Effect of Soaking Time of Indian Camphorweed (*Pluchea Indica* L.) Leaf Extract on the Growth of Seaweed (*Kappaphycus alvarezii)*

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Abstract. The growth of *Kappaphycus alvarezii* can be enhanced with biofertilizers. This study aimed to determine the optimal soaking time for *K. alvarezii* in camphor leaf extract (*Pluchea indica* L.) to improve growth. This study was conducted over 45 days, from May to July 2024, in the coastal waters of Rahia village, Central Buton district, Southeast Sulawesi. The study included four different soaking times (0, 6, 9 and 12 hours) as treatments with 12 replications. Observed variables included the daily growth rate (DGR), dry weight to wet weight ratio (DW: WW), clean anhydrous weed (CAW) content, as well as the presence of epiphytes and diseases. The highest DGR, which tended to be significantly different ($P< 0.05$), was observed in the 9-hour soaking times, ranging from 4.45% to 10.69% per day, while the lowest was in the 0-hour treatment, at 3.66% to 8.48% per day. No significant differences ($P> 0.05$) were found for DW: WW ratio and CAW content. Although *Ulva lactuca* were detected, no diseases or epiphytes were found in any extract treatments. A 9-hour soaking period in camphorweed leaf extract can effectively support the growth of *K. alvarezii* growth.

1 Introduction

Seaweed production in Southeast Sulawesi has shown a declining trend. Several factors contributing to this decline include the lack of high-quality seaweed seedlings, disease outbreaks, and changing water conditions due to climate change. Seaweed growth can be enhanced with the addition of growth hormones. However, a trend has emerged among seaweed farmers to use detergents to accelerate seaweed growth and reduce harvest time. Unfortunately, increased detergent residues in cultivation waters can inhibit seaweed growth. Therefore, it is essential to promote the use of natural materials available in the environment as safer alternatives.

Indian camphor (IC) leaf extract (*Pluchea indica* L.) has long been used as a biofertilizer. Stimulating effects on the radicle length of mustard greens (*Brassica chinensis*) and corn (*Zea mays*) have been observed with IC leaf extract concentrations of 1.3% and 2.5% w/w [1]. IC leaf extract can serve as a natural nutrient source and growth enhancer for seaweed

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through soaking, allowing the nutrients to be absorbed by the seaweed. Soaking duration is crucial for nutrient absorption, as different plant types have varying absorption capacities. Extending soaking time can enhance nutrient uptake through diffusion, accelerate physiological processes, and improve growth rates [2]. Since 2015, farmers in the Central Buton district of Southeast Sulawesi have been using IC leaf extract to nourish seaweed, resulting in healthier, stronger, and more disease-resistant crops (Karim, pers. comm., 2023). Unfortunately, no research has been conducted so far on the use of IC to stimulate the growth of *K. alvarezii*, and the optimal soaking time to maximize seaweed growth remains unknown. Therefore, this study aims to determine the ideal soaking time for IC leaf extract to enhance the growth of *K. alvarezii.*

2 Methods

This research was conducted over 45 days, from May to July 2024. Forty-five days is the standard duration for seaweed cultivation. The study took place in the waters of Rahia Village, Central Buton Regency, Southeast Sulawesi, Indonesia (122º39'0" N, 5º18'30" E). This coastal village is known for local cultivation of *K. alvarezii* seaweed. Both the seaweed seeds and IC leaves were sourced from this village.

2.1 Experimental design

The experimental design used was a Completely Randomized Design (CRD) with 4 treatments (0, 6, 9, and 12-hour soaking times) and 3 replicates per treatment, resulting in a total of 12 experimental units. All data were analyzed using ANOVA to assess treatment effects on the observed variables. Significant results were further analyzed using Tukey's test at a 95% confidence level in SPSS version 20.

2.2 The sample preparation of seaweed seeds and IC extract

The preparation was started with collection of *K. alvarezii* seaweed seeds. After colection, the seeds were cut into smaller parts, with an average seedling weight of 15 g.The preparation of IC extract as fertilizer involved first washing the IC leaves thoroughly to remove any dirt. The extract was made by weighing 100 g of IC leaves placing it into a grinder, and adding 100 ml of seawater (1:1 ratio). The mixture was then ground into a fine paste and filtered using a 60-mesh sieve to separate the seaweed from its extract.

