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Potential of *Kappaphycus alvarezii* (seaweed) extract as biostimulant on the growth of mangrove propagules (*Rhizophora mangle* L.) through foliar application

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Abstract
This study investigated the potential of seaweed extract of *Kappaphycus alvarezii* as biostimulant in 3% concentration on the relative growth rate, net assimilation rate, and root to shoot ratio of mangrove propagules of *Rhizophora mangle* L. through different day- intervals of foliar application under field experiment. There were two factors included: factor A- types of treatment (A1- water, A2- seaweed extract, A3- commercial biostimulant); and, factor B- different day-interval of application (B1- 4 days, B2- 8 days, B3- 12 days). It was revealed that 3% concentration of seaweed extract of *K. Alvarezii* had no significant effect on the rate growth rate, net assimilation rate, and root to shoot ratio of mangrove propagules of *R. Mangle* L. Moreover, seaweed extract of *K. Alvarezii* as biostimulant in 3% concentration had no significant difference to the commercial foliar biostimulant and to the different day-intervals of application. This study recommends to explore higher concentration of seaweed extract from *K. Alvarezii* on propagules of *R. Mangle* L. and other mangrove species in a greenhouse setting.

Keywords: Seaweed, mangrove, foliar application, biostimulant, propagules

1. Introduction
Kappaphycus alvarezii is a red seaweed of class Rhodophyceae having global recognition for its multiple benefits in food industry, agriculture, economics and others (Abbas *et al.*, 2011) [1]. In the Philippines, farming of this seaweed has been the source of income by the seaweed farmers because of its domestic and global market demand (Hurtado *et al.*, 2014) [24]. In agriculture, seaweed extract has been used as biostimulant through foliar application because of the presence of many nutrients and plant growth regulators (Das & Prasad, 2015) [12]. One of the research focus of Davao del Sur State College- DSSC (formerly known as Southern Philippines Agri- Business, Marine, Aquatic School of Technology- Digos Campus) is on *K. alvarezii* which they developed a foliar biostimulant (Argana, 2016; Perez, 2019) [4, 44].
Seaweed (*K. Alvarezii*) extract has micro and macro nutrients (i.e. potassium, calcium, magnesium, nitrogen & sulphur), growth stimulants, protective activities, cell division controllers as well as root formation stimulants for plants' growth and defense responses (Vasantharaja *et al.*, 2019; Mousavi *et al.*, 2018; Chojnacka *et al.*, 2012; Venkatesh *et al.*, 2011) [66, 36, 11, 67].
Mangroves are considered as one of the most important floras in the world that can easily adapt to extreme environmental conditions (Long & Giri, 2011) [72]. Across more than 100 countries, mangrove ecosystem has been threatened of rapid loss of destruction contributing immense ecological and economical damages (IPBES, 2019; Gevaña *et al.*, 2019; Duke *et al.*, 2007) [25, 23, 16]. In the Philippines, many studies had been reported that 50% of mangrove forest loss as of the year 2000 (Cervantes, 2021; Garcia *et al.*, 2014; Long & Giri, 2011; Spalding *et al.*, 2010; Yparraguire, 2008; Primavera *et al.*, 2004) [10, 22, 72, 62, 71, 50]. In Davao del Sur, mangrove loss in Malalag Bay was almost 100% that is primarily accounted to fishpond conversion (Valle *et al.*, 2000) [65]. Addressing this alarming decline of mangrove forest that attributed by settlements, aquaculture, salt pans, agriculture, industry as well as other conversions and uses, government agencies.

(i.e., Southern Philippines Agri-Business, Marine and Aquatic School of Technology- SPAMAST, Malita Campus) has been implementing programs like the Philippine National Aquasilviculture Program (PNAP) which aims for mangrove reforestation along the costal line of Malalag Bay (Pacyao & Macadog, 2018; Yparraguirre, 2008; Primavera *et al.*, 2004) [41, 71, 50]. Despite of the efforts (i.e. mangrove planting), many studies on mangrove reforestation recorded that results of past reforestation projects experienced high post-planting mortality (Pacyao & Llameg, 2018; Walters, 2004; Primavera & Agbayani, 1997; Pomeroy *et al.*, 1996; Calumpong, 1994; Saenger & Siddiqi, 1993; Lewis, 1990) [41, 69, 51, 45, 8, 55, 28]. These records show that the growth of mangrove propagules was affected by different major challenging factors such as anthropogenic activities and environmental stresses (Pacyao & Llameg, 2018) [41].

