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RESEARCH

# A *Kappaphycus alvarezii* aqueous extract improves yield and kernel quality in barley (*Hordeum vulgare* L.)

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## Abstract

The search for sustainable agricultural inputs has increased the interest in biostimulants based on macroalgae. An aqueous extract of *Kappaphycus alvarezii*, a red seaweed rich in bioactive compounds, has emerged as a promising biostimulant for various crops, in Brazil, but its effects on barley (*Hordeum vulgare* L.) remain underexplored. This study investigated the influence of foliar application of different concentrations, i.e., 0, 1, 2, 5, and 10% v/v, of a crude aqueous extract, derived from biomass cultivated in Santa Catarina, southern Brazil. The morphology, yield, and biochemical parameters of leaves and kernels, from treated plants in pots, in a greenhouse were measured. In contrast to the growth-promoting effects reported for other extracts of seaweeds, this *Kappaphycus* extract did not alter the plant architecture, but selectively enhanced reproductive output, suggesting an optimization of resource allocation towards grain development. It was seen that grain set increased at lower concentrations (1–2%) and the thousand-kernel weight at higher doses (5–10%). Biochemically, this specific extract elevated leaf amino acids by up to 47.4% at the 1% concentration, as compared to control. In addition, treatments selectively enhanced kernel qualities, including starch (+ 19.3%, at 5%), protein (+ 10.3%, at 10%), and amino acids (+ 56.6%, at 1–2%), while maintaining germination viability above 98.5%. PCAs revealed a clear segregation, isolating the control from all treatments, which formed distinct clusters in all parameters. The results demonstrated that the *K. alvarezii* extract used in this study could be an effective tool for enhancing barley kernel quality, particularly for the malting industry, by optimizing reserve accumulation, without compromising plant morphology and seed germination.

**Keywords** Seaweed aqueous extract · *Kappaphycus alvarezii* · Marine bioinputs · Rhodophyta · Sustainable agriculture · Physiological responses · Poaceae · Barley

## Introduction

The search for sustainable agricultural inputs has gained global prominence, driven by the need to reduce reliance on chemical fertilizers and mitigate environmental impacts (Brunelle et al. 2024). In this context, biofertilizers and biostimulants derived from macroalgal extracts have emerged as promising alternatives (Munaro et al. 2021; Nanda et al. 2022; Sario et al. 2025). These seaweed-derived inputs are recognized for their richness in bioactive compounds (e.g., plant hormones, polysaccharides, and micronutrients) that stimulate crop plant growth and enhance resistance (Ali et al. 2021a; Shukla et al. 2025), across all plant development stages, from germination to post-harvest (Ali et al. 2021b).

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Among species of macroalgae, the rhodophyte *Kappaphycus alvarezii* (Doty) L.M.Liao stands out as the fifth most cultivated seaweed in the world (Nunes et al. 2024). This macroalga has significant industrial applications, primarily due to the presence of the polysaccharide kappa carrageenan (Rudke et al. 2020). However, it has recently also begun to be used as an agricultural input (Layek et al. 2015; Kumar et al. 2020; Vaghela et al. 2022; 2023a; 2023b), due to its content of growth-promoting compounds (Vaghela et al. 2022), defense-inducing agents against stress (Patel et al. 2018; Trivedi et al. 2018), and high levels of soluble potassium (Karthikeyan and Shanmugam 2017; Gelli et al. 2020). Consequently, it has a pronounced effect on productivity across various crops, including sugarcane, soybean, and cereals such as maize, rice, and wheat (Nunes et al. 2024). It is crucial to note, however, that the biochemical composition and efficacy of such seaweed extracts are not uniform, as biogeographical and seasonal factors inherently influence them. The biochemical profile of the *K. alvarezii* biomass, and consequently its extracts, varies with the environmental conditions of its geographic origin (Nunes et al. 2025a) and the season of cultivation (Schneider et al. 2026). A clear understanding of this variability is therefore essential for developing standardized, reliable biostimulants that meet regulatory benchmarks and perform consistently in agricultural systems.