2.3 Treatment stage

The treatment stage began by weighing the seaweed seeds and tying them together with a rope. A soaking container with 20 liters of seawater was then prepared, along with 100 ml of *S. aquifolium* extract. The tied seaweed seeds were placed into the container with the seawater and *S. aquifolium* extract mixture. The seeds were soaked for varying durations. After the soaking treatment, the seaweed seeds were ready for planting. The planting was carried out using the long line method. The growth of the cultivated seaweed was monitored, along with the measurement of environmental parameters at the research site. Observations of seaweed growth were conducted for 45 days after planting, with the seaweed being weighed every 9 days. Water quality parameters, including temperature, salinity, pH, EC, TDS, and current speed, were also measured.

2.4 Observation stage

During this stage, the growth of the cultivated seaweed was monitored, along with the measurements of environmental parameters at the research site. Observations of seaweed growth were conducted for 45 days after planting, with the seaweed being weighed every 9 days. Water quality parameters, including temperature, salinity, pH, total dissolved solids (TDS), and current speed, were also measured. The observed variables include:

- *Daily Growth Rate (DGR).* The daily growth rate of seaweed was calculated using the formula [4]:

$$DGR = \left[\left(\frac{Wt}{W0}\right)^{\frac{1}{t}} - 1\right] \times 100\% \quad (1)$$

  where Wt=seedling weight at the end of the study (g), W0 = average seedling weight at the beginning time (g), and t=Maintenance time (days).
- *Dry Weight Ratio (DW): Wet Weight (WW) and Clean Anhydrous Weed (CAW) cleanliness level of seaweed*. The WW:DW ratio is obtained by measuring the wet weight (WW) of all harvested seaweed. After being sun-dried, each sample was weighed to get a dry weight (DW). The ratio was calculated for all samples. The cleanliness level of seaweed / CAW was determined by washing the dried samples using fresh water repeatedly until clean to remove dirt and salt. The washed samples were then dried in an oven at 80º for 18 hours. The CAW level was calculated using the following formula: CAW(%) = (final weight after rinsing/initial weight)*100.
- *Epiphytes and diseases*. Observations of pests and diseases found in seaweed were visually observed and analyzed descriptively.
- *Environmental parameters.* Temperature, salinity, pH, current speed and Total Dissolved Solid (TDS) were measured every 3 days using instruments (e.g., thermometer, refractometer, pH meter, current meter, and water quality tester). Turbidity, nitrate, and phosphate levels were assessed every 9 days using a turbidimeter and the Hanna instrument.

3 Results and discussion

3.1 Daily Growth Rate (DGR)

The 9-hour soaking treatment produced the highest DGR, ranging from 4.45±0.00%/day to 10.69±0.46%/day (Figure 1**)**. Following this was the 6-hour soaking treatment (3.97±0.04%/day), the 12-hour soaking (3.81±0.06%/day to 9.04±0.68%/day), and the control with no soaking (3.66±0.07%/day to 8.48±0.12%/day). In the 9-hour treatment, the peak DGR was observed on day 9 at 10.69±0.46%/day, gradually tapering to 4.45±0.00%/day by day 45. This DGR was significantly different ($P<0.05$) from the other treatments, except on days 27 and 36, where it showed no significant difference from the 6-hour treatment. Notably, the 9-hour treatment also produced the highest average DGR, reaching 6.91±2.38% per day, surpassing results from other studies. For example, [5] found that *K. alvarezii* seedlings soaked in Indian almond leaf extract (*Terminalia catappa* L.) achieved a DGR of 3.97%/day. The high DGR achieved in the 9-hour soaking treatment here suggests it provides an optimal period for nutrient absorption from IC leaf extract, enhancing growth by allowing more effective nutrient uptake, which likely accelerates and strengthens seedling development.

