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SUSTAINABLE APPLICATION OF PLANT BIOSTIMULANT DERIVED FROM *KAPPAPHYCUS ALVAREZII* EXTRACT RESIDUES FOR COCOA PLANTLET GROWTH ENHANCEMENT

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ABSTRACT – *The growing demand for sustainable agricultural practices has intensified interest in plant biostimulants as eco-friendly alternatives to chemical fertilizers, which pose risks to human health and the environment. This concern is particularly relevant to the cocoa industry, where optimizing plant growth while minimizing environmental impact remains a priority. Kappaphycus alvarezii, the primary cultivated seaweed species in Sabah, is widely utilized for carrageenan extraction and has garnered attention for its potential as a plant biostimulant due to its abundance of nutrients, phytohormones, and bioactive compounds. However, the large volume of residues generated during carrageenan extraction is often discarded, representing an underutilized resource. This study evaluates the efficacy of K. alvarezii extract residues which obtained after carrageenan extraction through chemical, physical, and biological methods, as plant biostimulants to enhance the growth performance of cocoa plantlets under nursery conditions. The residues, containing macronutrients and micronutrients at specific concentrations, were formulated into different biostimulant treatments and applied over a three-month plant trial. Results demonstrated that several formulations significantly improved key growth parameters, including plant height, leaf number, branch development, and root length, compared to the negative control. Among these, Treatment C1 (100% chemical residue extract) exhibited the most pronounced effect on cocoa plant growth. This study highlights the potential of seaweed extract residues as sustainable plant biostimulants, offering an eco-friendly approach to enhancing cocoa productivity while contributing to effective zero-waste management in the seaweed industry.*

Keywords: *Kappaphycus alvarezii*, carrageenan extraction, seaweed waste, plant biostimulant, cocoa plantlets

INTRODUCTION

Cocoa (*Theobroma cacao L.*) is known to be a perennial tropical tree and an important commodity crop in Malaysia. Cocoa cultivation is primarily driven by the economic value of its beans, which are processed into secondary products such as cocoa liquor, cocoa butter, and cocoa mass (Paparella *et al*., 2025). These products serve as essential raw materials in the food industry to produce chocolate and a wide range of cocoa-based products. Beyond food applications, these products are also utilized in non-food industries, including cosmetics, pharmaceuticals, and as sources of natural antioxidants (Nuraziawati *et al*., 2023). Given this wide spectrum of uses, cocoa plays a significant role in economic development. For instance, in the first quarter of 2025, the Department of Statistics Malaysia (DOSM) reported that the cocoa sector contributed approximately RM600 million to the national gross domestic product (GDP) (Ministry of Plantation and Commodities, 2025).

Up until today, national cocoa industry still faces a production-demand gap. As evidenced recently, the 2024 production figures showing only 445.37 tonnes of locally produced cocoa beans, whereas the volume of processed cocoa beans domestically used to manufacture secondary cocoa products (such as cocoa liquor, cocoa butter, and cocoa mass) reached 365,605 tonnes (Malaysian Cocoa Board, 2025), indicating the heavily dependance on imports cocoa beans.

Cocoa productivity is influenced by multiple factors, including pest and disease infestations, poor soil conditions, limited agricultural inputs, and climate change (Rozita, Tee, & Mohammed Helmi, 2024; Kongor *et al*., 2024). Among the major biological threats are the cocoa pod borer (*Conopomorpha cramerella*), *Phytophthora palmivora* (black pod disease), and *Oncobasidium theobromae* (vascular streak dieback). Climate change further exacerbates these challenges, with rising temperatures accelerating evapotranspiration and water deficits (Tee *et al*., 2022), while heavy rainfall and humidity favor fungal outbreaks, especially *P. palmivora* (Kongor *et al*., 2024). To sustain yields, good agricultural practices such as timely fertilizer and pesticide application are essential; however, the rising costs of agrochemicals (Boney *et al*., 2023) highlight the need for sustainable alternatives like plant biostimulants, which can enhance productivity while supporting smallholder livelihoods.

According to European Biostimulants Industry Council (EBIC), plant biostimulant is a product which stimulates plant nutrition processes independently of the product's nutrient content, with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere(i) nutrient use efficiency, (ii) tolerance to abiotic stress, (iii) yield and quality traits, and (iv) availability of confined nutrients in the soil or rhizosphere. This functional distinction differentiates plant biostimulants from fertilizers, as fertilizers primarily serve to supply nutrients, whereas biostimulants act to enhance plant growth and development through physiological stimulation (du Jardin, 2015; Rouphael & Colla, 2018).

Since the early 1980s, seaweeds have been extensively investigated for their potential as plant biostimulants and currently represent a major segment of the organic biostimulant market. Seaweed extracts are rich in essential macro- and micronutrients, amino acids, vitamins, and phytohormones such as auxins, cytokinins, and abscisic acid, all of which play critical roles in regulating cellular metabolism and enhancing plant growth and productivity (Patel *et al*., 2017). Empirical studies have consistently demonstrated the beneficial effects of seaweed extracts on key growth parameters of various crops such as medicinal plants, chili, tomato, and cowpea (Shamya Arokia Rajan *et al*., 2020; Suchithra *et al*., 2022; Uthirapandi *et al*., 2019; Vasantharaja *et al*., 2019). In addition, seaweed-based biostimulants have been shown to improve seed germination (Patel *et al*., 2018; Shamya Arokia Rajan *et al*., 2020) and to enhance plant tolerance against abiotic stresses including drought, low temperature, and salinity (Dehkordi *et al*., 2021; do Rosário Rosa *et al*., 2021; Patel *et al*., 2018).

In cocoa cultivation, the application of plant biostimulant is not widely adopted yet but recent studies have begun to demonstrate their potential benefits for both seedlings and mature cocoa plants. For example, an *Ascophyllum nodosum*-derived biostimulant was shown to enhance cocoa seedling growth by increasing shoot height, stem diameter, leaf number, and shoot and root dry mass, due to its rich composition of free amino acids and nitrogen compounds (Freitas *et al*., 2024). Similarly, polysaccharide-based biostimulants have been associated with improved cocoa productivity, a critical challenge in the sector (Torres-Rodriguez *et al*., 2024; Valleser *et al*., 2025). Chitosan- and carrageenan-based formulations have further demonstrated efficacy in reducing disease severity caused by pathogens such as *Moniliophthora roreri* and *Phytophthora palmivora* (Torres-Rodriguez *et al*., 2024; Valleser *et al*., 2025). In addition, a biostimulant derived from citrus industry waste significantly improved the rooting and survival of cocoa cuttings, serving as an effective alternative to indole-3-acetic acid (IAA) in Murashige and Skoog (MS) medium (Reyes-Perez *et al*., 2022).

