Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(6): 1713 – 1732 (2025)
www.ejabf.journals.ekb.eg

Formulation and Efficacy of Fermented Liquid to Enhance the Growth Performance of the Seaweed *Kappaphycus alvarezii*

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ARTICLE INFO
Article History:
Received: Aug. 20, 2025
Accepted: Nov. 5, 2025
Online: Dec. 4, 2025

Keywords:
Fermentation,
Nutrients,
Organic fertilizer,
Cultivation,
Seaweed growth,
Stability,
Synergy test,
Viability

ABSTRACT
This research aims to formulate and evaluate the efficacy of fermented mangrove leaf liquid in enhancing the growth performance of seaweed *Kappaphycus alvarezii*. The fermentation process utilized primary materials from the leaves of the mangrove *Avicennia marina*, brown sugar, and seawater. The fermentation results were formulated with macro (N, P, K) and micro (Fe, Mn, Mg) nutrients in accordance with the quality standards for liquid organic fertilizers. The study employed a completely randomized design with four treatments: C1 (1 L of fermented liquid/100 L of seawater), C2 (1 L of fermented liquid/50 L of seawater), C3 (1 L of fermented liquid/100 L of seawater), and a control (K), with three replications. Evaluation was conducted through nutrient content analysis, synergy testing among isolates, stability, viability, and seaweed growth tests at the laboratory scale. The results indicated that the formulated fermented liquid contained higher levels of macro (N, P, K) and micro (Fe, Mn, Mg) nutrients compared to the initial fermented liquid. Synergy tests indicated that the three isolates functioned synergistically without antagonistic interactions. Stability tests showed that the product remained homogeneous, without sedimentation, and was resistant to extreme temperature cycles (4-40°C). The viability of the liquid demonstrated organoleptic quality, pH, salinity, homogeneity, and viscosity that met application standards. Growth tests revealed that treatment C3 yielded the best results, with absolute growth of 3.56±0.97g and daily growth of 0.69±0.19%. Water quality during the study remained within optimal ranges for cultivation. Thus, the formulated fermented mangrove leaf liquid has the potential to serve as an effective liquid biofertilizer to enhance the growth of *K. alvarezii* and can be applied in sustainable cultivation systems.

INTRODUCTION

Seaweed is one of fishery commodities with high economic value, which is widely utilized in the food, pharmaceutical, and cosmetic industries. Indonesia, as a maritime country, possesses significant potential for seaweed cultivation, particularly the species *Kappaphycus alvarezii*. However, the primary challenge in seaweed cultivation is the variable growth rates and susceptibility to diseases, such as ice-ice (**Veenhof *et al.*, 2024**). Previous efforts have been made to control the bacteria responsible for the ice-ice disease

using fermented liquid. Mangrove leaves contain bioactive compounds and nutrients that have the potential to support disease control in seaweed and enhance seaweed growth (**Rahman *et al*., 2020a, b, c, 2024a**). The fermented liquid derived from the leaves of *Avicennia marina* contains secondary metabolites in the form of phytochemicals and bacteriocins, as well as potential endophytic bacteria that can inhibit the growth of pathogenic bacteria (**Rahman *et al*., 2020a, b, c**). Results from this study have reported the ability to inhibit the bacteria causing ice-ice at both *in vitro* (**Rahman *et al*., 2020a, b, c**) and semi-field scales (**Rahman *et al*., 2023a, b**). Although the mangrove leaf fermented liquid has demonstrated strong potential in controlling bacteria responsible for the ice-ice disease, its efficacy as a growth-promoting nutritional supplement (liquid biofertilizer) hasn't yet been optimized and requires formulation standardization. In this context, further research is needed to optimize its macro and micro nutrient content and to determine the most efficient dilution ratio for its application in *K. alvarezii* cultivation.

One of the factors influencing seaweed growth is the availability of nutrients in the water, particularly nitrogen (N), phosphorus (P), and potassium (K) (**Lubsch & Lansbergen, 2020**). A new strategy developed to enhance both the quality and quantity of seaweed is the optimization of nutrient content in fermented mangrove leaf liquid through the formulation of both macro and micro nutrients.

Although the mangrove leaf fermented liquid has demonstrated strong potential in controlling bacteria responsible for the ice-ice disease, its efficacy as a growth-promoting nutritional supplement (liquid biofertilizer) hasn't yet been optimized and requires formulation standardization. Hence, further research is needed to optimize its macro and micronutrient content and to determine the most efficient dilution ratio for its application in *K. alvarezii* cultivation.

