



Research Article

Impact of *Sargassum polycystum* and *Spirulina platensis* Extracts on Growth, Development and Morphological Characteristics of Wax Apple (*Syzygium samarangense*) Seedlings

Md. Tajol Faeiz Md. Tajudin, Mohammad Moneruzzaman Khandaker*, Nurul Elyni Mat Shaari and Nor Hasima Mahmod

School of Agriculture Science and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, Besut 22200, Malaysia.

Abstract | Utilising algal extracts as biostimulants presents a sustainable approach to enhancing plant growth and development. This study investigates the biostimulatory effects of *Spirulina platensis* and *Sargassum polycystum* extracts on the growth, development and morphological traits of wax apple seedlings. One month after transplanting, seedlings were foliar sprayed once per week for eight weeks with algal extracts at concentrations of 0% (control), 2.5%, 5.0%, and 10.0%, as well as a combined treatment of 5.0% (1:1) from both extracts. T6 (10.0% *S. polycystum* extract) exhibited the greatest fresh and dry weight increase by 55.93% and 51.76%, respectively. In contrast, moisture content was highest in the T3 (10.0% *S. platensis* extract) treatment (19.99%). T3 also showed the highest increase of plant height (19.17%), leaf height (266.67%), leaf number (379.28%), and stem diameter (142.79%) compared to control. At the same time, the combined treatment, T7 (5.0% *S. polycystum* + *S. platensis* extract), resulted in the largest leaf area, with a 31.66% enhancement. Additionally, the bud and flower formation was observed faster in T3 (30.33 and 19.00 days) and T6 treatments (32.33 and 20 days) while achieving the highest bud and flower numbers. Growth analysis revealed substantial effects of algal extracts on wax apple absolute growth rate (AGR), with T3 achieving a 472.79%, 428.01%, 230.69%, and 328.57% increase, respectively, in AGR of leaf number, leaf area, leaf weight, and plant height. T3 and T6 also recorded the highest relative growth rate (RGR) (0.080 and 0.074 g g⁻¹ day⁻¹, respectively). At the same time, specific leaf weight (SLW) improved to 0.016–0.017 g cm⁻² under higher concentrations while reducing the specific leaf area (SLA). These results highlight the significant ability of *S. platensis* and *S. polycystum* extracts as eco-friendly biostimulants for improving the growth, development, and morphological characteristics of wax apple seedlings, offering a promising solution for improving crop productivity while supporting environmental sustainability in agriculture.

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***Correspondence** | Mohammad Moneruzzaman Khandaker, School of Agriculture Science and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, Besut 22200, Malaysia. **Email:** moneruzzaman@unisza.edu.my

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Introduction

The wax apple (*Syzygium samarangense*), a sweet and crisp tropical fruit, has become a significant crop in Southeast Asia, with Indonesia, Thailand, and Vietnam among the leading producers, collectively yielding over 1.3 million tonnes annually (Shü *et al.*, 2008). This fruit, valued for its high vitamin C content and antioxidant properties, contributes to food security and economic development (Shamsudin *et al.*, 2012). However, its cultivation faces numerous biotic and abiotic challenges, such as pests, diseases, drought, and temperature fluctuations, which impede growth, productivity, and consistent quality. Efforts to address these constraints, including adopting innovative approaches like agricultural techniques, aim to enhance sustainability and efficiency in production. Despite its potential, achieving stable yields and quality remains a key challenge for the sustainable cultivation of wax apples (Shamsudin *et al.*, 2012). Applying mineral fertilisers has historically been a crucial strategy to counteract nutrient depletion and thus enhance yields. It is estimated that fertiliser usage contributed to a minimum 50% increase in crop yield throughout the 20th century (Choudhary *et al.*, 2021). The reliance on chemical fertilisers, while effective in boosting growth (Wang *et al.*, 2021), has raised concerns due to its long-term impacts on soil health, environmental pollution, and food safety through heavy metal contamination, acidification, and increased soil salinity (Das *et al.*, 2023; Levarity and Gustave, 2024).

As agriculture transitions to sustainability, there is an urgent demand for environmentally friendly solutions to improve crop output without detrimental effects on the ecosystem. Natural biostimulants derived from plants, microbes, and algae offer a promising approach. These non-toxic substances improve plant growth, optimise nutrient use, and strengthen resilience against environmental stresses (Halshoy and Sadik, 2024). Seaweed and algal extracts are particularly valuable due to their rich bioactive compounds essential for plant development and productivity (Chan *et al.*, 2022). As reported by the Food and Agriculture Organization (FAO), global algal production has grown nearly threefold over the past 20 years, expanding from 118,000 tons in 2001 to approximately 358,200 tons in 2019, reflecting remarkable progress in algal cultivation worldwide (Cai *et al.*, 2021; FAO, 2021). Beyond their role as food and industrial resources,

algae are emerging as crucial biostimulants, contributing significantly to sustainable agriculture and the bioindustry by enhancing crop yields and supporting economic growth.

Algal compositions are abundant in bioactive molecules, including phytohormones, amino acids, vitamins, and polysaccharides, making them highly effective in enhancing plant health and yield by stimulating root growth, improving nutrient absorption, and increasing photosynthetic efficiency (Abbas *et al.*, 2020; Mishra *et al.*, 2020). Among the most studied species, *Sargassum polycystum* (brown macroalga) and *Spirulina platensis* (cyanobacterium) are recognised for their nutrient-dense profiles and growth-promoting effects in various crops (Abd Tahar *et al.*, 2024; Ammar *et al.*, 2022).

Sargassum polycystum, a brown macroalga rich in polysaccharides, polyphenols, and minerals, enhances plant nutrient uptake and stress tolerance. Its extracts have proven effective in boosting plant growth and productivity across various crops. For instance, a 20% foliar application on Tadong rice increased growth and yields by 5.73–44.11%, offering a sustainable solution to low yields (Abd Tahar *et al.*, 2024). In *Vicia faba* and *Helianthus annuus*, *Sargassum polycystum* extract improved germination, photosynthetic efficiency, nutrient uptake, and biochemical traits like phenolics and flavonoids (Mohammed *et al.*, 2023). Furthermore, research on tomatoes revealed that *Sargassum* extracts promote vegetative growth, enhancing plant height, root length, flowering, and fruit weight (Fatimah and Daud, 2018). These results show that *Sargassum* extracts could be used as natural biostimulants to support sustainable farming and boost food yields.

Spirulina, renowned for its high nutritional content, has long been utilised as a dietary supplement due to its health benefits. Enriched with proteins, vitamins, minerals, and antioxidants, *Spirulina* has also gained attention in agriculture for its ability to enhance soil fertility and boost plant growth (Karkos *et al.*, 2011). Its bioactive compounds significantly facilitate essential physiological processes in plants, improving root elongation, shoot development, and biomass production (Shaari *et al.*, 2023). A study on *Spirulina platensis* has shown its effectiveness in enhancing the growth and productivity of tomato plants due to its nutrient-dense composition and bioactive character-

istics (Mostafa *et al.*, 2023). This finding highlights its potential as a sustainable biostimulant for agriculture.

