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Impact of Bio-foliar Application of Moringa (*Moringa oleifera*) on Foliage Yield and Quality of Mustard Green (*Brassica juncea* L.)

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ABSTRACT

Background: *Moringa oleifera* is a vegetable widely consumed because of its nutritional benefits such as proteins, vitamins minerals, antioxidant compounds and vitamin E. While the plant leaves have been utilized to produce organic fertilizer or bio-extract, other parts such as stems, branches, or leaf petioles have often been discarded as waste.

Methods: The present study evaluated different ratios (1:10; 1:20; 1:30; 1:40 and 1:50) and types of bio-foliar fertilizers (Moringa bio-foliar fertilizer, chitosan and seaweed fertilizers) on yield and quality in mustard green. The pot and field experiments were conducted in the spring seasons of 2019 and 2020 in completely randomized design using three replications.

Result: The results showed that the mustard green yield and quality were affected by ratio and type of bio-foliar fertilizer in both seasons. Highest yield and quality was found for application of moringa bio-foliar fertilizer. The nitrate accumulation value (147.3 mg kg^{-1}) found were also higher for moringa bio-foliar fertilizer but lower than the standard threshold. A strong relationship was found between economic yield (R^2= 0.82 to 0.95 in pots and field experiment (R^2= 0.78 to 0.81). In conclusion, moringa bio-foliar fertilizer at spraying ratio of 1:10 can be used to achieve higher yield and quality for mustard green.

Key words: Bio-foliar fertilizer, Moringa, Mustard green, Quality, Yield.

INTRODUCTION

Moringa oleifera Lam., a multipurpose tree from Moringaceae family and is widely grown in tropical and subtropical areas (Paliwal *et al.,* 2011; Balakumbahan and Kavitha, 2019). *Moringa oleifera* is rich in macro and micronutrients such as nitrogen, calcium, potassium, magnesium, copper, iron and other phytohormones like auxins (Hekmat *et al.,* 2015; Zaki and Rady, 2015). Therefore, the moringa plant is widely used as an organic source in agriculture recently and its extract are ragraded as an bio-foliar product that increase the growth, development and yield of many field crops at the lowest cost (Phiri, 2010; Basra *et al.,* 2011; Merwad, 2018). Also, the use of bio-foliar fertilizer orrginated from vegetative is increasing in because its reduces the nitrate accumulation in the leafy vegetables (Zheng *et al.,* 2016; Al-Redhaiman, 2023). Some studies on vegetables, fruits, flowers and ornamental crops focus on using extracting from apricot seaweed, seaweed, algae and macroalgae (Sharma *et al.,* 2014; Sa *et al.,* 2018). The using of moringa extracts provide inexpensive option for vegetable growner's in solving nitrate accumulation in recently.

Mustard green (*Brassica juncea* L.) belongs to the *Brassicaceae* family and is widely grown as leafy vegetables in Vietnam because of its high economic value (Huong *et al.,* 2013). To improve the yield of mustard green and reduce pests pressure and diseases, growers tend to apply heavy fertilizers and pesticides (Hoa *et al.,* 2018; Nguyen *et al.,* 2018). This not only enhances the input costs, also detrimental with the environment and human health. Therefore, organic agriculture with the application of bio-foliar fertilizer can be the alternative strategy to protect the environment, humans and product quality from the residual effects of chemical usage.

In Thua Thien Hue province, using foliar fertilizer from other plant sources like seaweed has been investigated but there is little research on producing and using bio-foliar fertilizer from moringa tree. Growers are lacking knowledge and experience in using and producing plant extract as organic fertilizer. Thus, this research aimed to evaluate the effect of Moringa bio-foliar fertilizer in improving the yield and quality of mustard green towards sustainable organic products.

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MATERIALS AND METHODS

Moringa bio-foliar fertilizer preparation

The moringa and its plant parts (stems, leaves, and leaf petioles) were collected and washed with clean water to eliminate all adhered particles. All plant samples were chopped into small particles before incubation. A plastic container with a capacity of 100 L consisted of 70 kg of moringa chopped material was added with 0.2 kg of EM products and 5 litters of molasses, all this were stirred, covered tightly and stored until sampling. Moringa bio-foliar fertilizer was extracted after composting for 4 months by squeezing water then filtered to remove the residue and only the solution was taken and preserved in an airtight container until use.