One key of successful mangrove restoration is the establishment of mangrove nurseries (Sinohin & Baconguis, 2000) [60]. Mangrove nursery is a place where seeds and propagules are grown under optimal condition that ensures growth and high survival rate limiting the factors of nutrient deficiency (Damasco *et al.*, 2017; Reef *et al.*, 2010; Feller *et al.*, 2003; Feller *et al.*, 1999) [13, 54, 19, 21]. Moreover, many studies on nutrient addition of mangroves used systemic application of biostimulant than foliar application elaborating the function of the root system in the distribution of nutrients to the stem and leaves of plants (Mangora, 2016; Chen & Ye, 2014; Lovelock *et al.*, 2009; Martin, 2007; Lovelock *et al.*, 2004; Parida & Das, 2004; Feller *et al.*, 2002; Feller *et al.*, 1999; Naidoo, 1987) [33, 72, 28, 33, 30, 42, 19, 21, 36]. On the other hand, seaweed (K. alvarezii) extract has been reported that promotes plant growth in various crops but limited on marine plants like mangroves (Babu & Rengasamy, 2012; Karthikeyan & Shanmugam, 2014; Pramanick *et al.*, 2014; Devi & Mani, 2015; Trivedi *et al.*, 2018) [5, 25, 46, 13, 63]. From these points, it could be hypothesized that the seaweed extract of *K. Alvarezii* as biostimulant can significantly increase the growth of mangrove propagules through foliar application. Thus, this study investigated the influence of seaweed extract on the growth of mangrove propagules through foliar application.

Objectives

The study assessed the efficacy of crude extract of *K. alvarezii* as biostimulant on the growth of mangrove (*R. Mangle* L.) propagules through foliar application. Specifically, it determined the: (1) effect of seaweed extract in 3% concentration on the growth of mangrove propagules in terms of relative growth rate (RGR), net assimilation rate (NAR), and root: shoot ratio (R/S); (2) significant difference of seaweed extract to commercial foliar biostimulant; and, (3) significant difference of seaweed extract to differentday-intervals of application.

Materials and Methods

Entry protocol

Permits including the permit for the collection of mangrove propagules and study site were secured from the Office of Punong Barangay of Bulacan that manages the mangrove patches and owns the study site. Moreover, laboratory permit was secured from University of Southeastern Philippines (USeP) - Obrero for the use of dry oven and other laboratory apparatus and equipment.

Establishment of the study site

The location of the study site was 6° 34'06.5"N, 125° 24'46.8"E. It was an open space with two houses present near the site. It was located near the water source that is an advantage when watering the mangrove propagules. There were tall trees in distance that can shade the site starting from 3:00 PM in the afternoon. Establishment of perimeter fence of the 5m x 5 m study area was done using bamboo and hog wires to protect the mangroves from stray animals and coconut fronds was used in three- layer as shade for acclimatization (Melana *et al.*, 2000) [35].

Collection of mangrove propagules

A total of 100 mature and healthy *Rhizophora mangle* L. propagules considerably in same length size were collected from mangrove patches in Sitio Bulo, Bulacan, Malalag, Davao del Sur. Mature propagules were identified having a ring-like mark (abscission layer) below the pericarp (ERDB-DENR, 2010) [17].

Seaweed concentration

Seaweed concentration at 3% concentration was done by diluting 30mL of seaweed extract per 1000 mL of water. This concentration was based on the average result through meta-analysis of 13 studies.

Soil sterilization

Soils were sterilized by roasting method where soils were placed in a pan, kept mix using wood until it reach 100 °C for 1 hr. Temperature was checked using a kitchen thermometer. Soils were set to cool for 24 hr. before planting.