Seaweeds are widely exploited for the extraction of high-value bioactive compounds such as alginate, carrageenan, alginic acid, and fucoidan. However, these processes generate substantial amounts of residues, which are often discarded as waste. For example, carrageenan extraction from *Kappaphycus alvarezii* via alkaline treatment has been reported to leave behind 60–70% of solid residues (Uju *et al*., 2015), most of which are typically disposed of in landfills, contributing to environmental pollution. Valorizing these residues as plant biostimulants offers a dual benefit of promoting sustainable agriculture and improving waste management within the seaweed industry. Notably, such residues may retain considerable amounts of nutrients and bioactive compounds, yet their biostimulant potential remains underexplored compared to raw seaweed.

In this study, *Kappaphycus alvarezii* was subjected to carrageenan extraction using three methods: chemical, physical, and biological treatments. The chemical method employed alkaline reagent, namely potassium hydroxide, which induced desulphation of carrageenan by removing sulphate groups at the 6-position of galactose units, forming recurring 3,6-anhydro-D-galactose, with potassium ions stabilizing the charges. Potassium chloride was then added to precipitate the extracted carrageenan. For physical extraction, microwave-assisted treatment was applied to disrupt algal cells, enabling efficient carrageenan release while reducing solvent and energy use. Biological extraction utilized cellulase enzymes to degrade cell walls and release carrageenan. All three methods generated substantial residues, which formed the basis of this study to evaluate the potential of *K. alvarezii* extract residue-based biostimulants in improving cocoa seedling growth under nursery conditions.

MATERIALS AND METHODS

Carrageenan Extraction

Fresh *Kappaphycus alvarezii* samples were carefully selected and washed to remove the impurities. The selected seaweed was firstly rinsed with tap water followed by distilled water to ensure thorough cleaning. Subsequently, the samples were air-dried in an oven at 40 °C for approximately four days. The dried material was then subjected to an additional washing step by soaking in distilled water for 45 minutes to eliminate residual salts, followed by oven drying at 60 °C for 24 hours. The prepared samples were then used for carrageenan extraction (Tye *et al*., 2018).

Chemical Extraction Method

Carrageenan extraction via chemical treatment was performed following the methods of Solorzano-Chavez *et al*. (2019) and Liu *et al*. (2022), with slight modifications. Approximately 35 g of dried seaweed were pretreated with 6% potassium hydroxide (KOH) for 24 hours at 25 °C. The pretreated seaweed was then separated from the alkaline solution by sieving and subsequently oven-dried overnight. To remove residual KOH, the dried samples were washed twice with 1 L of distilled water for 10 min each and further dried at 60 °C.

For extraction, 5 g of the dried seaweed were milled using a crusher and transferred into an Erlenmeyer flask containing 400 mL of distilled water. The mixture was incubated in a shaker incubator at 65 °C and 120 rpm for 2 hours. The extract was filtered through a strainer to separate the residue from the filtrate. The filtrate was then precipitated with 5% potassium chloride (KCl) at a 1:1 volume ratio. The carrageenan obtained in gel form was separated from the solvent using a filter cloth. Both carrageenan and the remaining residue were oven-dried, with the residue being washed with distilled water prior to drying to remove excess KOH.

Physical Extraction Method

Microwave-assisted extraction was employed as the physical method for carrageenan recovery, following Ponthier *et al*. (2020) with modifications. Briefly, 5 g of dried, milled *K. alvarezii* were mixed with 150 mL of distilled water in a beaker at a solid-to-liquid ratio of 1:30 (w/v). The mixture was subjected to microwave heating using a commercial digital microwave oven (SHARP R607EK) at 50P power for 6 minutes, during which the temperature reached approximately 100 °C (boiling point of water). Following heating, the sample was cooled to 55 °C and subsequently filtered through a strainer to separate the liquid fraction containing carrageenan from the solid residue. Carrageenan was then precipitated from the liquid extract by adding ethanol at a 1:1 (v/v) ratio, with continuous stirring to ensure complete precipitation. The precipitated carrageenan was further collected by straining.

Biological Extraction Method

Biological extraction of carrageenan was conducted based on the methods of Tarman *et al*. (2020) and Varadarajan *et al*. (2009), with minor modifications. Dried seaweed (20 g) was initially heated at 60 °C for 20 minutes to promote cell expansion and facilitate cell wall disruption. Subsequently, 0.1 g of cellulase was added, and the mixture was incubated in a shaking water bath at 60 °C for 1 hour. The resulting suspension was centrifuged at 5000 rpm for 15 minutes at 4 °C to separate the filtrate from the residue. Carrageenan was precipitated from the filtrate by adding 2-propanol at a 1:1 (v/v) ratio, followed by centrifugation at 12,000 rpm.

Effectiveness of Seaweed Extract Residues as Plant Biostimulant on Cocoa Plantlets

Experimental design

The aqueous extract of seaweed residues was prepared according to the methods described by Fayzi *et al*. (2020) and Mohanty & Adhikary (2018), with slight modifications. Dried seaweed residue samples were suspended in distilled water at a 1:100 (w/v) ratio and incubated at 37 °C with continuous shaking at 150 rpm for 72 h. The mixtures were filtered through Whatman filter paper, and the resulting supernatants were designated as 100% stock solutions.

The experiment was arranged in a Completely Randomized Design (CRD) with four different formulations prepared for each extraction method— Chemical (C1–C4), Physical (P1–P4), and Biological (B1–B4)—as outlined below and summarized in Table 1. All formulations were tested on cocoa plantlets (Helaly, 2021; Prakash *et al*., 2018).

