Revista Verde de Agroecologia e Desenvolvimento Sustentável
Green Journal of Agroecology and Sustainable Development

Biofertilizer from *Kappaphycus alvarezii* enhances rooting in basil (*Ocimum basilicum* L.) plants

Biofertilizante de *Kappaphycus alvarezii* melhora o enraizamento de plantas de manjericão (*Ocimum basilicum* L.)

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ARTICLE
Recebido: 18-04-2025
Aprovado: 12-07-2025

Key words:
Aqueous extract
Seaweed
Rhodophyta

ABSTRACT
The use of biofertilizers in agriculture has proven to be a promising strategy to optimize plant rooting, promoting a more vigorous and healthier root development. In this context, this study evaluated the effect of the biofertilizer derived from *Kappaphycus alvarezii*, cultivated in São Paulo (Kal-SP) and Santa Catarina (Kal-SC), on the rooting of basil plants (*Ocimum basilicum* L.). An entirely randomized design was used, with ten plants per treatment in a growth chamber. Four concentrations of the biofertilizer (1%, 3%, 5%, and 7%) were tested, using distilled water as a control. At the end of the experiment, morphological parameters (fresh and dry mass and root length) and biochemical parameters (total carbohydrates, total soluble sugars, and total starch) were evaluated. The root length showed significant increases with Kal-SC at concentrations of 3% (58%), 5% (55%), and 7% (54%) compared to the control. For fresh weight, the increases were 262%, 287%, and 312% at the same concentrations, and 200% for Kal-SP at 5%. In terms of dry weight, the increases were 257%, 277%, and 284% for Kal-SC and 204% for Kal-SP. Biochemically, higher amounts of total starch were observed in plants treated with Kal-SC at 3%, 5%, and 7%, as well as with Kal-SP at 1%, 3%, and 5%, with maximum increments of 82% (Kal-SP 3%). Thus, it is verified that the biofertilizer from *K. alvarezii* not only stimulates the rooting of basil but also alters the carbohydrate balance in the treated plants.

Palavras-chave:
Algas marinhas
Extrato aquoso
Rhodophyta.

RESUMO
O uso de biofertilizantes na agricultura tem se mostrado uma estratégia promissora para otimizar o enraizamento das plantas, promovendo um desenvolvimento radicular mais vigoroso e saudável. Nesse contexto, este estudo avaliou o efeito do biofertilizante derivado de *Kappaphycus alvarezii*, cultivado em São Paulo (Kal-SP) e Santa Catarina (Kal-SC), no enraizamento de plantas de manjericão (*Ocimum basilicum* L.). Usou-se um delineamento inteiramente casualizado, com dez plantas por tratamento em câmara de crescimento. Foram testadas quatro concentrações do biofertilizante (1%, 3%, 5% e 7%), com água destilada como controle. Ao final do experimento, foram avaliados parâmetros morfológicos (massa fresca e seca e comprimento da raiz) e bioquímicos (carboidratos totais, açúcares solúveis totais e amido total). O comprimento da raiz apresentou aumentos significativos com Kal-SC nas concentrações de 3% (58%), 5% (55%) e 7% (54%) em relação ao controle. Para o peso fresco, os aumentos foram de 262%, 287% e 312% nas mesmas concentrações, e de 200% para Kal-SP a 5%. Em termos de peso seco, os aumentos foram de 257%, 277% e 284% para Kal-SC e 204% para Kal-SP. Bioquimicamente, foram observadas maiores quantidades de amido total em plantas tratadas com Kal-SC a 3%, 5% e 7%, bem como com Kal-SP a 1%, 3% e 5%, com incrementos máximos de 82% (Kal-SP 3%). Assim, verifica-se que o biofertilizante de *K. alvarezii* não apenas estimula o enraizamento do manjericão, mas também altera o balanço de carboidratos nas plantas tratadas.

INTRODUCTION

The red alga *Kappaphycus alvarezii* (Rhodophyta) is the sixth most cultivated macroalga species in the world (FAO, 2022). This species is particularly valued in the food industry due to the presence of κ-carrageenan, a polysaccharide with important physicochemical properties, such as gelling, thickening, emulsifying, and stabilizing. In addition, its application also extends to other industries, e.g., cosmetics, animal nutrition, human food, health/medicine and agriculture (NUNES et al., 2024).

The commercial cultivation of this macroalga in Brazil, specifically in the states of Santa Catarina (Southern Brazil), Rio de Janeiro, and São Paulo (Southeastern Brazil), was authorized through Normative Instruction 01/2020 by the Brazilian Institute of Environment and Renewable Natural Resources (BRAZIL, 2020). Initially, the primary goal of this cultivation was the production of κ-carrageenan for the national market. However, a new opportunity emerged among algae farmers: the production of biostimulants and biofertilizers (GELLI et al., 2020; HAYASHI et al., 2024).

