Plant Cell Biotechnology and Molecular Biology 21(71&72):208-214; 2020 ISSN: 0972-2025

FERMENTED SEAWEED (*Kappaphycus alverezii*) BY-PRODUCT PROMOTES GROWTH AND DEVELOPMENT OF LETTUCE (*Lactuca sativa* VAR. CURLY GREEN)

PET ROEY L. PASCUAL, DANNY E. CARABIO, MARLENE E. RONDINA, NONNA FATIMA H. ABELLO* AND VALERIE U. PASCUAL

Department of Crop Science, College of Agriculture, Cebu Technological University - Barili Campus, Barili, Cebu, Philippines [PRLP, DEC, MER].

Crop Biotechnology Unit, Center for Studies in Biotechnology, Cebu Technological University at Barili Campus, Barili, Cebu, Philippines [PRLP, NFHA].

Department of Agricultural Economics and Development Studies, College of Agriculture, Cebu Technological University - Barili Campus, Barili, Cebu, Philippines [VUP].

[*For Correspondence: E-mail: nonnafatima.abello@ctu.edu.ph]

Article Information

Editor(s):
(1) Dr. Ahmed Medhat Mohamed Al-Naggar, Professor, Cairo University, Egypt.
Reviewers:
(1) Sviatoslav Pavlyshyn, University of Horticulture, Ukraine.
(2) Indriyani Nur, Halu Oleo University, Indonesia.

Received: 25 October 2020
Accepted: 30 December 2020
Published: 31 December 2020

Original Research Article

ABSTRACT

Seaweed processing into juice extraction produces a large volume by product which from a kilo of seaweed (*Kappaphycus alverezii*), 800 grams (80%) of residue goes to waste after the extraction. The aim of this study was to improve the growth and yield of lettuce as: height, growth rate, leaf length, above and below ground fresh weight through the use of Fermented Marine Macro Algae Residue. Four treatments were compared; solid seaweed sludge (by-product of fermentation at 10tons/ha), fermented liquid seaweed (10ml/L) and the combination of both against a commercially available seaweed-based fertilizer (10ml/L). For lettuce (*Lactuca sativa* var. Curly Green) plant height after five days from transplanting showed that application of seaweed sludge (solid seaweed fertilizer) outperformed the commercial fertilizer at 10.01±0.72SE cm and 6.28 ±0.0.57SE cm respectively. On the 20th day, the combination of both liquid and solid seaweed fertilizer produced the tallest plants (plant height) with the longest leaves at 13.87±0.42SE cm and 15.43±0.54SE cm, respectively. The fastest growth rate was achieved from 16-20 days with the combined application of liquid and solid seaweed fertilizer at 5.31±0.44SE cm. From transplanting (0-5 days), highest growth rate was achieved by applying solid seaweed fertilizer alone (4.08±0.44SE cm/5-days). At comparable below ground weight, liquid fertilizer application resulted to almost three times heavier above ground weight (270.17±163.61SE g/plant) than all of the treatments. Thus, waste products from processing seaweed-based beverage can effectively be utilized as fertilizer through fermentation in both liquid and in solid form, and more importantly in combination for the production of lettuce.

Keywords: Seaweed; organic agriculture; fermentation; sludge; liquid fertilizer.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is widely cultivated throughout the world (Silva et al. [1]) for their innumerable benefits as development and regulation of the body through its high vitamins and minerals [2]. This plant is low in calories, fat and sodium and is a good source of fiber, iron, folate, and vitamin C. It is also a good source of various other health-beneficial bioactive compounds. *In vitro* and *in vivo* studies have shown anti-inflammatory, cholesterol-lowering, and anti-diabetic activities attributed to the bioactive compounds in lettuce [3]. In the Cebu Philippines, its cultivation is done traditionally and more recently, an integral part of organic farms.

Mineral fertilization, despite its advantages, has several negative effects like increase soil salinity, decrease fertility, decline in soil organic matter and accumulation of chemicals in the soil [4]. Organic fertilizer due to their greater availability of nutrients, increase soil pH along with other benefits presents a good alternative to mineral fertilizer [5]. Organic fertilizers have a greater impact on the microbial community structure than chemical fertilizers. Application of organic fertilizers reduced the occurrence of soil diseases and remodelled the structure and function of the soil bacterial community [6].