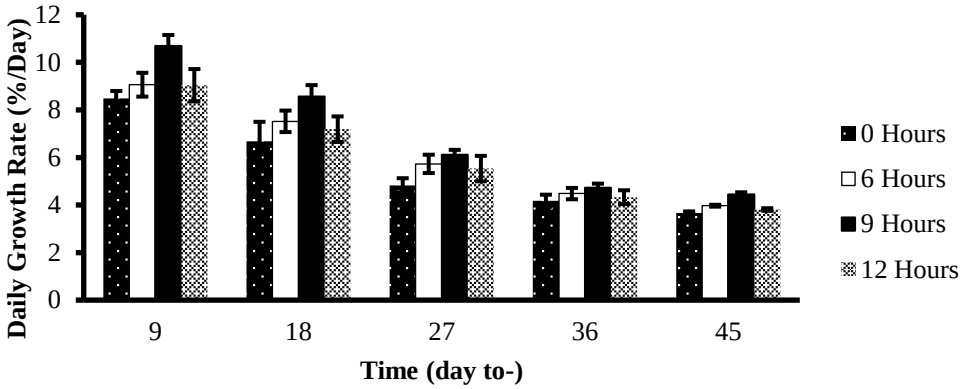

Fig. 1. Daily Growth Rate of *K. alvarezii* Seaweed using different soaking times 0, 6, 9 and 12 hours.

This study further highlights that the IC extract produced a much higher DGR compared to using commercial biostimulants such as Acadian Marine Plant Extract Powder AMPEP). Previous studies reported lower average DGRs using AMPEP for *K. alvarezii* during a 45-day growth period: 3.7±0.2–5.6±0.1% [6], and 3.43–4.25% per day [7]. The variation in daily growth rates among soaking treatments (6, 9, and 12 hours) is likely due to differences in soaking times, which influence the amount of nutrients absorbed from IC leaves by the seaweed's cell walls, thereby impacting *K. alvarezii* growth.

This study also revealed that the durations of the lag and exponential growth phases in *K. alvarezii* differ from those observed in subtropical regions. In India, the lag phase, an acclimatization period during which *K. alvarezii* prepares for growth, typically lasts about 15 days, followed by an exponential growth phase that maximizes biomass until around day 30 [8]. In contrast, our study found that these phases occur much more quickly, completing within just nine days. This difference is likely due to climatic conditions: [8]'s study was conducted in India's subtropical climate, while ours took place in a hot tropical climate.

3.2 Dry weight (dw) and wet weight (ww) ratio

The DW: WW ratio indicates the amount of wet weight needed to produce 1 kg of dry weight. In this study, the ratios for soaking treatments of 0, 6, 9, and 12 hours were 1:10.23, 1:10.32, 1:10.53, and 1:10.26, respectively (Figure 2). This means that approximately 10 kg of fresh material is required to obtain 1 kg of dried seaweed. Moreover, the ratio found in this study is very similar to those reported in previous studies, where [9] and [10] observed ratios of 1:9.85 and 1:9.89, respectively.

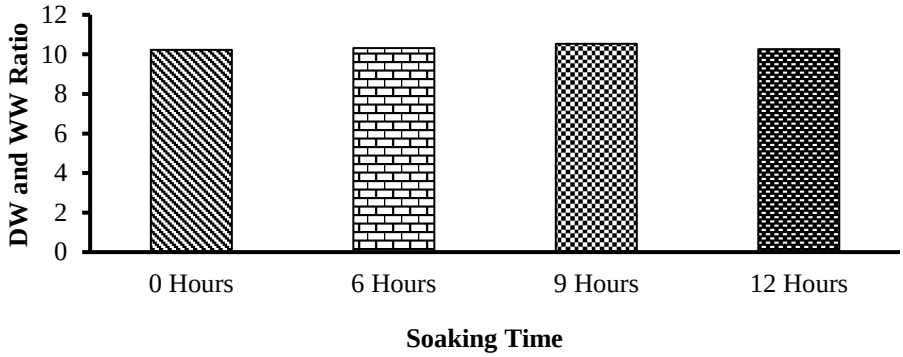

Fig. 2. Ratio of DW:WW of *K. alvarezii* with varying soaking times (0, 6, 9, and 12 hours)

3.3 Clean Anhydrous Weed (CAW)

CAW is a parameter used to determine the salt-free dry matter content in seaweed [27]. In this study, the CAW percentages for the different soaking times were 6.49±6.32%, 45.94±2.11%, 45.69±1.45%, and 50.51±8.97% for 0, 6, 9, and 12 hours, respectively (Figure 3). No significant differences were observed among the CAW values of the samples (P>0.05).