Despite the proven benefits of algae-derived biostimulants in promoting plant growth, their application in wax apple (*Syzygium samarangense*) cultivation remains underexplored. Most studies have focused on staple crops, leaving a critical research gap for high-value fruits like wax apple. Thus, this study investigated the influence of *Sargassum polycystum* and *Spirulina platensis* extracts on the morphological and physiological development of wax apple seedlings. Through weekly foliar applications for eight weeks, the study hypothesises improved nutrient uptake, enhanced photosynthesis, and stimulated leaf and shoot development. This research aimed to demonstrate the positive influence in wax apple seedling's growth, development and morphological characteristics by validating the efficacy of these algal extracts as natural biostimulants. Additionally, the findings will be one of sustainable alternatives to chemical fertilisers. These measures enhance crop health and productivity, promote soil fertility, and mitigate the environmental impact of agricultural activities (Chabili *et al.*, 2024).

Material and Methods

Algal collection and identification

S. polycystum was harvested during low tide from Blue Lagoon, Port Dickson, Malaysia (2°24'56.5" N, 101°51'17.5" E). The thalli were rinsed with seawater to remove impurities, packed in polyethylene bags with slush ice, and transported to the laboratory. Species identity was confirmed based on morphological traits following Yip *et al.* (2018). *S. platensis* was obtained from Algaeliving SDN. BHD. and cultured in Zarrouk's medium at 25°C under continuous illumination (1020 lux) with aeration. The pH was adjusted to pH 8, and optical density at 730 nm was used to measure growth as described by Sivalingam (2020). Biomass was extracted after one month using centrifugation at 5000 rpm and subsequently sun-dried under a glass cover for four hours. The *S. platensis* biomass was stored at -18°C for further experiment (Figure 1).

Algae extraction

The preparation of algal extracts involved air-drying fresh algal samples at a temperature of 20°C to maintain uniformity for further processing. Based on a modified protocol from Pise and Sabale (2010), 50 g

of dried algae biomass was combined with 500 mL of distilled water in a 1 L flask. The mixture was heated to 70°C and continuously stirred for two hours using a hot plate stirrer to extract bioactive compounds. After heating, the solution was centrifuged at 4000 rpm for 10 minutes to separate solid residues, followed by vacuum filtration to obtain a clear and uniform extract. The filtrate's pH and colour were recorded for characterisation. The resulting solution was designated a 100% concentrated extract, which was subsequently diluted to the required concentrations for the experimental treatments.

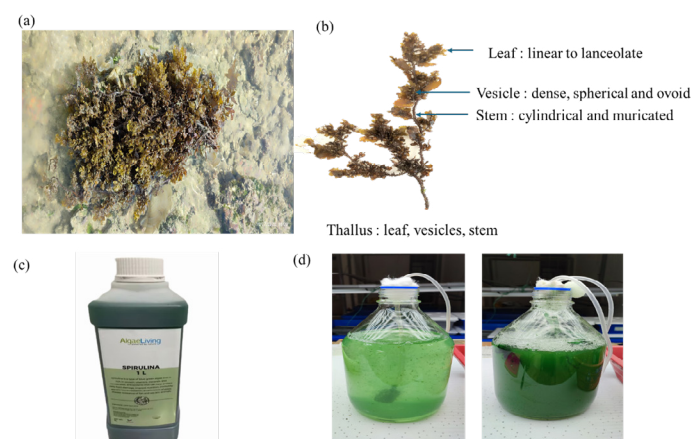


Figure 1: The images of algae used for the experiment. (a) *Sargassum polycystum* collected in Blue Lagoon, Port Dickson, (b) morphological detail of *Sargassum polycystum*, (c) *Spirulina platensis* obtained from AlgaeLiving and (d) cultured *Spirulina platensis* in Zarrouk's medium.

Experimental design and plantation

The study was conducted at the Faculty of Biore-sources and Food Industry shade house, Universiti Sultan Zainal Abidin (UniSZA), Besut Campus, Terengganu, Malaysia. Wax apple seedlings propagated via air layering were selected after two months, with forty uniform-sized cuttings transplanted into polybags. Soil for planting was sourced from an agricultural farm in Jerteh, Terengganu, and prepared by air-drying and sieving through a 2 mm mesh to ensure uniformity. Key soil properties included a pH of 6.2, 0.15% nitrogen, 1.50% carbon, 21 mg/kg phosphorus, a cation exchange capacity (CEC) of 10.8 cmol (+)/kg, and base cation levels of 2.2, 1.50, and 1.05 cmol (+)/kg for potassium, calcium, and magnesium, respectively. The soil's textural composition was 35.8% coarse sand, 10.1% fine sand, 40.0% clay, and 12.5% silt, deemed suitable for plant growth. The shade house provided partial sunlight exposure, with light intensity ranging from 600-1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ during the day. One month after transplant-

ing, the seedlings received weekly foliar treatments for two months with a total of eight sprays at 0%, 2.5%, 5.0%, and 10.0% of *S. polycystum* and *S. platensis* extracts and a 1:1 combined treatment (5.0% each) (Table 1). Each treatment, including a 100 mL foliar spray, was manually applied to the seedlings, with untreated plants as the control group. The experiment followed a Randomized Complete Block Design (RCBD) with five replicates per treatment, ensuring robust experimental results (Table 1).

Table 1: The design of experiments to examine the effects of *S. polycystum* and *S. platensis* extracts on wax apple seedlings.

Treatment	Description
T0	0 % extracts
T1	2.5% <i>S. platensis</i> extracts
T2	5.0% <i>S. platensis</i> extracts
T3	10.0% <i>S. platensis</i> extracts
T4	2.5% <i>S. polycystum</i> extracts
T5	5.0% <i>S. polycystum</i> extracts
T6	10.0% <i>S. polycystum</i> extracts
T7	5.0% <i>S. platensis</i> + <i>S. polycystum</i> extracts

Growth characteristics

The height of the leaf was determined by determining the distance from the soil surface to the node connected with the leaf. The fresh weight (FW) of wax apple leaf was weighed using an electronic balance. Following two days of oven drying at 80 °C, the leaf's dry weight (DW) was determined using the identical balance. The leaf moisture content was calculated using the following formula;

$$\text{Leaf moisture content (\%)} = \frac{ww - wd}{ww} \times 100$$

Where;

ww: Leaf's wet weight; Wd: Dry weight.

Morphological parameters

Algal extracts from *S. platensis* and *S. polycystum* were investigated for their influence on the morphological and growth traits of wax apple seedlings. The study commenced in March 2021, and treatments were applied for two months. Morpho-physiological parameters were measured at one-month intervals, totalling two observations. The plant height of each seedling was measured using a meter tape for precise assessment. Leaf area was calculated using the formula:

$$\text{Leaf area} = \text{Leaf length} \times \text{Mean leaf width}$$

Stem diameter was measured using a vernier calliper for accuracy, ensuring consistent measurement across all samples. Meanwhile, the leaf number per seedling was manually counted and recorded.

