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Enhanced assimilation rate due to seaweed biostimulant improves growth and yield of rice bean (*Vigna umbellata*)

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Abstract. Rice beans are traditionally planted as intercrop to corn or as the main crop during dry season when corn production is difficult. The use of biostimulants is widely studied to ameliorate the adverse effects of biotic and abiotic stresses. Three possible fermented biostimulants: seaweed, bamboo shoot, and Japanese snail were compared to a commercial organic liquid fertilizer (10 mL L^{-1}) based on morphological, photosynthetic, and yield responses. Fermented seaweed-treated rice bean registered the greatest average vapor pressure deficit (VPD) at 4.33 KPa on the first month and is comparable to the highest average VPD of 4.39 KPa registered by plants applied with fermented Japanese snail on the second month. This interestingly, did not result in difference of transpiration rate (μmol H_2O m^{-2} s^{-1}). Such could be attributed to the plants reduced stomatal aperture when applied with fermented seaweed at 406.80 μmol CO_2 mol stomatal conductance and 38.59 Pa total conductance on the second month. Despite this, the average carbon dioxide assimilation rate of rice beans still increased in both the first (15.26 μmol CO_2 m^{-2} s^{-1}) and second (16.51 μmol CO_2 m^{-2} s^{-1}) month. This increased assimilation rate of fermented seaweed-treated rice beans resulted to about 12 cm increase in height at 128.53 cm (R^2 = 0.894), 0.02 g pod^{-1} (R^2 = 0.978) heavier and 0.90 seeds pod^{-1} (R^2 = 0.978) more when compared to those applied with the commercial liquid organic fertilizer. Thus, by limiting stomatal conductance, despite the differences in VPD, transpiration rate was not affected while significantly increasing assimilation rate to improve production of rice beans, thereby taking full advantage of available seaweed by-products.

Key words: japanese snail liquid fertilizer, morphological responses, organic foliar fertilization, photosynthetic responses.

INTRODUCTION

Rice bean is a neglected crop cultivated in small areas by subsistence farmers in Nepal, Northern India and Southeast Asia. However, it can be grown in diverse conditions and is well known among farmers because of its wide adaptation and productivity even in marginal lands, drought-prone sloping areas, and flat rainfed tars (Joshi et al., 2007). The nutritional quality of rice beans is higher than many other legumes of the Vigna family. The plant has appreciable levels of crude protein with 59–93% protein digestibility, and all essential amino acids (especially methionine, tryptophan, lysine, tyrosine, and valine), minerals, vitamins, and a relatively high proportion of healthy, unsaturated fatty acids make it a nutritious health package (Katoch, 2012).

Farmers commonly use inorganic fertilizers because these are known to increase yield, which also increases income. All the same, it is observed to have severe and detrimental effects on soil, the environment, and other species. For that reason, many farmers turn back to using organic fertilizers. Nevertheless, the organic matter application is essential in improving and sustaining land productivity by enhancing soil's physical, chemical, and biological properties (Lasmini et al., 2019).

Seaweed biostimulant is one of the known organic fertilizers. It is a beneficial marine resource that is affordable and is also rich in various bioactive compounds. Some of those are lipids, proteins, carbohydrates, mineral nutrients, and antimicrobial compounds (Raghunandan et al., 2019). It increases the biochemical constituents of every plant, and this possesses environmental stress mitigating potential. Application of seaweed liquid fertilizer to soil improves soil health by enhancing micronutrient quantity and quality, and microbial activity.

Net assimilation rate (NAR) is used to determine the photosynthetic or growth efficiency in plants. NAR has been used as a growth predictor for some woody and herbaceous plants (Poorter & Nagel, 2000; Shipley, 2006). Photosynthesis results from the assimilation of water and carbon dioxide. The plant absorbs them and converts them into a plethora of organic molecules directly in the plant's numerous cells. A reduced assimilation rate meant that CO_2 and water are less utilized for the production of essential biomolecules. Carbon dioxide assimilation in plants is the most critical key for crop production (Lawlor, 2002).

Thus a study to determine the physiological, morphological and overall yield performance of rice beans is deemed necessary considering that Japanese snail is a major pest of most crops; bamboo shoot is widely abundant in the Philippines; and seaweed by-product is abundant for fermentation since the University is engaged in the production of wellness blends from seaweed. Moreover, rice bean is an integral component in crop rotation mostly utilized as legume in the farming system used by corn farmers in Cebu, Philippines.