Selection of appropriate ratio of moringa bio-foliar fertilizer

The pot experiments were conducted under vinyl house of Institute of Biotechnology, Hue University from January to May 2019. The average tempeature and precipitation were ranging from 20-28.3°C and 152.4 mm per month during the experiments. The moringa bio-foliar fertilizer extracts was performed after 4 months of incubation following standard methods and showed that extracts had pH= 5.04, OM= 32.77%, N= 11.90%; P= 2.63% and K= 5.07% contents.

There were 5 treatments with different ratio of moringa bio-foliar fertilizer with diluted into water as: 1:10, 1:20, 1:30, 1:40 and 1:50, respectively. The pot experiments were ranged in completely randomized design (CRD) comprising five application extraction ratios replicated three-times in 2 seasons. The seedlings at 3-4 leaf stage were planted with a single plant per pot. The pot (20 × 20 cm) contained 2.5 kg of alluvial soil (pH_{KCl}: 5.5; OC: 1.42%; total N: 0.06%; total P: 0.07% and total K 0.29%) treated with lime and mixed with cattle manure before planting. The moringa bio-foliar fertilizer according to the ratio were sprayed at 10 ml per plant with 5-day interval and finished before harvesting at least 5 days.

Comparison of moringa bio-foliar fertilizer with other commercial foliar fertilizer

The field experiment was carried out in Phu Thuong ward, Phu Vang district, Thua Thien Hue province, Central Vietnam from January to May 2020 in two growing seasons to compare the best moringa bio-foliar fertilizer rate with other commercial foliar fertilizers. Climatic condition in this region belongs to tropical monsoon condition with the temperature 24.6°C and rainfall is approximately 63.3 mm per month during the experiments. The experiment were conducted in completely randomized design following four treatments including control (water spray); moringa bio-foliar fertilizer (1:10); chitosan fertilizer (N: 3.0%, P_2O_5: 3.6%; K_2O: 5.4%; B: 2500 mg kg^{-1}; Zn: 400 mg kg^{-1}; Mn: 200 mg kg^{-1}; Fe: 100 mg kg^{-1} and pH: 5.82) and seaweed fertilizer (N: 0.63%, P_2O_5: 0.18%; K_2O: 15.3%; Mg: 0.18%; Ca: 0.18% and pH: 10). The plot size of each treatment was 10 m^2. Before planting, the soil was plowed and applied with 2 kg of farmyard manure m^{-2}. The seedlings at 3-4 leaf stage were planted at adensity of 25 plant m^{-2}. Foliar fertilizers were sprayed at 1 L $plot^{-1}$ and the number of sprays was 5-day interval and finished before harvest at least 5 days.

Yield determination

Some parameters as fresh weigh, biomass yueld and economis yield of mustard green yied was measured after harvesting the plants in both pot and field experiments. The plants were harvested at 32-35 days after planting. In pot experiments, all plants were collected to determine fresh weight and yields. Mean yield was calculated from yield in 2 planting times.

Mustard green quality

The 5 plants with average weight were selected to measure for Brix, vitamin C content and nitrate accumulation. The Brix content was measured by a Brix meter. Vitamin C content was determined by redox titration using iodine solution following the method described by Ciancaglini *et al.* (2001).

The nitrate accumulation in mustard green leaves was measured based on the method by Kmecl *et al.* (2007) using a Segmented Flow Analyzer (AA II, Bran + Luebbe) at the wavelength of 540 nm.

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.

RESULTS AND DISCUSSION

Yield response of mustard green

Pot experiment

The results indicated that moringa bio-foliar fertilizer strongly affected the fresh weight of mustard green at harvesting during both seasons. The fresh weight ranged from 77.37 g to 123.33 g. $plant^{-1}$ in the 1st season and 86.98 g. to 138.21 g. $plant^{-1}$ during 2nd season. The highest fresh weight was found at the ratio of 1:10 in both seasons and tended to decrease with the ratio from 1: 20 to 1:50. Highest biomass yields of mustard green was also obtained at Moringa bio-foliar fertilizer ratio of 1:10 that ranged from 63.10 g to 101.77 g. pot^{-1} during 1st and 71.90 g to 111.27 g. pot^{-1} in 2 seasons, respectively (Table 1).

The highest economic yield in both seasons were also observed at similar ratio compared to other ratios. The highest yield obtained was 77.27 g and 97.77 g. pot^{-1} in both seasons, followed by other ratios 1:20, 1:30, 1:40 and 1:50, respectively. During both seasons, the economic yield were higher at 1:20 ratio than 1:30 and 1:40, but there were no significant differences among differences in the ratios of 1:30 and 1:40.