Bud and flower formation

The reproductive growth of wax apple seedlings was evaluated by tracking bud and flower formation. The duration till bud formation was measured as the number of days from the initiation of treatment to the emergence of a discernible swelling at leaf axils or nodes, as assessed through daily visual inspections. For flower formation, the number of days from the first observed bud to the emergence of the first flower with visible petals was recorded. Flower formation was confirmed by the presence of a fully developed flower. Daily observations documented the transitions from bud initiation to flower emergence, providing insights into reproductive development.

Growth analysis

The Specific Leaf Area (SLA) was determined to assess leaf expansion and thickness across treatments. Fully developed, mature leaves were selected from each treatment group and excised for measurement. Fresh leaf was collected and measured for leaf area. A constant dry weight was achieved by drying the leaf in an oven at 80°C for 48 hours. SLA was calculated and interpreted in cm²g⁻¹ (Kvet *et al.*, 1971) using the following equation.

$$\text{Specific Leaf Area (SLA)} = \frac{\text{Leaf area}}{\text{Leaf dry weight}}$$

The Specific Leaf Weight (SLW) was assessed to determine leaf density and thickness changes resulting from algal extract treatments. Fully expanded, mature leaves were sampled from each treatment group, and their leaf area was measured. Next, the leaves were dried for 48 hours at 80°C to maintain a consistent dry weight. To determine SLW, the dry weight was divided by the appropriate leaf area. The findings were then presented as g cm⁻², following the method described by Pearce *et al.* (1968).

$$\text{Specific Leaf Weight (SLW)} = \frac{\text{Leaf dry weight}}{\text{Leaf area}}$$

To compare biomass formation rates in wax apple seedlings among treatments, the Relative Growth Rate (RGR) was computed accordingly. The seedlings' starting and ending dry weights were noted at the start and end of the experiment, correspondingly. RGR was determined and expressed in $\text{g g}^{-1} \text{day}^{-1}$ (Tylova-Munzarova *et al.*, 2005) using the formula:

$$\text{Relative Growth Rate (RGR)} = \frac{\text{Final DW} - \text{Initial DW}}{\text{Time interval}_{(\text{final}-\text{initial})}} \times \frac{1}{\text{Initial DW}}$$

The Leaf Area Index (LAI) was determined to evaluate the canopy density and leaf distribution across treatments. The total leaves number was recorded and multiplied by individual leaf area to identify the total leaf area of the plant. The ground area was calculated based on the planting space allocated to each seedling. The value of the total leaf area and ground were substituted in the following equation (Williams, 1946) to determine LAI.

$$\text{Leaf Area Index} = \frac{\text{Total leaf area of plant}}{\text{Ground area occupied by plant}}$$

The Absolute Growth Rate (AGR) was determined to measure biomass accumulation in wax apple seedlings throughout the experimental period for all treatments. This was achieved by recording the seedlings' dry weights at the beginning and termination of the study. AGR was calculated using the formula:

$$\text{Absolute Growth Rate (AGR)} = \frac{\text{Final DW} - \text{Initial DW}}{\text{Time interval}_{(\text{final}-\text{initial})}}$$

Statistical Analysis

The experiment followed a Randomized Complete Block Design (RCBD) with five replications for each treatment. Data analysis was conducted using SPSS-17 software, employing two-way ANOVA to determine significant differences among the parameters. Tukey's HSD test was used for pairwise comparisons of the effects of varying algal extract concentrations, with a significance threshold set at $p = 0.05$.

Results

Growth characteristics

Figure 2 illustrates the effects of algal extracts on the fresh weight (FW), dry weight (DW), and moisture content of wax apple leaves. Increasing extract concentrations significantly boosted FW and DW,

with the highest values recorded in the T6 (10.0% *S. polycystum*) treatment, showing 55.93% and 51.76% increases, respectively. T4 and T5 (2.5% and 5.0% *S. polycystum* respectively) also demonstrated notable improvements, with FW increases of 32.63% and 43.22% and DW increases of 24.71% and 45.88%, respectively. While treatments with 2.5%, 5.0% and 10.0% *S. platensis* (T1, T2, and T3) exhibited smaller gains, they still surpassed the control, with FW increases ranging from 11.44% to 23.31% and DW increases from 11.76% to 23.53%. The combined treatment, 5.0% *S. polycystum* + *S. platensis* extract (T7) significantly enhanced FW by 30.08%, though its 4.71% increase in DW was not statistically significant.

For moisture content, T3 showed the highest improvement with a 19.99% increase over the control, followed by T2 (12.42%), T7 (9.58%), and T5 (7.65%), while T1 and T6 showed minimal gains (1.29% and 0.26%, respectively). Except for T3, differences in moisture content were not statistically significant. These results underscore the potential of higher concentrations, particularly *S. polycystum* treatments, to enhance FW, DW, and moisture content in wax apple leaves. (Figure 2).

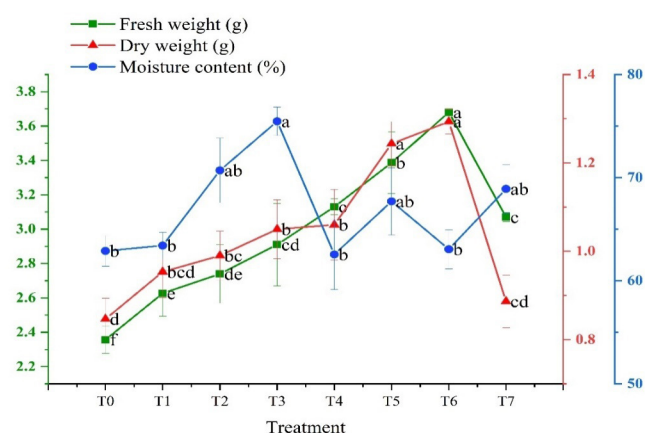


Figure 2: The fresh weight (green line), dry weight (red line) and moisture content (blue line) of wax apple leaf after being treated with algal extracts at different concentrations. Repeated letters did not exhibit a significant difference in values ($n = 5$) at $p < 0.05$. A standard error of five replicates is represented by the error bars. Treatments are as detailed in Table 1.

Morphological parameters

Figure 3 presents the wax apple seedlings after treatments with *S. platensis* and *S. polycystum* extracts (T0-T7). A linear correlation was observed between algal extract concentrations and plant height.

Table 2: The plant height, leaf height, leaf area, leaf number and stem diameter of wax apple seedlings after algal extract treatment.