MATERIALS AND METHODS

Collection, Preparation, and Fermentation of Organic Fertilizers

The materials used for fermentation were collected at Cebu Technological University - Barili Campus, Barili, Cebu, Philippines. All of the materials were washed to remove dirt.

The seaweed pulp by-product from wellness blend production was added with molasses. A ratio of 1:1 was used following the procedure of Rosit et al. (2015). After seven days of fermentation, all materials were squeezed and filtered through a strainer. Lastly, it was stored in a cool, dry place.

The outer covering of bamboo shoots were peeled off and were chopped into approximate less than 1 cm per piece. Next, water was added at a 1:1 ratio. After 24 hours of soaking, these were squeezed and filtered through a strainer. Lastly, the material was stored in a proper place.

The Japanese snails were smashed, including the shell. These were placed in a container and added with molasses at a ratio of 1:1. The mixture was stirred thoroughly. The container was covered with manila paper and was placed in a cool, dry place. After seven days of fermentation, the mixture was squeezed and filtered through a strainer.

Experimental Site and Plant Samples

The research was conducted inside the greenhouses of Cebu Technological University - Barili Campus (10°7'53" N, 123°32'45" E) with an area of 8.95 m × 6 m. Rice bean seeds, used as the planting material, were secured from a local farmer. To manage possible leakage of the applied foliar fertilizer, 2.5 kg garden soil as growing media were placed in pots spaced 30 cm apart.

Experimental Design and Treatments

This research used a randomized complete block design with four treatments at 15 samples per treatment and replicated three times. The treatments include T0 – commercial natural liquid fertilizer, T1 – fermented seaweed, T2 – fermented bamboo shoot, and T3 – Japanese snail. The concentration of 10 mL L^{-1} biostimulant at a weekly rate used was based on the study of Pascual et al. (2020). Data were analyzed using Analysis of Variance (ANOVA), and a further test was done using Tukey's test at $p < 0.05$ to test for differences between means of treatments using SPSS version 18.

Data Gathered

Li-6800 Portable Photosynthesis System was used to measure plant photosynthetic parameters such as; average vapor pressure deficit, average transpiration rate, average stomatal conductance, average total conductance and average assimilation rate. Morphological parameters such as plant height (from the base to the tip of the plant held vertically), leaf length (from the base of the petiole to the tip of the leaf blade of the middle leaf), and the number of leaves were also recorded together with the yield parameters of grain weight (the weight of grain per pod), pod diameter (the diameter of the base of the pod), number of pods (counting the pods per plant), number of seeds per pod and pod length (from the base to the tip of the pod).

RESULTS AND DISCUSSION

Growth and Yield Responses

Based on Table 1, seaweed biostimulant application significantly produced the tallest plants at 128.53 ± 3.9 cm. at week 8. This resulted by a 12 cm difference in height with the commercial liquid organic fertilizer. Yield response (Table 2) with the application of seaweed biostimulant significantly increased the grain weight at 0.72 ± 0.09 g, number of pods per plant at 95.80 ± 2.14, number of seeds per pods at 9.5 ± 0.31, and pod diameter 0.53 ± 0.31 cm. This result coincides with Pascual et al. (2020) study that the length of shoots outperformed other treatments due to the auxins present in the seaweed extracts that have an influential role in cell division and enlargement. In the study of Das

& Prasad (2015), 18% of the total indole-3-acetic acid (IAA) was present in *Kappaphycus alvarezii* seaweed, responsible for the enhanced growth of the plant. This is the essential auxin in plants, which controls many important physiological processes that include cell enlargement and division, tissue differentiation, and responses to light and gravity (Leveau & Lindow, 2005).