Germination of mangrove propagules

Each mangrove propagules was planted in 50:50 ratio of sandy-loam and organic material (coco-peat) in 0.15 m x 0.20 m polybag. Propagules were sown about 1/3 of its length (Melana *et al.*, 2000) [35]. Propagules were elevated 0.71 m from the ground and placed in rectangular gutter measuring 0.61 m x 1.52 m (50 pots per gutter). These propagules were watered twice a day with groundwater and nursed until 4 to 6 fully developed leaves. This is a significant number of leaves for foliar application (Schreel *et al.*, 2019) [55]. Mangrove propagules having 4 to 6 fully developed leaves were acclimatized by removing one layer of coconut fronds each day.

Experimental set-up

This experiment conducted outside greenhouse (Erftemeijer *et al.*, 2021; Devi & Mani, 2015) [17, 13].

Mangrove propagules were transplanted to pots, randomly placed in each quadrant, and elevated by 0.71 m from the ground. There were two factors on this experiment (factor A: types of treatments and factor B: different day- interval of application). There were 36 mangrove propagules (factor A: 3 treatments x factor B: 3 treatments x 4 replications). Seaweed extract of *K. Alvarezii* was outsourced from DSSC, Digos City. Commercial foliar biostimulant and water (control) were the other treatments. The foliar application was done in the morning (5:00 AM – 6:00 AM) and in the afternoon (5:00 PM – 6:00 PM) using hand-held sprayers with course nozzle as categorized by Southcombe and colleagues (1997) [60]. Improvised three-sided barrier made from transparent cover was used to ensure that the treatments will be applied only to each respective treatment limiting the chance of droplets to

reach the leaves of other treatments. All propagules were watered twice a day (Morning and afternoon) with groundwater (Damasco *et al.*, 2017; Barnuevo & Asaeda, 2018) [12, 6]. This factorial experiment was laid in Randomized Complete Block Design (RCBD) for the field trial experiment with Factor A as type of treatments: A1- water as control, A2- seaweed Extract and A3- commercial foliar biostimulant while different day- intervals: B1- 4 days (Singh, 2018) [58], B2- 8 days and B3- 12 days (Kumar, 2015) [26] of application were served as Factor B.

After 60 days from the start of application, data were gathered to determine the growth rate (RGR), net assimilation rate (NAR), and root to shoot ratio (R./S). All 36 mangrove propagules were harvested and dried oven for 72 h at 60 ˚C. Weights were measured in grams (g) before and after the drying process. Separation of roots and top part and measuring of leaf area using the counting grid method were done prior to the drying process. In addition, separate 18 mangrove propagules were harvested to determine the initial total average biomass including the dried weights for the root and shoot as well as the total average of leaf area in the beginning of the experiment. These are the formulas to be used for each growth key indices suggested by Price and Munns (2016) [51].

For rate of growth rate (RGR),

$$RGR = \frac{\ln W2 - \ln W1}{t2 - t1}$$

Where W1 and W2 are the initial and terminal biomass, respectively. On the other hand, t1 and t2 are the time intervals.

For net assimilation rate (NAR),

$$NAR = \left[\frac{W2 - W1}{t2 - t1}\right]\left[\frac{\ln A2 - \ln A1}{A2 - A1}\right]$$

Where A1 and A2 are the initial and terminal leaf area, respectively.

For root: shoot ratio (R/S), dry weight for roots was divided by dry weight for top of plant. The final R/S was derived by getting the difference of the terminal R/S minus initial R/S.

Statistical design

Normality of data was determined and all data were normal. Parametric test like the two- way analysis of variance (ANOVA) using R was done.

Results and Discussion Effect of seaweed extract on the growth of *Rhizohora mangle* L.

Seaweed extract had been used as biostimulant in 3% concentration on the growth of mangrove propagules (*Rhizophora mangle* L.) through foliar application showing that relative growth rate obtained 0.65% as the highest percentage value among selected growth indices (Table 1).

Table 1: Types of treatments and percentage increase of treatments in comparison to A1 on the growth of mangroves

Type of treatments	RGR	NAR	R/S
A1- water	0.0070	0.0013	-0.0873
A2- seaweed extract	0.0065	0.0012	-0.1082
A3-commercial biostimulant	0.0067	0.0014	-0.1036
Percent increase of treatments in comparison to A1			
A2 against A1	-7.0328	-7.4584	23.9638
A3 against A1	-4.8354	2.0313	18.7287

Table 2: Relative growth rate in two-way ANOVA using R.