Treatment	Plant height (cm)	Leaf height (cm)	Leaf area (cm ²)	Leaf number/plant	Stem diameter (cm)
T0	60.00±1.41c	7.62±0.29e	63.46±1.94d	74.00±3.46c	2.08±0.19d
T1	61.00±3.19c	15.24±0.58cd	67.08±3.11bcd	100.33±2.60c	2.38±0.03cd
T2	69.25±4.15ab	12.25±0.60d	64.75±8.2cd	134.00±9.45b	2.98±0.16bc
T3	71.50±2.60a	27.95±1.53a	82.27±14.49ab	354.67±20.50a	5.05±0.45a
T4	66.00±11.45b	20.35±1.15b	60.63±3.44d	152.33±7.22b	2.75±0.28bcd
T5	67.25±4.71b	17.75±0.58b	72.42±2.34abcd	187.00±6.51c	3.25±0.30b
T6	68.50±1.71ab	26.25±0.88a	80.31±2.97abc	323.00±14.43a	4.45±0.28a
T7	67.00±9.02b	20.35±1.15b	83.52±5.66a	134.00±13.45b	2.47±0.25bcd

Repeated letters did not exhibit a significant difference in values ($n = 5$) at $p < 0.05$. A standard error of five replicates is represented by the error bars. Treatments are as detailed in Table 1.

Table 2 shows that, except for T1 (2.5% *S. platensis*), all treatments substantially enhanced plant height in comparison to the control. The highest plant height was recorded in T3 (10.0% *S. platensis*), with a 19.17% increase over the control, followed by T2 (5.0% *S. platensis*) (15.42%), T6 (10.0% *S. polycystum*) (14.17%), T5 (5.0% *S. polycystum*) (12.08%), T7 (5.0% *S. polycystum* + *S. platensis*) (11.67%), T4 (2.5% *S. polycystum*) (10.00%), and T1 (1.67%).

Similarly, leaf number showed a positive correlation with algal extract concentrations. T3 recorded the highest increase at 379.28% over the control, with other treatments also showing significant increases, T6 (336.49%), T5 (152.70%), T4 (105.85%), T2 and T7 (81.08%), and T1 (35.14%). For leaf area, the most significant increase was observed in T7 (31.66%), followed by T3 (29.61%) and T6 (26.55%). Other treatments also showed an increment, including T5 (14.11%) and T2 (2.03%), while T4 decreased leaf area by 4.46% compared to the control. These findings emphasise the effectiveness of higher algal extract doses, particularly 10.0% of *S. platensis* and *S. polycystum*, in enhancing the growth parameters of wax apple seedlings.

Significant variations in leaf height were observed across treatments, with the highest increase recorded in T3, showing a remarkable 266.67% increase over T0 (Table 2). T6 followed closely with a 244.33% increase, while T2 showed a 161.00% improvement. Treatments T4, T7, and T5 also significantly enhanced leaf height by 166.67%, 166.67%, and 133.33%, respectively. T1 exhibited the smallest increase at 100.00%, effectively doubling the control's value. These results highlight those higher concentrations, particularly in T3 and T6, were most effective in enhancing leaf

height. Likewise, stem diameter responded positively to the treatments, with T3 showing the most remarkable improvement of 142.79% over the control (Table 2). Substantial increases were also observed in T6 (113.46%), T2 (43.27%), T5 (56.73%), T4 (32.21%), and T7 (18.75%). These findings confirm that higher treatment concentrations, especially in T3 and T6, were the most effective in promoting leaf height and stem diameter growth. (Table 2 and Figure 2).

Bud and flower formation

The results showed significant differences in bud and flower formation of wax apple seedlings when treated with algal extracts, as shown in Table 3. The control (T0) had the longest bud formation time (46.67±0.88 days) and the lowest bud number (6.00±0.58), while T3 (10.0% *S. platensis*) and T6 (10.0% *S. polycystum*) had the shortest bud formation times (30.33±0.33 and 32.33±1.45 days) and the highest bud numbers (12.00±1.15 and 11.00±1.53). Following this, T2 (5.0% *S. platensis*), T5 (5.0% *S. polycystum*), and T7 (5.0% *S. polycystum* + *S. platensis*) also improved bud formation duration and count.

For flower formation, T3 achieved the shortest duration (19.00±2.52 days) and the highest flower number (9.00±1.15), followed by T6 (20.00±0.58 days, 8.67±0.88 flowers). In contrast, the control had the longest time to flowering (28.67±2.33 days) and the fewest flowers (2.67±0.33). Treatments T2, T5, and T7 showed moderate improvements, while T4 (2.5% *S. polycystum*) exhibited delayed flowering (26.33±0.88 days) and a lower flower count (3.00±1.00). Conclusively, T3 and T6 were the most effective in enhancing bud and flower development in wax apple seedlings. (Table 3).

Table 3: The buds and flowers formation and their number after the introduction of various concentrations of algal extracts.

Treatment	Bud formation (days)	Number of buds	Flower formation (days)	Number of flowers
T0	46.67±0.88a	6.00±0.58c	28.67±2.33a	2.67±0.33d
T1	41.67±1.45b	6.33±1.20c	25.67±2.19abc	3.33±0.33cd
T2	36.33±0.88c	7.67±1.45c	22.33±0.67bcd	5.00±0.58bc
T3	30.33±0.33d	12.00±1.15a	19.00±2.52d	9.00±1.15a
T4	36.33±0.33c	5.67±1.20c	26.33±0.88ab	3.00±1.00cd
T5	37.67±0.88c	7.33±0.33c	26.00±1.53ab	4.67±0.67bcd
T6	32.33±1.45d	11.00±1.53ab	20.00±0.58cd	8.67±0.88a
T7	36.33±0.88c	8.33±0.67bc	21.33±0.88bcd	6.33±0.33b

Repeated letters did not exhibit a significant difference in values ($n = 5$) at $p < 0.05$. A standard error of five replicates is represented by the error bars. Treatments are as detailed in Table 1.