Currently, commercializing wellness blends based mainly on *Kappaphycus alverezii,* of which 800 grams of sludge remains after juice extraction from one kilo of fresh seaweed. This normally would go to waste if not processed. Fermented seaweed extracts are commercially sold as liquid fertilizer mainly because they contain growth promoting hormones like auxins and gibberellins. Seaweed extracts contain trace elements and growth stimulators required by plants. It is also reported that these extracts are rich in vitamins, amino acids, trace elements (Fe, Cu, Co, Ni, Zn and Mn). It contains nitrogen, phosphorus and higher amount of water soluble potash, other minerals and trace elements in a form readily absorbable by plants [7]. They control deficiency diseases [8]. The most commonly used is brown seaweed (*Ascophyllum nodosum*) [9].

Considering the current situation of the production of seaweed-based juice and the potential benefits of seaweed-based fertilizer, the present study was undertaken to improve the growth and yield of lettuce using solid and liquid fertilizer from seaweed.

METHODOLOGY

Collection and Production of Marine Macro Algae Residue

Seaweed extracts were obtained from alga *Kappaphycus alverezii* and samples were collected from Argao, Cebu, Philippines. Seaweeds were washed three times with tap water to remove the dirt and any unwanted particles that were included in the collection. These were blanched for 30 seconds and were washed again with running water. The seaweed samples were homogenized using electrical blender. The samples were blended with a ratio of 1:2. For every 250 grams of seaweeds, these were added with 500 mL water and were strained using filter cloth. The extracts were collected by squeezing the cloth.

Fermentation of Marine Macro Algae Residue

The Marine macro algae residues were washed three times to remove the unwanted particles after the extraction process. These were added with Molasses with a ratio of 1:1 and were fermented for seven days. The study used 5 kg of seaweed waste residue and 5 L of Molasses in the fermentation process following the procedure used by Pascual et al. [10] for fermented plant juice. The fertilizers prepared were the following; solid seaweed sludge (by-product of fermentation at 10 tons/ha), fermented liquid seaweed (10 ml/L) and the combination of both against a commercially available seaweed-based fertilizer (10ml/L). These were compared against a commercial seaweed-based organic fertilizer which is described to also contain natural fermentation extracts of plants / herbs known to have natural insecticidal / fungicidal properties such as neem, jatropha, kakawati, hot pepper, garlic, onions, etc. that doesn't harm the plants, animals and farmers/consumers (product label). The rate of application was based on the commercial liquid fertilizer and on Pascual et al. [10] for the solid organic fertilizer.

Experimental Site and Plant Sampling

The experiment was conducted in the field microplots measuring 1 sq. m on the vegetable area of Cebu Technological University – Barili Campus (10°7'53" N, 123°32'45" E). Lettuce seedlings were planted in each micro plot two week after emergence at 20 x 20 cm spacing.

Experimental Design and Treatments

This was laid out in Completely Randomized Design with four treatments; commercial seaweed-based fertilizer, solid seaweed sludge, fermented liquid seaweed and the combination of both solid and liquid seaweed fertilizer. Each treatment was replicated three times.

Treatment	
T0	Commercial Seaweed-based Fertilizer
T1	Solid Seaweed Sludge
T2	Fermented Liquid Seaweed
T3	$\frac{1}{2}$Solid seaweed sludge + $\frac{1}{2}$Fermented Liquid Seaweed

Application of Treatments

The commercial seaweed-based fertilizer and formulated fermented liquid seaweed fertilizer was applied as foliar spray for four times; first at 4^{th} day, second at 8^{th} day, third at 12^{th} day and fourth at 16^{th} day. This was applied at a rate of one liter spray into the leaves of the growing lettuce per square meter at 10 ml/L concentration. Seaweed sludge solid fertilizer amounting to 1 kg/m^{2}based on the 10 tons/ha application was added directly into the soil one week before transplanting for Treatment 1, while for Treatment 3, only 500 g/m^{2}was used as basal and 5 ml/L concentration was used as foliar. Supplemental watering using tap water was done uniformly across all treatments whenever necessary.

Data Gathered

The following parameters were gathered in this study: Plant height (cm) – which was measured using a ruler for 20 days with a 5 day interval after planting; growth rate – which was accounted as the initial height subtracted to the actual height at the time of data gathering; leaf length – which was measured using a ruler from the tip of the longest leaf at one end of the leaf to the point where the leaf joins the stalk at the other end; above ground fresh weight and below ground fresh weight – this was measured using a digital balance right after the plants were removed from the soil, washed off any loose soil and was wiped off gently with soft paper towel to remove any free surface moisture and have the accurate amount.