MATERIALS AND METHODS

Preparation of fermented mangrove leaves liquid

The preparation of fermented liquid enriched with *Bacillus subtilis* MSAR-01, *Bacillus vietnamensis* MSAR-06, and *Bacillus* sp. MSAR-07, each with a density of 10^5 cell/ mL, was conducted following the procedures outlined in the method of **Rahman *et al*. (2019, 2020a, b, c, 2023a, b, 2024a, b)**.

Provision of test seaweed and acclimatization container

Healthy seaweed *K. alvarezii* was obtained from a cultivation center in Banggai Regency. A sample weighing 100kg was immersed in a 1% povidone iodine solution for 3-4 minutes, then placed into an acclimatization tank measuring 2x 1x 0.5m containing 1000L of water, equipped with aeration and lighting, and maintained for 3 days (**Rahman *et al*., 2019, 2020a, b, c, 2023a, b, 2024a, b**).

Formulation of fermented mangrove leaves liquid

The harvested fermented liquid was formulated with nitrogen (5.2%), phosphorus (3.50%), potassium (4.50%), magnesium (0.80%), iron (1200 ppm), and manganese (350 ppm) in accordance with the quality standards for liquid organic fertilizers (Ministery of

Agriculture Regulation No. 70/2011) and the nutrient requirements for seaweed (**Basmal, 2009**). The solution was filtered (200-micron mesh) to remove solid residues and was subsequently tested for its nutrient content prior to application.

Experimental design

This study employed a completely randomized design consisting of four treatments and three replications. The research design implemented in this study is presented in Table (1).

Table 1. Experimental design of the present study

Treatment	Treatments	
	Fermented Liquid (L)	Dilution with Seawater (L)
C1	1	100
C2	1	50
C3	1	25
Control (K)	-	-

Phase I: Nutrient content analysis, synergy testing, stability, viability

Nutrient content analysis

The analysis of nutrient content in the fermented liquid was conducted before and after formulation, encompassing nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), and manganese (Mn). Nitrogen was tested using the Kjeldahl method, phosphorus via spectrophotometry, while potassium, iron, magnesium, and manganese were analyzed using atomic absorption spectroscopy (AAS) (**Hermawati *et al*., 2021; Simamora *et al*., 2022; Musa *et al*., 2024**).

Synergy testing

Isolates in the fermented liquid were streaked to intersect on agar to facilitate interaction. The fermented liquid was centrifuged (10.000 rpm, 10 minutes), and the supernatant was filtered (0.22μm). The isolates were combined with the filtrate, incubated for 24 hours, and then observed for clear zones or inhibition zones (**Hartantai, 2020; Rahman *et al*., 2020c**).

Stability testing of fermented liquid

The initial fermented liquid was evaluated and then stored at 4°C for 24 hours, followed by being transferred to 40°C for 24 hours. This storage was counted as one cycle and was repeated six times (**Slamet *et al*., 2020; Rachmawati *et al*., 2022**).

Viability testing of fermented liquid

a. Organoleptic testing

The fermented liquid was placed in a transparent container to facilitate the observation of color and form. Subsequently, the shape, color, odor, and taste were assessed (**Hujjatusnaini *et al*., 2022; Szalai *et al*., 2023; Devi *et al*., 2024**).

b. pH and salinity testing

The filtered fermented liquid, which had been processed to remove solid particles, was measured for pH using a pH meter and for evaluating salinity, a refractometer was utilized

(**Sukmawati *et al*., 2022; Maulidayanti *et al*., 2024**).

c. Homogeneity testing

Homogeneity testing was conducted by applying 5mL of the fermented liquid onto a glass slide aligned with visual observation. The preparation was considered homogeneous if the particles were evenly distributed without sedimentation or clumping (**Maulidayanti *et al*., 2024; Musa *et al*., 2024**).

d. Viscosity testing

The sample was introduced, and the spindle was immersed to a specified depth, after which the device was activated. The viscosity value was recorded in centipoise (cP); higher viscosity indicated greater thickness (**Irawati, 2018; Evadewi & Tjahjani, 2021; Magalingan *et al*., 2024**).