Barley (*Hordeum vulgare* L.) is one of the oldest crops and most widely cultivated cereals globally. It is used extensively for human consumption, animal feed, and malt production (Ross et al. 2023). Despite their widespread use in agricultural research, the potential of seaweed extracts in barley studies remains underexplored. A particular area of interest lies in barley's responsiveness to different types and concentrations of seaweed extracts. For instance, applications of extracts from the brown seaweed *Ascophyllum nodosum* (L.) Le Jolis have been shown to enhance germination rates, seedling vigor, and overall plant growth in barley (Möller and Smith 1999; Rayorath et al. 2008; Goñi et al. 2021), and to influence germination, radicle and plumule growth, and seedling development (Kaya et al. 2024). In another study, the extract of *Ecklonia maxima* (Osbeck) Papenfuss improved nitrogen-use efficiency, resulting in higher yields and lower fertilizer use (Cozzolino et al. 2021). These findings underscore the significant role of seaweed extracts in enhancing barley performance, demonstrating their potential as effective tools for sustainable crop management. However, despite the extensive use of *K. alvarezii* in other cereals, its effects on barley yield, partitioning, and grain biochemical quality remain unknown.

This study aimed to evaluate the effects of a specific aqueous extract of *K. alvarezii* biomass cultivated in Santa Catarina, southern Brazil, on the yield components and kernel quality in treated barley (var. Rubi). To this end, plants were treated with different concentrations (i.e., 0, 1, 2, 5, and 10% v/v) of the aqueous extract. Agronomic parameters, including plant architecture and kernel formation, were assessed. Furthermore, comprehensive biochemical analyses were conducted on both vegetative tissues and mature kernels to determine changes in quality-related metabolites.

## Material and methods

### Experimental conditions

The experiment was conducted in a greenhouse at the Epagri Training Center (CETRE) in Florianópolis, Santa Catarina, Brazil, from 19 June to 23 October 2024. Seeds of barley (*Hordeum vulgare* var. Rubi) were purchased from Ambev S.A. (Companhia de Bebidas das Américas) and, when tested, showed a germination rate of 98–100%. Before sowing, the seeds were surface-disinfected with 2% sodium hypochlorite for 6 min, followed by 6 rinses with distilled water. This standardized protocol effectively eliminated surface pathogens without compromising seed vigor or germination potential. The disinfected seeds were dried overnight in an oven at 30 °C with forced-air circulation (Brasil 2018).

The seeds were sown in 10-L (30 cm diameter) pots filled with a commercial medium (TN Mix, Agrinobre, Brazil) pre-fertilized with 5 g of NPK (10:10:10) per pot. Sowing was performed by placing 8 seeds per pot, distributed in four equidistant holes. Seedlings were thinned to a final density of four plants per pot on the seventh day after emergence.

The aqueous extract of *K. alvarezii* was provided by VerdeMar® (Palhoça, Santa Catarina, southern Brazil), from biomass produced locally during the autumn. This raw liquid, obtained directly from the mechanical processing of fresh algal biomass, was used as the 100% stock solution for preparing the treatment dilutions. The biochemical characterization of the extract was performed according to the methods described by Nunes et al. (2025a). A detailed profile of its biochemical and nutrient composition is provided in Table 1.

A completely randomized design was adopted with eight replicates (pots) per treatment. The treatments consisted of foliar applications at concentrations of 1, 2, 5, and 10% (v/v), extract diluted in water, with a control (0%, water only). Based on the total solid content of the stock solution ($3.85 \pm 0.18$%, Table 1), the estimated solid content delivered per treatment was approximately: 0.039% solids (1% v/v), 0.077% solids (2% v/v), 0.193% solids (5% v/v), and 0.385% solids (10% v/v). Foliar sprays were applied at 14-day intervals using a manual sprayer calibrated to deliver a specific volume to promote leaf runoff without waterlogging the substratum (2.5 mL per plant; 5 sprays). The first application was made 14 days after sowing (DAS),

**Table 1** Biochemical and macro- and micronutrient analysis of the aqueous extract of *Kappaphycus alvarezii* applied to barley plants (*Hordeum vulgare* L.), from seaweed biomass produced during autumn, in Palhoça, Santa Catarina, Southern Brazil