	DF	Sum SQ	Mean SQ	F value	Pr (>F)
Factor_ A	2	1.5000e-06	7.6900e-07	0.0500	0.9510
Factor B	2	3.6500e-05	1.8230e-05	1.1880	0.3200
Residuals	27	4.1440e-04	1.5350e-05		

Moreover, it shows that 23.96% increase was observed on the root to shoot ratio of mangrove propagules when applied with seaweed extract in comparison to the control group (A1- water). However, seaweed extract does not have significant effect on the relative growth rate (RGR), net assimilation rate (NAR), and root to shoot ratio (R/S) of mangrove propagules of *R. mangle* L.

In terms of RGR, results showed that there is no significant difference among treatments that can be observed in Table 2 where factor A had higher p-values than the set significant value of 0.05.

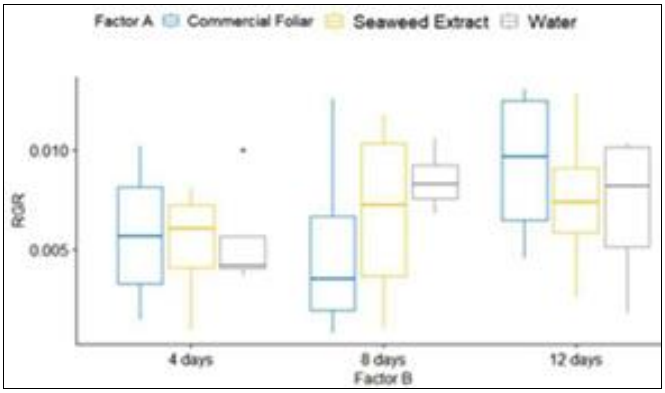

Fig 1: Box plot for relative growth rate under Factor A and Factor B using R

However, the data among factor A (A1- water, A2- seaweed extract, & A3- commercial biostimulant) is shown in Figure 1 presenting that seaweed extract had consistently varied among other treatments.

The determinant of RGR is the increment of plant's biomass over a period of time. Moreover, foliar application could possibly be one of the determinants of the result. It was suggested that correct diagnosis of nutrient deficiency in soil media is a fundamental procedure in determining the success of foliar application of nutrients. Conversely, there was no soil analysis conducted since soil analysis for foliar application is not necessary (Alshaal & El-Ramady, 2017) [3]. The response of *R. mangle* L. in terms of its RGR under different treatments of nutrient addition (i.e., seaweed extract,

commercial biostimulant, and water) was consistent to the result of the study of Manea and colleagues (2018) [31] that reported seaweed extracts and commercial biostimulant were ineffective to influence significant increase in total biomass of broccoli. Moreover, Ponteras (2020) [45] reported that the total biomass of cucumber in field experiment under different biostimulants including the extract of K. alvarezii through systemic and foliar application had no significance. Furthermore, these findings are also similar to the mesocosm experiment of Mangora (2016) [32] where it was found that nutrient addition of N, P, K (20-20-20) had no influence on biomass accumulation of mangrove seedlings (Heritiera littoralis Dryand). All of the above studies suggest that nutrient addition under different level of nutrient concentration (i.e., low concentration and high concentration), methods of application (i.e., foliar application and systemic application) and the type of experiment (i.e., field experiment and potted experiment) of any plant species remarked no influence on relative growth rate of plant. According to Nemali and van Iersel (2004) [37], the application of nutrient addition in high concentration under high light intensity has low effect on plant and this could not be confirmed by plant's biomass. In this statement, this can strongly support the result of this present study that seaweed extract of K. alvarezii has no influence to the relative growth rate of *R. Mangle* L. Since the experimental setting of this study is outside laboratory where contributing factors like high light intensity can also be observed specifically the concentration of seaweed extract was low. Moreover, the efficacy of seaweed extract is attributed to the presence of bioactive chemicals including potassium, cytokinin and auxin (Prasad *et al.*, 2010; Chojnacka *et al.*, 2012) [47, 10]. Conversely, Norrie and Keathley (2006) reported that seaweed extract (A. nodosum) had a significant effects on the yield of grape (*Vitis vinifera* L.) in terms of fruits size (13% increase), yields (60.4% increase), and weight (39% increase). It was found also that there is a significant increase on the yield of grain by 11.80% for grain plants that received foliar application of K. alvarezii and 9.52% when applied with *Gracilaria* sp.