Table 1: Treatment formulations

Treatments	Formulation
F1	No treatment (negative control)
F2	100% chemical fertilizer (positive control)
C1	100% chemical extract residue
C2	80% chemical extract residue + 20% chemical fertilizer
C3	99.5% chemical extract residue + 0.5% humic acid

C4	79.5% chemical extract residue + 20% chemical fertilizer + 0.5% humic acid
P1	100% physical extract residue
P2	80% physical extract residue + 20% chemical fertilizer
P3	99.5% physical extract residue + 0.5% humic acid
P4	79.5% physical extract residue + 20% chemical fertilizer + 0.5% humic acid
B1	100% biological extract residue
B2	80% biological extract residue + 20% chemical fertilizer
B3	99.5% biological extract residue + 0.5% humic acid
B4	79.5% biological extract residue + 20% chemical fertilizer + 0.5% humic acid

The stock solution was subsequently utilized to prepare treatment formulations, as outlined in Table 1. These treatments comprised different combinations of seaweed residue extracts with chemical fertilizer, humic acid, or both. Stock solutions of chemical fertilizer (YaraTera KRISTALON 18-18-18 + 3MgO + micronutrients, a balanced NPK formulation suitable for foliar application and greenhouse cultivation) and humic acid (Sigma-Aldrich) were prepared according to the manufacturers' instructions and diluted to the required concentrations for the respective treatments (Helaly, 2021; Prakash *et al*., 2018).

Plant Treatments

Prior to germination, the mucilage of wet cocoa seeds was completely removed using sawdust, followed by thorough rinsing with tap water. The cleaned seeds were subsequently sterilized with a fungicide to prevent fungal contamination. After sterilization, the seeds were placed on wet sackcloth to promote germination, which was maintained in a shaded area to avoid direct sunlight exposure. Radicle emergence was observed after three days. Germinated seeds were carefully removed from the sackcloth to prevent damage to the developing roots and transferred to the nursery for transplantation into polybags (5" × 7") containing organic black soil. Small holes were created in the soil, and the seedlings were positioned with roots at the base to ensure proper establishment (Biosci *et al*., 2023). One month after transplantation, seedlings had developed new leaves and were considered ready for treatment with chemical fertilizers (positive control) and different formulations of plant biostimulants.

Treatments and Irrigation Scheduling

Treatments were applied to cocoa plantlets through foliar spraying of all plant parts at monthly intervals for three consecutive months. Throughout the experimental period, plants were watered daily using tap water, except on the day following the application of the plant biostimulants, to ensure optimal absorption. The positions of all replicates were kept fixed to maintain consistent environmental conditions. Growth parameters of the cocoa plantlets, including plant height, number of leaves, and number of branches, were recorded monthly. At the end of the observation period, root lengths were also measured to evaluate overall plant development.

Data Analysis

Statistical analyses were performed using SPSS version 16. Differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Data are presented as mean ± standard deviation (SD), and differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 2 highlights the nutrient composition of the seaweed extract residues, confirming the presence of nitrogen, phosphorus, potassium, and other trace elements that may have contributed to the growth response of cocoa plantlets. The growth performance results after three months of treatment with chemical, physical, and biological residues are presented in Tables 3. Visual observations revealed that plantlets in the negative control group exhibited reduced leaf and branch development, along with shorter and less vigorous root systems, compared to those receiving residue-based treatments. Nevertheless, statistical analysis indicated no significant differences in plant height among the treatment groups.

Table 2: Nutrient composition of the seaweed extract residues generated from the chemical, physical and biological extraction methods.

Nutrient Content	Chemical Residue	Physical Residue	Biological Residue
Total Nitrogen (% w/w)	<0.1	<0.1	<0.1
Phosphorus (mg/kg)	840	750	675
Potassium (mg/kg)	1325	1470	1335
Magnesium (mg/kg)	85	81.5	53.5
Calcium (mg/kg)	410	625	310
Iron (mg/kg)	<0.5	3.1	<0.5
Manganese (mg/kg)	<0.5	<0.5	0.6
Boron (mg/kg)	3.0	1.2	1.0
Molybdenum (mg/kg)	<0.5	<0.5	<0.5
Sodium (mg/kg)	615	745	525

Plant Height

Based on Figure 1, certain seaweed extract residue formulations significantly enhanced the plant height of cocoa plantlets after three months of treatment. Treatments C1 (66.3 cm) and P3 (68.1 cm) showed marked increases compared to the negative control (49.6 cm). Among the biological-based treatments, B4 (60.3 cm) recorded the highest plant height, although the improvement was not statistically significant. Overall, plant height across all treatments was comparable to the positive control (54.6 cm), with no significant differences observed at the end of the study.

Nitrogen is a key nutrient needed for vegetative growth in cocoa seedlings (Tarigan *et al*., 2020). Although macronutrient analysis (Table 2) indicated relatively low concentrations of nitrogen and other essential nutrients in the residues, the soil likely supplied sufficient nitrogen to support growth. This aligns with findings by Carmona Rojas *et al*. (2022), who reported that cocoa seedlings at the nursery stage (0–6 months) have relatively low nutrient demands, and even 50% of the recommended nitrogen and potassium inputs can promote substantial biomass accumulation. Nevertheless, nutrient requirements may vary by genotype, though macronutrients remain universally essential. The notable increase in plant height observed in treatment C1, a 100% chemical extract residue, suggests that its nutrient composition was adequate to sustain vegetative growth. In contrast, biological-based residues, which contained the lowest macronutrient levels, did not significantly enhance plant height, likely due to limited nutrient availability.

Among all treatments, P3 (physical extract residue combined with humic acid) was the most effective, yielding the greatest plant height. Humic acid is a well-established plant biostimulant that enhances nutrient uptake, stress tolerance, and overall agronomic performance (du Jardin, 2015). The synergistic effect observed here is particularly noteworthy, as applications of seaweed residues in combination with other materials remain underexplored in cocoa cultivation. This result is consistent with findings by Gopi Krishna and Shanmugam (2024), who demonstrated that concentrated seaweed extract combined with humic liquid composites significantly improved plant height in maize.

Table 3: Growth performance of cocoa plantlets after three months of treatment with chemical-, physical- and biological- based seaweed extract residue formulations.

