International Journal of Agriculture Innovations and Research
Volume 9, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 09/12/2020 | Accepted on: 14/01/2021 | Published: 28/01/2021

Green Microalgae, *Chlorella sorokiniana* Promotes the Growth of Chinese Cabbage, *Brassica rapa chinensis* (L.) Hanelt

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***Abstract* – The present study evaluated the growth of *Brassica rapa chinensis* watered with different treatments — T1: tap water; T2: *Chlorella sorokiniana*; T3: tap water with inorganic fertilizer (14% NH_3PO_4, 14% P_2O_3, 14% K_2O); and T4: *C. sorokiniana* with inorganic fertilizer. The *B. rapa chinensis* seeds were planted in a pot and were watered daily. After 10 days, twenty (20) sprouts were randomly selected and transplanted to bigger pots with a ratio of 1 plant per pot. Then, the growth performance of the plant was evaluated for a month. After one month of evaluation, statistical analyses revealed that T4 attained the significantly highest growth performance among the treatments garnering the largest leaf (length = 17.06 ± 0.05; width = 8.89 ± 0.04), and the highest number of leaves (8.6 ± 0.24) ($p < 0.05$). This was followed by T2 and T3, whilst poor growth performance was observed in T1. Overall, the combination of *C. sorokiniana* and chemical fertilizer significantly improved the morphometric growth of *B. rapa chinensis*, however, the effect of this treatment on the plant and soil nutrient content requires further investigation.**

***Keywords* – Bataan, Bio-Fertilizer, Leaf Length, Orani, Pechay.**

I. Introduction

Algae represent a large group of microorganisms that are beneficial for enhancing soil productivity. They fix atmospheric nitrogen and synthesize plant growth [1]. Algae can serve as bio-fertilizers and are known to be a cost-effective replacement for chemical fertilizers [2]. They contain plant-growth regulators including auxins, cytokinins, abscisic acid, and ethylene [3]. *Scenedesmus* spp. and *Chlorella* spp. are good examples of green algae that synthesize plant growth-promoting compounds. The indirect positive effect of algae includes improving the water-holding capacity of soils, and the prevention of anaerobiosis in the root systems [4].

Several studies have been conducted utilizing microalgal treatments as growth promoters for various important crops [5], [6]. Despite the potential of green microalgae for improved soil fertility, there is no known research conducted on the utilization of *C. sorokiniana* as a natural growth promoter for *Brassica. rapa chinensis* (L.) Hanelt. The *B. rapa chinensis* is one of the most important commercial vegetables in the Philippines [7] that has a short cultivation period and can be produced near the market. There is a high demand for this vegetable due to its good taste and high nutritional value. Despite being habitat-generalist, like other cruciferous species, optimum growth can be achieved by planting them in appropriate soil quality (loosely high fertile, well-drained soil, friable and rich in organic matter), and normal weather condition. [8], [9].

To provide new information, this preliminary study investigated the potential of *C. sorokiniana* as a biofertilizer for *B. rapa chinensis*. Specifically, the present research aimed to determine the effect of different fertilizers on height, the diameter of the largest leaf and, the number of leaves of *B. rapa chinensis.*

II. Materials and Methods

A. *Algal Culture*

The *C. sorokiniana* media were prepared (1 inoculant: 4 distilled water ratio) in an Erlenmeyer flask with fertilizer 14-14-14 NPK (14% NH_3PO_4, 14% P_2O_3, 14% K_2O) as a source of the nutrient. The fertilizers were dissolved in water at a rate of 0.1 g L^{-1}. Afterward, the culture was exposed to the sunlight continuously with mild artificial aeration. The salinity was maintained at 6–7 ppt. The chlorella densities were estimated using a hemocytometer following the direct plankton enumeration technique [10]. The cultured chlorella was harvested after 4 days following the protocol [10].