Table 1. Effects on Plant Height (cm), Leaf Length (cm), and Number of Leaves (± *SD*) on Rice beans with the Application of Different Foliar Fertilizers

Parameters	Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Plant Height	T0	12.30 ± 0.12	21.09 ± 0.80	27.90 ± 2.60 a	34.60 ± 1.22 b	92.40 ± 10.23 a	106.33 ± 10.44	107.60 ± 6.92 b	116.00 ± 6.97 b
	T1	12.09 ± 0.13	19.03 ± 1.10	23.40 ± 2.08 ab	30.47 ± 0.70 c	67.27 ± 5.22 b	96.13 ± 3.74	123.80± 3.67 a	128.53 ± 3.91 a
	T2	12.41 ± 0.64	20.82 ± 0.88	26.37 ± 1.59 a	39.93 ± 0.42 a	97.60 ± 2.88 a	113.40 ± 7.8	114.60 ± 1.56 ab	117.33 ± 2.23 b
	T3	11.95 ± 0.50	18.78 ± 0.83	20.67 ± 0.31 b	28.27 ± 2.58 c	66.53 ± 2.87 b	100.33 ± 5.60	113.27 ± 3.70 ab	118.30 ± 1.31 ab
Leaf Length	T0	6.61 ± 0.53 a	7.73 ± 0.37	9.79 ± 0.69 ab	12.77 ± 0.86	13.37 ± 1.20 a	11.40 ± 0.10 ab	11.70 ± 0.35	11.27 ± 0.29
	T1	6.05 ± 0.15 ab	6.88 ± 0.19	8.43 ± 0.32 b	10.46 ± 3.00	11.28 ± 0.48 ab	10.87 ± 0.15 ab	11.43 ± 1.21	10.60 ± 0.56
	T2	6.49 ± 0.03 ab	7.14 ± 0.19	9.99 ± 0.57 a	12.37 ± 0.25	12.63 ± 0.29 ab	11.63 ± 0.55 a	12.10 ± 0.62	10.60 ± 0.56
	T3	5.97 ± 0.19 b	6.85 ± 0.54	8.60 ± 0.57 ab	10.64 ± 0.55	10.67 ± 1.17 b	10.50 ± 0.44 b	10.79 ± 0.61	11.23 ± 0.83
Number of Leaves	T0	4.73 ± 0.23	10.73 ± 0.31	16.87 ± 1.70	25.53 ± 1.81 ab	33.33 ± 1.70	59.13 ± 3.31 b	66.13 ± 5.03 c	69.27 ± 5.61 a
	T1	4.47 ± 0.50	9.73 ± 0.90	15.60 ± 3.47	24.33 ± 2.66 ab	34.60 ± 3.49	61.33 ± 3.20 ab	83.00 ± 1.56 b	85.00 ± 3.49 b
	T2	4.87 ± 0.23	9.33 ± 1.14	24.40 ± 12.41	28.53 ± 1.72 a	35.93 ± 3.10	71.47 ± 3.52 a	103.27 ± 2.53 a	97.07 ± 7.40 b
	T3	4.40 ± 0.53	8.93 ± 0.92	15.13 ± 2.66	22.27 ± 2.73 b	31.53 ± 2.72	51.13 ± 8.33 c	72.57 ± 4.10 c	116.73 ± 5.22 c

Means within a column followed by a common letter is not significantly different from each other at 5% level of significance using Tukeys *HSD*.

Legend: T0 – Commercial Natural Liquid Fertilizer; T1 – Fermented Seaweed; T2 – Fermented Bamboo Shoot; T3 – Fermented Japanese Snail.

Table 2. Growth and Yield Components of Rice bean (± *SD*) as Affected by Different Liquid Organic Fertilizer

Treatments	Grain weight per pods (g)	Number of pods per plant	Number of seeds per pods	Pod diameter (cm)	Pod length (cm)
T0	0.68 ± 0.03a	122.03 ± 3.20a	8.60 ± 0b	0.45 ± 0.12b	8.94 ± 1.2b
T1	0.72 ± 0.09a	95.80 ± 2.14b	9.5 ± 0.31a	0.53 ± 0.31a	9.42 ± 2.90ab
T2	0.61 ± 0.05b	129.47 ± 2.32a	9.47 ± 0.46a	0.44 ± 0.2b	9.57 ± 3.25a
T3	0.52 ± 0.02c	129.36 ± 3.29a	9.00 ± 0.35a	0.46 ± 0.2b	9.11 ± 2.57ab

Means within a column followed by a common letter is not significantly different from each other at 5% level of significance using Tukeys *HSD*.

Legend: T0 – Commercial Natural Liquid Fertilizer; T1 – Fermented Seaweed; T2 – Fermented Bamboo Shoot; T3 – Fermented Japanese Snail.