| Biochemical and Nutrient Analysis | Mean ± SD |
|---|---|
| Total Solid Content (%) | 3.85 ± 0.18 |
| pH | 6.57 ± 0.01 |
| Conductivity (mS $cm^{-1}$) | 60.56 ± 14.45 |
| Total Carbohydrate (mg $g^{-1}$) | 4.96 ± 1.60 |
| Total Soluble Sugar (mg $g^{-1}$) | 1.79 ± 0.76 |
| Total Starch (mg $g^{-1}$) | 5.51 ± 1.17 |
| Total Phenolic content (mg (100 g)$^{-1}$) | 16.78 ± 5.69 |
| Total Protein (mg $g^{-1}$) | 29.38 ± 16.65 |
| Total Amino Acid (mg (100 g)$^{-1}$) | 3.23 ± 1.61 |
| Total Lipid (%) | 3.73 ± 1.21 |
| Organic Carbon (%) | 0.80 ± 0.91 |
| N (%) | ND |
| P (%) | 0.01 ± 0.00 |
| K (%) | 1.62 ± 0.29 |
| Ca (%) | 0.01 ± 0.00 |
| Mg (%) | 0.03 ± 0.01 |
| S (%) | 0.05 ± 0.02 |
| Bo (mg $kg^{-1}$) | 1.18 ± 0.55 |
| Cu (mg $kg^{-1}$) | 0.08 ± 0.04 |
| Fe (mg $kg^{-1}$) | 5.29 ± 2.11 |
| Mn (mg $kg^{-1}$) | 0.39 ± 0.36 |
| Na (mg $kg^{-1}$) | 2470.40 ± 845.84 |
| Zn (mg $kg^{-1}$) | 0.59 ± 0.31 |

*ND* Not detected.

corresponding to the tillering stage, and the final application at 74 DAS, corresponding to the end of the vegetative stage, and beginning of heading (i.e., a total of 5 applications). After each application, the plants were randomly rearranged in the greenhouse.

## Parameters analyzed

The morphological and yield parameters evaluated were plant height (PH – cm), measured from the base of the stem to the tip of the longest leaf blade; number of total tillers per pot (TTP), number of total spikes per pot (TSP), total number of kernels per pot (NKP), average number of kernels per spike (NKS), and the grain set (i.e., percentage of florets that turned into kernels – GS) as a percentage. At 74 DAS, most plants had initiated the booting process; tissue samples were collected from the third leaf below the flag leaf for biochemical analyses. These included chlorophyll *a*, chlorophyll *b*, total chlorophyll (TChl), and total carotenoids (TCN), as well as primary metabolites including totals of protein (TP), carbohydrates (TC), and amino acids (TAA).

Additionally, the influence of the dilution of seaweed aqueous extract on grain reserve accumulation in the kernels produced (filial generation – F1) was assessed using thousand-kernel weight (TKW) and a germination index (G%), both following standardized protocols (Brasil 2018). To evaluate G%, the germination of 200 kernels from each treatment was assessed using a Germitest paper roll assay (ProLab, 28 × 38 cm, 65 g $m^{-2}$ grammage) moistened with water equivalent to 2.5 times the weight of the paper. Kernels were arranged in four replicates of 50, incubated in a germination chamber at 23 °C and 12 h/12 h light/dark, for 8 days, after which the germination percentage was quantified. For biochemical analysis, a composite kernel sample from each treatment was ground into flour using a knife mill, sieved (50 mesh), and analyzed for primary metabolites: total soluble sugars (F1-TSS), total starch (F1-TS), total protein (F1-TP), and total amino acids (F1-TAA).

## Chlorophyll and total carotenoids

For the analysis of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (TChl), and total carotenoids (TCN), 100 mg of leaf samples were incubated in a water bath with 7 mL of dimethyl sulfoxide (DMSO) for 2 h at 65 °C. The extract was recovered by filtration and adjusted to a final volume of 10 mL with DMSO, following the protocol of Hiscox and Israelstam (1979). The absorbance values at 480, 649, and 665 nm were measured and the concentrations were calculated using the formulae provided by Wellburn (1994):

$$\text{Chl}a = \left[12.19 * \left(A_{665}\right) - 3.45 * \left(A_{649}\right)\right]$$
$$\text{Chl}b = \left[21.99 * \left(A_{649}\right) - 5.32 * \left(A_{665}\right)\right]$$
$$\text{TCN} = [1{,}000 * \left(A_{480}\right) - 2.14 * (\text{Chl}a) - 70.16 * (\text{Chl}b)]/220.$$

## Total carbohydrate

Total carbohydrate (TC) was determined only for the leaf sample, using a method adapted from DuBois et al. (1956). Briefly, a 50 mg sample of pooled leaves (n = 3) for each treatment was mixed with 20 mL of distilled water, centrifuged (10 min, 4000 rpm), and 2 mL of the supernatant was collected. To this, 50 µL of 80% phenol and 5 mL concentrated sulfuric acid were added. After 10 min the mixture was vortexed and incubated in a water bath (30 °C, 15 min). Absorbance was measured at 490 nm. A calibration curve was prepared using galactose (Sigma-Aldrich, USA) at concentrations from 7.80–500 µg $mL^{-1}$ ($y = 0.0026x$, $R^2 = 0.999$) and the levels were expressed in mg of galactose equivalent per g of dry weight (mg $g^{-1}$).