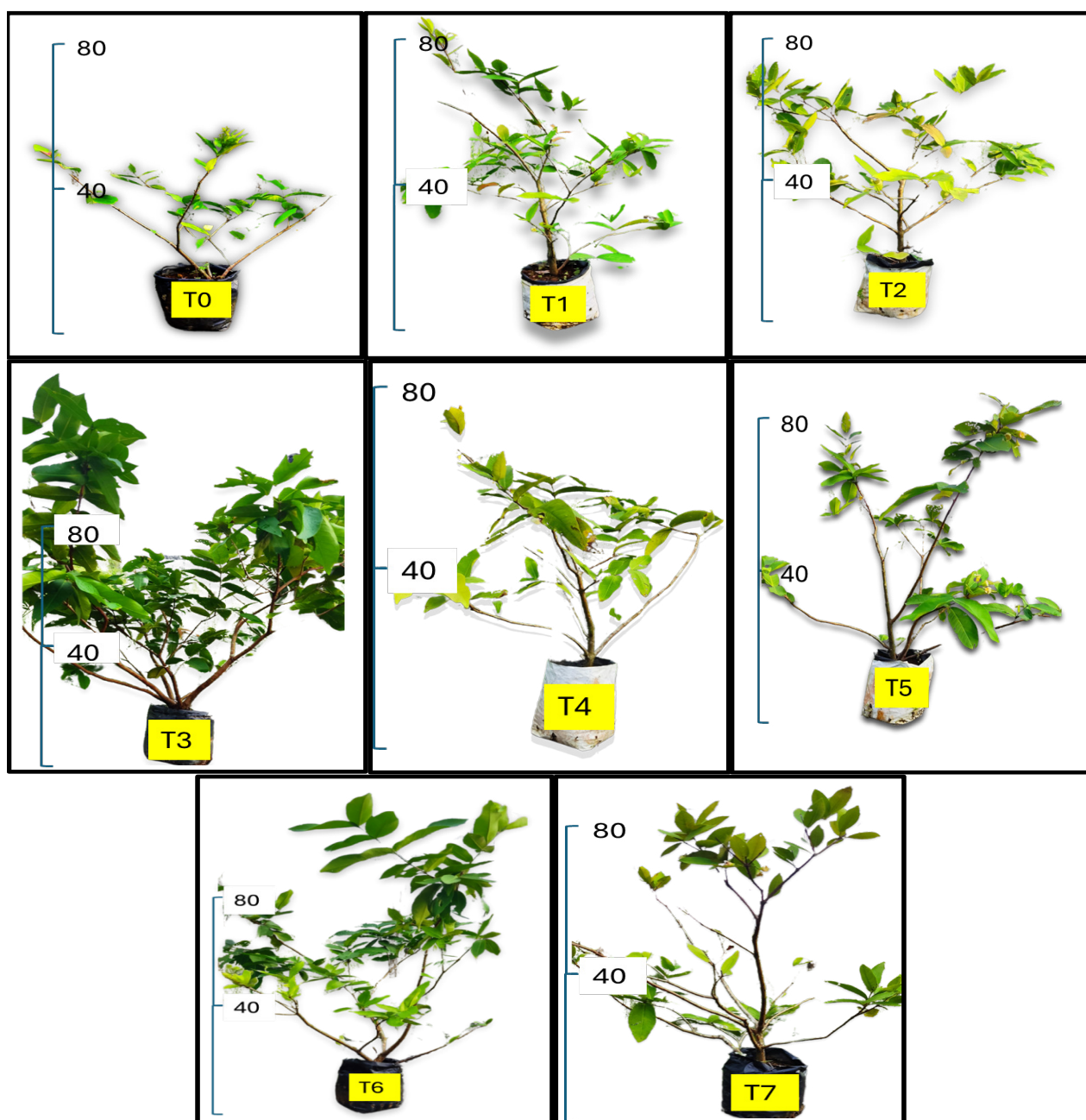


Figure 3: The images of wax apple seedlings after treatment of *S. platensis* and *S. polycystum* extracts. Treatments are as detailed in Table 1.

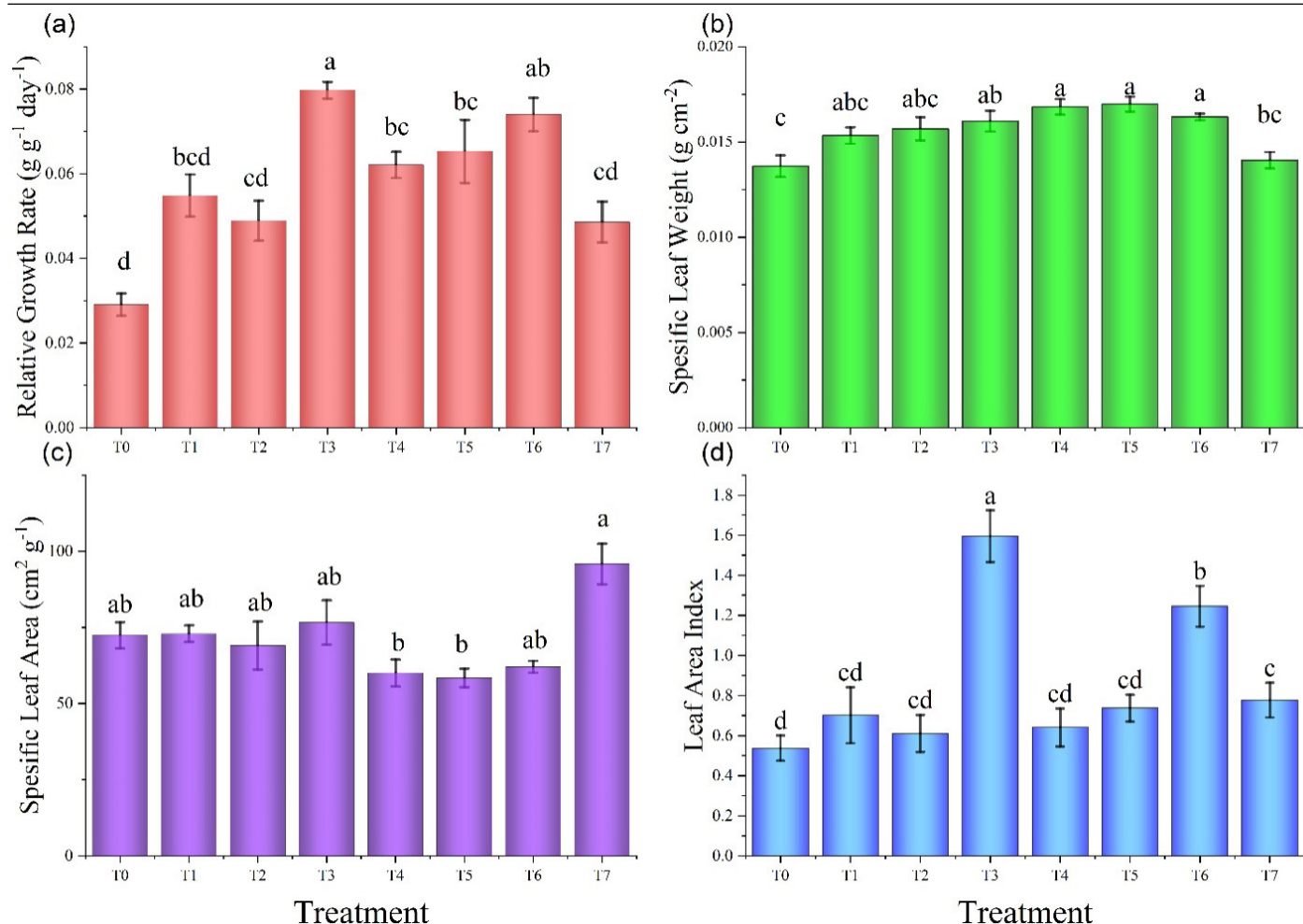


Figure 4: The growth analysis of leaf of wax apple, (a) RGR, (b) SLW, (c) SLA and (d) LAI, after exposure to different concentrations of *S. platensis* and *S. polycystum* extracts. Repeated letters did not exhibit a significant difference in values ($n = 5$) at $p < 0.05$. A standard error of five replicates is represented by the error bars. Treatments are as detailed in Table 1.