A systematic review of researches conducted between 2017 and 2023 identified 34 manuscripts highlighting the use of *K. alvarezii* in agriculture, showcasing significant results in enhancing production and quality, reducing biotic and abiotic stresses, and acting as an elicitor of resistance (NUNES et al., 2024). Furthermore, the biofertilizer may promote root development and seedling growth. However, research on the use of *K. alvarezii* biofertilizer for crop rooting remains limited, highlighting the need to analyze this product across various crops (NUNES et al., 2024).

Additionally, given the relatively recent cultivation of *K. alvarezii* in Brazil, along with distinct soil and climate conditions compared to other countries where studies on the usage of the algal biofertilizer are more advanced (HAYASHI et al., 2024), agronomic research is essential to demonstrate the benefits of using *K. alvarezii* for biofertilizer production. Therefore, this study aimed to evaluate whether the biofertilizer derived from *K. alvarezii* could enhance the rooting of basil (*Ocimum basilicum*) plants.

MATERIAL AND METHODS

The experiment was conducted with samples of *K. alvarezii* (red and green strains) were collected in February 2024 (summer) from the Fisheries Institute in Ubatuba, São Paulo (23° 27'07" S, 45° 02'49"W) and in a marine farm in Florianópolis, Santa Catarina (27° 42' 32.724" S, 48° 33' 35.5" W) (NUNES et al., 2025a).

The collected algal biomass was washed with chlorinated water to remove salt and impurities. The red and green strains were weighed separately to ensure equal amounts, i.e., 500 g, for the biofertilizer production. An industrial blender was used for 15 minutes to chop and blend the algal biomass into a paste with a particle size of approximately 200 micrometers, which was then filtered, retaining only the aqueous portion. The biofertilizer samples from São Paulo and Santa Catarina were labeled as Kal-SP and Kal-SC, respectively, and stored in plastic containers suitable for aqueous extracts, maintained at -20ºC until use (NUNES et al., 2025a).

Basil seeds were purchased from Isla Sementes® (Porto Alegre, Rio Grande do Sul state, southern Brazil) and showed a germination rate of 97% and 100% purity. The seeds were sown in plastic trays with 200 cells (10 cm deep) in a controlled cultivation room (~25ºC with a photoperiod of 12 hours of darkness and 12 hours of light) for 21 days. After the pilot test to evaluate the germination and development of the species, a commercial substrate (TN Mix Agrinobre) enriched with NPK fertilizer (10:10:10) was used. After this period, the roots of all plants were trimmed to a standardized length of approximately 5 cm to maintain experimental homogeneity. Subsequently, each plant was individually transplanted into Styrofoam cups containing 80 mL of the following treatments: control (distilled water); Kal-SC at 1%, 3%, 5%, and 7%; and Kal-SP at 1%, 3%, 5%, and 7%. A total of ten plants per treatment were used, resulting in 90 plants overall.

The plants were kept in Styrofoam cups containing the biofertilizers Kal-SC and Kal-SP at different concentrations in the cultivation room (~25ºC with a photoperiod of 12 hours of darkness and 12 hours of light) for two weeks before determining their morphological parameters, namely root length (cm) and fresh and dry weights of the roots (g) for all plants. Regarding the biochemical analysis of the roots, total carbohydrates (DUBOIS et al., 1956), total soluble sugars, and total starch (UMBREIT; BURRIS, 1964) were determined in triplicates ($n = 3$), pooling samples from the same treatment.

Statistical analysis of the data set used the Scott & Knott test ($p \leq 0.05$), allowing for a comparison of means with a 5% probability of error. The data were analyzed using AgroEstat software (version 1.1.0.712; BARBOSA; MALDONADO JUNIOR, 2015). A principal component analysis (PCA) was also applied to the morphological and biochemical data set. For that, the singular value decomposition (SVD) algorithm available in the The Unscrambler® X software (v. 10.4; CAMO SOFTWARE AS, 2016) was used for the calculation of the latent variables.

RESULTS AND DISCUSSION

The analysis of the root morphological data, including length, fresh weight, and dry weight, revealed statistically significant differences among as shown in Table 1. The biofertilizer samples from Santa Catarina (Kal-SC) treatments at 3%, 5%, and 7% showed meaningful ($p < 0.05$) root length increased by 59%, 55%, and 54%, respectively, compared to control. However, Kal-SC at 1% and biofertilizer samples from São Paulo (Kal-SP) in all concentrations tested did not differ from the control. Regarding the root fresh weight, Kal-SC at 3%, 5%, and 7%, as well as Kal-SP at 5%, determined increases of 262%, 287%, 312%, and 200%, respectively, in respect to control. Similar trends were observed for the root dry weight, with increases of 258%, 278%, 282%, and 204%, respectively (Table 1).

