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Influence of different concentrations of ammonium phosphate on nitrogen assimilation of red seaweed *Kappaphycus striatus*

Albaris Beteh TAHILUDDIN[1,2,*], Euphraim Jayson DICIANO[1], Rizal Jhunn Falcatan ROBLES[1], Jainal Potong AKRIM[1]

[1]Mindanao State University-Tawi-Tawi College of Technology and Oceanography, College of Fisheries, Sanga-Sanga, Bongao, Tawi-Tawi/PHILIPPINES
[2]Kastamonu University, Institute of Science, Department of Aquaculture, Kastamonu/TURKEY

[*]Corresponding author: albarist20@gmail.com
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Abstract

Eucheumatoid farming is among the most popular source of livelihood for coastal dwellers, especially in the southern Philippines. *Kappaphycus* and *Eucheuma* are the two major cultured eucheumatoid species. However, seaweed farmers have been experiencing low seaweed production for the past years. The slow growth of *Kappaphycus* spp. in the farm is linked to extensive farming, causing nutrient depletion in the seawater. Hence, seaweed farmers in the province of Tawi-Tawi, southern Philippines, enrich their seaweeds, *Kappaphycus striatus*, with a wide range of inorganic nutrient solution concentrations with an average of nearly 9 g L^{-1}. To provide standard concentration (g L^{-1}) of inorganic nutrient solution for soaking before farming the seaweed, this study investigated nitrogen assimilation using the Kjeldahl method. Treatments were the concentrations of commonly used inorganic fertilizer [ammonium phosphate, $(NH_4)_3PO_4$] dissolved in filtered seawater namely; T1 = 0 g L^{-1} as control, T2 = 3 g L^{-1}, T3 = 6 g L^{-1}, and T4 = 9 g L^{-1}. Results showed that the total nitrogen (%) of T4 (0.68±0.01) and T3 (0.59±0.07) were significantly higher ($p<0.05$) than T2 (0.48±0.02) and T1 (0.46±0.02). This suggests that using inorganic nutrients as enrichment to *K. striatus* at a concentration of 6 or 9 g L^{-1} is effective for obtaining high nitrogen assimilation. Therefore, considering the cost-effective inorganic nutrient enrichment practice, this study recommends using 6 g L^{-1} of inorganic nutrient concentration to help seaweed farmers boost production by improving the growth performance of *K. striatus*, especially when planted in nutrient-depleted farms.

Keywords: Ammonium phosphate, Inorganic nutrient enrichment, *Kappaphycus striatus*, Kjeldahl method, Nitrogen assimilation

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1. Introduction

Kappaphycus is a member of red algae (Rhodophyta) largely cultivated worldwide (Kreckhoff et al., 2015; Tahiluddin and Terzi, 2021a). *Kappaphycus striatus* (Schmitz) Doty ex Silva, 1996 is one of the sources of kappa-carrageenan and is commercially produced for the global seaweed industry (Mendoza et al., 2006). The third most significant hydrocolloid after gelatin and starch is the carrageenan (Mustapha et al., 2011), which is utilized and processed for different commercial applications in food, cosmetic, pharmaceutical industries as gelling, thickening, and stabilizing agents (Hayashi et al., 2010). The production of seaweed globally amounting to 35.8 million tons in 2019 was contributed by 49 countries, wherein the *Kappaphycus* and *Eucheuma* comprised 33.5% out of 95% of seaweed cultivation (excluded wild) (FAO, 2021). China, Indonesia, the Republic of Korea, and the Philippines are among the major seaweed-producing countries with a total volume of 20.12, 9.92, 1.81, and 1.5 million tons, respectively, in the same year (FAO, 2021).

In the Philippines, nearly 70% of seaweed production in the second quarter of 2020 was contributed by Bangsamoro Automatous Region in Muslim Mindanao (BARMM), where almost half of the production (53%) was coming from Tawi-Tawi province (PSA, 2020).

In the Philippines, seaweed farmers have been complaining that their *Kappaphycus* seedlings grow slowly, thereby obtaining lower production as compared to previous years (Luhan & Sollesta, 2010; Luhan et al., 2015). They also experienced insufficient seedling materials for the cultivation and lack of supply and for the farming brought by the changes in the environment such as seasonal changes, water temperature, pests, destructive epiphytes (Luhan et al., 2015; Tahiluddin et al., 2021a), and ice-ice disease outbreaks, which have significant effects on the seaweed production (Trono, 1993; Arasamuthu & Edward, 2018; Tahiluddin & Terzi, 2021a; Tahiluddin & Terzi, 2021b).