Moreover, Mazhar et al. (2020) stated in their study that the application of seaweed extracts significantly increase the bulb weight of onion cultivars by 5.8% (Lambada), 5.4% (Red Bone), 2.4% (Phulkari), and 2.0% (Nasar Puri) at 0.5% SWE, respectively. This may be due to the seaweed's auxin, which improved cell division, elongation, and differentiation and the uptake of higher proteins and nucleic acid reserves, leading to an increased grain weight. In addition, nutrient manipulation - such as soil drenches, fertilization, foliar sprays, and systemic nutrient implants - is one way and commonly used to enhance plant health and, as a result, minimize disease severity. (Nadeem et al., 2018). Ahmed et al. (2012) mentioned in their study that the use of seaweed extract drastically improved the growth and yield components of snap beans through spraying or foliar application. His research also stated that the highest values of photosynthetic pigments, N, P, K, and Mg content of leaves, were obtained by spraying seaweed extract at a higher rate.

Bamboo shoot significantly enhanced the leaf length at 12.10 ± 0.62 cm and pod length at 9.57 ± 3.25 cm. This is because bamboo shoots have a high potassium content. Potassium is an essential fertilizer ingredient that is needed for plant development. This factor aids in the development of a good root system and the retention of water. This is in line with Alimento et al. (2021) findings that foliar application of 10 g per 1 L potassium improves soybean yields compared to other treatments. Bamboo shoot is believed to have a plant growth regulator such as gibberellin, stimulating and influencing cell division. Auxin and gibberellins promote vessel tissue growth and cell division, resulting in stem cell enlargement. The study of Carabio et al. (2021) supported this result, where bamboo shoot-based liquid fertilizer alone produced the longest leaves that which is also comparable with the control. It was reported by Gamuyao et al. (2017) that the young tissues of bamboo shoots were reported to have an enrichment of genes associated with the synthesis of DNA and DNA precursors, the cell cycle, cell division, and cell organization kinesins and microtubules. The leaf primordium in plants grows mainly through cell proliferation which will be replaced with an alternative mode of cell cycle activity and endoreduplication that enhances endopolyploidy linked to increased cell size (Gonzalez et al., 2010). This might be the reason why bamboo liquid fertilizer resulted to increased leaf length compared with the other treatments.

For the number of leaves, Japanese snail fertilizer has the highest among all the treatments with 116.73 ± 5.22. In the study of Jatto et al. (2010), the Japanese snail's shell has a chemical composition that includes proteins (amino acids), carbohydrates, fats, and minerals such as iron, zinc, and copper, all of which are essential for the growth as well as the number of leaves. Catubis et al. (2013) found that applying a minimal amino acid (100 ppm) to a native tomato garnered the most leaves on unflooded and minimally flooded conditions. This is also found to be rich in phosphorus, an essential element affecting plants' growth like plant height, leaf number, and shoot dry biomass (Malhotra et al., 2018). The application of organic fertilizer is of great help to soil with crop rotation implemented. In addition, biological means are taken to fight diseases and pests, thus resulting in improved plant growth (Česonien & Rutkoviene, 2009).

Physiological Responses

Result showed based on Fig. 1, A, fermented seaweed-treated rice beans registered the greatest average vapor pressure deficit at 4.33 KPa on the first month and is comparable to the highest average VPD of 4.39 KPa registered by plants applied with fermented Japanese snail. This, interestingly, did not result in differences in average transpiration rate (μmol H_2O m^{-2} s^{-1}) (Fig. 1, B). However, in the study reported by Schoppach et al. (2017) the limited whole-plant transpiration under high VPD in wheat has resulted in advantageous water conservation and crop yield increase which relates to this study's result in which plant treated with seaweed biostimulant increased the plant height with high VPD. This was also supported by Gholipoor et al. (2010) in which studies in species other than wheat also indicate that limited transpiration at high VPD in water-limited environments results in yield increases. Limiting transpiration in this situation will help the plant to conserve water for later use in the crop growing seasons when drought develops (Mura & Vangimalla, 2018).