Table 3: Net assimilation rate in two-way ANOVA using R.

	DF	SUM SQ	Mean SQ	F value	Pr (>F)
Factor_ A	2	1.0700e-07	5.3600e-08	0.0710	0.9320
Factor B	2	2.6800e-05	1.3400e-06	1.7650	0.1900
Residuals	27	2.0500e-04	7.5940e-07		

In terms of NAR (Table 1), results show that seaweed extract only obtained a numerical mean value of 0.12% that is a higher value than root to shoot ratio; moreover, seaweed extract obtained no percentage increase in comparison to the control group (A1- water). Futhermore, seaweed extract had no significant effect on the NAR of mangrove propagules of *R. mangle* L. that can be observed where factor A have higher p-values than the set significant value of 0.05 (Table 3). However, data for factor A is shown in Figure 2 presenting that seaweed extract had considerably varied.

In terms of NAR (Table 1), results show that seaweed extract only obtained a numerical mean value of 0.12% that is a higher value than root to shoot ratio; moreover, seaweed extract obtained no percentage increase in comparison to the control group (A1- water). Furthermore, seaweed extract had no significant effect on the NAR of mangrove propagules of *R. Mangle* L. that can be observed where factor A have higher p-values than the set significant value of 0.05 (Table 3). However, data for factor A is shown in Figure 2 presenting that seaweed extract had considerably varied.

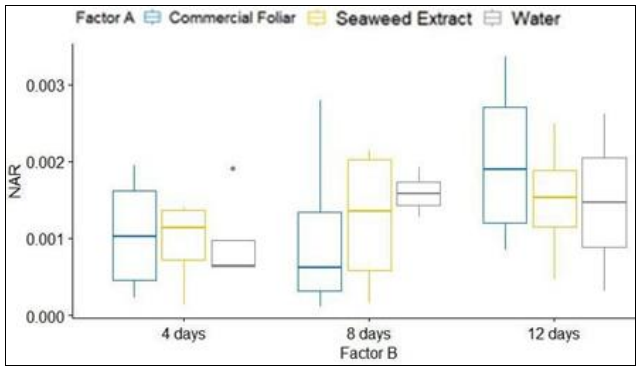

Fig 2: Box plot for net assimilation rate under Factor A and Factor B using R

In terms of R/S (Table 1), results show that seaweed extract obtained the numerical mean value of -10.82% that is the least value among selected growth indices of mangrove propagules of *R. Mangle* L.; however, R/S obtained the highest percentage increase of 23.96% among selected growth indices of mangrove propagules of *R. mangle* L. when applied with 3% concentration of seaweed extract in comparison to the control group (A1-water).

Table 4: Root and shoot ratio in two- way ANOVA using R

	DF	Sum SQ	Mean SQ	F value	Pr (>F)
Factor_ A	2	2.9000e-03	1.4510e-03	0.6100	0.5500
Factor B	2	2.7300e-03	1.3640e-03	0.5740	0.5700
Residuals	27	6.4190e-02	2.3770e-03		

Moreover, seaweed extract had no significant effect on the R/S of mangrove propagules of *R. mangle* L. that can be observed where factor A have higher p-values than the set significant value of 0.05 (Table 4). However, data for factor A shown in Figure 3 presenting that seaweed extract had considerably varied. This result can be attributed to the investment of nutrient between root and shoot. Possible factors includes plant's anatomy and physiology as well as environmental factors affecting foliar application including light, time of day, amount and intensity of precipitation and others. Beckett and Van Staden (1989) [7] reported that an adequate of K supply did not show significant increase on the roots of plants. The role of potassium in plants is essential in growth and development of root morphology (Du *et al.*, 2017) [14]. Moreover, cytokinin enhances shoot proliferation and auxin enhances the root proliferation (Wang & Charles, 1991; Ngomuo *et al.*, 2013) [69, 38].