The quality of seedlings gradually degrading and the overstocking of seaweed farming caused nutrient depletion (Vairappan, 2006; Luhan et al., 2015; Robles, 2020). Many studies have attempted to innovate and improve the production of seaweed by various cultivation techniques (Raven & Geider, 1988; Brault & Queguiner, 1989; Rui et al., 1990; Burfeind & Udy, 2009; Ali et al., 2014; Wenno et al., 2015; Zuldin & Shapawi, 2015; Zuldin et al., 2016; Rustam et al., 2017; Nian et al., 2018; Aslin et al., 2019) and through nutrient enrichment using both organic and inorganic fertilizers (Dawes et al., 1994; Hayashi et al., 2008; Hurtado & Cheney, 2003; Yunque et al., 2011; Luhan et al., 2015; Tahiluddin et al., 2018; Sahir et al., 2019; Illud et al., 2020; Tahiluddin & Terzi, 2021a).

In Tawi-Tawi, southern Philippines, inorganic nutrient enrichment has been practiced by most seaweed farmers for the past years with the aim of increasing seaweed production by improving growth rate and reducing the ice-ice disease outbreak using different inorganic fertilizer concentrations (Tahiluddin et al., 2018; Tahiluddin et al., 2021b). However, although Tahiluddin et al. (2019) showed that a concentration of almost 9 g L^{-1}, an average concentration used by the seaweed farmers (Tahiluddin et al., 2018), significantly increased the growth of cultured *K. striatus* and reduced the ice-ice disease occurrence, it is still unclear which concentrations will obtain the highest assimilated nitrogen from the inorganic nutrient enrichment, important enough for the growth of farmed *Kappaphycus*. Hence, this study aimed to determine the influence of different concentrations of ammonium phosphate [$(NH_4)_3PO_4$] on nitrogen assimilation to standardize the used concentration of inorganic fertilizer in nutrient enrichment of *K. striatus*.

2. Material and Methods

The study was carried out at the College of Fisheries (COF), Mindanao State University-Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao, Tawi-Tawi, Philippines, from March 1 to April 1, 2019, including the sample analysis. The seaweeds enriched with inorganic nutrient was done based on the practice of the seaweed farmers in Sibutu, Tawi-Tawi, as surveyed by Tahiluddin et al. (2018) using various concentrations of ammonium phosphate [$(NH_4)_3PO_4$]: T1 = 0 g L^{-1}, T2 = 3 g L^{-1}, T3 = 6 g L^{-1}, and T4 = 9 g L^{-1}. Seedlings of *K. striatus* obtained randomly from the local seaweed farm were cut into 50-100 grams, dipped into respective concentrations of inorganic nutrient solutions for 30 s, and were covered and left overnight. These were performed in the hatchery of COF, MSU-TCTO in Completely Randomized Design (CRD). Nutrient-enriched seedlings of *K. striatus* were sun-dried for 5 days. All seaweed samples were in triplicates. Dried samples were sent to the Department of Science and Technology-Region IX, Zamboanga City, Philippines, to determine the nitrogen assimilation or the total nitrogen using the Kjeldahl method (AOAC, 2016). To determine the significant differences, a One-way Analysis of Variance (ANOVA) was employed using IBM SPSS version 20. Post hoc test (Duncan) was used to rank the means.

3. Results and Discussion

Table 1 shows the total nitrogen of nutrient-enriched *K. striatus*. Statistical analysis revealed that T4 and T3 were significantly higher ($p<0.05$) than T2 and T1. Our result indicates that higher concentrations of ammonium phosphate [$(NH_4)_3PO_4$] (6 or 9 g L^{-1}) had a greater total amount of nitrogen assimilated in the thallus of *K. striatus*.

Table 1. Total nitrogen of nutrient-enriched *Kappaphycus striatus*

Treatment	Total nitrogen (%±SE)*
T1 (0 g L^{-1})	0.46 ± 0.02^b
T2 (3 g L^{-1})	0.48 ± 0.02^b
T3 (6 g L^{-1})	0.59 ± 0.07^a
T4 (9 g L^{-1})	0.68 ± 0.01^a

*different superscripts are significantly different ($p<0.05$).