Such could be attributed to a reduced stomatal aperture of rice beans applied with fermented seaweed at 406.80 μmol CO_2 mol average stomatal conductance (Fig. 1, C) and 38.59 Pa average total conductance (Fig. 1D) on the second month. The feedback hypothesis states that stomatal conductance decreases as VPD increases because of an increase in transpiration that lowers the leaf water potential. The results available for wheat are not consistent with stomatal closure at high VPD being a response to an increased whole leaf transpiration rate or lower leaf water potential. The lack of conductance response to VPD in CO_2 - free air suggests that ABA may mediate the response (Medina et al., 2019). This was also supported by Grossiord et al. (2020), where it was stated that there is an abundance of evidence that suggests that stomatal conductance declines under high VPD, just the same with the total conductance to CO_2.

Photosynthetic carbon assimilation (Fig. 1, E) is directly related to stomatal conductance. Still, this relationship is mediated by the intrinsic water-use efficiency (iWUE = A/gs), so that the response of photosynthesis to VPD depends on the stomatal sensitivity to VPD but also on the extent to which iWUE itself changes as VPD rises. In this case, this study's assimilation rate increases because vapor pressure deficit was also higher in this treatment. The average assimilation rate of rice beans was increased in both the first (15.26 μmol CO_2 m^{-2} s^{-1}) and second (16.51 μmol CO_2 m^{-2} s^{-1}) when applied with fermented seaweed.

This increased assimilation rate of fermented seaweed treated rice beans resulted in about 12 cm taller plants at 128.53 cm (R^2 = 0.894), 0.02 g pod^{-1} (R^2 = 0.978) heavier and 0.90 seeds pod^{-1} (R^2 = 0978) more when compared to those applied with the commercial liquid organic fertilizer, thereby showing strong positive correlation (Fig. 2). In the result conducted by González et al. (2013), Oligo-carrageenans stimulate growth of 3-year-old *Eucalyptus globulus* trees by increasing photosynthesis, nitrogen assimilation, and basal metabolism. This means that application of seaweed liquid organic fertilizer increases assimilation rate which indeed is the reason for the increase on plant height of Rice Bean. In the study of Li et al. (2016), forest trees always had high net assimilation rates and the individuals with high assimilation rates always grew fast. It is one of the reasons why it was reported that net assimilation rate and sustainable growth rate were strongly positively associated with both maximum photosynthetic rate and leaf N concentration, especially when expressed on the basis of leaf area.