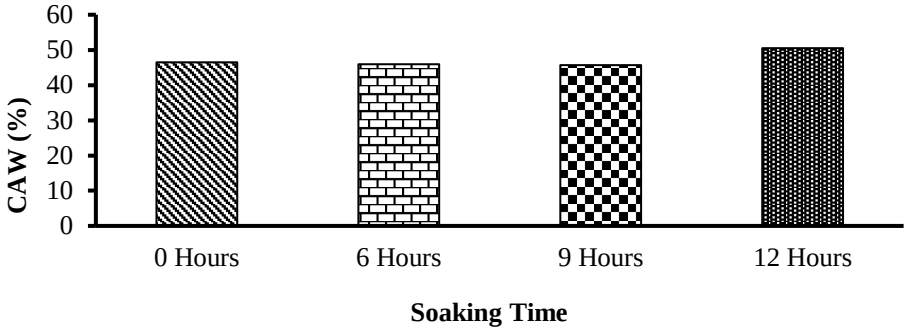

Fig. 3. Clean Anhydrous Weed (CAW) of *K alvarezii* with varying soaking times (0, 6, 9, and 12 hours)

Additionally, the samples meet the international standard, which sets a minimum CAW of 40% [11]. The results of this study are also consistent with other published findings, where CAW from dried *Kappaphycus* and *Eucheuma* ranged from 39-45% [12].

3.4 Epiphytes and disease

Observations of epiphytes and diseases on seaweed during the study showed differences between seaweed treated with IC leaves extract soaking (6, 9, and 12 hours) and those not treated (0 hours).

Fig. 4. Epiphytes and diseases observed in seaweed during the study. (A) *K. alvarezii* soaked in IC leaf extract (left) and *K. alvarezii* without soaking, showing signs of ice-ice disease (right); (B) *Ulva lactuca* growing as epiphytes on *K. alvarezii* (highlighted within the circle); (C) Ice-ice disease on *K. alvarezii* from local farmers (indicated by an arrow); (D) Unidentified epiphytes on *K. alvarezii* from local farmers.

These extracts clearly protect seaweed from pests and diseases, positively impacting its growth. Seaweed growth is closely linked to its overall health; the healthier the seaweed, the better its growth rate. Epiphytes on the seaweed thallus can cause significant harm, as they may carry diseases that infect the seaweed, impairing its growth and reducing productivity. In contrast, a previous study using the biostimulant AMPEP still found epiphytes and diseases, albeit at a low incidence [7]. This finding is particularly interesting regarding the use of Indian camphorweed leaf extract for reducing disease and epiphyte presence in cultivated *K. alvarezii*. To optimize the use of this extract, further research is needed to evaluate its effectiveness in mitigating the impact of diseases and epiphytes on *K. alvarezii*. Future studies should also compare the effects of this leaf extract with those of commercial biostimulants like AMPEP.

3.5 Environmental parameters

All environmental parameters—temperature, salinity, total dissolved solids (TDS), current velocity, pH, turbidity, nitrate, and phosphate—at the study site were suitable for the growth of *K. alvarezii* (Figure 5).