Thus, it was concluded that biomass yield and economic yield in mustard green were obtained at the ratio of 1:10 than the other ratios and was further used for field

investigation. A strong relationship between the economic yield of mustard green (R2=0.82 to 0.95) and different ratios of moringa bio-foliar fertilizer in both seasons (Fig 1).

Field experiment

There was significant differences among treatments for fresh weight. Application of moringa bio-foliar fertilizer showed the highest fresh weight in both season, followed by seaweed and chitosan fertilizers. In the 2nd season, there was no significant differences in fresh weight in treatment spraying moringa bio-foliar fertilizer and seaweed fertilizer. The highest biomass yield and economic yields was observed with spraying moringa bio-foliar fertilizer at 29.45, 23.29 and 28.71, 23.33 t.ha^{-1} during 1st and 2nd season, respectively (Table 2). There was strong relationship of economic yield with the difference types of foliar fertilizers under field condition in mustard green (R^2=0.78 to 0.81) (Fig 2).

Quality of mustard green in pot and field experiments

Pot experiment

Application of moringa bio-foliar fertilizers also improved the quality of mustard green. The leaf and stem of mustard green exhibited varied responses to different ratios and types of fertilizers for vitamin C, nitrate accumulation and Brix content (Table 3 and 4).

The highest vitamin C (0.336%) contents was found using moringa bio-foliar fertilizer at a ratio of 1:10 while no significant differences was observed for other commercial stimulants in both seasons. The vitamin C contents in mustard green ranged from 0.280 to 0.313% in pot and 0.293 to 0.336% in both seasons in the field experiment.

Under field condition, the lowest vitamin C content was found with foliar spray of water in both seasons, with no significant difference between water spray and Chitosan foliar application except in 2nd season. A higher vitamin C contents was observed in moringa bio-foliar fertilizer and seaweed fertilizer while a significant difference was found with other treatments in both seasons.

Nitrate accumulation in mustard green leaves varied in pot and field experiments. For the pot experiment, the highest nitrate contents (147.3 mg. kg^{-1} and 143.0 mg. kg^{-1}) were found for moringa bio-foliar application at 1:10 ratio in both seasons as compared to other ratios. In field experiment, the nitrate content were higher for foliar fertilizers than control, however, nitrate accumulation were safe compared with the standard threshold (Table 3 and 4).

The highest brix content (7.9-8.2%) was found with moringa bio-foliar fertilizer spray at a ratio of 1:10 in both seasons as compared to other ratios in the pot experiment.

Table 1: Effect of Moringa bio-foliar fertilizer on yield of mustard green in the pot experiment.

Moringa bio-foliar fertilizer	Fresh weight (g plant^{-1})		Biomass yield (g pot^{-1})		Economic yield (g pot^{-1})	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
1:10	123.33a	138.21a	101.77a	111.27a	77.27a	97.77a
1:20	112.00b	122.50b	89.63b	98.00b	65.87b	76.63b
1:30	104.67b	105.73b	83.77bc	84.53c	58.88c	66.83c
1:40	105.73b	104.71c	82.36c	85.10c	54.74c	63.76c
1:50	77.37c	86.98d	63.10d	71.90d	48.18d	54.92d
$LSD_{0.05}$	7.47	4.86	5.96	4.77	6.19	3.92

