squeezing water, then filtered through a small hole sieve to remove the residue and only the solution was taken and preserved it in airtight container until use. Quality analysis of extracts was performed after 60 days of incubation following standard methods and showed that soil contained pH= 6.87, EC=1.78 dS m^{-1}, OM=33.34%, N=0.45%; P =0.17% and K =1.34% contents only.

***Experimental layout*:** Two experiments with soil filled pots were conducted on lettuce (*Lactuca sativa*) cv. Mo, a cash crop usually grown by small landholders, during the spring season (February to May, 2020) with five application extraction ratio (bio-foliar fertilizer: water) (0:1, 1:10, 1:20 and 1:30). The experiments were conducted in a randomized completed block design in three replications. The pot size was 15 x 15 cm. The air-dried, sieved and treated soil collected from a 0–20 cm depth was analyzed for chemical characteristics. Each experimental pot contained 2 kg of soil, treated with lime (0.3 g of lime pot^{-1}) and mixed with cattle manure (8.3 g pot^{-1}) containing C: 35%, total N, P and K of 1.1%, 0.4% and 0.6% respectively before t sowing. Each pot was grown with one plant with 2-3 true leaves (20 days old). Spraying time was from 5-6 pm. Foliar fertilizer was mixed with water according to the above ratio and sprayed directly into plants with the amount of 5 ml plant^{-1} (plants from 3 to 10 days old); 10 ml plant^{-1} (plants from 11-15 days old); 15 ml plant^{-1} (plants from 16 - 20 days old). Plants were sprayed at 3-day interval and ended before harvest at least 5 days.

Based on the pot experiments, two best ratios of treatments (one from seaweed and one from water hyacinth) were selected to compare with commercial foliar fertilizers used frequently on lettuce (Mo variety) for growth and yield under field condition. Field experiments were comprised of following five treatments including (1) control (water spraying); (2) bio-foliar fertilizer (ratio 1:10 from water hyacinth); (3) bio-foliar fertilizer (ratio 1:10 from seaweed); (4) Seaweed fertilizer imported from Japan (N 0.63%; P 0.08%; K 12.7%); (5) Growmore (N 15%; P 13%; K 12%) based on 15 tons of manure and 500 kg lime ha^{-1}. Under field condition, randomized complete block design (RCBD) was used in three-replications and with a net plot area of 10 m^2. Foliar fertilizers were sprayed at 1 L plot^{-1} following the ratio in each treatment, number of sprays were at 3-day interval and ended at least 5 days before harvest.

***Determination of lettuce yield and quality*:** Lettuce yield was measured after harvesting the plants in both pot and field experiments. The plants were harvested at 30–39 days after planting. In pot experiments, all plants were collected. In the field experiment, we made a sample of total of 30 plants from the middle of four seed rows by selecting in a row with every eighth marketable plants. A composite sample of ten plants was used to determine a whole-plant weight. At physiological maturity stage, an area of 2 m^2 was harvested to measure the marketable yield. Mean yields were calculated in two cropping seasons.

***Lettuce quality*:** The brix content was measured from 5 plants by Brix meter and nitrate content by colorimetric method. The nitrates in the extracts of the analyzed samples were converted to nitrites by the enzyme nitrate reductase. The nitrites were converted together with nitrites available in the analyzed samples to react with sulfanilamide and N-1-naphtyl-ethylene diamine hydrochloride. The color intensity of red-form chemical was measured on a spectrophotometer at 540 nm wavelength. The difference between the values was used to compute the nitrate content.

***Statistical analysis*:** The statistical analyses were carried out following ANOVA and Tukey's test for comparing differences among treatments at 5% probability using the SPSS 20.0 analytical software package.

RESULTS

***Response of bio-foliar fertilizer on yield of lettuce*:** Results showed that type and ratio of bio-foliar from aquatic plants

Table 1. Effect of bio-foliar fertilizer from aquatic plants on yield of lettuce in pot experiment.

Treatment	Dry matter (g plant^{-1})		Biomass yield (g pot^{-1})		Economic yield (g pot^{-1})	
	Season 1	**Season 2**	**Season 1**	**Season 2**	**Season 1**	**Season 2**
Control (water spray)	1.20b	1.20b	27.30b	24.20b	23.90b	21.60b
BT 1:10	2.20a	2.10a	42.10a	37.90ab	38.60ab	36.80a
BT 1:20	1.90ab	1.60ab	40.20a	34.30ab	36.00ab	33.40ab
BT 1:30	1.70ab	1.50ab	38.50ab	31.40ab	34.70ab	30.10ab
RB 1:10	2.40a	2.20a	44.40a	41.10a	40.20a	39.10a
RB 1:20	2.10ab	2.00a	41.30a	35.40ab	38.20ab	34.30ab
RB 1:30	1.90ab	1.80ab	39.30ab	33.40ab	35.40ab	32.00ab
$LSD_{0.05}$	0.70	0.80	7.80	8.50	8.10	9.20