Formulation	**Negative Control**	**Positive Control**	**Formulation 1**	**Formulation 2**	**Formulation 3**	**Formulation 4**
Chemical - based seaweed extract residue formulations			 C1	 C2	 C3	 C4
Physical - based seaweed extract residue formulations			 P1	 P2	 P3	 P4
Biological - based seaweed extract residue formulations			 B1	 B2	 B3	 B4

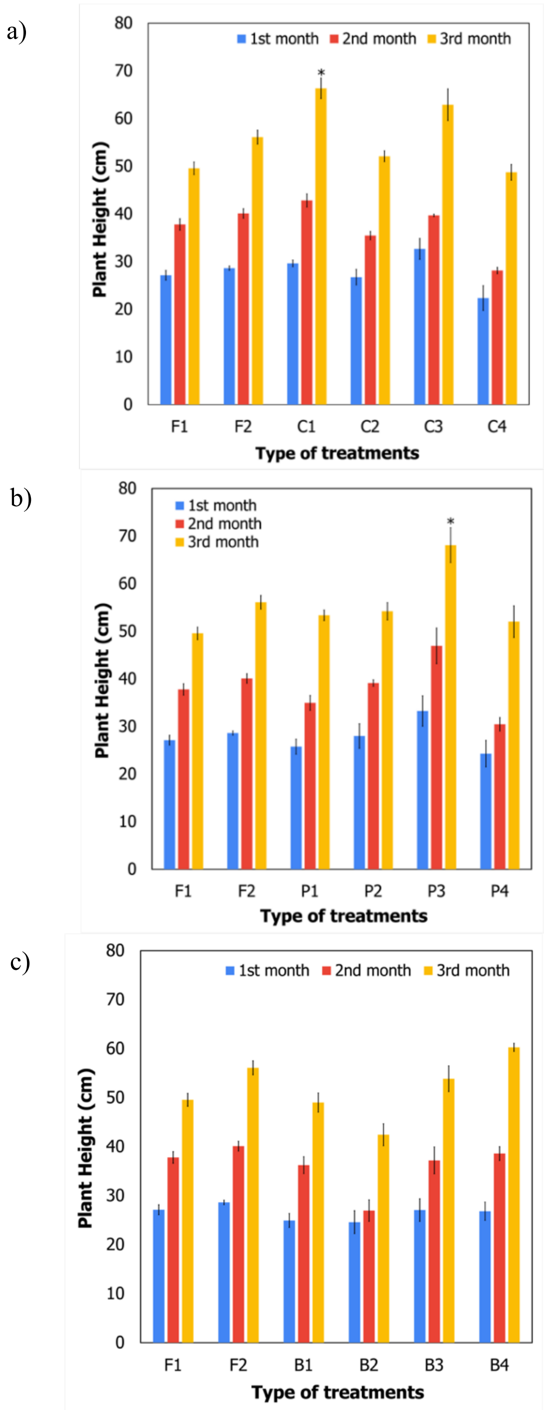


Figure 1: Effect of different seaweed extract residue formulations on cocoa plantlet height over three months of treatments. Error bars indicate standard deviation (n=3). Significant differences from the negative and positive controls are denoted by (*) and (**), respectively. (a) Chemical-based extraction formulations, (b) Physical-based extraction formulations, (c) Biological-based extraction formulations.

Number of Leaves

Based on Figure 2, after three months of treatment, the P3 formulation recorded the highest number of leaves (32) among all treatments. For the chemical- and biological-based extraction, the best-performing formulations were C1 (27 leaves) and B4 (29 leaves), respectively. Nevertheless, statistical analysis revealed that differences in leaf number among all treatments were not significant when compared with the negative control (18 leaves) and the positive control (21 leaves). This observation is consistent with the findings of Quiñones-Cabezas *et al.* (2024), who reported that variations in fertilizer dosage did not significantly affect leaf number in cocoa seedlings at the nursery stage. A possible explanation is the restricted root growth within polybags, which may have limited nutrient uptake efficiency and thereby constrained leaf development.

It is also important to note that the development of vegetative organs is strongly influenced by specific nutrient requirements. For cocoa seedlings, Leiva-Rojas and Ramírez-Pisco (2017), as cited in Carmona-Rojas *et al.* (2022), demonstrated that macronutrient uptake in certain Colombian cultivars follows the order N > K > Ca > Mg > P > S. Although the seaweed extract residues utilized in this study contained these macronutrients, biological-based residues exhibited comparatively lower levels of calcium and magnesium. Calcium plays a vital role in the initiation and elongation of young shoots, while magnesium is critical for chlorophyll synthesis and photosynthetic efficiency. Hence, relying solely on biological extract residues may lead to imbalanced nutrient availability. Integrating 100% biological residues with supplemental NPK fertilizer and humic acid could therefore provide a more balanced nutrient supply to support enhanced leaf growth in cocoa seedlings. In line with this, Reyes-Perez *et al.* (2022) reported that the application of a biostimulant derived from citrus industry waste, in which oligogalacturonides were identified as the primary active compounds, significantly promoted leaf development in cocoa cuttings.

a)

b)

c)