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

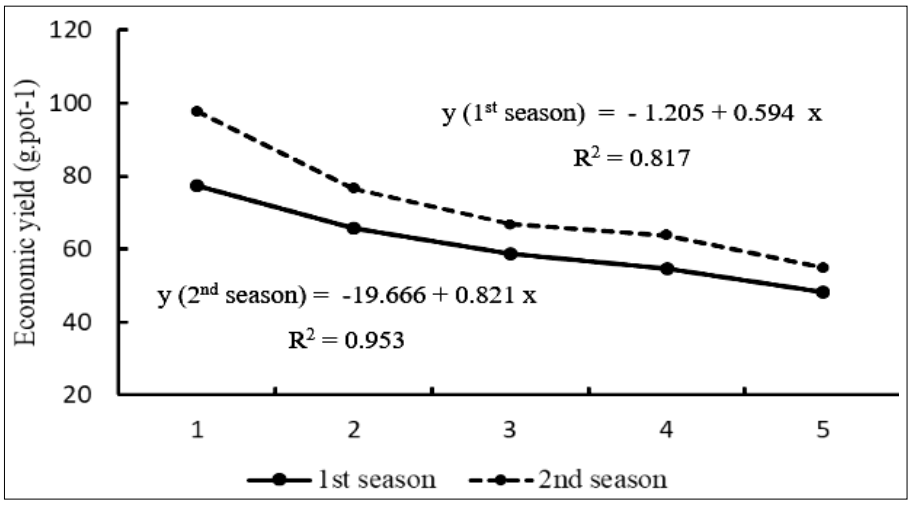

Fig 1: Relationship between differences ratios of Moringa bio-foliar fertilizer.

However, in the field experiment, there were no significant differences among the foliar fertilizers, except control treatment.

Moringa extract has attained much interest as a bio-foliar fertilizer for improving the growth and yield of plants (Hasan *et al*., 2021; Brockman and Brennan, 2017). These extracts can be used through irrigation or directly spraying to plants, especially leafy vegetables. The influence of these extracts is based on the nutrient availability and other biological products. In this study, Moringa leaf and its stems, and petioles as extracts were used in comparison with others kind of fertilizers. The increase in plant performances and yield of mustard green by foliar application of bio-foliar moringa may be presence of higher nutrients as evident from Yaseen and Hájos (2021) in lettuce cultivar.

Higher fresh weight, biomass yield and economic yield at 1:10 ratio of bio-foliar fertilizer in pot and field experiments during both seasons compared with other ratios and types of foliar fertilizers. The better crop response at low ratio than other the ratios and types of fertilizers may be due to presence of nutrients in higher amount present than other types. Significant response with application of moringa bio-foliar fertilizer in improving growth, development, yield and quality of mustard green might be due to presence of macro- and micro-nutrients such as nitrogen, phosphorus, potassium, manganese, zinc and iron. Hassan *et al*. (2021) report that Moringa leaf extract (20 per cent) resulted in the highest production, minerals and nutraceutical-related characteristics in stevia plants (Hasan *et al*., 2021). The moringa leaves are also rich in growth hormone regulators like auxins, cytokinins and zeatin to improve growth, yield and quality of crops (Fuglie, 1999). For instance, cytokinins are involved in the stimulation of cell division, cell elongation, shoot initiation, chlorophyll content, nutrient uptake and also delay senescence and aging in different plant tissues (Sardar *et al.,* 2021). The increases in yield might have come from plant regulators in foliar fertilizers. Plant hormones play an important role in increasing the yield of plants since it influences every stage of plant growth and development of plant (Maishanu *et al*., 2017). Zeatin is a natural form of cytokine that can be found at a high level (5 µg and 200 µg/g) in fresh moringa leaves (Basra *et al*., 2011; Abdalla, 2013) that due to rich in zeatin, the moringa leaves, can be an

Table 2: Effect of different bio-foliar fertilizers on yield of mustard green in the field experiment.

Treatment	Fresh weight (g plant^{-1})		Biomass yield (tons ha^{-1})		Economic yield (tons ha^{-1})	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control (water spray)	85.33c	90.41c	21.62c	22.34d	18.14c	16.65c
Chitosan fertilizer	95.42b	96.42b	24.65b	24.74c	19.72b	19.32b
Seaweed fertilizer	96.70b	109.38a	24.47b	27.05b	21.92a	19.58b
Moringa bio-foliar fertilizer (1:10)	117.73a	113.35a	29.45a	28.71a	23.29a	23.33a
$LSD_{0.05}$	4.58	4.27	1.75	1.51	1.55	1.12

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

Table 3: Effect of moringa bio-foliar fertilizer quality of mustard green in the pot experiment.

Treatment	NO^{3-} content (mg kg^{-1})		Brix content (%)		Vitamin C (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
1:10	147.3a	143.0a	8.2a	7.9a	0.313a	0.310a
1:20	122.6b	116.1b	7.6b	7.3b	0.290ab	0.307ab
1:30	121.7b	105.2d	6.7c	7.1bc	0.293ab	0.297abc
1:40	110.5c	109.1c	7.3b	7.1bc	0.283b	0.287bc
1:50	108.0c	115.6b	7.4b	7.0c	0.287ab	0.280c
$LSD_{0.05}$	8.45	0.69	0.43	0.29	0.03	0.02

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

Table 4: Effect of different bio-foliar fertilizers on quality of mustard green in the field experiment.