Growth analysis

Calculations of relative growth rate (RGR), specific leaf weight (SLW), specific leaf area (SLA), and leaf area index (LAI) revealed significant influences of algal extracts on wax apple leaves, as depicted in Figure 4. For RGR, T3 (10.0% *S. platensis*) ($0.080 \text{ g g}^{-1} \text{ day}^{-1}$) and T6 (10.0% *S. polycystum*) ($0.074 \text{ g g}^{-1} \text{ day}^{-1}$) showed the highest values relative to the control, with no significant difference observed between them (Figure 4a). T4 (2.5% *S. polycystum*) ($0.062 \text{ g g}^{-1} \text{ day}^{-1}$) and T5 (5.0% *S. polycystum*) ($0.065 \text{ g g}^{-1} \text{ day}^{-1}$) also exhibited notable effects, while T1 (2.5% *S. platensis*), T2 (5.0% *S. platensis*), and T7 (5.0% *S. polycystum* + *S. platensis*) were not significantly different from the control. SLW results (Figure 4b) indicated significant improvements in T3, T4, T5 and T6 compared to the control. T3 and T6 shared the same value (0.016 g cm^{-2}), while T4 and T5 recorded 0.017 g cm^{-2} each, with no substantial differences among these treatments. These findings suggest that a

higher concentration of *S. platensis* (10.0%) effectively increased SLW, while *S. polycystum* extracts influenced SLW across all concentrations.

For SLA (Figure 4c), T7 achieved the highest value ($95.89 \text{ cm}^2 \text{ g}^{-1}$), followed by T3 ($76.70 \text{ cm}^2 \text{ g}^{-1}$) and T1 ($72.97 \text{ cm}^2 \text{ g}^{-1}$) over the control. T2, T4, T5, and T6 showed lower SLA values than the control, but the differences were not statistically significant, indicating that SLA was less influenced by the treatments. LAI analysis (Figure 4d) showed substantial enhancements with algal extract treatments. T3 (1.60) and T6 (1.25) achieved the highest LAI, demonstrating significant improvements in canopy development. The combined treatment, T7 (0.78), also contributed to LAI improvement but was less effective than T3 and T6. Lower-concentration treatments (T1, T2, T4, and T5) showed improvements without significant differences from the control. These results indicate that higher doses of algal extracts, especially in T3 and T6,

are most effective in enhancing LAI, promoting light interception and overall plant growth. (Figure 4).

In the study of plant growth, Absolute Growth Rate (AGR) is a key metric to measure changes in plant biomass over time, capturing the effects of different treatments on growth dynamics. The findings revealed a linear correlation between algal extract concentrations and AGR for leaf weight, area, number, and plant height, as illustrated in Figure 5. For leaf weight AGR, the most significant increases were recorded in T3 (230.69%) and T6 (213.47%) compared to the control (T0). Other treatments showed notable, albeit smaller, improvements: T5 (133.47%), T4 (100.40%), T1 (70.30%), T7 (66.73%), and T2 (44.16%). However, T2 did not significantly differ from the control (Figure 5a). These findings suggest that higher concentrations of algal extracts significantly enhance leaf weight growth, with the most pronounced effects seen in T3 and T6.

Leaf area AGR followed a similar trend, with T3 demonstrating the highest increase (428.01%) over the control. Other notable treatments included T6

(272.04%), T7 (190.38%), T2 (187.92%), and T5 (184.79%), as shown in Figure 5b. T1 and T4 did not show significant effects on leaf area AGR. These results highlight that lower concentrations of algal extracts have limited effects, whereas higher concentrations yield substantial improvements. For leaf number AGR, T3 again stood out with the greatest increase (472.79%), followed by T6 (384.56%), T2 (96.32%), and T4 (67.65%). This pattern underscores the potent effect of T3 and T6 treatments on stimulating leaf production. Regarding to plant height AGR, T3 achieved the highest increase (328.57%) compared to the control, as shown in Figure 5c. T5 (271.43%) and T6 (264.29%) also demonstrated significant enhancements. Lower concentrations exhibited minor effects, indicating a dose-dependent response. In summary, the study confirms that higher concentrations of algal extracts, particularly in T3 and T6 treatments, significantly enhance AGR for various plant growth parameters, including leaf weight, leaf area, leaf number, and plant height. These results emphasised the potential of algal extracts as effective growth promoters in wax apple cultivation (Figure 5).