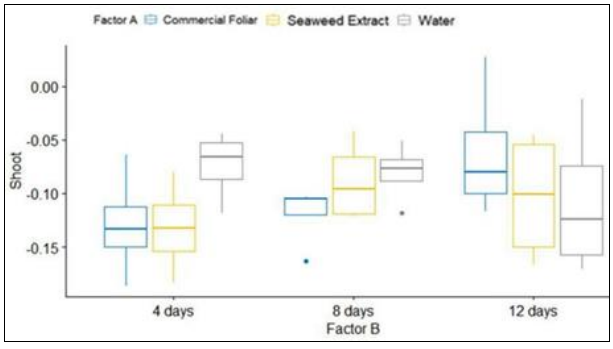

Fig 3: Box plot for root and shoot ratio under Factor A and Factor B using R

Furthermore, cytokinin and auxin protect plant from unfavorable temperature, responsible for controlling bud and cell division, and initiates root formation and elongation (Tarakhovskaya *et al.*, 2007) [62]. Generally, the biomass of root of the plant can be affected when there is a greater demand of energy from the above-ground parts of the plants. In the same manner, above-ground biomass is higher when there is a sufficient supply of nutrient from the roots.

Difference of seaweed extract to commercial foliar biostimulant

Seaweed extract had been obtained lesser total numerical mean value than to commercial foliar biostimulant where it obtained 0.49% as total numerical mean value of the three growth indices. In comparison to seaweed extract, commercial foliar biostimulant obtained higher numerical mean differences: 0.015%, 0.013% and 0.45% for RGR, NAR, and R/S, respectively. Unlike to the seaweed extract that only obtained percentage increase on R/S, commercial foliar biostimulant obtained percentage increase on the NAR (2.03%) and R/S (18.73%) in comparison to the control group (A1- water). Despite of the number of percentage increase with respect to the control group for both seaweed extract and commercial foliar biostimulant, R/S of mangrove propagules of *R. Mangle* L. had a higher percentage increase of 23.96% when applied with seaweed extract than the commercial foliar biostimulant (Table 1). However, seaweed extract had no significant difference to commercial foliar biostimulant on the RGR, NAR, and R/S of mangrove propagules of *R. Mangle* L. that can be observed where factor A have higher p-values than the set significant value of 0.05 (Table 2, 3, & 4). Furthermore, data for factor A shown in Figures 1, 2, and 3 presenting that commercial foliar biostimulant had considerably varied across RGR, NAR, and R/S of mangrove propagules of *R. Mangle* L.

One major factor influencing the different results on the RGR, NAR, and R/S of mangrove propagules of *R. mangle* L. is the difference of nutrient contents of the two treatments (A2- seaweed extract & A3- commercial foliar biostimulant) wherein seaweed extract of K. alvarezii contains N (0.07%), P (0.028%) and K (1.70%) whereas commercial foliar biostimulant contains N (3%), P (16%), and K (9%). Generally, nitrogen has been attributed on the development of plant structures where plant intakes nitrogen in producing amino acid and protein. Phosphorus also plays a crucial role in carbon assimilation by participating as adenosine diphosphate (ADP) and adenosine triphosphate (ATP) in energy storage and transfer. Potassium is used for plants as a requirement in the transport of sugars as well as regulates the entry of carbon dioxide that is important in photosynthesis. This means that these nutrients directly affect RGR, NAR, and R/S of plant knowing that nitrogen, phosphorus and potassium participate on carbon assimilation and photosynthetic efficiency of plant where RGR and R/S determines the carbon assimilation of plant while NAR determines the photosynthetic efficiency of plant; moreover, these growth indices linearly affected one another (Du *et al.*, 2017; Chojnacka *et al.*, 2012; Prasad *et al.*, 2010) [14, 10, 47]. The result of this present study agrees to the report of discussing the morphological characters and growth indices of plant can be affected by the presence of macro and micro nutrients as well as growth promoting substances in seaweeds. El-Hadidi and colleagues (2010) [16] said that plant biomass of crops (i.e., cucumber) can be influenced by the different levels of nutrients from organic biostimulants; moreover, Prakash and Arora (2020) [48] claimed that commercial biostimulants can bring a significant change on plant's growth. Conversely, Shehata and colleagues (2016) [57] noted that nutrients of seaweed extract had significant increase on the plant biomass of celeriac plants. Moreover, Shafeek and colleagues (2015) [56] reported plant biomass of onion had significant increase when applied with seaweed extract. It is also interesting to note that when there is an increase of plant biomass it has direct effect on RGR, NAR, and R/S of plants since plant biomass is a requirement in determining these three growth indices.