Data Analysis

The experiment was laid out in Randomized Complete Block Design. Each treatment was replicated three times. The data were subjected to analysis of variance (ANOVA) using SPSS. Tukey's test at $p<0.05$ was utilized to test for variances between treatment means.

RESULTS AND DISCUSSION

The result of the current study on the plant height after five days from transplanting showed that application of seaweed sludge (solid seaweed fertilizer) outperformed the commercial fertilizer by 10.01±0.72SE cm and6.28 ±0.0.57SE cm respectively. On the 20^{th} day, the combination of both liquid and solid seaweed fertilizer produced the tallest plant at 13.77±0.54SE cm. This means that the fermented liquid seaweed and the combination of both have beneficial effect to the plant compared to the commercial seaweed-based fertilizer (Fig. 1). This was in relation with the study of Gollan and Wright, [11] that length of shoots increases due to the auxins present in the seaweed extracts which have an effective role in cell division and enlargement.

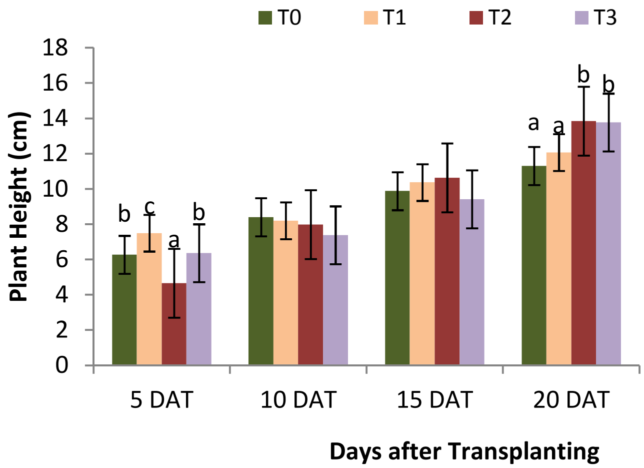

Fig. 1. Effects of seaweed-based fermented fertilizer on the plant height of Lettuce (*Lactuca sativa* var. Curly Green) grown under different treatments. Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

The result as shown in Fig. 2 indicated that the growth rate of the lettuce increased with an addition of the fermented seaweed-based fertilizer. The treatment that has the highest growth rate within the first and second five-day interval was those basally applied with the rate of 4.08±0.67SE cm and 1.62±0.38SE cm, respectively. On the 20th day, foliar application produced the highest growth rate at 5.31±0.19SE cm. Studies indicate that seaweed extracts can improve the growth rate of plants including vegetables, trees, cut flower crops and grain crops. This may be due to the effect of plant hormones in the extract or the effect on soil microorganisms, soil structure and macro and micronutrient availability [12].

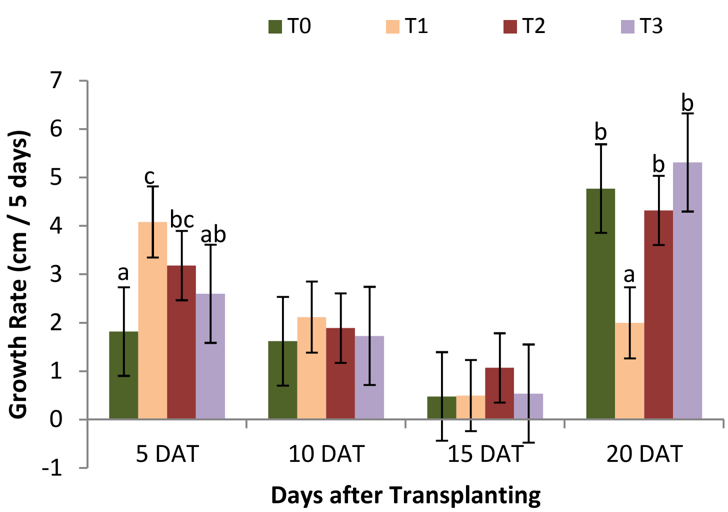

Fig. 2. Effects of seaweed-based fermented fertilizer on the growth rate of Lettuce (*Lactuca sativa*). Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

Leaf length on the 5th to 15th day, application of solid seaweed fertilizer constantly exceeds other treatment at 8.81±0.81SE cm, 10.94±0.38SE cm and 11.44±0.51SE cm, respectively. On the 20th day, liquid fertilizer tops all other treatments with a leaf length of 15.44±0.54SE cm (Fig. 3). In the study of Munisamy, [13] it was reported that seaweed extracts increase the physiological activities of the plants which includes the size of leaves. This was also reported by Abas et al. [14], that leaf blade of onions increases in the four cultivars used after the application of seaweed extraction. The highest increase between cultivars reached up to 65%.