Phase II: Seaweed growth testing at the laboratory scale

The acclimatized seaweed seedlings were maintained in 1-liter bottles according to the treatments outlined in Table (1), under controlled conditions. The temperature was maintained between 20-28°C, with a pH of 7.5-8.5, light intensity ranging from 1000 to 5000 lux, and a photoperiod of 12 hours of light and 12 hours of darkness. Aeration was provided to facilitate nutrient circulation and oxygen supply, while the maintenance medium was replaced every 2-3 days to prevent the accumulation of metabolites. Growth measurements were taken at the time of seeding and weekly thereafter (**Sulistiani & Yani, 2014; Jiksing *et al*., 2022; Obando *et al*., 2023; Rahman *et al*., 2023a, b**).

Data analysis

Nutrient, synergy, stability, and viability data were analyzed descriptively, while seaweed growth was tested using one-way ANOVA. If significant effects were observed ($P<0.05$), further analysis was conducted using Duncan's test with SPSS 29.

RESULTS

Nutrient content analysis

The analysis of the fermented mangrove leaf liquid and the formulated fermented mangrove leaf liquid revealed the presence of nitrogen (N), phosphorus (P), potassium (K), iron (Fe), manganese (Mn), and magnesium (Mg) compounds (Table 2). The macro (N, P, K) and micronutrients (Fe, Mg, Mn) found in the fermented liquid are essential for seaweed growth.

Table 2. Compounds of mangrove leaves fermentation liquid

Compound	Quantitative results	
	Fermentation liquid	Formulation of fermentation liquid
Nitrogen (%)	0.02	0.87
Phosphorus (P_2O_5) (%)	0.01	0.22
Potassium (K_2O) (%)	0.14	2.61
iron (Fe) (ppm)	18.10	174.61

Manganese (Mn) (ppm)	8.82	113.22
magnesium (Mg) (%)	0.12	0.27

The nitrogen content in the fermented liquid was recorded at 0.02%, while in the formulated fermented liquid, it increased to 0.87%. Phosphorus, in the form of P_2O_5, was detected at 0.01% in the fermented liquid and rose to 0.22% in the formulated fermented liquid. Potassium, in the form of K_2O, was found at 0.14% in the fermented liquid and significantly increased to 2.61% in the formulated fermented liquid. The Fe measured 18.10ppm in the fermented liquid and increased to 174.61ppm in the formulated fermented liquid. Additionally, Mn was detected at 8.82ppm in the fermented liquid and increased to 113.22ppm in the formulated fermented liquid, while Mg was measured at 0.12% in the fermented liquid and increased to 0.27% in the formulated fermented liquid.

The analysis results indicate that the formulation of the fermented mangrove leaf liquid possesses a higher nutrient content compared to the initial fermented liquid. The increase in macro (N, P_2O_5, K_2O) and micronutrients (Fe, Mn, Mg) levels suggests that the formulation process effectively enriches the nutrient composition, thereby enhancing its potential to improve the quality and effectiveness in supporting seaweed growth under controlled cultivation conditions.

Synergy

The results of the synergy tests among the bacterial isolates used in the fermented mangrove leaf liquid and the formulated fermented liquid were evaluated. Three potential isolates were tested, including *Bacillus subtilis* MSAR-01 (A), *Bacillus vietnamensis* MSAR-06 (B), and *Bacillus* sp. MSAR-07 (C), which exhibited the best inhibition against the bacteria responsible for the ice-ice disease in seaweed. After a 24-hour incubation period, none of the interacting isolates formed clear zones or inhibition zones (Fig. 1 & Table 3).

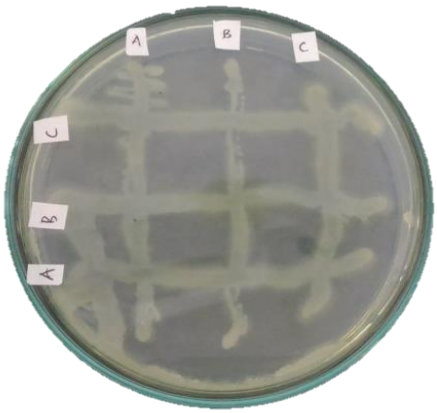

Fig. 1. Synergy between isolates

Table 3. Synergy among the best fermentation liquid isolates and the synergy between isolates and the fermentation liquid

Isolate	A	B	C
A	+	+	+
B	+	+	+
C	+	+	+
Fermentation liquid	+	+	+
Formulation of fermentation liquid	+	+	+

Note: Synergistic (+); Antagonistic (-), *Bacillus subtilis* MSAR-01 (A), (*Bacillus vietnamensis* MSAR-06 (B), and *Bacillus* sp. MSAR-07 (C).