### Total protein

The total protein (TP) content was determined using the Bradford (1976) method with some adaptations. Briefly, 200 mg of the leaves or flour samples was extracted with 10 mL phosphate-buffered saline (PBS), pH 7, vortexed, allowed to stand for 15 min, and centrifuged (10 min, 4000 rpm). An aliquot of 100 µL of the supernatant was mixed with 5 mL of a 1:5 dilution of the Bradford reagent in water and allowed to stand for 5 min. The absorbance was measured at 595 nm using a microplate reader. A calibration curve was prepared using bovine serum albumin (Sigma-Aldrich) at concentrations ranging from 3.90 to 250.0 µg $mL^{-1}$ ($y = 0.0032x$, $R^2 = 0.977$). The results were converted to mg $g^{-1}$ of dry weight.

### Total amino acid

The total amino acids (TAA) was determined using a method adapted from Silveira and Furlong (2007). To each 100 mg of macerated leaves or flour, 2 mL of water was added, vortexed, and centrifuged (4000 rpm, 10 min). A 1 mL aliquot of the supernatant was collected and 3 mL of a 0.2% ninhydrin solution in sodium phosphate buffer (pH 7.0) was added. After stirring, the mixture was placed in a water bath at 100 °C for 30 min. After cooling to room temperature, the absorbance of the reaction was read at 570 nm. An external calibration curve was produced with proline (Sigma-Aldrich) at concentrations ranging from 1.95 to 500 µg $mL^{-1}$ ($y = 0.0018x$, $R^2 = 0.996$). The results were converted to mg $g^{-1}$ of dry weight.

### Total soluble sugars and total starch

Total soluble sugars (TSS) and total starch (TS) were analyzed only in the kernels. The TSS were determined using a method adapted from Umbreit and Burris (1964). Briefly, 50 mg of the kernel flour was extracted with 2 mL of methanol, chloroform, and water (MCW, 12:5:3) solution. The mixture was centrifuged (10 min, 4000 rpm) and the supernatant was collected. A second extraction was performed on the pellet and the supernatants were combined. To the mixture were added 1 mL of chloroform and 1.5 mL of water. After stirring and definition of bi-phases, 1 mL of the aqueous phase was collected and to this portion were added 2 mL of 0.2% anthrone in sulfuric acid, followed by further vortexing. The solution was heated in a water bath (100 °C) for 3 min and then cooled to room temperature. Absorbance was measured at 630 nm. Glucose (Sigma-Aldrich) was used to generate a calibration curve with concentrations ranging from 7.8 to 500 µg $mL^{-1}$ ($y = 0.0018x$, $R^2 = 0.992$). The results were expressed in mg as the equivalent amount of glucose per gram of dry weight (mg $g^{-1}$).

The TS was determined using the same method adapted from Umbreit and Burris (1964). The remaining pellet from the TSS analysis was used for extraction with 2 mL of 30% perchloric acid, centrifuged (10 min, 4000 rpm), and the supernatant was collected. This extraction was repeated and the supernatants were combined. To a 1 mL aliquot, 2 mL of 0.2% anthrone in sulfuric acid was added and the mixture was vortexed. The reaction was heated in a water bath (100 °C, 3 min) and cooled to room temperature. Absorbance was measured at 630 nm. The starch content was calculated from an external calibration curve at concentrations ranging from 1.9 to 500 µg $mL^{-1}$ ($y = 0.0016x$, $R^2 = 0.992$), and the levels were expressed in milligrams of starch equivalent per gram of dry weight (mg $g^{-1}$).

### Statistical analysis

The morphological and productivity variables were performed in eight replicates ($n = 8$) per treatment, as average per pot (PH and NKS) and total per pot (TTP, TSP, NKP). Furthermore, for kernel analysis, a pooled sample of the 8 pots per treatment was used: thousand-kernel weight (TKW) was performed in eight replicates ($n = 8$), while the germination index (G%) of harvested kernels was performed in four replicates ($n = 4$). For the biochemical analyses, composite samples (leaves or kernel flour) were pooled from all eight pots per treatment to ensure representativeness and reduce analytical costs, with technical triplicates ($n = 3$) to account for analytical variability. All results are expressed as mean ± standard deviation (SD).