Table 5: Difference of growth indices applied with seaweed extract and commercial biostimulants

Growth indices	A2- Seaweed Extract	A3- Commercial Biostimulants	Percentage difference of commercial biostimulants to seaweed extract
RGR	0.6540	0.6695	0.0155
NAR	0.1238	0.1365	0.0127
R/S	-10.8198	-10.3628	0.4569
Total mean	-10.0420	-9.5569	0.4851

Difference of seaweed extract to different day- intervals of application

Seaweed extract had been obtained the highest numerical mean value of 0.76% among the three different day- intervals of application when applied with seaweed extract in terms of RGR of mangrove propagules of *R. Mangle* L. However, results in 12 days- interval that seaweed extract obtained the least numerical mean value of 0.65% across different day- intervals of application in comparison to other treatments. In terms on the NAR of mangrove propagules of *R. Mangle* L., results in 12 days- interval obtained the highest numerical mean value of 0.15% among the three different day- intervals of application when applied with seaweed extract; however, seaweed extract obtained the least numerical mean value of 0.12% across different day- intervals of application in comparison to other treatments. In terms on the R/S of mangrove propagules of *R. Mangle* L., results in 8 days- interval of application obtained the highest numerical mean value of -0.09% among the three different day- interval of application when applied with seaweed extract; however, seaweed extract obtained the least numerical mean value of -10.82% across different day- intervals of application in comparison to other treatments Moreover, seaweed extract had no significant difference to different day- intervals of application on RGR, NAR, and R/S of mangrove propagules of *Rhizophora mangle* L. that can be observed in Figure 1, 2 and 3 where factor B have higher p-values than the set significant value of 0.05.

The result of the study considers that timing of foliar application could influence the effect to the RGR of mangrove propagules of *R. mangle* L. because according to Alexander (1986) [2] noted that time should be considered a critical factor in relation to the optimum efficacy of the foliar treatment; however, all three different day- intervals of application in this present study had no significant effect on the RGR, NAR, and R/S of mangrove propagules of *R. mangle* L. This result could be attributed in the rate of absorption of a particular element to the leaf tissue. The optimal success of foliar application of nutrient can be affected also by different endogenous factors (i.e., leaf anatomical structure). This means that leaf absorption

efficiency applied with foliar nutrients depends on the thickness of the cuticle, lower and upper leaf surfaces, green shoots, number of cuticular pores as well as ectodesmata or ectoteichodes (Alshaal & El-Ramady, 2017) [3]. Time is crucial in determining net assimilation rate specifically the time interval of data gathering of leaf area from the initial data to terminal data. This is because if the plant has numerous senesced leaves, net assimilation rate could not be accurate (Vernon & Allison, 1963) [67]. Moreover, investment of nutrients between root and shoot is greatly influence of the age of the plant. Conversely, it could not be possible that the result can be attributed to the time duration since there were no senesced leaves observed and all mangrove propagules have the same number of leaves. Furthermore, precipitation after foliar application could be the major attributor of the result as remarked that precipitation within 48 hours after application may reduce foliar efficiency such that not all nutrient materials are immediately absorbed into the plant tissue.

Conclusion

The application of seaweed extract (*Kappaphycus alvarezii*) as biostimulant under different day -intervals of foliar application had no significant increase on the relative growth rate (RGR), net assimilation rate (NAR), and root to shoot ratio (R/S) of mangrove propagules (*Rhizophora mangle* L.).

Recommendation

This present study recommends the following: (1) apply of 3% concentrations of seaweed extract (*Kappaphycus alvarezii*) on mangrove propagules of *Rhizophora mangle* L. under greenhouse set-up; (2) validate and evaluate the same test plant to be applied with different concentrations of seaweed extract (*K. alvarezii*); and, (3) test other mangrove species to be applied with seaweed extract (*K. alvarezii*).

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