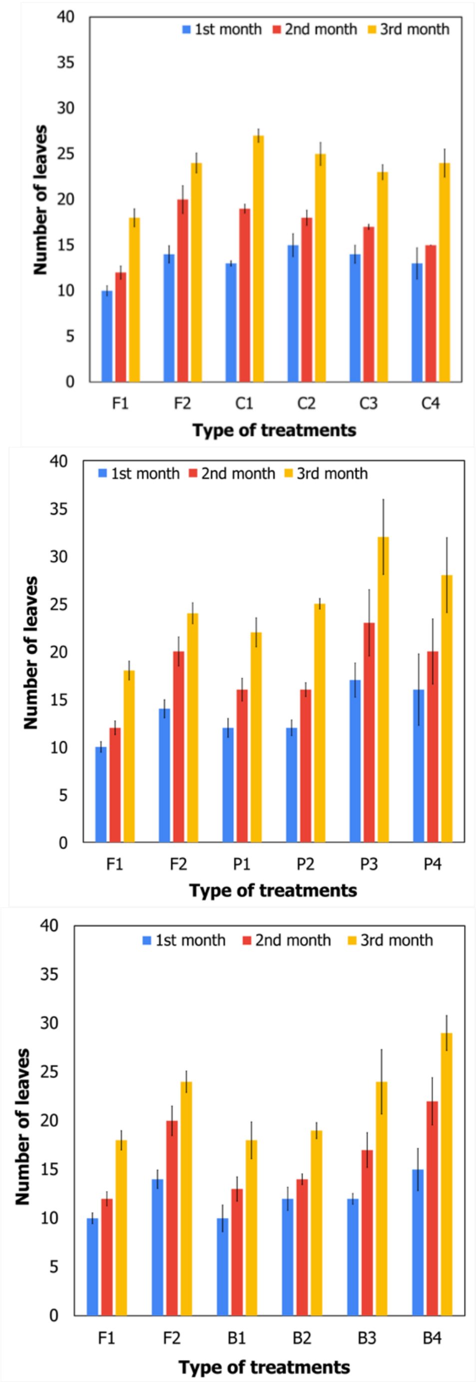


Figure 2: Effect of different seaweed extract residue formulations on the number of leaves in cocoa plantlets over three months of treatments. Error bars indicate standard deviation (n=3). Significant differences from the negative and positive controls are denoted by (*) and (**), respectively. (a) Chemical-based extraction formulations, (b) Physical-based extraction formulations, (c) Biological-based extraction formulations.

Number of Branches

Figure 3 shows that among the chemical-, physical-, and biological-based extraction formulations, treatments C1 (25 branches), P3 (32 branches), and B4 (27 branches) produced the highest number of branches, respectively, after three months of application. However, only treatment P3 exhibited a statistically significant increase in branch number compared to the negative control (18 branches), while no significant difference was observed relative to the positive control (21 branches).

In cocoa plantlets, branch development is important for cocoa productivity, as each branch has the potential to support multiple leaves and subsequently form flower buds and cocoa pods during the reproductive stage (Niemenak *et al*., 2010). Nutrient availability plays a central role in this process. Nitrogen is a critical macronutrient that promotes vegetative growth and branching (Carmona-Rojas *et al*., 2022; Tarigan *et al*., 2018). Potassium, typically stored in stem tissues, serves as a readily mobilizable nutrient reserve during periods of high physiological demand, while calcium, which was most abundant in the physical extract residue, is essential for structural development, including branch formation.

In this study, all seaweed extract residues contained appreciable amounts of potassium and calcium, with the physical extract residue showing the highest concentrations. The significant enhancement in branch numbers observed in treatment P3 may therefore be attributed to the synergistic effects of these nutrients in the seaweed residues and those inherently present in the soil, highlighting their biostimulatory potential. Additionally, the incorporation of humic acid in this treatment may have further improved nutrient uptake efficiency. This finding aligns with Ichwan *et al*. (2022), who demonstrated that applying 25% humic acid in combination with 75% NPK fertilizer increased soil nutrient availability and promoted chili plant growth.

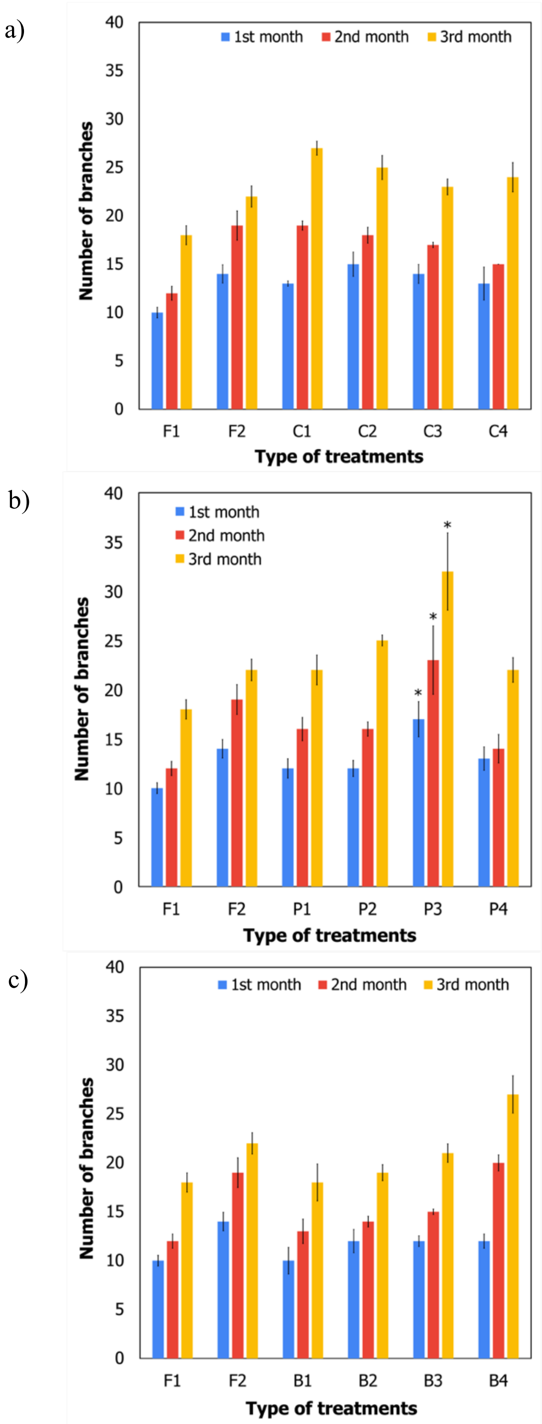


Figure 3: Effect of different seaweed extract residue formulations on the number of branches in cocoa plantlets over three months of treatments. Error bars indicate standard deviation (n=3). Significant differences from the negative and positive controls are denoted by (*) and (**), respectively. (a) Chemical-based extraction formulations, (b) Physical-based extraction formulations, (c) Biological-based extraction formulations.

Root Length

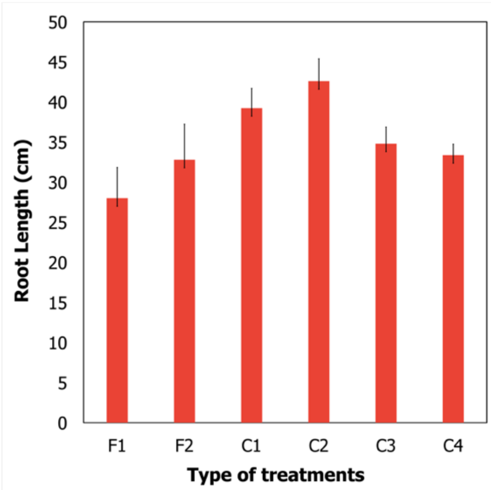
Figure 4 illustrates that treatments C2 (42.6 cm), P4 (36.9 cm), and B3 (34.8 cm) produced the greatest root lengths among the chemical-, physical-, and biological-based seaweed extract residue formulations, respectively. These values represent the highest root development recorded within each treatment group after three months of application. Nevertheless, statistical analysis showed no significant differences in root length between any treatment group and the negative (28.0 cm) or positive (32.8 cm) controls.