The extract concentrations were analyzed as categorical variables using the non-parametric Kruskal–Wallis test, with post-hoc comparisons using Dunn's test with Bonferroni adjustment for multiple comparisons ($p_{holm} < 0.05$), performed with the rstatix package (Kassambara 2023) in RStudio software (version 2023.06.0). Graphical representations were generated using the ggplot2 package (Wickham 2016). The parameters were also analyzed using principal component analysis (PCA), employing the singular value decomposition (SVD) algorithm, using Unscrambler X software (v. 10.4).

## Results

### Morphological and yield parameters

The application of the aqueous extract of *K. alvarezii* via foliar spray to barley resulted in no significant changes to key vegetative morphological parameters across the tested concentrations (0–10% v/v). Specifically, PH ranged from 87.43 ± 8.33 cm in the control to 91.13 ± 3.18 cm at the 1% concentration ($\chi^2(4) = 1.343$, $p = 0.854$). TTP values

varied between 39.4 ± 4.34 (1%) and 52.5 ± 16.03 (control) ($\chi^2(4) = 9.419$, $p = 0.051$), while TSP ranged from 35.13 ± 6.06 (5%) to 44.25 ± 13.11 (control) ($\chi^2(4) = 4.773$, $p = 0.311$). Similarly, the extract did not significantly alter the numerical yield components: total kernels per pot (NKP) ranged from 550.63 ± 79.02 (10%) to 581.00 ± 86.42 (control) ($\chi^2(4) = 0.918$, $p = 0.922$), and average kernels per spike (NKS) varied between 15.23 ± 3.92 (control) and 17.21 ± 1.49 (1%) ($\chi^2(4) = 2.297$, $p = 0.681$). Complete graphical representations of these non-significant parameters are available in supplementary material (Figs. S1 and S2).

In contrast, the extract induced significant, concentration-dependent improvements in grain quality parameters. Grain set (GS) was significantly enhanced ($\chi^2(4) = 17.515$, $p = 0.002$), with the 1% treatment showing an 18.24% increase (81.30 ± 4.09%) compared to the control (68.76 ± 7.74%). Thousand-kernel weight (TKW) also exhibited a significant positive dose–response ($\chi^2(4) = 12.143$, $p = 0.016$), with the highest values recorded at 5% (53.37 ± 0.92 g) and 10% (53.30 ± 1.50 g), representing an increase of approximately 4.2% over the control (51.13 ± 1.39 g) (Fig. 1).

A principal component analysis (PCA, Fig. 2) was applied to morphological and yield data to effectively capture the treatment-induced variations. The first two principal components (PCs) accounted for 94% of the total variance (PC1: 78%, PC2: 16%). The projection of the data on the PC1-PC2 plane revealed a clear segregation among the treatments. The control (0%) was distinctly isolated in the positive extreme of PC1 +, while the extract treatments formed separate clusters on PC1-. The parameters plant height (PH), number of kernels per spike (NKS), and grain set (GS) were grouped to the treatment 1%, on PC1- and PC2-, while thousand-kernel weight (TKW) was grouped to treatments 2, 5, and 10%, on PC1- and PC2 + (Fig. 2).

## Biochemical composition of plants

Regarding photosynthetic pigments (Chl *a*, Chl *b*, TChl, and TCN), the Kruskal–Wallis test revealed a significant overall effect of extract concentration ($\chi^2(4) = 13.500$; $p = 0.009$). However, after Bonferroni correction for multiple comparisons, only the 5% concentration differed significantly from the control ($p < 0.05$), while intermediate concentrations (1, 2, and 10%) did not reach statistical significance in pairwise comparisons (Fig. 3). The lowest values occurred at 5% concentration, a reduction of approximately 50.5% as compared to control ($\chi^2(4) = 13.500$; $p = 0.009$) for all pigments.

Regarding the primary metabolites (Fig. 4), TC peaked at 5% *Kappaphycus* extract concentration (27.20 ± 0.25 mg $g^{-1}$), which was significantly higher than the 10% treatment (20.33 ± 1.30 mg $g^{-1}$) but did not differ statistically from the control or other concentrations ($\chi^2(4) = 11.733$; $p = 0.019$). TP showed no significant differences across treatments ($\chi^2(4) = 7.138$; $p = 0.129$), with values ranging from 0.08 ± 0.03 mg $g^{-1}$ at 1% to 0.25 ± 0.10 mg $g^{-1}$ at 5% concentration. TAA presented the highest value at 1% concentration (58.12 ± 1.34 mg $g^{-1}$), with an increase of 47.44% compared to the control (39.42 ± 1.91 mg $g^{-1}$; $\chi^2(4) = 13.500$; $p = 0.009$).