B. *Experimental Set-Up*

The experimental plant used in this study was *B. rapa chinensis*. The seeds were obtained from a local agricultural office in Orani, Bataan. The *B. rapa chinensis* seeds were planted in a pot and were watered daily. After 10 days, twenty (20) sprouts were randomly selected and transplanted to bigger pots with a ratio of 1 plant per pot [11]. The soil used in the pots was mixed in a large container and 5 kg of soils were put in every pot. The pots with soils were randomly positioned in a 4 m^2 lot [12]. Data such as the number of leaves, diameter of the largest leaf, and height of the largest leaf were determined weekly. The study evaluated the growth of *B. rapa chinensis* for one month.

C. *Treatments*

Treatments used in the study are presented in Table 1. Each treatment was replicated five times and was assigned in Completely Randomized Design (CRD). The first treatment application was done 10 days after the emergence [6].

Table 1. Fertilizer treatments used in the study.

Treatments	Description
1	Tap water (control)
2	*C. sorokininia* culture (ca. 50 x 10^7 cells ml^{-1})
3	Commercial inorganic fertilizer (14-14-14) solution
4	*C. sorokininia* culture (ca. 50 x 10^7 cells ml^{-1}) + Commercial inorganic fertilizer solution

In T1, the plants were watered using tap water. In T2, the plants were watered with *C. sorokininia* culture. The cell density of *C. sorokininia* in T2 was estimated using a hemocytometer. In T3, the plants were fertilized biweekly at a rate of 7.5 g of 14-14-14 in every 3.75 L of water [13]. In T4, the plants were treated using the approach used in T2 and T3.

D. *Data Gathered*

Data on the following growth parameters were documented - length of the first leaf (cm), length of the largest leaf (cm), diameter of the largest leaf (cm), and number of leaves of each plant [14].

E. *Statistical Analyses*

Data were analyzed using analysis of variance (ANOVA) to determine the effect of the different fertilizers on the morphometric growth of *B. rapa chinensis* ($p < 0.05$). If ANOVA indicated significant effects, the Tukey's posthoc test was used to determine differences among individual treatment means. All statistical analyses were performed by SPSS (Windows version 15.0).

III. Results and Discussion

The means of the different growth parameters measured are presented in Table 2. In the 1st sampling, the longest 1st leaf (7.22 ± 0.06 cm), largest leaf (10.1 ± 0.04 cm), and the highest number of leaves (3.6 ± 0.24 cm) were all recorded in T2 and T4. Significantly widest leaf was also recorded in T4 (4.3 ± 0.04 cm).

In the final sampling, T4 had the significantly longest 1st leaf (10.2 ± 0.04 cm; $F = 26.38$), largest leaf (17.06 ± 0.05 cm; $F = 68.01$), and widest leaf (8.89 ± 0.04 cm; $F = 19.09$). Leaf width of T4 is statistically homogenous with T2. The number of leaves was significantly highest in T4 (8.6 ± 0.24) and followed by T2 (7.4 ± 0.24).

The present study demonstrated that microalgal treatment using *C. sorokiniana* has a positive effect on leaf morphometry and leaf counts of *B. rapa chinesis*. The finding conforms to the reported growth improvements observed in algal-treated (*C. vulgaris*) food crops including *Lactuca sativa* [5], maize, wheat, and bean [6]. Furthermore, the application of green microalgae, *Nannochloropsis* sp. improved the leaf attributes of tomato and the sugar and carotenoid content of its fruit [15]. It was reported that the application of seaweed liquid fertilizer of *Enteromorpha clathrata* and *Hypnea musciformis* in the soil increases the growth of green gram, black gram, and rice [16]. Similarly, seaweed extract improves the chlorophyll level of cucumber and tomato plants [17].

Apart from the rich nutrient contents of algal fertilizer, microalgae contain cytokinins which induce physiological activities and increase the total chlorophyll count in the plant, which could cause increase photosynthesis activity [12], [17]. They also have auxins that facilitate vitamin and hormone production in the treated plants [18]. In another study published elsewhere [19], the presence of algae tends to improve the nutrient status of the soil that resulted in the increased fresh and dry weight of plants.