Treatment	NO^{3-} content (mg kg^{-1})		Brix content (%)		Vitamin C (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control (water spray)	80.0c	83.4d	6.8c	6.8c	0.293c	0.297b
Chitosan fertilizer	108.7b	148.2a	7.8a	7.3b	0.313b	0.310ab
Seaweed fertilizer	132.8a	117.3b	7.6ab	7.5a	0.330a	0.333a
Moringa bio-foliar fertilizer (1:10)	105.2b	103.3c	7.4b	7.5a	0.334a	0.336a
$LSD_{0.05}$	11.1	12.7	0.23	0.11	0.02	0.03

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

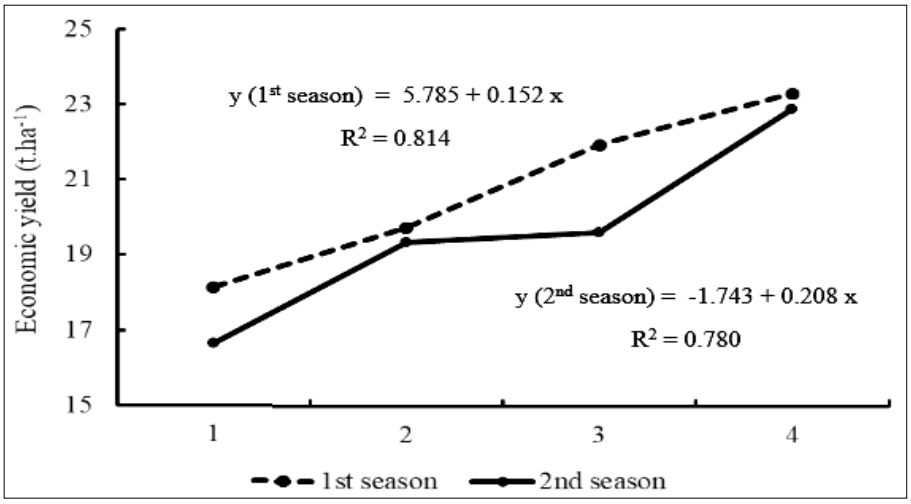

Fig 2: Relationship between treatments of bio-foliar fertilizers on economic yield of mustard green in the field experiment.

effective plant growth hormone to improve 25-30% crop yields in soybean, maize and coffee. The present study found significant response of mustard green growth to foliar fertilizer application as compared with other ratios and type of foliar fertilizers that was due to nutritional composition of this bio-foliar fertilizer compared with other market foliar fertilizers.

The nitrate accumulations found in present study was in safe limit compared with the standard threshold (MARD, 2007). Nazaryuk *et al*. (2002) revealed nitrate accumulation in plants and showed that the process of nitrate accumulation depends on three main factors: application of fertilizers, treatment with physiologically active substances and sorbents and the natural and anthropogenic changes in the soil environment. Besides, nitrate accumulation in vegetables depends on the amount and kind of nutrients present in the soil and is closely related to the time of application, the amount and composition of the fertilizers applied (Zhong *et al*., 2002; Huang *et al*., 2010; Qiu *et al*., 2014). Yaseen and Hájos (2021) reported that foliar application of moringa leaf extract (6%) reduced the leaf nitrate content by 16-44% in lettuce. Some other studies with application of biostimulants as double irrigation with 1.0% Bio-algeen S-90 (200 mL per plant) extracted from the brown seaweed and 0.3% Megagreen (100 mL m^{-2}) reduced nitrate content in lettuce leaves (Dudaš *et al*., 2016). Baitilwake *et al*. (2012) also reported that, organic fertilizer application on leafy vegetables reduced nitrate accumulation than conventional fertilizer method. In this study, the application of organic fertilizer as a basal dressing and bio-foliar fertilizers lowered nitrate accumulation than in the other studies. Thus, using moringa bio-foliar fertilizer in green mustard may improve its nutritional quality.

CONCLUSION

The present study showed that moringa bio-foliar fertilizer affected the productivity and quality of mustard green. The ratio of moringa bio-foliar application (1:10) showed the highest yield compared with other ratios and types of foliar fertilizers. The result is expected to give addtional options for vegetables production by using bio-foliar fertilizers toward organic production.

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Conflict of interest

All authors declare that they have no conflict of interest.

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