When using PCA on biochemical parameters, a total variance of 98% was found (PC1: 58%, PC2: 24%). Two distinct clusters were observed on PC1- quadrant, with 5% and 10%

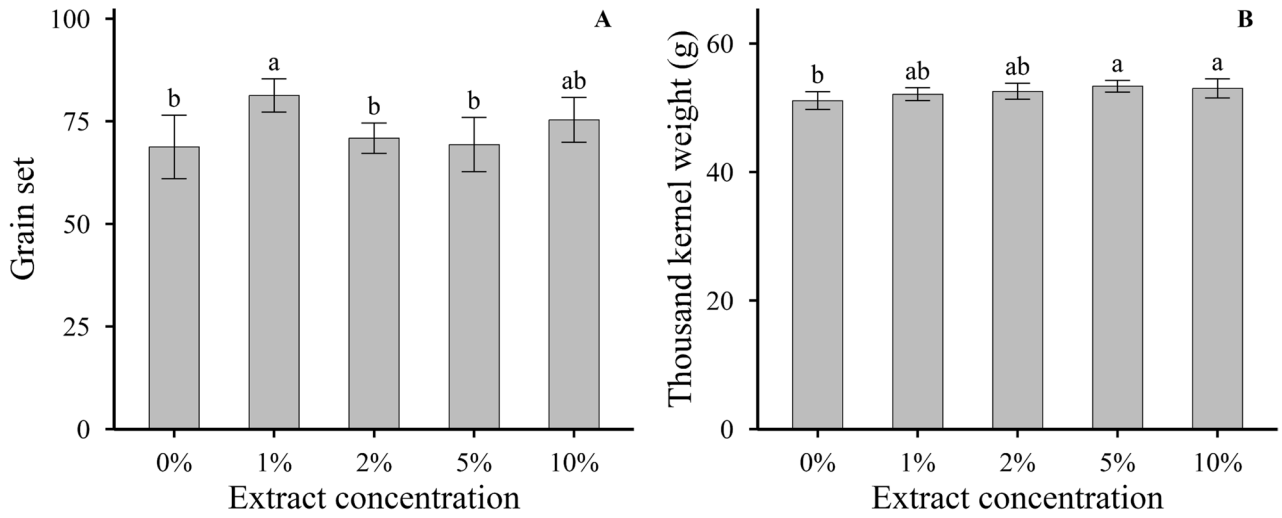



**Fig. 1** Yield components of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – grain set (GS); B – thousand kernel weight (TKW – g). Data are presented as mean + standard deviation. Values followed by different letters differ significantly for Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