The results reflected in Fig. 4 indicated that on the 20th day above ground fresh weight reached 270.17±163.61SE g/ plant when applied with seaweed liquid fertilizer. Promising results were obtained from all formulated fertilizer, either singly as basal or in combination. The result coincides with the study of Rathore, [15] where there's an increase in vegetative growth after the application of seaweed extracts. The increased growth was reported to be due to the presence of growth promoting hormones of seaweeds.

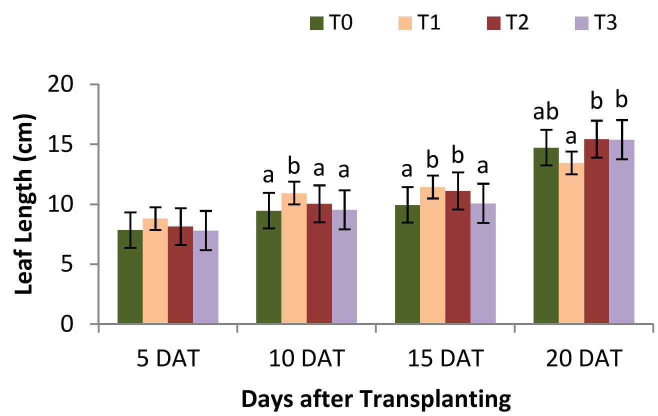

Fig. 3. Leaf length of Lettuce (*Lactuca sativa*) affected by seaweed-based fermented fertilizer. Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

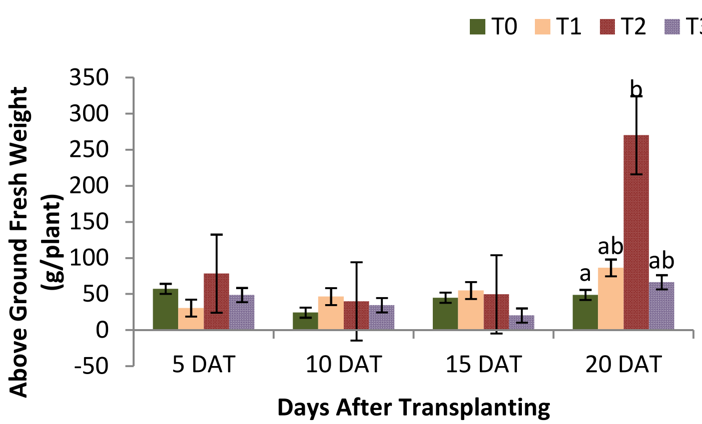

Fig. 4. Above ground fresh weight of Lettuce (*Lactuca sativa*) affected by seaweed-based fermented fertilizer. Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

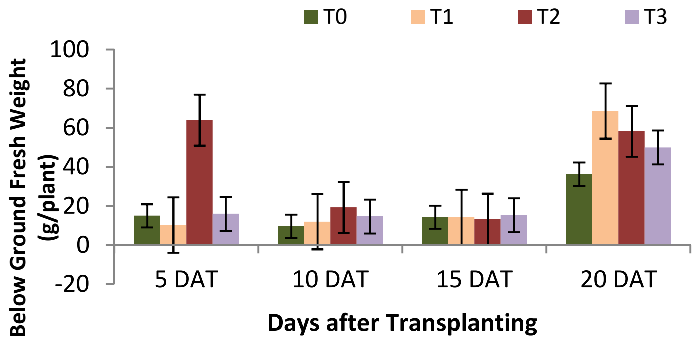

Fig. 5. Below ground fresh weight of Lettuce (*Lactuca sativa*) affected by seaweed-based fermented fertilizer

Based on the results presented in Fig. 5, all of the treatments from 5th day to the 20th day were not significant different from each other. It can be observed that below ground fresh weight increased with the addition of the fermented seaweed-based fertilizer just like in the result reported by Thomas, [16] that seaweed extracts improved the growth of roots of Rosemary.

CONCLUSION

The use of waste products from processing seaweed-based beverage can effectively be utilized as fertilizer through fermentation in liquid and in solid form and in combination. If done singly, solid form fertilizer is effective in the early stages on plant height, growth rate and leaf length. While the liquid form is effective during the later stages on the same parameters to include above ground fresh weight. For these outcomes, these fertilizers can undergo utility model to be commercialized.