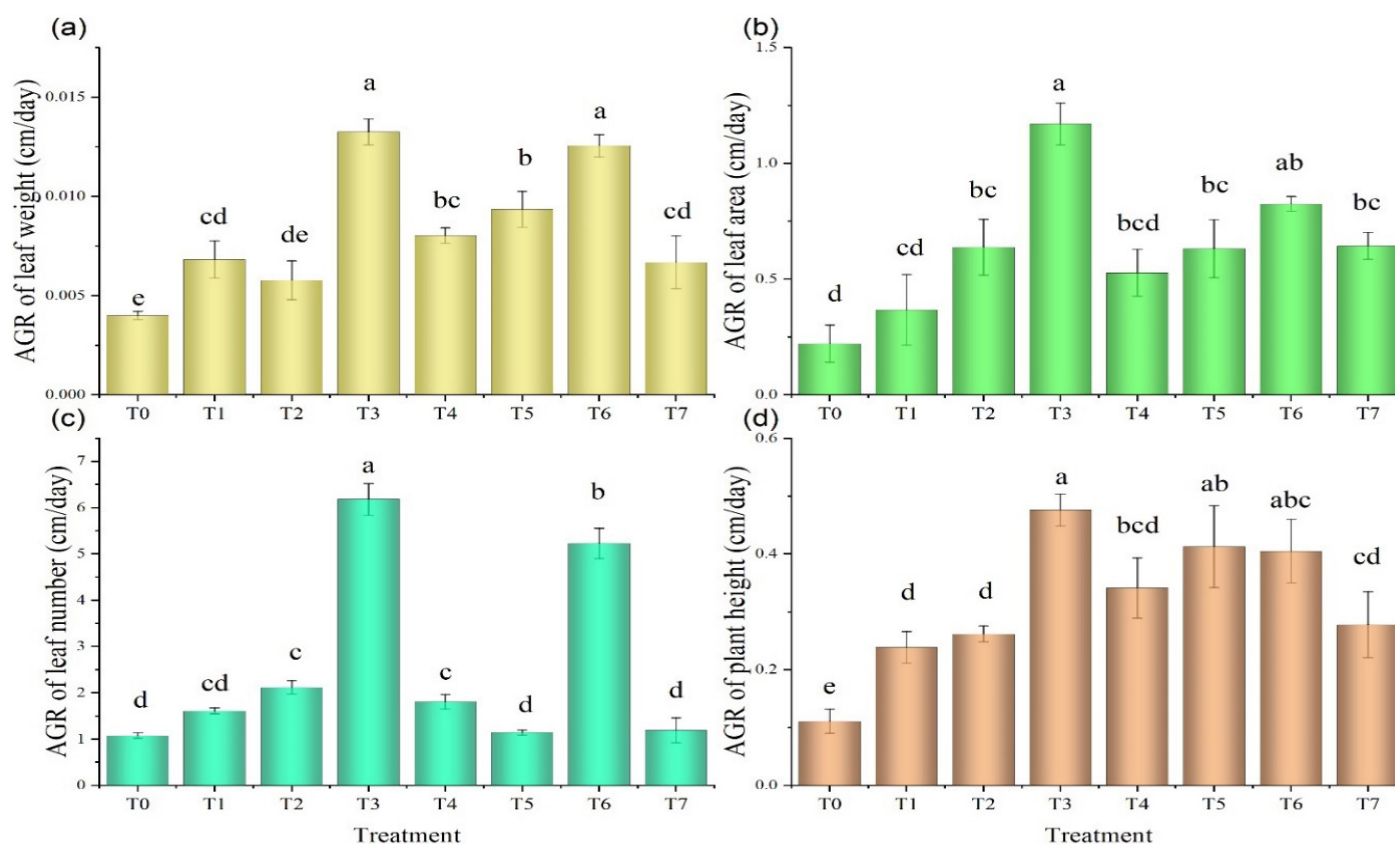


Figure 5: AGR of (a) leaf weight, (b) leaf area, (c) leaf number and (d) plant height of wax apple seedling after exposure to different concentrations of *S. platensis* and *S. polycystum* extracts. Repeated letters did not exhibit a significant difference in values ($n = 5$) at $p < 0.05$. A standard error of five replicates is represented by the error bars. Treatments are as detailed in Table 1.

Discussion

Growth characteristics

Results showed that algal extracts significantly affected wax apple's leaf moisture content, dry weight (DW), and fresh weight (FW), which may enhance plant biomass and water retention. The 10.0% *S. polycystum* extract treatment increased the FW over control, which aligns with previous research that attributes such improvements to the bioactive compounds in algal extracts, including phytohormones, polysaccharides, and micronutrients (Michalak *et al.*, 2016). In various studies, algal extracts have consistently demonstrated positive effects on plant growth characteristics. For example, extracts from *Nostoc calcicole* and *Anabaena vaginicola* significantly increased the FW and DW of tomato, squash, and cucumber leaves (Solomon *et al.*, 2023), while *Cenedesmus quadricauda* extracts enhanced the FW and DW of lettuce seedlings (Puglisi *et al.*, 2020). Furthermore, extracts from *Ulva lactuca* and *Ascophyllum nodosum* increased the FW and DW of wheat (*Triticum aestivum*) and amaranth (*Amaranthus tricolor*), respectively (Carillo *et al.*, 2020). *Ascophyllum nodosum* has also improved water retention in olive plants under water stress, enhancing their water-holding capacity (Dias *et al.*, 2024). It has been suggested that algal extracts stimulate carotenoid and chlorophyll pigment biosynthesis, which likely increases photosynthetic activity and contributes to the enhanced FW and DW of plants (Chiaiese *et al.*, 2018). Additionally, the growth promoting substances and bioactive compounds found in algal extracts, including phytohormones, polysaccharides, and micronutrients, play a critical role in improving plant growth. These compounds stimulate cell division and expansion, which help increase leaf biomass and promote better overall growth and development of plant tissues (Parmar *et al.*, 2023). It has been also reported that the growth promoting chemicals and plant growth regulators improved the plant growth and development (Jamaludin *et al.*, 2020; Khandaker *et al.*, 2018).

Morphological parameters

The present study found a significant positive correlation between the concentration of algal extracts and various growth parameters of wax apple seedlings, including plant height, leaf number, leaf area, and stem diameter. The higher doses, particularly 10.0% *S. platensis* and 10.0% *S. polycystum*, showed the most noticeable improvements, suggesting that algal extracts

can effectively promote plant growth. This outcome highlights the potential of algal extracts as a tool for enhancing agricultural productivity. Similar findings have been reported in other studies, where algal extracts, such as those from *S. platensis*, *Ascophyllum nodosum*, and *Chlorella vulgaris*, significantly improved plant growth in various crops. For instance, *S. platensis*, *Ascophyllum nodosum*, and Baltic green macroalgae enhanced crop height in winter wheat (Michalak *et al.*, 2016), while *Aphanothece* sp., *Chlorella ellipsoidea*, and *Scenedesmus obliquus* promoted plant height and leaf number in tomatoes (Mutale-Joan *et al.*, 2020). *Ascophyllum nodosum* extracts also improved plant height, leaf number, and stem diameter in *Amaranthus tricolor* (Carillo *et al.*, 2020), and *S. platensis* extracts enhanced growth and yield in *Lupinus luteus* at a 0.25% concentration (Shedeed *et al.*, 2022). Additionally, extracts from *S. platensis* and *Chlorella vulgaris* increased plant height and leaf area in tomatoes after 45 days of treatment (Mostafa *et al.*, 2023), while foliar sprays of *Chlorella vulgaris*, *Nannochloropsis salina*, and *Arthrospira platensis* promoted plant height, leaf area, and root length in common beans (Gharib *et al.*, 2024).

These effects are likely attributed to the presence of phytohormones such as gibberellins, auxins, and cytokinins, which promote cell division and elongation, thereby accelerating plant growth (Hamouda *et al.*, 2022). Algal extracts mimic or enhance the plant's natural hormonal balance, boosting growth responses (Rathod *et al.*, 2023). Additionally, they are rich in essential macronutrients like nitrogen (N), phosphorus (P), and potassium (K), as well as micronutrients such as iron, manganese, and zinc, which are vital for enzymatic processes and overall plant metabolism (Carillo *et al.*, 2020; Michalak *et al.*, 2016). The synergistic action of these bioactive compounds significantly enhances plant vigour and growth. Khandaker *et al.* (2017) also reported that the macro and micro-nutrients of organic fertilisers may be responsible for higher plant growth and development.

Bud and flower formation

The introduction of algal extracts from *S. platensis* and *S. polycystum* has significantly reduced the time needed for bud and flower development. Higher doses, such as 10.0% *S. platensis* and 10.0% *S. polycystum* significantly accelerated bud and flower production compared to smaller doses and the control. While research on the impact of algae extracts on bud and flower formation is limited, the findings of this study

suggest promising results. The budding and flowering phases are crucial for fruit set and overall fruit yield. [Gene Albrigo and Galán Saúco \(2002\)](#) highlighted that the yield of fruit tree crops is primarily determined by the flowering intensity and subsequent fruit set. The processes involved in flowering, such as bud induction and differentiation, are closely linked to the number of flowers that can develop into fruits. Therefore, an intense flowering phase is essential for maximising fruit production ([Gene Albrigo and Galán Saúco, 2002](#)). In line with this, *Ascophyllum nodosum* extracts have been shown to enhance fruit set, yield, and quality in winter guava (*Psidium guajava* L.) ([Ali et al., 2021](#)).