The test results indicate that among the isolates and when mixed into the fermented liquid or the formulated fermented liquid, synergistic outcomes were observed. This finding suggests that no antagonistic interactions were present among the isolates. This condition underscores that the isolates utilized can collaborate harmoniously to support fermentation activity. Such positive synergy is significant as it indicates the potential for enhanced effectiveness of the fermented liquid when used as a nutrient source, particularly for applications in seaweed cultivation or other microorganisms. The presence of multiple supporting isolates will strengthen the stability of the metabolites produced, improve nutrient quality, and prevent the dominance of a single strain that could disrupt the fermentation process.

Stability

The stability test of the formulated fermented mangrove leaf liquid was conducted by storing the liquid at 4°C for 24 hours, followed by a transfer to 40°C for another 24 hours. This process was repeated for a total of six cycles to evaluate any changes occurring in the liquid, including sedimentation, color alteration, or texture modification. This test aimed to ensure the product's stability under varying storage conditions and its effectiveness after long-term storage.

Table 4. Stability of the formulated mangrove leaves fermentation liquid

Sample	Cycle	Storage Temperature (°C)	Storage Time	Observation Results
Sample 1	Cycle 1	4	24 hours	No change in color or sedimentation in the liquid.
Sample 1	Cycle 2	40	24 hours	The liquid remained homogeneous, with no change in texture or odor.
Sample 1	Cycle 3	4	24 hours	No clumping (coagulation) or change in taste occurred in the liquid.
Sample 1	Cycle 4	40	24 hours	The liquid could still be used without significant changes.
Sample 1	Cycle 5	4	24 hours	No difference in texture or odor even though it had been heated and cooled.
Sample 1	Cycle 6	40	24 hours	The liquid remained stable after being stored at high and low temperatures for six cycles.

Viability

Organoleptic

The results of the organoleptic testing of the formulated fermented liquid were based on color, odor, taste, texture, and viscosity. These parameters are essential for assessing the quality and effectiveness of the fermented mangrove leaf liquid, as well as its impact on seaweed growth.

Table 5. Organoleptic of the formulated fermentation liquid

Parameter	Score (1-5)	Observation Results
Color	3	The color is predominantly dark brown, not very clear/transparent.
Odor	4	The fermentation odor is quite sharp, pungent but not overly bothersome (or, not too unpleasant).
Taste	2	The taste is slightly sour and bitter, not too strong.
Texture	3	Liquid texture with moderate viscosity (thickness), non-foaming.

pH and salinity

The measurement of pH and salinity of the liquid was conducted to determine the acidity or alkalinity levels and the concentration of dissolved salts in the liquid used as fertilizer. Appropriate pH is crucial to ensure that organic fertilizers can be effectively absorbed by plants, while suitable salinity is necessary to prevent osmotic stress on plants and to maintain healthy growth conditions.

Table 6. pH and salinity of the mangrove leaves fermentation liquid

Sample	pH	Salinity (ppt)
Fermentation liquid	2.74	64
Formulation of fermentation liquid	4.64	75
C1	5.27	35
C2	4.96	41
C3	4.69	45

The initial fermented liquid exhibited a very acidic pH of 2.74, attributed to the accumulation of organic acids resulting from microbial activity during the fermentation process. After formulation, the pH increased to 4.64, indicating a reduction in acidity, thus rendering the liquid more stable and safer for application. The dilution treatments from C1 to C3 showed a trend of increasing pH corresponding to the level of dilution. Treatment C1 exhibited the highest pH of 5.27, approaching neutral conditions, while treatments C2 and C3 tended to have lower pH values.

The salinity of the initial fermented liquid was relatively high at 64ppt, which increased to 75ppt following the formulation process, likely due to the addition of mineral materials or salts during formulation. The dilution of the liquid resulted in a significant decrease in salinity, with values ranging from 35ppt in C1 to 45ppt in C3. These results indicate that the concentration of the fermented liquid directly affects the salt content, where higher dilution levels in treatment C1 resulted in lower salinity.

Homogeneity

The results of the homogeneity observation of the formulated fermented mangrove leaf liquid are presented in Table (7). This assessment is crucial for determining the uniformity and stability of the liquid, particularly concerning sedimentation and clumping, which can affect the quality of the formulated fermented liquid.