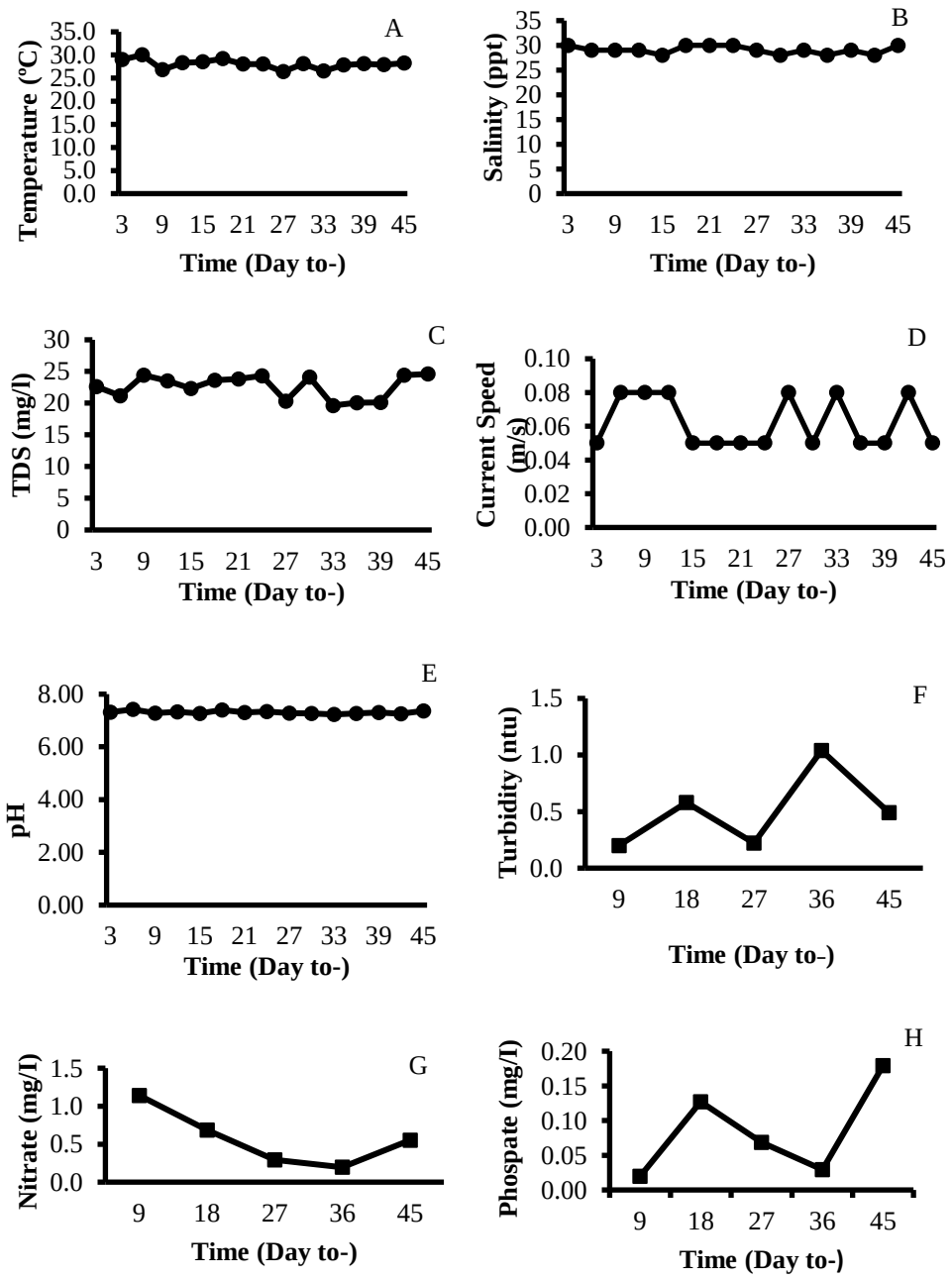

Fig. 5. Water Quality Parameters During the Study: (A) Temperature (ºC); (B) Salinity (ppt); (C) Total Dissolved Solids (TDS) (mg/L); (D) Current Speed (m/s); (E) pH; (F) Turbidity (NTU); (G) Nitrate (mg/L); (H) Phosphate (mg/L)

The seawater temperature varied from 26-30^{0}C during the study period (Figure 5a). The average temperature was 28.1^{0}C This range and average seawater temperature are considered ideal for the growth of *K. alvarezii*. High seaweed production and growth are primarily observed within the temperature range of 27 ºC-30 ºC. The high growth rates found in this study coincided with a favorable growth season, which aligns with the peak growth period

of *K. alvarezii* from April/May to June/July. This high DGR was supported by an optimal temperature of 29.5±1.0 ºC [13].