As expected, plants treated with fertilizer also showed positive results. Previous works observed that the experimental plants treated with inorganic fertilizer (14–14–14) grew with more leaves and thicker stems compared to those untreated plants [14], [20]. However, there are some prejudices on the use of chemical fertilizers as they can adversely affect plant soil and the environment. Although there is a balanced use of chemical fertilizer, a high yield level could not be maintained over the years because of deterioration in soil physical and biological properties [21]. It is for this reason why organic fertilizers are being used for sustainable farming practices [15].

Significantly greater results in all of the measured growth parameters were achieved in T4. These promising results might be attributed to the complementary effects of microalgae and fertilizer on plants. The nutrients provided by the inorganic fertilizer and the different nutrients in microalgae lead to increased plant height, leaves number, and diameter [22]. Proximate analyses for both the organic fertilizer and the experimental crops are highly recommended. Further study can be done using other highly valuable crops, with emphasis on seasonal variation of other growth parameters, and economic feasibility. Microalgae can also be beneficial not only to plants but also to the environment. The microbial fertilizers from microalgae help to maintain the soil pH and showed that the amount of soil organic matter and water holding capacity were improved and has positive effects on soil, plants, and therefore to the environment [2], [23]. The present study, however, focused only on the efficacy of treatments on leaf attributes of *B. rapa chinensis*. The influence of algal treatments on soil quality is open for further study. Moreover, the application of green algae as a biofertilizer can be included in the integrated farming systems being promoted for sustainable agri-fisheries production in Central Luzon, Philippines [24], [25].

IV. Conclusion

The present study demonstrated the efficacy of *C. sorokiniana*-based fertilizer for improved growth of experimental *B. rapa chinensis* under semi-controlled conditions. This preliminary finding provides a promising option for sustainable farming for mustard using algal-based fertilizers. The study is also hoped to contribute to the conservation of the environment, aiming to lessen the impact of synthetic fertilizer which is consequently spread out to the environment through runoff.

Table 2. Means (± SE) of different parameters of *Brassica rapa chinensis* as affected by different fertilizer treatments. In a row, means with different superscript letter are significantly different ($p < 0.05$). a > b > c > d.

Parameters	Weekly Sampling	Treatments			
		T1	T2	T3	T4
Length of the 1st leaf (cm)	2nd	4.0 ± 0.04b	7.22 ± 0.06a	3.9 ± 0.04b	7.22 ± 0.04a
	3rd	4.48 ± 0.04d	7.84 ± 0.05b	6.18 ± 0.07c	8.98 ± 0.04a
	4th	5.38 ± 0.04d	8.38 ± 0.04b	7.38 ± 0.04c	10.2 ± 0.04a
Length of largest leaf (cm)	2nd	7.36 ± 0.05b	10.1 ± 0.04a	7.46 ± 0.04b	10.1 ± 0.04a
	3rd	9.88 ± 0.04d	13.36 ± 0.04b	10.52 ± 0.07c	14.16 ± 0.07a
	4th	11.18 ± 0.04d	15.42 ± 0.07b	13.92 ± 0.04c	17.06 ± 0.05a
Diameter of the largest leaf (cm)	2nd	2.56 ± 0.07d	4.28 ± 0.03a	3.52 ± 0.04c	4.3 ± 0.04a
	3rd	4.88 ± 0.09c	7.02 ± 0.08a	6.94 ± 0.06b	6.86 ± 0.08b
	4th	7.5 ± 0.01b	8.78 ± 0.09a	7.92 ± 0.06b	8.89 ± 0.04a
Number of leaves	2nd	3.2 ± 0.2b	3.6 ± 0.24a	3.4 ± 0.24ab	3.6 ± 0.24a
	3rd	3.8 ± 0.2c	5.4 ± 0.24b	5.4 ± 0.24b	7.6 ± 0.24a
	4th	4.4 ± 0.2d	7.4 ± 0.24b	6.6 ± 0.24c	8.6 ± 0.24a

Acknowledgments

This project is funded by the Research and Development Office of Bataan Peninsula State University. The authors are appreciative to the Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems (CRAABS) Natural Production Laboratory for the green algae used in the experiment. To the Orani Municipal Agricultural Office for the seeds of experimental *pechay*, and to the anonymous reviewers who devoted their time to improve this manuscript.

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