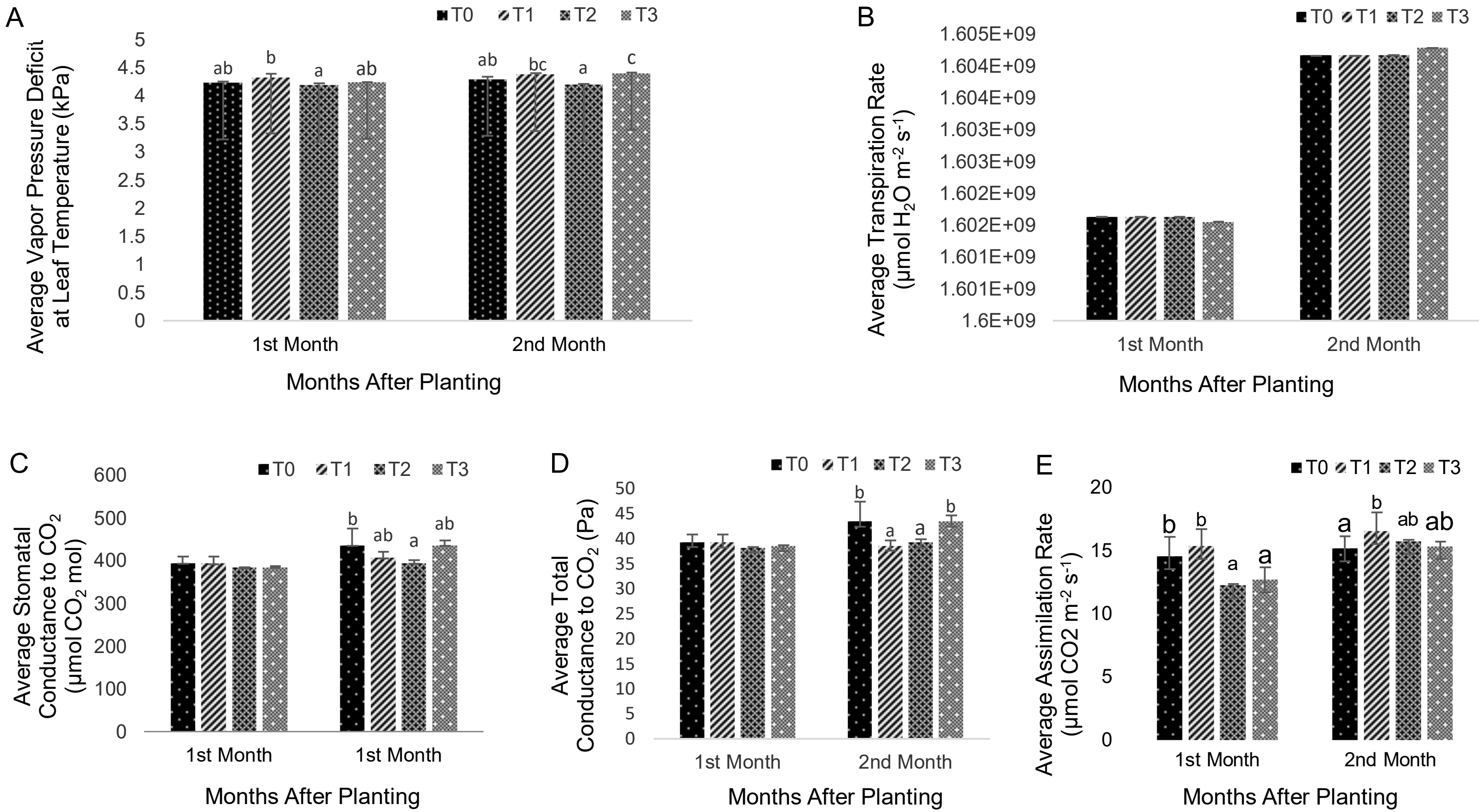
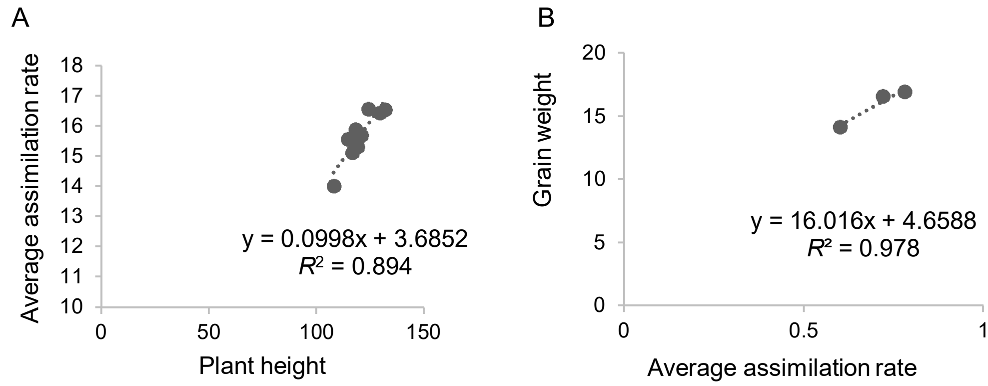


Figure 1. Photosynthetic Responses after application of different treatments on Corn: (A) Average vapor pressure deficit at leaf temperature (B) Average Transpiration Rate (C) Average Stomatal Conductance to CO_2 (D) Average Total Conductance to CO_2 (E) Average Assimilation Rate.



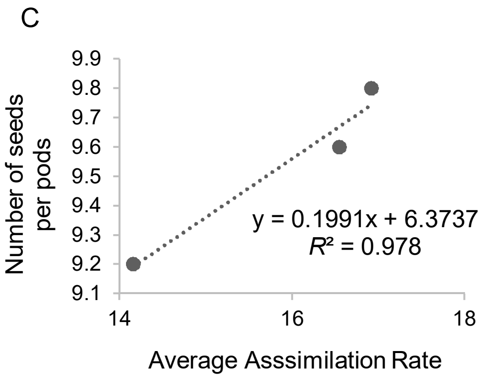


Figure 2. Relationship of Assimilation after the application of Seaweed liquid organic fertilizer; (A) plant height, (B) grain weight and (C) number of seeds per pods.

CONCLUSION

Application of Seaweed biostimulant significantly enhanced the assimilation rate of rice beans thereby resulting to increase in height, heavier pods and more seeds per pod. Bamboo shoot extract produced longer leaves and pods. Fermented Japanese snail increased the number of leaves.

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