Salinity during the study averaged 29.07 ppt, ranging from 28 to 30 ppt (Figure 5b). This range falls within the optimal salinity level for seaweed cultivation. The average total dissolved solids (TDS) level was 22.59 mg/L, with a range of 19.63 to 24.60 mg/L (Figure 5c), which is highly suitable for supporting marine life, including seaweed farming. Water current speed averaged 0.06 m/s, ranging from 0.05 to 0.08 m/s (Figure 5d). Conducted from May to July, the study showed favorable conditions for seaweed production and growth, indicating the beginning of an optimal planting season. The pH at the research site ranged from 7.23 to 7.42, with an average of 7.31 (Figure 5e), which falls within the ideal range for the growth of *K. alvarezii*. Turbidity levels averaged 0.51 NTU, with a range of 0.20 to 1.04 NTU (Figure 5f), also considered suitable for *K. alvarezii* cultivation. Nitrate concentrations at the site ranged from 0.19 to 1.14 mg/L, with an average of 0.57 mg/L (Figure 5g). Phosphate levels ranged from 0.02 to 0.17 mg/L (Figure 5g), placing the site within the highly suitable range for seaweed farming.

The high growth observed in this study suggests that the site is highly favorable for seaweed cultivation, supported by both optimal water conditions and the application of Indian camphor (IC) leaf extract. Similar favorable water conditions have also been reported in other areas, such as Bontang, East Kalimantan, Indonesia, where seaweed production was approximately five times higher than in less suitable regions like Manado, North Sulawesi [14].

4 Conclusion

The conclusion on this research highlights the promising potential of Indian camphorweed leaf extract in supporting the growth of *K. alvarezii*, reducing epiphytes, and combating diseases, particularly Ice-Ice. The use of Indian camphorweed leaf extract presents a viable alternative to chemical fertilizers, which are harmful to the aquatic environment.

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References

1. M. R. Salim, W. Z. Aieeda ,W. A. Halim JAB **13** (2022)
2. R. S. Patadjai, I. Nur, S. Kamri IOP Conf Ser Earth Environ Sci **370**, 1 012026 (2019)
3. E. Tamala, A, Slamet, Jumiati . Scientific Journal of Life Sciences **7**, 1 (2022)
4. W. T. L. Yong, J. Y. Y. Chin, S. Yasir S, Phycol. Res. **62** (2014).
5. P. M. Kurniawan, R. L. Kreckhoff, E L A Ngangi and B T Wagey, Journal of Aquaculture. **8,** 2 (2020)
6. M. K. M. Ali, S. Md. Yasir, A. T. Critchley, A. Q. Hurtado, J Appl Phycol **30**, 1,
7. H Cahyani, Hardianti, D P Kurnia and L O M Aslan, IOP Conf. Ser.: Earth Environ. Sci. **473**, 012009 (2020)
8. A. Seth, M. Shanmugam, Seaweeds as Agricultural Crops in India: New Vistas. In: Dagar, J., Sharma, P., Sharma, D., Singh, A. (eds) Innovat(ive Saline Agriculture. (Springer, New Delhi, 2016)
9. L. O. M. Aslan, W. Iba, A.B. Patadjai, M. Rahim, F. Febriyanti, Raznawati, H. Hafid, Armin, IOP Conf. Ser.: Earth Environ. Sci **382** 012007 (2019)

10. Armin, L.O.M Aslan, A.B. Patadjai, W Iba, Yusnaeni, W.H Muskita, IOP Conf. Ser.: Earth Environ. Sci **370** 012043 (2019)
11. C. Periyasamy, P. V. S. Rao, P. Anantharaman, J. Appl. Phycol **28,** 1 (2015)
12. M. Shanmugam, K. Sivaram, E. Rajeev, V. Pahalawattaarachchi, P. N. Chandraratne,J. M. Asoka, Abhiram Seth, J. Appl. Phycol. **29**, 6 (2017)
13. L.O.M. Aslan, R.S. Patadjai, Ruslaini, I.J. Effendy, A.H. Sarita, S. Amina, Nuraeni, Armin, IOP Conf. Ser.: Earth Environ. Sci. **37** (2019)
14. N. F. Simatupang, P. R. Pong-Masak, P. Ratnawati, Agusman, N. A. Paul, M. A. Rimmer, Aquac. Res. **20** (2021)