The results indicate that the algal biofertilizer positively affected the rooting of basil plants, enhancing both root length and weight. The increase in root weight might be attributed to the development of new roots, a phenomenon not observed in the control group (Figure S1 – supplementary material). In a previous study conducted by our research group (NUNES et al., 2025b), it was demonstrated that the foliar application of Kal-SP at 3% on basil plants cultivated in a hydroponic system increased root length by 54.76% compared to the control. Additionally, Kal-SC at 5% stimulated root dry mass development, resulting in an increase of 37.44% compared to the control.

Table 1. Heatmap of mean root length (cm), fresh weight, and mean dry weight (g) of basil plants treated with *Kappaphycus alvarezii* biofertilizer produced in São Paulo (Kal-SP) and Santa Catarina (Kal-SC) states, Brazil.

Treatment	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control	6.88 ± 1.48 b	0.08 ± 0.04 c	0.0045 ± 0.0020 c
Kal-SC 1%	8.31 ± 1.37 b	0.18 ± 0.05 b	0.0101 ± 0.0027 b
Kal-SC 3%	10.88 ± 3.56 a	0.29 ± 0.09 a	0.0161 ± 0.0036 a
Kal-SC 5%	10.70 ± 3.21 a	0.31 ± 0.07 a	0.0170 ± 0.0038 a
Kal-SC 7%	10.62 ± 2.83 a	0.33 ± 0.12 a	0.0172 ± 0.0071 a
Kal-SP 1%	8.15 ± 0.89 b	0.16 ± 0.06 b	0.0098 ± 0.0029 b
Kal-SP 3%	8.05 ± 1.40 b	0.21 ± 0.09 b	0.0116 ± 0.0040 b
Kal-SP 5%	7.80 ± 1.42 b	0.24 ± 0.08 a	0.0137 ± 0.0042 a
Kal-SP 7%	7.98 ± 1.24 b	0.19 ± 0.08 b	0.0115 ± 0.0043 b

Means followed by different letters in the columns are statistically different according to the Scott & Knott test ($p < 0.05$). Heatmap colors were calculated per column (variable) through the highest values (red), medium values (yellow), and the lowest one (green).

Studies with other plant species also support the findings herein described with *O. basilicum*. Castro et al. (2023) reported that foliar application of *K. alvarezii* biofertilizer at 5% and 10% in rice plants promoted increases in root dry mass, with the higher dose also increasing the number of roots. Additionally, a root application of the algal biofertilizer at 3% stimulated fresh and dry root mass production by 60% and 28%, respectively. In their turn, Amatuzzi et al. (2020) demonstrated that 50 mg L^{-1} of the *K. alvarezii* biofertilizer induced rooting and the growth (fresh mass) of seedlings of *Epidendrum secundum* orchids.

The positive results found for the morphological parameters of *O. basilicum* can be attributed to the presence of bioactive compounds, such as growth hormones (particularly indole acetic acid, zeatin, kinetin, and gibberellic acid) and micro- and macronutrients (especially potassium) in the algal biofertilizer, which promotes plant rooting (RUDKE et al., 2020).

In the biochemical analysis, the control (distilled water) showed higher content of total carbohydrates (155.24 mg/g), statistically differing from all treatments. A similar result was found for the total soluble sugar content, where the control (196.39 mg/g) differed ($p < 0.05$) from all the treatments, except for Kal-SC at 1% concentration (184.39 mg/g). However, the opposite was found regarding the total starch content, where plants treated with Kal-SP 3% exhibited the highest content (80.53 mg/g) of that polysaccharide, significantly differing from the other treatments (Table 2).

Table 2. Total carbohydrates (mg/g), total soluble sugars (mg/g), and total starch (mg/g) of basil plants treated with *Kappaphycus alvarezii* biofertilizer, cultivated in São Paulo (Kal-SP) and Santa Catarina (Kal-SC), Brazil.