Table 7. Homogeneity of the formulated mangrove leaves fermentation liquid

Sample	Liquid Volume (mL)	Observation Results
Sample 1	5	Homogeneous, no sedimentation or clumping/coagulation
Sample 2	5	Homogeneous, no sedimentation or clumping/coagulation
Sample 3	5	Homogeneous, no sedimentation or clumping/coagulation
Sample 4	5	Homogeneous, slight sedimentation
Sample 5	5	Not homogeneous, there is sedimentation and clumping/coagulation

Viscosity

The results of the viscosity measurements of the formulated fermented mangrove leaf liquid across various samples are shown in Table (8). Viscosity is an important parameter that indicates the thickness of the liquid, influencing the ease of flow and application of the formulation in cultivation or fertilization processes. These measurement results illustrate the variation in thickness among the tested samples.

Table 8. Viscosity of the formulated mangrove leaves fermentation liquid

Sample	Liquid Volume (mL)	Spindle Used	Viscosity (cP)	Observation Results
Sample 1	100	Spindle 1	12.5	Low viscosity, the liquid is somewhat fluid (thin) and flows easily.
Sample 2	100	Spindle 2	18.7	Medium viscosity, the liquid is slightly thicker than sample 1.
Sample 3	100	Spindle 3	30.2	High viscosity, the liquid is thick and takes longer to flow.
Sample 4	100	Spindle 4	45.6	Very high viscosity, the liquid is very thick and difficult to flow.
Sampel 5	100	Spindle 2	20.3	Medium viscosity, the liquid flows easily but is slightly thick.

Growth

The results of the study regarding absolute growth and daily specific growth of seaweed reveal the influence of the dilution treatment of the solution on both parameters (Table 9).

Table 9. Seaweed growth (absolute & daily) in different treatments

Treatment	Absolute growth (g) ($\bar{X}$±SD)	Daily growth (%) ($\bar{X}$±SD)
Control (K)	1.51±0.40a	0.30±0.07a
C1	3.42±1.18b	0.61±0.16b
C2	3.48±0.89b	0.63±0.18b
C3	3.56±0.97b	0.69±0.19b

Different letters beside the mean value (standard deviation) within the same column indicate a significant difference at the 5% test level.

Based on the analysis of variance concerning absolute weight growth, a significant effect (P<0.05) was observed with different dilutions of the formulated fermented liquid. Duncan's test results showed that the K differed significantly from treatments C1, C2, and C3, while treatment C1 did not differ significantly from treatments C2 and C3. The highest average weight gain was recorded in treatment C3 (3.56±0.97 g), followed by treatment C2 (3.48±0.89 g), treatment C1 (3.42±1.18 g), and treatment K (1.51±0.40 g). The substantial increase in weight in treatment C3 is presumed to be due to the effectiveness of the formulated fermented liquid used, which adequately supplied the nutrients absorbed by the seaweed. The low growth observed in the control treatment is thought to result from the seaweed relying solely on nutrients from the maintenance medium, leading to suboptimal availability of required compounds.

Based on the analysis of variance regarding specific weight growth, a significant effect (P<0.05) was also noted with different dilutions of the formulated fermented liquid. Duncan's test results indicated that the K differed significantly from treatments C1, C2, and C3, while treatment C1 did not differ significantly from treatments C2 and C3. The highest average specific weight gain was observed in treatment C3 (0.69±0.19%), followed by treatment C2 (0.63±0.18%), treatment C1 (0.61±0.61%), and K (0.30±0.07%). The increase in growth in treatment C3 is presumed to be ascribed to the seaweed's enhanced ability to absorb the macro and micronutrients present in the formulated fermented liquid, thereby facilitating better and faster growth activity. This is evident as each treatment exhibited different responses to the growth rate of the seaweed.

Water quality parameters

The data on water quality measurements during the study can be found in Table (10). The displayed data represent the range of water quality throughout the study for each treatment.

Table 10. Values of water quality parameters in different treatments

Treatment	Temperature (°C)	Salinity (ppt)	pH	Dissolved Oxygen (mg/L)
K (Control)	27.56-28.30	30-31	7.47-7.18	5.8-6.30
C1	27.63-28.60	30-31	7.50-7.24	5.4-6.20
C2	27.23-28.60	30-31	7.60-7.66	5.5-6.39
C3	27.45-28.80	30-31	7.35-7.65	5.8-6.43

The observations indicated that the treatments applied for temperature, salinity, pH, and dissolved oxygen (DO) resulted in relatively similar environmental conditions. The temperature across all treatments ranged from 27.23 to 28.80°C, indicating minimal fluctuations that did not significantly affect the variability of other parameters. Salinity remained stable across all treatments, ranging from 30 to 31ppt, reflecting consistent aquatic conditions typical of marine habitats. The pH values were within a neutral to slightly alkaline range, from 7.18 to 7.66, with treatment C2 exhibiting a slightly more alkaline pH (7.60-7.66). Although the pH of treatment C1 tended to be lower, this difference did not indicate significant fluctuations affecting oxygen solubility. The measured dissolved oxygen (DO) levels across all treatments ranged from 5.4 to 6.43mg/L, with the K and C3 showing the highest DO levels. Treatment C1 had the lowest DO, although it remained within a safe range for aquatic life. Overall, despite minor variations among treatments, the environmental conditions remained within ranges that support aquatic life.