Additionally, extracts from *Hypnea cornuta*, *Ulva ohnoi*, and *Sargassum muticum* significantly increased the number of flowers and their longevity in *Mammillaria prolifera* ([Prisa and Spagnuolo, 2022](#)). A study on *Freesia hybrid* L. demonstrated that seaweed extracts promoted growth and flowering in treated plants ([Al-Karimjassim et al., 2019](#)). These findings suggest that algae can enhance plant growth by influencing mineral concentrations in tissues, increasing flower output, and improving overall plant quality ([Tarraf et al., 2015](#)). Plant hormones are bioactive compounds that are found in algae. These hormones play an essential part in the growth and development of plants. However, their concentrations are often too low to be detected using standard analytical instruments ([Rathod et al., 2023](#)). [Prisa and Spagnuolo, \(2022\)](#) noted that some algal extracts contain trace amounts of ethylene precursors, which can stimulate flowering and fruit development in plants.

Growth analysis

An enhanced RGR is directly associated with improved nutritional status, as better nutrient availability fuels metabolic processes, boosts photosynthetic activity, and promotes biomass accumulation ([Gent, 2017](#)). In this study, the RGR of wax apple leaves demonstrated significant improvements with the application of algal extracts, particularly in T3 (10.0%*S. platensis*) and T6 (10.0%*S. polycystum*) treatments. The foliar applications of algae extract increased the RGR of *Solanum quitoense* cv. Septentrionale. Another study employed *Chlorella vulgaris* and *Scenedesmus quadricauda* extracts, which successfully increased the RGR value in lettuce (*Lactuca sativa*) ([Santoro et al., 2023](#)). This study discovered that the biological pathways responsible for improving RGR were 'bio-

synthesis of amino acids' and 'plant hormone signal transduction, signifying that algal extract plays a crucial role in causing a profound rearrangement of hormone biosynthesis pathways ([Santoro et al., 2023](#)). *Chlorella vulgaris* extract also increased the growth traits, chlorophyll and protein content, stimulating both primary and secondary metabolic pathways in the same plant ([La Bella et al., 2021](#)). The enhanced growth rates are also highly associated with larger leaf areas, which improve light capture and increase photosynthetic activity ([Dambreville et al., 2015](#)). Larger leaves can absorb more photosynthetically active radiation (PAR), crucial for maximising carbon gain ([Yang et al., 2022](#)).

This study found that algal extracts increased specific leaf weight (SLW) while decreasing particular leaf area (SLA). Similar results were reported in sweet pepper by [Abdelaziz \(2014\)](#). An increase in SLW often corresponds with a decrease in SLA, as thicker leaves with higher SLW tend to have smaller areas per unit weight ([Frąszczak et al., 2024](#)). SLW and SLA are frequently influenced by dry weight and leaf area. A 50% algal extract in wheat increased dry weight, contributing to higher biomass and SLW ([Michalak et al., 2016](#)). Algal extract exposure typically enhances leaf structures, boosting SLW ([Díaz-Leguizamón et al., 2016](#)). Improved leaf structures also enhance photosynthesis, resulting in larger leaf areas relative to biomass, as bioactive compounds in algal extracts improve nutrient uptake and leaf development ([Lefi et al., 2023](#)).

The leaf area index (LAI) analysis showed significant improvements with algal extract treatments, particularly T3 and T6, indicating a positive effect on canopy development and plant vigour. These treatments provided optimal conditions for leaf expansion, increasing the photosynthetic surface area. This finding aligns with studies where *Chlorella vulgaris* and *Nannochloropsis salina* enhanced leaf area and chlorophyll content, improving light interception and photosynthetic efficiency ([Gharib et al., 2024](#)). As [Keane \(2019\)](#) noted, high LAI is crucial for maximising light interception and carbon assimilation, leading to better plant growth and productivity.

The findings highlight the fundamental role of algal extracts in promoting plant growth, particularly with significant increases in absolute growth rate (AGR) parameters such as leaf weight, leaf area, leaf number,

and plant height in T3 and T6 treatments. Similar findings have been reported that algal extracts from *S. platensis* and *Ascophyllum nodosum* positively impact crop productivity and quality (Michalak *et al.*, 2016; Kang *et al.*, 2021). In addition, tomato plants treated with algal extracts exhibited more branches and flowers, indicating improved growth (Kang *et al.*, 2021). Microalgal extracts also promote biostimulant effects, enhancing plant length, fresh weight, dry weight, photosynthetic components, and metabolite profiles in *Solanum lycopersicum* (Mutale-Joan *et al.*, 2020). These extracts are rich in essential nutrients and bio-active compounds, facilitating better nutrient uptake and promoting growth (Van Oosten *et al.*, 2017). Studies also highlighted their role in increasing the absorption of K, Fe, and Cu in olive plants (Shukla *et al.*, 2019) and boosting rapeseed (*Brassica napus*) growth through enhanced nitrogen and sulphate uptake (Jannin *et al.*, 2013). Furthermore, algal-derived polysaccharides contribute significantly to nutrient availability, directly supporting growth metrics like AGR (Rachidi *et al.*, 2021).

Conclusions and Recommendations

This study highlighted the substantial potential of *S. platensis* and *S. polycystum* extracts as effective biostimulants for improving the growth and development of wax apple seedlings. The application of these algal extracts, especially at higher concentrations, resulted in significant enhancements in significant growth parameters such as plant height, leaf number, stem diameter, and leaf area. Treatments also promoted faster bud and flower formation, with higher numbers of buds and flowers observed. Moreover, increases in Absolute Growth Rate (AGR) and Relative Growth Rate (RGR) demonstrated enhanced biomass accumulation and overall plant vigour. Improvements in specific leaf weight (SLW) and reductions in specific leaf area (SLA) further emphasise the extracts' positive impact on leaf structure. These findings reinforced the role of algal extracts as eco-friendly alternatives to synthetic fertilisers, advancing sustainable agricultural practices and boosting crop productivity. This research added to the evidence supporting the use of biostimulants for sustainable crop production and environmental conservation.

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Novelty Statement

The study provides novel information on algal extract's effects on the vegetative and reproductive growth of wax apple seedlings.

Author's Contribution

Md. Tajol Faeiz Md. Tajudin: Experiment conduction, data curation and analysis, manuscript writing.

Mohammed Moneruzzaman Khandaker: Supervising, project planning and manuscript editing.

Nurul Elyni Mat Shaari: Data analysis, manuscript writing and editing.

Nor Hasima Mahmood: Supervising and reviewing of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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