Further study may be done to include Photosynthetic parameters such as chlorophyll content, transpiration rate, assimilation rate, vapor pressure deficit, stomatal conductance and total conductance.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support provided by Cebu Technological University for the realization of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Silva MRP, Pinheiro FC, De Paula MT, Prigol M. Parasitological evaluation of lettuce (*Lactuca sativa*) marketed in a municipality of western frontier, RS Brazil Tropical Pathology Magazine. 2015;44(2): 163-169. Available:Https://doi.org/10.5216/rpt.v44i2.36646
2. Martins ACA, Silva L, Santos J, Andrade L, Martins LP. Quality assessment microbiological analysis of lettuce (*Lactuca sativa*) sold in the city of Bananeiras-PB. National Day From Agroindustry, 3 Bananeiras,PB. Annals. Banana Tree UFPB; 2008.
3. Kim MJ, Moon Y, Tou J, Mou B, Waterland N. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). Journal of Food Composition and Analysis. In Press; 2016. DOI: 10.1016/j.jfca.2016.03.004
4. Pedrotti A, Chagas RM, Ramos VC, Prata APN, Lucas AAT, Santos PB. Causes and consequences of the process of soil salinization. Electronic Magazine on Environmental Management, Education and Technology; 2015.

5. Santi A, Carvalho MA, Campos OR, Silva AF, Almeida JL, Monteiro S. Effect of organic material on the production and characteristics of lettuce cultivars. Brazilian Horticultural Magazines. 2010;2(8):87-90. Available:Https://doi.org/10.1590/S0102-05362010000100016
6. Wu L, Jiang Y, Zhao F, He X, Liu H, Yu K. Increased organic fertilizer application and reduced chemical fertilizer application affect the soil properties and bacterial communities of grape rhizosphere soil. Sci Rep. 2020;10:9568.
7. Emer Shannon, Nissreen Abu-Ghannam. Seaweeds as nutraceuticals for health and nutrition, Phycologia. 2019;58:5:563-577 DOI: 10.1080/00318884.2019.1640533
8. Jayasinghe Pradeepa. Effect of seaweed liquid fertilizer on plant growth of *Capsicum annum*. Discovery an International Journal. 2016;52:723-728.
9. Rajkumar Immanuel S, Subramanian SK. Effects of fresh extracts and seaweed liquid fertilizers on some cereals and millets. Seaweed Research Utilization. 1999;21:91.
10. Pascual P, Jarwar A, Nitural PS. Fertilizer, fermented activators, and EM utilization in pechay (*Brassica pekinensis* L.) production. Pak. J. Agri., Agril. Engg. Vet. Sci. 2013;29(1):56-69. Available:https://agris.fao.org/agris-search/search.do?recordID=PK2014000384 ISSN: 1023-1072.
11. Gollan JR, Wright JT. Limited grazing by native herbivores on the invasive seaweed *Caulerpa taxifolia* in a temperate. Australia Estuary Marine and Fresh Water Research. 2006;57:685-694.
12. Yusuf R, Kristiansen P, Warwick N. Effect of two seaweed products and equivalent mineral treatments on lettuce (*Lactuca sativa* L.) Growth. Journal of Agronomy. 2019;18:100-106.
13. Munisamy S. Seaweed plant nutrients to enhance the population and health of earthworm eudriluseugeniae along with crop betterment in chilli *Capsicum annum* (var. Kkm-ch1) under pot culture. Journal of Agriculture and Environment; 2018.
14. Abbas R. Mazhar, Anwar Jahanzeb, Zafar-ul-Hye, Muhammad Khan, Rashid, Saleem, Muhammad, Rahi, Ashfaq, Danish, Subhan, Datta, Rahul. Effect of seaweed extract on productivity and quality attributes of four onion cultivars. Horticulturae. 2020;6. DOI: 28. 10.3390/horticulturae6020028
15. Rathore SS, Chaudhary DR, Boricha GN, Ghosh A, Bhatt BP, Zodape ST, Patolia JS. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. South African Journal of Botany. 2009;75(2):351–355. DOI: 10.1016/j.sajb.2008.10.009
16. Thomas SCL. Production development of sea buckthorn L. T.S.C. product development of sea buckthorn. In: J. Janik and A. whipke (Eds), Trendsin New Crops and New Uses. ASHS, Alexandria. VA. 2002;393-398.

© Copyright International Knowledge Press. All rights reserved.