DISCUSSION

The fermented liquid from mangrove leaves, both before and after formulation, exhibited the presence of essential macro (N, P_2O_5, K_2O) and micronutrients (Fe, Mn, Mg), magnesium) crucial for seaweed growth, as presented in Table (2). The nitrogen content in the fermented liquid was 0.02%, which increased to 0.87% in the formulated fermented liquid, playing a significant role in protein synthesis and supporting vegetative growth in plants (**Marschner, 2012**). P_2O_5, which is vital for photosynthesis and the formation of roots and reproductive organs in seaweed, was found at 0.01% in the fermented liquid and increased to 0.22% in the formulated liquid, thereby supporting the physiological development of marine plants (**Fageria *et al*., 2010**).

K_2O, which regulates water balance and enhances resistance to environmental stress, was present at 0.14% in the fermented liquid and rose to 2.61% in the formulated liquid. The increase in K_2O content up to 2.61% in this formulation significantly exceeds the minimum requirements for liquid organic fertilizers (LOF) and supports the role of K as an osmotic regulator. This finding is consistent with the research by **Marschner (2012)**, which states that potassium is essential for regulating water balance and enhancing the tolerance of plants (including macroalgae) to environmental stress. The Fe content reaching 174.61ppm after formulation is crucial. This high concentration ensures an adequate supply of Fe, a factor that likely explains the enhanced growth observed compared to the control treatment, which relied solely on the Fe naturally present in the seawater. This is vital as Fe is a limiting

micronutrient essential for chlorophyll synthesis and photosynthetic efficiency in algae, a mechanism confirmed by various studies (**Oura *et al*., 2003; Paine *et al*., 2023**). Mn, found at 8.82ppm in the fermented liquid and 113.22ppm in the formulated liquid, plays a role in photosynthesis and carbohydrate metabolism (**Marschner, 2012**). Mg, a key element in chlorophyll formation, was measured at 0.12% in the fermented liquid and increased to 0.27% in the formulated liquid, supporting overall plant metabolism (**Fageria *et al*., 2010**). The increase in nutrient content in the formulated fermented liquid indicates that the formulation process effectively enriches essential nutrients, thereby allowing this formulation to provide greater benefits in supporting optimal seaweed growth, particularly under controlled cultivation conditions (**Zakaria *et al*., 2023; Salido *et al*., 2024**).

The synergy among *Bacillus* isolates is crucial as it demonstrates the ability of the three isolates to work harmoniously without inhibiting one another. The absence of an inhibition zone (antagonism) between the *Bacillus* isolates demonstrates that the consortium is synergistic. This condition aligns with the findings of **Aslamyah *et al.* (2017)**, who suggested that probiotic consortia can yield a richer and more stable metabolite profile through positive interactions, unlike the risks associated with single-strain dominance. This condition also prevents the dominance of a single strain that could disrupt the fermentation process, thereby maintaining the microbiological balance during production. Such synergy holds significant potential in seaweed cultivation, as stable and nutrient-rich fermentation will optimally support seaweed growth and health (**Hsu *et al*., 2025; Tayyab *et al*., 2025**).

Stability is essential to ensure that the formulated fermented liquid can be stored under various conditions without losing its effectiveness. According to similar studies in the field of biofertilizers and biological fermentation products, stability against temperature changes ensures the consistency of nutrient quality and contained metabolites, which directly impacts the performance of the product in the field. Furthermore, this stability indicates protection against the degradation of enzymes or active compounds that may occur at both high and low temperatures during long-term storage (**Allouzi *et al*., 2022; Khan *et al*., 2023**). Therefore, the results of this test provide confidence that the formulated mangrove leaf fermented liquid possesses sufficient resilience for use in seaweed cultivation applications and other plant needs.