The means with similar lower case letter within columns did not differ significantly at 5% probability; BT: Bio-foliar fertilizer from water hyacinth; RB: Bio-foliar fertilizer from seaweed; Feb – March, 2020 (season 1); March – April, 2020 (season 2).

impacted dry matter of lettuce at harvesting in pot experiment (Table 1). The dry matter varied from 1.2 to 2.4 g plant^{-1} in season 1 and from 1.2 to 2.2 g plant^{-1} in season 2. The highest dry matter was found at a ratio of 1:10 in both types of bio-foliar (2.1–2.2 g plant^{-1} at BT type-water hyacinth and 2.2–2.4 g plant^{-1} at RB type-seaweed) than control from 0.9-1.0 g plant^{-1} and 0.7 g plant^{-1} in BT and RB types, respectively.

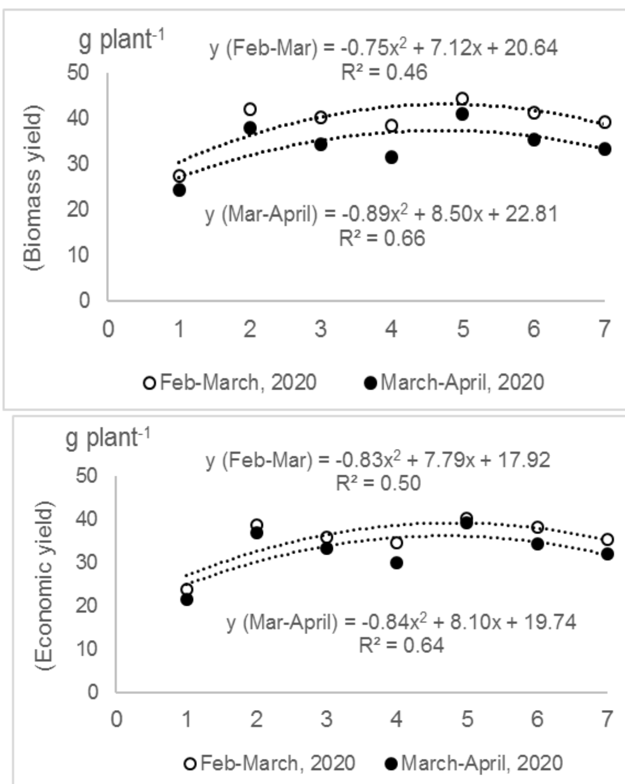

Figure 1. Relationship between types and spraying ratio of bio-foliar fertilizer to biomass and economic yields of lettuce.

The water hyacinth bio-foliar fertilizer and seaweed at ratio of 1:10, had the maximum biomass yield in both seasons ranging from 37.9 to 42.1 g pot^{-1} and 41.1 to 44.4 g pot^{-1} respectively. Nonetheless, in both different types of bio-foliar fertilizer, the higher biomass yield was found in seaweed than in water hyacinth at 1:10 ratio (Table 1).

The types of bio-foliar fertilizer extracted from BT and RB at a spray ratio of 1:10 had the highest economic yield during both seasons, followed by a 1:20 spray ratio, respectively (Table 1). Thus, in both types of bio-foliar fertilizer, the spray ratio of 1:10 gave the highest economic yield, in which the RB type had higher than BT type (1.6–2.3 g pot^{-1}).

There was also a close relationship between biomass yield of lettuce (R^2=0.46 to 0.66) and economic yield (R^2 =0.50 to 0.64), type and ratio of bio-fertilizer from aquatic plants (Fig. 1).

Data in field experiment indicated that dry matter content of lettuce varied significantly (Table 2) and maximum dry matter was found with Growmore application and followed by application of bio-foliar fertilizer from seaweed at a ratio of 1:10.

Highest biomass yields were observed with spraying seaweed at a ratio 1:10 which ranged from 11.34–13.11 t ha^{-1}, however, differences in biomass yield was non-significant during crop season 1 and 2 respectively. Similarly, economic yields of lettuce also showed significant differences for bio-fertilizer sprays and crop seasons. Economic yield was the highest for bio-foliar fertilizer from seaweed with a ratio of 1:10 in both crop seasons.

Interestingly, a close relationship between biomass yield (R^2 = 0.45 to 0.77) and economic yield of lettuce (R^2 = 0.54 to 0.84) and types of foliar fertilizer in experimental treatments was also found under field condition (Fig. 2).