Root system development is fundamental for water and nutrient uptake, which directly influences cocoa plant productivity. As highlighted by Carmona-Rojas *et al*. (2022), a well-developed root system, comprising both thick and lateral roots, is essential for efficient nutrient absorption. Among macronutrients, phosphorus is particularly critical for root initiation and elongation, while calcium promotes the formation and stability of young roots. Bustamante González *et al*. (2022) demonstrated that applying a vegetable-based biostimulant to cocoa seedlings enhanced nutrient uptake and nutrient use efficiency, with phosphorus identified as the most extensively absorbed element. In the present study, treatment C2, which is comprising 80% chemical extract residues and 20% chemical fertilizer, yielded the longest root length. This outcome is likely attributable to the high phosphorus concentration in the chemical residues, further supplemented with phosphorus derived from both the fertilizer and the soil.

The absence of statistically significant differences in root length among treatments in this study is consistent with the findings of Quiñones-Cabezas *et al*. (2024), who also observed no significant variations in root length across fertilizer treatments. One plausible explanation is the restricted rooting volume provided by the experimental containers. As root biomass accumulates in confined spaces, competition for oxygen and nutrients intensifies, while pore space availability diminishes, ultimately limiting further root elongation and lateral expansion.

Based on the results, although some treatments appeared to enhance plant growth parameters such as height, leaf number, branch number, and root length compared to both the negative and positive controls, statistical analysis showed that most improvements were not significant. This may be due to the relatively low nutrient requirements of cocoa plantlets at the nursery stage, where nutrients available in the soil or supplied through seaweed residue-based treatments were sufficient to sustain growth. In addition, the restricted rooting volume of the experimental containers may have limited oxygen and nutrient uptake, thereby reducing treatment effects. Future studies should incorporate larger sample sizes to strengthen statistical power and minimize the potential physiological or environmental variation. Extending the observation period into the fruiting stage is also recommended, as this would provide more comprehensive evaluation of biostimulant effects in cocoa. Furthermore, assessing qualitative parameters such as nutrient accumulation, chlorophyll content, and bioactive compound levels in harvested fruits would offer deeper insights into the efficacy of seaweed extract residues as biostimulants.

a)



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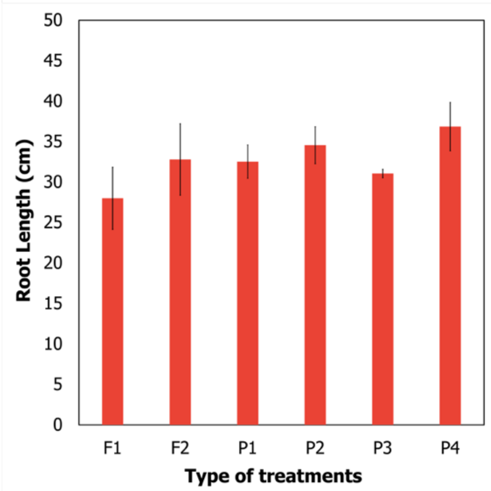

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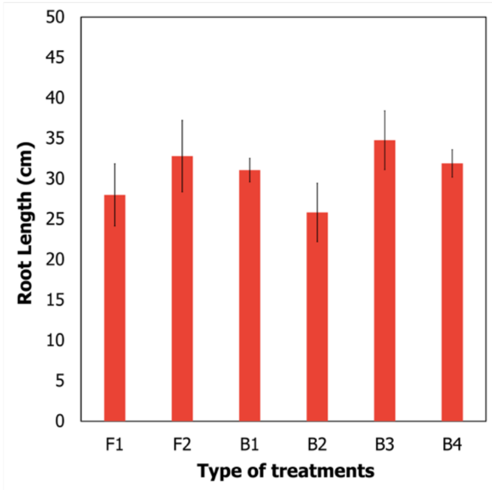

Figure 4: Effect of different seaweed extract residue formulations on the root length of cocoa plantlets over three months of treatments. Error bars indicate standard deviation (n=3). Significant differences from the negative and positive controls are denoted by (*) and (**), respectively. (a) Chemical-based extraction formulations, (b) Physical-based extraction formulations, (c) Biological-based extraction formulations.

CONCLUSIONS

The nursery-based plant trials demonstrated that incorporating seaweed residue extracts into specific formulations can significantly enhance the growth performance of cocoa plantlets compared to untreated controls. Among the tested treatments, the C1 formulation (100% chemical residue extract) was the most effective, producing the greatest plant height (66.3 cm), number of leaves (27), number of branches (27), and root length (39.2 cm). Notably, these growth parameters were comparable to those achieved with conventional chemical fertilizer, indicating that the nutrient composition of the chemical residue extract alone was sufficient to support robust plantlet development.

These findings highlight the potential of seaweed residue extracts as sustainable biostimulants for cocoa cultivation. Future studies should extend beyond the nursery stage to evaluate their efficacy throughout the reproductive phase and to determine their effects on yield attributes and the physicochemical properties of cocoa beans.

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SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://tinyurl.com/4bxyc7tc.

REFERENCES

Biosci, I. J., Gonzales, A. T., Mariano, D., Memorial, M., Union, L., & Biosci, I. J. (2023). Seed germination and seedling performance of Cacao (*Theobroma cacao L.*) varieties using vermicompost based potting. *International Journal of Biosciences* (IJB), **6655**, 255–261. https://doi.org/10.12692/ijb/23.1.255-261

Boney, M., Jenrry, S., Sairul, J., & Mohd. Yusof, M. Y. (2023). Effect of organic fertilizers combination on yield and soil properties of cocoa (Theobroma cacao L.). *Malaysian Cocoa Journal*, **15(1).** 31 – 37.