The results of the organoleptic testing of the organic fertilizer based on mangrove leaves indicate characteristics that reflect product quality and its potential to support plant growth. The color of the fermented liquid, rated 3, exhibited a deep brown hue and was not overly clear, which is typical of organic fermentation products due to the degradation of organic materials and the formation of color metabolites during the fermentation process. This color reflects a sufficiently prolonged fermentation period and the presence of dissolved organic compounds (**Maryanti & Wulandari, 2023; López-Rubio *et al*., 2025**). This color indicates a sufficiently prolonged fermentation period and the presence of dissolved organic compounds **(Jamarun *et al*., 2020)**.

The aroma of the formulated fermented liquid received a score of 4, characterized by a sharp and pungent smell that was not overly disruptive. This aromatic characteristic is common in fermented liquids due to the activity of acid-producing microbes and volatile compounds during the fermentation process (**López-Rubio *et al*., 2025; Wardana *et al*., 2025**). The fermentation odor serves as an indicator of microbial activity that is crucial in

the degradation of organic materials and nutrient production, suggesting that fermentation is proceeding well and that microbes are producing bioactive metabolites (**Sarah *et al*., 2023**).

The taste of the fermented liquid was rated 2, with a mild sour and bitter sensation. This sour taste is typically caused by organic acids such as acetic acid or lactic acid produced during microbial fermentation. Although this taste is not very strong, the presence of sourness indicates that fermentation is proceeding well and producing bioactive compounds that may stimulate plant growth (**Andesta *et al*., 2023; Suryani *et al*., 2023**). Organic acids in liquid fertilizer fermentation play a crucial role in enhancing nutrient availability and beneficial microbial activity for plants (**Ji *et al*., 2017**).

The texture of the fermented liquid was rated 3, indicating moderate viscosity and no foaming. This moderate viscosity reflects a good balance between dissolved solids and liquid, which is essential for ease of application in the field and optimal nutrient penetration into plants (**Bogusz *et al*., 2021; Senou *et al*., 2025**). The absence of foam indicates that fermentation is stable without excessive gas-producing microbial activity that could compromise product quality (**Mariska *et al*., 2024**). Overall, these organoleptic results depict the mangrove leaf fermented liquid as a product with suitable physical and sensory characteristics, making it effective and acceptable as a liquid organic fertilizer in cultivation practices (**Mariska *et al*., 2024; Yosilia *et al*., 2025**).

The initial fermented liquid had a very acidic pH of 2.74, attributed to the accumulation of organic acids resulting from microbial activity during the fermentation process **(Dominguez *et al*., 2023; López-Rubio *et al*., 2025)**. This high acidity indicates active fermentation; however, excessively low pH may pose risks to plants or disrupt soil pH balance if applied without dilution **(Meng *et al*., 2022)**. The increase in pH from 2.74 (initial liquid) to 4.64 (formulated liquid) indicates a reduction in acidity. **Mariska *et al.* (2024)** noted that excessively low pH can inhibit nutrient uptake by plants and algae. Thus, the formulation has been improved to ensure the pH is within a safer range, supporting optimal nutrient absorption by *K. alvarezii*. Further dilution processes from treatments C1 to C3 showed a trend of increasing pH, with the highest dilution C1 reaching 5.27, approaching neutral conditions, while more concentrated dilutions maintained low acidic pH. This dilution is important as it can reduce the acidity of the liquid, making it safer for plants **(Yosilia *et al*., 2025)**.

The salinity of the fermented liquid was very high at 64ppt and increased to 75ppt after formulation, likely due to the addition of mineral materials or salts during the formulation process to enrich nutrients **(Kechasov *et al*., 2021; Vargas-Cuy *et al*., 2025)**. With dilution, salinity significantly decreased from 35ppt in C1 to 45ppt in C3, where higher dilution reduced salt concentration, thus preventing potential salinity stress on plants **(Khan *et al*., 2024; Guedes *et al*., 2025)**. Adjusting pH and salinity through formulation and dilution is crucial to ensure that the formulated fermented liquid can be applied safely and efficiently in the field without harming soil and plant conditions **(Novriyansyah, 2025)**.

Homogeneity is critical in liquid products as it ensures that the contained nutrients and metabolites can be evenly distributed throughout the plants, thereby supporting optimal growth and preventing uneven nutrient distribution **(Novriyansyah, 2025)**. Sedimentation or clumping observed in some samples may be due to insufficient stabilization or suboptimal formulation processes **(Bogusz *et al*., 2021; Mariska *et al*., 2024)**, which could potentially reduce application effectiveness and complicate field use. Therefore, maintaining the

homogeneity of the fermented liquid through good process control is essential for the safety and efficiency of organic fertilization **(Bahri *et al*., 2025)**, especially in applications for seaweed cultivation that require consistent nutrient distribution.