Tahiluddin et al. (2018) surveyed that there were 87% of seaweed farmers in Sibutu, Tawi-Tawi, a province with high production of seaweeds in the Philippines (PSA, 2019), using various concentrations of ammonium phosphate [$(NH_4)_3PO_4$] ranging from 2.26 to 25 g L^{-1} with an average of 8.82 g L^{-1} concentration applied by dipping the seaweeds for 5-60 s, left covered overnight, and planted the following day. They further reported that farmers observed an increase in seaweed growth as well as a decrease in an ice-ice disease outbreak and likewise obtained similar observations in the field experiment (Tahiluddin et al., 2019). In the study of Luhan et al. (2015), *K. alvarezii* was short-immersed to 10 ppm of sodium nitrate for 12 h and obtained a significant result of total thallus nitrogen (1.19±0.16%) using this method compared to control (0.55±0.05%). Although the value of total thallus nitrogen obtained was comparatively lower than to the study of Luhan et al. (2015) due to different methods, inorganic fertilizer used, and seaweed species, but the high significant total thallus nitrogen of *K. striatus* obtained in the present study is beneficial in enriching the cultured *K. striatus* with nutrients in a faster and feasible way.

The two elements (nitrogen and phosphorus) are very scarce in the water and are considered as limiting factors for seaweed growth (Littler et al., 1991; Lapointe et al., 1992; McGlathery et al., 1992; Delgado & Lapointe, 1994; Yunus et al., 2010; Sahir et al., 2019). Increased nutrient supply or the application of fertilizer can improve the physiological process of seaweed as well as increase assimilation and photosynthetic activity (Yu & Yu, 2008; Roleda & Hurd, 2019). Optimum growth of seaweed can be achieved if there are enough nutrients like phosphate and nitrogen (Yu & Yu, 2008; Sahir et al., 2019).

Nutrient uptake of seaweeds is affected by abiotic and biotic factors such as water movement, light, temperature, carbon dioxide, salinity, desiccation, and life stage of seaweeds (Roleda & Hurd, 2019). Nutrient uptake of seaweeds may also be dependent on the concentrations of available nutrients in the surrounding water. For instance, *K. alvarezii* was reported to have a greater ammonium uptake in a higher concentration of ammonium (Dy & Yap, 2001). In this study, *K. striatus* was shortly dipped to a high nutrient-enriched solution for a few seconds and allowed the nutrients to assimilate during the overnight storage while covered and desiccated under total dark conditions. Therefore, this method used in the present study is considered efficient enough to have high total thallus nitrogen assimilated, which may result in high growth when planted on the farm, as what the farmers observed. This is parallel to previous studies where inorganic nutrient enrichment of seaweeds (*K. striatus*, *Pelvetia canaliculata*, *Fucus serratus*, and *F. spiralis*), under the dark and desiccation conditions, obtained better and similar results of nutrient uptakes (Thomas & Turpin, 1980; Hurd, 1990; Hurd & Dring, 1991; Dy & Yap, 2001; Harrison & Hurd, 2001).

Inorganic nutrient enrichment is one way of increasing the nitrogen assimilation of farmed *K. alvarezii*, thereby utilizing the assimilated nitrogen for growth and protecting against ice-ice disease-causing pathogens when planted in a nutrient-deficient farm (Luhan et al., 2015). In addition, numerous studies for the past decades showed that the supplementation of inorganic nutrients to seaweeds enhanced nitrogen content, photosynthesis, and growth (Topinka & Robbins, 1976; Neish et al., 1977; Rui et al., 1990; Harrison & Hurd, 2001; Menéndez et al., 2002; Martins et al., 2011), thereby increasing seaweed productivity.

4. Conclusion

In conclusion, our result suggests that the inorganic enrichment nutrient of *K. striatus* at the concentrations of 6 or 9 g L^{-1} is effective in providing additional nutrients, especially nitrogen, as evident from our result. In addition, assimilated nitrogen is vital in increasing the growth of *K. striatus*, especially when planted in nutrient-limited farms. Considering cost-effective inorganic nutrient enrichment practice, a practical choice of 6 g L^{-1} is recommended to avoid the extra cost of inorganic fertilizer.

Conflict of interest

The authors declare that there is no conflict of interest.

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