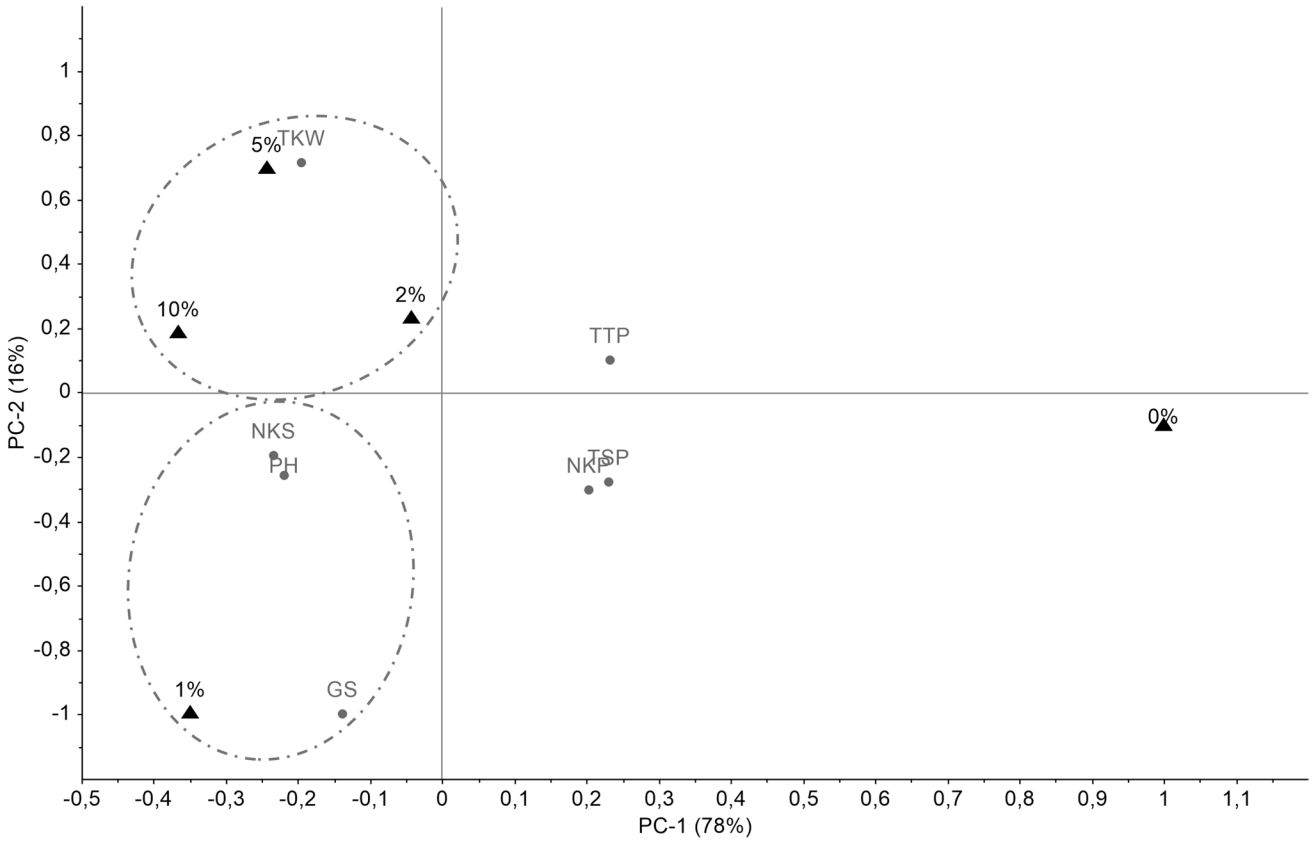



**Fig. 2** Principal component analysis (PC1 and PC2) calculated from the data of plant height (PH), total tillers per pot (TTP), total spikes per pot (TSP), total kernels number per pot (NKP), number of kernels per spike (NKS), grain set (GS), and thousand-kernel weight (TKW), of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii*, at different doses (control, 1, 2, 5, and 10% v/v). The seaweed raw materials were cultivated in Santa Catarina, Brazil

grouping with total protein (TP), and 2% grouping with total amino acids (TAA) and total carbohydrates (TC) (Fig. 5).

## Germination and biochemical composition of kernels

The germination index (G%) of grains produced by treated plants was consistently high (98.5–100%) and not significantly different across all treatments ($\chi^2(4) = 5.146$; $p = 0.273$). However, all the biochemical components of the kernels were significantly affected (Fig. 6). Total starch content (F1-TS) presented the highest value at 5% concentration ($21.79 \pm 0.25$ mg $g^{-1}$), differing statistically from the control ($18.26 \pm 0.23$ mg $g^{-1}$), representing an increase of 19.33% of TS ($\chi^2(4) = 12.033$; $p = 0.017$). Conversely, total soluble sugar content (F1-TSS) was significantly lower at the 5% concentration ($161.54 \pm 2.64$ mg $g^{-1}$) compared to the control ($188.01 \pm 3.24$ mg $g^{-1}$), a downgrade of 14.08% ($\chi^2(4) = 10.867$; $p = 0.028$). The total protein (F1-TP) increased in 10% concentration ($1.82 \pm 0.06$ mg $g^{-1}$), differing statistically from the control ($1.65 \pm 0.06$ mg $g^{-1}$), representing an increase of 10.30% ($\chi^2(4) = 9.951$; $p = 0.041$). The amino acid content (F1-TAA) was higher in 1% and 2% concentrations ($17.30 \pm 0.77$ and $17.70 \pm 0.16$ mg $g^{-1}$, respectively), resulting in a significant increase of 54.87% compared to the control ($11.30 \pm 0.75$ mg $g^{-1}$; $\chi^2(4) = 12.300$; $p = 0.015$).

When employing PCA on the kernel parameters, a total variance of 100% was observed between PC1 (61%), PC2 (35%), and PC3 (4%) (Fig. 7). Four distinct clusters were observed across three quadrants, with the 10% treatment grouping with F1-TSS on PC1 + and PC2 +; the 1% treatment grouping with F1-TS and 2% grouping with F1-TP and F1-TAA, on PC1- and PC2 +; and 5% grouping with germination index (G%).

## Discussion

The applications of the aqueous extracts of *K. alvarezii* elicited a concentration-dependent response in barley, significantly altering key yield components and biochemical profiles, while leaving basic plant architecture largely unchanged.