Moderate viscosity enhances handling and application efficiency, which in turn increases nutrient availability and absorption by plants (**Allouzi *et al*., 2022; Xia *et al*., 2024**). Liquids with higher viscosity tend to have lower flow and homogeneity, limiting their practical use, while fermented liquids formulated with very low viscosity may lead to rapid runoff from plant surfaces (**Fahlivi *et al*., 2015**). Additionally, physicochemical properties such as viscosity directly affect fertilizer transport, adhesion, and interaction with plant surfaces, influencing overall fertilizer effectiveness (**Yin *et al*., 2023**).

The maximum daily growth rate achieved in the C3 treatment was 0.69±0.19%, which demonstrates a significant advantage. The increased growth observed in treatment C3 is presumed to be due to the more optimal availability of nutrients from the nitrogen-rich, phosphorus, potassium, iron, manganese, and magnesium-rich fermented liquid **(Nasmia *et al*., 2021; Pangaribuan *et al*., 2022)**, which supports the metabolic and physiological activities of marine plants better than the control, which relied solely on nutrients from the maintenance medium. The substantially low growth observed in the control group (0.30% day^{-1}) highlights the critical role of nutrient supplementation. This is particularly relevant because *K. alvarezii* frequently faces oligotrophic conditions (low nutrient concentration) in tropical cultivation waters, a challenge emphasized by **Jiksing *et al.* (2022)** in their discussion of cultivation sites. Our results thus confirm that the formulated liquid successfully eliminates this key limiting factor, facilitating faster and more efficient growth activity, which is not possible when the seaweed relies solely on ambient nutrient levels. The control treatment, which depended solely on the growth medium, exhibited low growth, indicating the need for additional nutrients from external sources such as fermented liquid to stimulate seaweed growth **(Singh *et al*., 2025; Yasmeen *et al*., 2025)**. These results underscore the importance of providing mangrove leaf fermented liquid with appropriate formulation and optimal concentration to enhance seaweed cultivation productivity through more efficient nutrient absorption and faster growth **(Yusuf *et al*., 2023)**.

The water temperature across all treatments ranged from 27.23°C to 28.80°C, indicating minimal temperature fluctuations that did not significantly affect other variables or seaweed growth conditions (**Boyd & Tucker, 2012**). Salinity remained stable within a range consistent with natural marine habitat conditions, which is important for maintaining the physiological balance of seaweed and its supporting microorganisms, as significant fluctuations could lead to physiological stress and hinder growth **(Tilaar *et al*., 2025)**.

The pH values of the water were within a neutral to slightly alkaline range, with treatment C2 showing the highest pH (7.60-7.66). This range is common and safe for marine life and supports the metabolic and photosynthetic processes of seaweed **(Jalil *et al*., 2020; Hengjie *et al*., 2023)**. Although there were differences in pH among treatments, they were not significant enough to negatively impact oxygen solubility **(Mulyono *et al*., 2025)**.

Dissolved oxygen levels measured between 5.4 and 6.43mg/ L, with the control (K) and treatment C3 showing the highest levels. This is sufficient to meet the respiratory needs of marine organisms, including seaweed, and indicates that biological processes in the system are functioning well without low oxygen stress **(Jalil *et al*., 2020; Kasnir *et al*., 2023)**. Treatment C1 had the lowest levels but remained within a safe range. The water

quality conditions during the study supported a stable and healthy cultivation environment for seaweed, with physical and chemical parameters remaining within optimal limits that support growth and optimal metabolic activity **(Zhang *et al*., 2025)**.

CONCLUSION

This research successfully formulated and evaluated the efficacy of fermented *Avicennia marina* leaf liquid as a nutrient-rich biofertilizer for *Kappaphycus alvarezii* cultivation. The formulation significantly enriched macro and microelement content, while demonstrating thermal stability and synergism among *Bacillus subtilis* MSAR-01, *Bacillus vietnamensis* MSAR-06, and *Bacillus* sp. MSAR-07 isolates. Laboratory-scale application showed significant growth enhancement ($P<0.05$), with the 1:25 dilution (Treatment C3) optimal, achieving the highest specific daily growth rate (0.69 ± 0.19%) and largest absolute biomass increase, statistically different from the control (0.30 ± 0.07%).

ACKNOWLEDGEMENTS

The authors acknowledge with appreciation the support of the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology, as well as the Aquaculture Study Program, University of Muhammadiyah Luwuk, with the laboratory facilities provided.

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