Bustamante González, C. A., Ferras, Y., Morán-Rodríguez, N., Pérez- Almaguer, A. F., Selva-Hernández, F. F., & Clappe-Borges, P. (2022). Effect of Enerplant® doses on the development and nutrient use by cacao (*Theobroma cacao L.*) seedlings. *Agro Productividad*, **15(4),** 79–87. https://doi.org/10.32854/agrop.v15i4.2130

Carmona Rojas, L. M., Gutiérrez Rodríguez, E. A., Henao Ramirez, A. M., & Urrea Trujillo, A. I. (2022). Nutrition in cacao (*Theobroma cacao L.*) crops: What determining factors should be considered? *Revista de La Facultad de Agronomía*, **121(Especial 2),** 101. https://doi.org/10.24215/16699513e101

Dehkordi, R. A., Roghani, S. R., Mafakheri, S., & Asghari, B. (2021). Effect of biostimulants on morpho-physiological traits of various ecotypes of fenugreek (Trigonella foenum-graecum L.) under water deficit stress. *Scientia Horticulturae*, **283(January 2020),** 110077. https://doi.org/10.1016/j.scienta.2021.110077

do Rosário Rosa, V., Farias dos Santos, A. L., Alves da Silva, A., Peduti Vicentini Sab, M., Barcellos Cardoso, F., Marin, M. A., & de Almeida Silva, M. (2021). Enhanced Tolerance to Cold in Common Bean Treated with Biostimulant. *Gesunde Pflanzen*, **73(1)**, 39–50. https://doi.org/10.1007/s10343-020-00526-2

du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, **196**, 3–14. https://doi.org/10.1016/j.scienta.2015.09.021

Fayzi, L., Dayan, M., Cherifi, O., Boufous, E. H., & Cherifi, K. (2020). Biostimulant effect of four moroccan seaweed extracts applied as seed treatment and foliar spray on maize. *Asian Journal of Plant Sciences*, **19(4),** 419–428. https://doi.org/10.3923/ajps.2020.419.428

Freitas, J. L. de, Jardim, I. N., & Guedes, M. N. S. (2024). Development of cacao seedlings (*Theobroma cacao L.*) as a function of D'Raz® biostimulant concentration. *Diversitas Journal*, **9(2),** 660–671. https://diversitasjournal.com.br/diversitas_journal

Gopi Krishna, R., & Shanmugam, M. (2024). Synergistic Effect of Tropical Seaweed Based Biostimulant With Humic Products on Grain Yield of Maize (Var. Syngenta Nk-6240) Farmed Under Semi-Arid Region. *Journal of Sustainable Agricultures*, **8(2),** 126–132. https://doi.org/10.26480/mjsa.02.2024.126.132

Helaly, A. (2021). Green Seaweed Extract (*Ulva Lactuca*) as a Model for Bio Stimulating and Reducing of Chemical Fertilization on the Growth and Yield of Sweet Potato Plants. *Scientific Journal of Agricultural Sciences*, https://doi.org/10.21608/sjas.2021.60170.1067

Ichwan, B., Eliyanti, E., Irianto, I., & Zulkarnain, Z. (2022). Combining humic acid with NPK fertilizer improved growth and yield of chili pepper in dry season. *Advances in Horticultural Science*, **36(4),** 275–281. https://doi.org/10.36253/ahsc12816

Kongor, J. E., Owusu, M., & Oduro-Yeboah, C. (2024). Cocoa production in the 2020s: challenges and solutions. *CABI Agriculture and Bioscience*, **5(1),** 1–28. https://doi.org/10.1186/s43170-024-00310-6

Liu, Y., An, D., Xiao, Q., Chen, F., Zhang, Y., Weng, H., & Xiao, A. (2022). A novel κ-carrageenan extracting process with calcium hydroxide and carbon dioxide. *Food Hydrocolloids*, **127**, 107507. https://doi.org/10.1016/j.foodhyd.2022.107507

Ministry of Plantation and Commodities. (2025, May 28). GDP performance (Prestasi KDNK). Kementerian Perladangan dan Komoditi. Retrieved from https://www.kpk.gov.my/kpk/sidebar-prestasi-kdnk

Mohanty, D., & Adhikary, S. P. (2018). Standardization of protocol for preparation of liquid extracts from seaweeds, quantification of their phytohormones and application for crop improvement. *Indian Journal of Geo-Marine Sciences,* **47(7),** 1364–1372.

Niemenak, N., Cilas, C., Rohsius, C., Bleiholder, H., Meier, U., & Lieberei, R. (2010). Phenological growth stages of cacao plants (*Theobroma sp*.): Codification and description according to the BBCH scale. *Annals of Applied Biology*, **156(1),** 13–24. https://doi.org/10.1111/j.1744-7348.2009.00356.x

Nuraziawati, M. Y., Ghizan, S., Rita, M. A., Khairul Bariah, S., Albert, L., Haya, R., & Aizat, J. (2023). A study on flavour profile of selected cacao germplasm collection in Cocoa Research and Development Centre Bagan Datuk, Perak. *Malaysian Cocoa Journal*, **15(1)**. 11 – 22.

Patel, K., Agarwal, P., & Agarwal, P. K. (2018). Kappaphycus alvarezii sap mitigates abiotic-induced stress in *Triticum durum* by modulating metabolic coordination and improves growth and yield. *Journal of Applied Phycology*, **30(4),** 2659–2673. https://doi.org/10.1007/s10811-018-1423-4

Paparella, A., Schirone, M., & López, C. C. (2025). The Health Impact of Cocoa from Cultivation to the Formation of Biogenic Amines: An Updated Review. Foods, 14(2), 255. https://doi.org/10.3390/foods14020255

Patel, R., Pandya, K., Jasrai, R., & Brahmbhatt, N. (2017). a Review: Scope of Utilizing Seaweed As a Biofertilizer in Agriculture. *International Journal of Advanced Research*, **5(7),** 2046–2054. https://doi.org/10.21474/ijar01/4941

Prakash, P., Mitra, A., Nag, R., & Sunkar, S. (2018). Effect of seaweed liquid fertilizer and humic acid formulation on the growth and nutritional quality of *Abelmoschus esculentus*. *Asian Journal of Crop Science*, **10(1),** 48–52. https://doi.org/10.3923/ajcs.2018.48.52