Treatment	Carbohydrates	Soluble sugars	Starch
Control	155.24 ± 2.01 a	196.39 ± 14.96 a	44.31 ± 1.61 c
Kal-SC 1%	114.48 ± 8.73 c	184.39 ± 9.23 a	47.02 ± 1.74 c
Kal-SC 3%	99.43 ± 10.95 d	174.40 ± 12.19 b	59.77 ± 0.50 b
Kal-SC 5%	93.33 ± 2.31 d	138.90 ± 0.72 c	61.50 ± 7.22 b
Kal-SC 7%	100.00 ± 4.88 d	161.42 ± 6.78 b	62.69 ± 2.70 b
Kal-SP 1%	129.33 ± 7.61 b	178.31 ± 15.03 b	60.43 ± 2.95 b
Kal-SP 3%	73.71 ± 6.59 e	117.42 ± 1.29 d	80.53 ± 6.89 a
Kal-SP 5%	76.00 ± 1.14 e	103.64 ± 2.54 d	60.88 ± 2.89 b
Kal-SP 7%	62.67 ± 4.58 f	74.49 ± 2.97 e	37.54 ± 1.17 c

Means followed by different letters in the columns are statistically different according to the Scott & Knott test ($p < 0.05$). Heatmap colors were calculated per column (variable) through the highest values (red), medium values (yellow), and the lowest value (green).

The biochemical analysis revealed that the control group presented higher amounts of total carbohydrates and total soluble sugars, both of which were statistically different from all the other treatments. This may indicate that the application of the algal biofertilizer to *O. basilicum* could lead to a reallocation of metabolic resources within the plant, directing energy towards root development rather than immediate carbohydrate storage (MEENA et al., 2025). However, it is important to clarify that the higher total starch content observed in biofertilizer-treated plants (except for two treatments) suggests a complex metabolic response. While the biofertilizer appears to prioritize root growth, it also facilitates the conversion of carbohydrates and soluble sugars into starch. This starch serves as a more specific energy reserve, which can be stored in plant tissues and mobilized to support root growth over time (APRIYANTO et al., 2022).

Therefore, the initial reallocation of energy towards root development does not negate the eventual conversion of excess sugars into starch for storage. This strategy might optimize the use of metabolic resources, ensuring that energy is available for both immediate root growth and future demands (DONG et al., 2018; BOUTAHIRI et al., 2024). Furthermore, the reduced starch content and elevated levels of soluble carbohydrates/sugars in control plants (water-only) likely resulted from nutrient deprivation-induced stress, which triggered carbon reallocation towards osmoprotectants and immediate energy reserves at the expense of starch storage. This mechanism aligns with stress-responsive carbon partitioning (DONG et al., 2018).

In the principal component analysis (PCA), a total variance of 85% was captured by PC1 (58%) and PC2 (27%) (Figure 1). The control, Kal-SC and Kal-SP 1%, were found to be grouped in PC1-/PC2-, due to the total carbohydrate and soluble sugar content. In their turn, the Kal-SP at 3% and 5% samples were clustered in PC1+/PC2+, but without grouping with morphological or biochemical variables. Notably, the treatments Kal-SC at 3%, 5%, and 7% clustered together (PC1+/PC2-), due to their morphological variables root length, fresh weight, and dry weight, as well as their contents of total starch.