Response of bio-foliar fertilizer on quality of lettuce in pot and field experiments: There was found a significant difference in brix content at $p<0.05$ in both seasons and highest brix contents of 2.2–2.3% with foliar spray of RB 1:10 in pot condition. The highest nitrate content in the leaves of lettuce was found for bio-foliar fertilizer extracted from water hyacinth than the extract from seaweed. However, all treatments had very low nitrate content in the leaves (< 200 mg kg^{-1}) (Table 3).

Table 2. Effect of bio-foliar fertilizer from aquatic plants on yield of lettuce under field.

Treatments	Dry matter (g plant^{-1})		Biomass yield (t ha^{-1})		Economic yield (t ha^{-1})	
	2020	2021	2020	2021	2020	2021
Control (water spray)	1.99b	2.24b	9.04d	9.86a	7.64b	7.88c
Water to hyacinth extract (1:10)	2.29ab	3.18a	11.95c	10.32a	8.93a	8.96ab
Water to seaweed extract (1:10)	2.37ab	3.58a	13.11a	11.34a	9.40a	9.41a
Seaweed fertilizer	2.25ab	3.27a	11.47c	10.25a	8.37ab	8.93ab
Growmore 15-13-12	2.47a	3.60a	12.04b	10.88a	9.13a	9.12ab
$LSD_{0.05}$	0.64	0.59	0.91	1.53	1.09	1.12

The means with similar lower case letter within columns did not differ significantly at 5% probability; BT: Bio-foliar fertilizer from water hyacinth; RB: Bio-foliar fertilizer from seaweed.

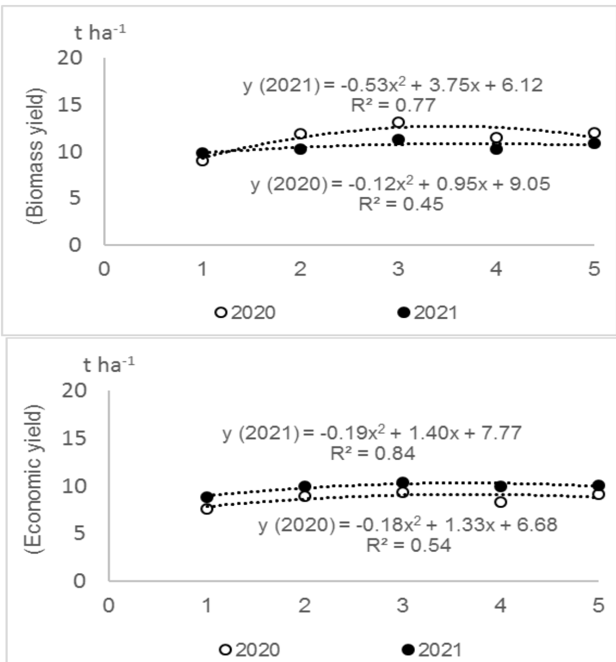

Figure 2. Relationship between different treatments on types of bio-foliar fertilizer and biomass and economic yields of lettuce.

Table 3. Effect of bio-foliar fertilizer from aquatic plants on quality of lettuce in pot experiment.

Treatments	Brix content (%)		NO_3^- content in leaf (mg kg^{-1})	
	Season 1	Season 2	Season 1	Season 2
Control (water spray)	1.7b	1.5b	55c	87c
BT 1:10	2.2a	2.0ab	155a	181a
BT 1:20	1.8b	2.0ab	114b	131ab
BT 1:30	1.8b	2.0ab	100b	125b
RB 1:10	2.3a	2.2a	135ab	159a
RB 1:20	2.0ab	1.9ab	122b	121b
RB 1:30	2.2a	1.8ab	110b	118b
$LSD_{0.05}$	0.5	0.6	45	56

The means with similar lower case letter within columns did not differ significantly at 5% probability; BT: Bio-foliar fertilizer from water hyacinth; RB: Bio-foliar fertilizer from seaweed; Feb – March, 2020 (season 1); March – April, 2020 (season 2).

Under field condition, different types of foliar fertilizers affected brix and nitrate contents of lettuce. There were significant differences in brix and nitrate contents of leaf in lettuce. The highest brix content was found with bio-foliar fertilizer spray of seaweed at ratio 1:10 (2.53–2.65%) and the highest nitrate content ranged from 201–214 mg kg^{-1} with application Growmore fertilizer (Table 4).

Table 4. Effect of bio-foliar fertilizer from aquatic plants on quality of lettuce under field condition.

Treatments	Brix content (%)		NO_3^- content in leaf (mg kg^{-1})	
	2020	2021	2020	2021
Control (water spray)	2.12a	2.27b	76a	81a
Water to hyacinth extract (1:10)	2.21b	2.23b	101b	112b
Water to seaweed extract (1:10)	2.65c	2.53a	96b	105b
Seaweed fertilizer	2.41b	2.36b	115b	120b
Growmore 15-13-12	2.33b	2.30b	201c	214c
$LSD_{0.05}$	0.31	0.29	58	76

The means with similar lower case letter within columns did not differ significantly at 5% probability.