Ponthier, E., Domínguez, H., & Torres, M. D. (2020). The microwave assisted extraction sway on the features of antioxidant compounds and gelling biopolymers from *Mastocarpus stellatus. Algal Research*, **51**, 102081. https://doi.org/10.1016/J.ALGAL.2020.102081

Quiñones-Cabezas, J. A., Quiñones-Quiñones, J. L., & Ballesteros-Possú, W. (2024). Effect of fertilization on cocoa plants, *Theobroma cacao L.*, in the southern Colombian region. *Revista de Ciencias Agrícolas,* **41(2),** e2231. https://doi.org/10.22267/rcia.20244102.231

Reyes-Perez, J. J., Llerena-Ramos, L. T., Chila, V. H. R., Torres-Rodriguez, J. A., Farouk, S., Hernandez-Montiel, L. G., & Tezara, W. (2022). Effect of Pectimorf on the rooting ability, and morpho-physiological trials of national cocoa (*Theobroma cacao L.*) under different substrates. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **50(3),** 1–14. https://doi.org/10.15835/nbha50312847

Rouphael, Y., & Colla, G. (2020). Editorial: Biostimulants in Agriculture. *Frontiers in Plant Science,* **11(February)**, 1–7. https://doi.org/10.3389/fpls.2020.00040

Rozita, O., Tee, Y. K., & Mohammed Helmi, S. (2024). Application of advanced technology in cocoa cultivation: A Malaysian Cocoa Board Journey. *Malaysian Cocoa Journal*, **16**. 7 – 14.

Shamya Arokia rajan, M., Thriunavukkarasu, R., Joseph, J., & Aruni, W. (2020). Effect of seaweed on seed germination and biochemical constituents of *Capsicum annuum*. *Biocatalysis and Agricultural Biotechnology*, **29**, 101761. https://doi.org/10.1016/j.bcab.2020.101761

Solorzano-Chavez, E. G., Paz-Cedeno, F. R., Ezequiel de Oliveira, L., Gelli, V. C., Monti, R., Conceição de Oliveira, S., & Masarin, F. (2019). Evaluation of the *Kappaphycus alvarezii* growth under different environmental conditions and efficiency of the enzymatic hydrolysis of the residue generated in the carrageenan processing. *Biomass and Bioenergy*, **127**, 105254. https://doi.org/10.1016/j.biombioe.2019.105254

Suchithra, M. R., Muniswami, D. M., Sri, M. S., Usha, R., Rasheeq, A. A., Preethi, B. A., & Dinesh kumar, R. (2022). Effectiveness of green microalgae as biostimulants and biofertilizer through foliar spray and soil drench method for tomato cultivation. *South African Journal of Botany*, **146,** 740–750. https://doi.org/10.1016/j.sajb.2021.12.022

Tarigan, D., Siregar, H., Utami, S., Basyuni, M., & Novita, A. (2020). Seedling Growth in Response To Cocoa (*Theobroma Cacao L.*) For The Provision of Guano Fertilizer And Mycorrhizal Organic Fertilizer In The Nursery. *Agricultural and Food Sciences*, **2(1),** 290–294.

Tarman, K., Ain, N. H., Sulistiawati, S., Hardjito, L., & Sadi, U. (2020). Biological Process to Valorise Marine Algae. *IOP Conference Series: Earth and Environmental Science*, **414(1),** 12026. https://doi.org/10.1088/1755-1315/414/1/012026

Tee, Y. K., Tun, Y. L., & Karunanithi, K. (2022). An empirical analysis of the climate and price of cocoa production in Malaysia. *Malaysian Cocoa Journal*, **14**. 77 – 91.

Tye, Y. Y., Khalil Hps, A., Kok, C. Y., & Saurabh, C. K. (2018). Preparation and characterization of modified and unmodified carrageenan-based films. *IOP Conference Series: Materials Science and Engineering*, **368(1)**. https://doi.org/10.1088/1757-899X/368/1/012020

Torres-Rodriguez, J. A., Reyes-Pérez, J. J., Medel-Rodriguez, G. J., Rangel, P. P., Laiño, K. N. F., & Hernandez-Montiel, L. G. (2024). Chitosan: Biocontrol of *Moniliophthora roreri* and a Biostimulant of *Theobroma cacao L*. *Journal of Global Innovations in Agricultural Sciences*, **12(4),** 993–1001. https://doi.org/10.22194/JGIAS/24.1503

Uthirapandi, V., Boomibalagan, P., Eswaran, S., Ramya, S. S., Vijayanand, N., Rathinavel, S., & Kathiresan, D. (2019). Biofertilizing potential of seaweed liquid extracts of marine macro algae on growth and biochemical parameters of *Andrographis paniculata*. *Plant Archives*, **19(2),** 3201–3206.

Valleser, V. C., Aguilar, E. A., Ocampo, E. T. M., & Protacio, C. M. (2025). Biostimulants and Paclobutrazol Foliar Sprays for the Alleviation of Black Pod Rot (*Phytophthora palmivora* Butler) Stress in Cacao (*Theobroma cacao L*.). *Journal of the Saudi Society of Agricultural Sciences*, **24(4).** https://doi.org/10.1007/s44447-025-00028-5

Vasantharaja, R., Abraham, L. S., Inbakandan, D., Thirugnanasambandam, R., Senthilvelan, T., Jabeen, S. K. A., & Prakash, P. (2019). Influence of seaweed extracts on growth, phytochemical contents and antioxidant capacity of cowpea (V*igna unguiculata L. Walp*). *Biocatalysis and Agricultural Biotechnology*, **17**, 589–594. https://doi.org/10.1016/J.BCAB.2019.01.021

Uju, Wijayanta, A. T., Goto, M., & Kamiya, N. (2015). Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acid–ionic liquid pretreatment. *Biomass and Bioenergy*, **81,** 63–69. https://doi.org/10.1016/J.BIOMBIOE.2015.05.023

Varadarajan, S. A., Ramli Nazaruddin, Arbakariya, A., & Mamot, S. (2009). Development of high yielding carragenan extraction method from *Eucheuma Cotonii* using cellulase and *Aspergillus niger*. *Prosiding Seminar Kimia Bersama*, 461–469.