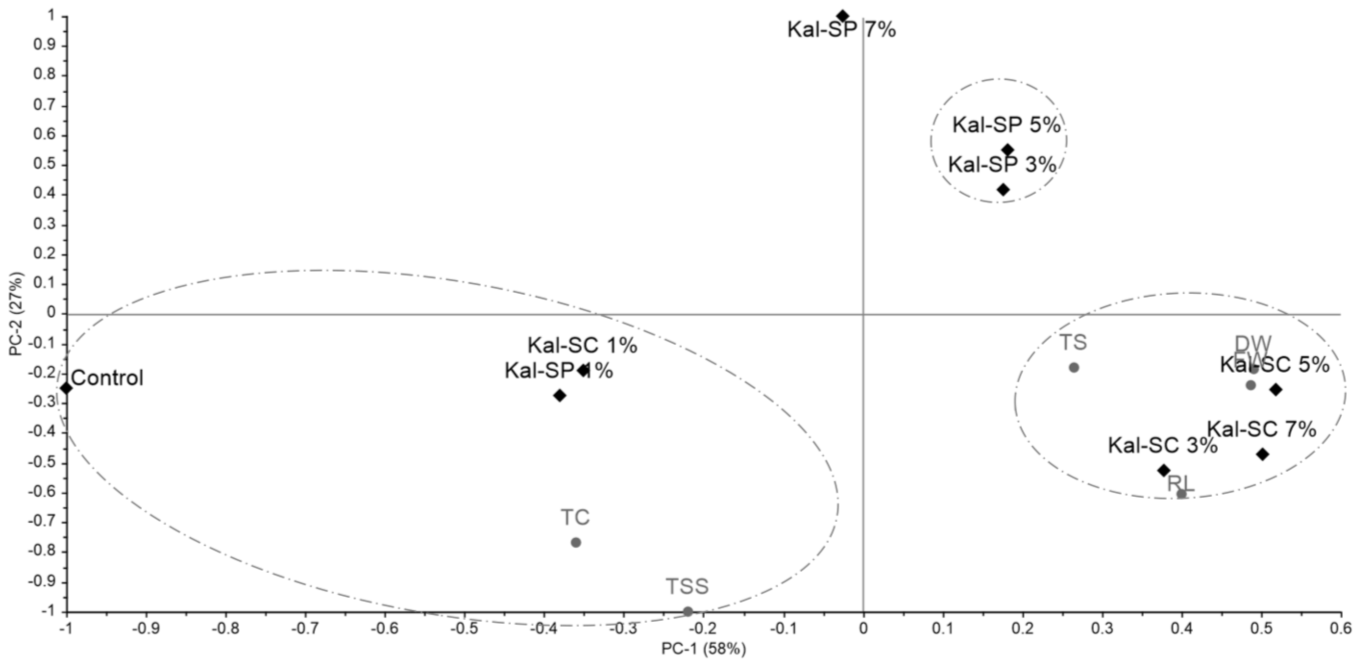

Figure 1. Scores scatter plot of the latent variables PC1 and PC2 calculated from the data set of root length (RL), fresh weight (FW), dry weight (DW), total carbohydrates (TC), total soluble sugars (TSS), and total starch (TS) contents of basil plants treated with the *Kappaphycus alvarezii* biofertilizer (1%, 3%, 5%, and 7%) produced in São Paulo (Kal-SP) and Santa Catarina (Kal-SC) states, Brazil.

The PCA indicates that the treatments of Kal-SC at 3%, 5%, and 7%, which were clustered with the morphological variables and total starch, had the most significant impact on the roots of basil plants. These findings suggest that the cultivation sites of *K. alvarezii* can influence the biofertilizer composition, and, consequently, the biological effects detected in basil plants. In a previous study (NUNES et al., 2025a) it was showed that the biochemical composition, particularly the contents of phenolics, carotenoids, and proteins, is distinct between Kal-SC and Kal-SP samples. In fact, the content of total phenolic compounds and proteins was higher in Kal-SP samples, contrary to the carotenoid amounts.

Similarly, discrepancies were also detected in the metabolic profiles determined by 1D- and 2D- nuclear magnetic resonance spectroscopy, where higher amounts of acetate, formate, lactate, and succinate were found in Kal-SC samples, while betaine, glutamate, and glycine were higher in the Kal-SP ones (NUNES et al., 2025a). This distinct composition of metabolites in the biofertilizer product may occur primarily due to temperature changes (KUMAR et al., 2020) and salinity during the growth cycle of that macroalga species (SIDDIQUI et al., 2024).

It is important to emphasize that, despite the differences observed between Kal-SC and Kal-SP, both treatments showed positive responses in root development, particularly in the formation of new roots. This approach proves to be particularly valuable for seedling production, especially in challenging rooting situations. The positive effects observed on root development indicate that the *K. alvarezii* biofertilizer can help increasing the production of basil plantlets by horticulturists. By optimizing application protocols and understanding the molecular mechanisms involved in the morphogenetic and biochemical responses found will be essential for effectively integrating biofertilizers into sustainable agricultural practices under field conditions. Therefore, further research is essential in this area, as the existing literature on promoting rooting remains limited.

CONCLUSION

The *Kappaphycus alvarezii* biofertilizers impact the rooting of basil plants by influencing the morphological and biochemical parameters of the roots, altering the morphogenetic and metabolic characteristics. In general, the 5% biofertilizer is the most suitable for the rooting of the plants, regardless of the origin.

ACKNOWLEDGEMENT

The authors thank Algas Brasil Ltda (Florianópolis, Santa Catarina) and the Fisheries Institute of the State of São Paulo for providing the samples that made the experiments possible.

DECLARATION

Funding: Grant 2023/03886−1, São Paulo Research Foundation (FAPESP), on behalf of A. Nunes. The research fellowship from CNPq on behalf of M. Maraschin (process no. 405949/2022-7), G.P.P. Lima (process no. 311719/2023-6), and E.R. Oliveira (process no. 303956/2023-2). The research fellowship from CAPES on behalf of F.S. Dutra (process no. 88887.696139/2022-00).

Conflict of interest: The authors declare no conflict of interest.

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Supplementary material

Figure S1 – Basil plants (*Ocimum basilicum* L.) treated with a biostimulant derived from *Kappaphycus alvarezii* to enhance rooting.