DISCUSSION

The rate and type of bio-foliar fertilizers used in this study significantly affected lettuce growth, yield, and quality. The presence of macronutrients like nitrogen, phosphorus, and potassium in crude extracts of aquatic plants might had influence on growth. Moreover, many plant growth regulators, including auxins, cytokines, gibberellins, abscise acid, and others are also present in extracts of seaweeds (Garcia *et al.*, 2020). Because water hyacinth gathers nitrogen and phosphate in its roots, it is considered as an organic fertilizer source (Parra and Hortenstine, 1974). The application of 10% kelp waste extracts (KWE) to *Brassica chinensis* plants improved plant growth via increase in shoot and root lengths, leaf length, fresh and dry weights, however, higher concentrations of kelp waste extracts (20–100%) inhibited growth (Zheng *et al.*, 2016; Ghaderiardakani *et al.*, 2019). The increase in growth of lettuce may be plausible due rich in growth substances, macro- and microelements, amino acids, and vitamins present in seaweed extracts. Ganapathy *et al.* (2013) found that seaweed extract is used as a natural organic fertilizer with a high nutritional value, and it promotes seed germination, enhances yields and resistant ability in many crops.

Dry matter and economic yields were found higher with application of bio-foliar fertilizer at ratio of 1:10 from seaweed than water hyacinth. Because the chemical properties of the bio-fertilizer extracted from seaweed are better than water hyacinth, which improves plant's height and the lettuce leaves number (Tran *et al.,* 2018). Many studies suggest that seaweeds were used as a bio-fertilizer to reduce soil nitrogen, phosphorous, and potassium deficiencies (Nabti *et al.,* 2017; Hassan *et al.,* 2021). Paraikovi *et al.* (2018) found that using a plant-derived biostimulant had a greater impact on developmental traits such as leaf number, plant height, mass head and vitamin C in lettuce leaves and dry matter content as compared with Megagreen foliar fertilizer application. Furthermore, according to Davari *et al.* (2012), seaweed bio-fertilizers including organic matter have provided nutrient content to increase plant development.

Seaweeds were used as organic fertilizers in large quantities to improve field crop development (Chbani *et al.,* 2015). Bradáčová *et al.* (2016) found that adding micronutrients to seaweeds enhanced plant biomass that might be attributed to the high Zn content. A combination of macroalgae application according to Nabti *et al.,* (2017) provided significant levels of organic matter and several nutrients, including NH_4^-, NO_3^-, and NO_2^-, as well as phosphate.

The nitrate contents found in lettuce leaves of present study are within safe limits compared to the standard threshold (MARD, 2007). Likely, Paraikovi *et al.* (2018) reported the effects of brown seaweed *Ascophyllum nodosum* (L.) including the biostimulant Bio-algeen S-90 on plant development and total yield. Both treatments reduced nitrate contents in leaves and non-marketable harvest. Nonetheless, Adriatic algae extracts could also be explored as potential biostimulants.

Likely, Nenseigné *et al.* (2007) reported that lettuce plants treated with inorganic fertilizers grew at a similar rate to the control and had the greatest nitrate concentrations. Soil application of organic fertilizer showed more lettuce growth and yield than soil applied with inorganic fertilizer, however, inorganic, and organic fertilizer-treated lettuce had higher nitrate concentrations from 5000–6100 and 4300–5200 mg kg^{-1}, respectively (Liu *et al.,* 2014). Nevertheless, reduced nitrate concentration by 4–10% was observed for soil application of organic fertilizers in lettuce compared with liquid fertilizers. Previously, it has been reported that organic fertilizer application on lettuce accumulated lower nitrate concentrations than conventionally treated lettuce (Malmauret *et al.,* 2002; Williams *et al.,* 2002; Hajslova *et al.,* 2005). Thus, using biostimulants derived from seaweed extracts in lettuce production may aid to improve lettuce nutritional quality, as demonstrated in the current study.

***Conclusion*:** The present study showed that bio-foliar fertilizers made from seaweed and water hyacinth affected productivity and quality of lettuce crop. In both pot and field studies, the type of bio-foliar fertilizer extracted from seaweed with a spraying ratio of 1:10 produced the maximum production and quality of lettuce crop. Management approach in producing foliar bio-fertilizers produced from aquatic plants to supply natural nutrients to counteract the drawbacks of chemical fertilizers and pesticides, boost crop yield and quality, decrease pollution, and promote long-term agricultural development.

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