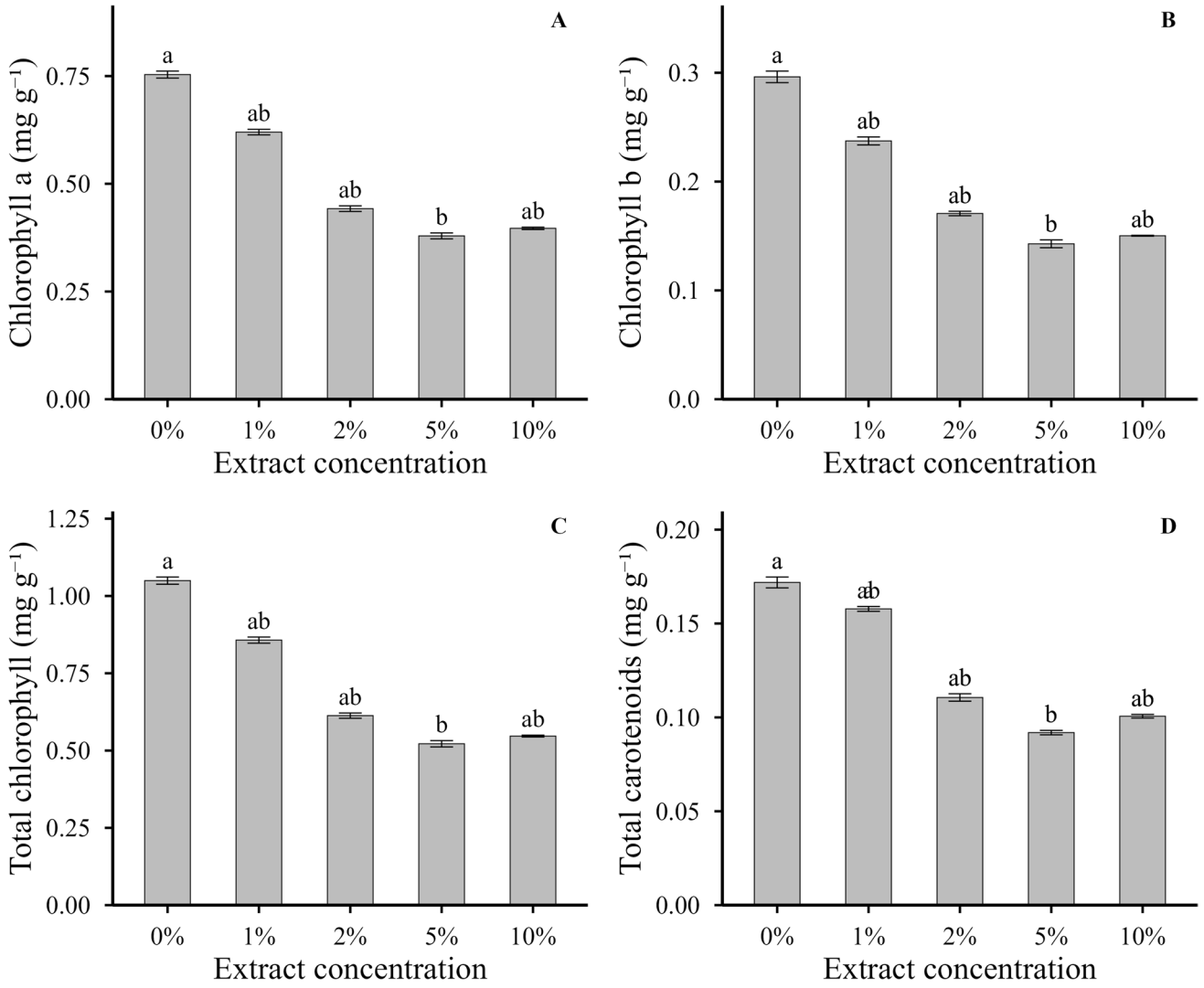



**Fig. 3** Leaf pigments (mg $g^{-1}$) of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – chlorophyll *a*; B – chlorophyll *b*; C – total chlorophyll; D – total carotenoids (TCN). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

The extract had a minimal direct impact on vegetative growth under the controlled greenhouse environment. The findings suggested that the commercial potting medium pre-fertilized with NPK (10:10:10) at 5 g per pot provided sufficient baseline nutrition to support optimal vegetative development of the barley plants in the greenhouse, potentially diminishing the extract's additive effects on these traits. However, there have been more consistent, positive responses reported in other cereals, e.g., in wheat, rice, and maize, foliar applications of *K. alvarezii* extracts have been shown to promote growth metrics such as height, biomass, and tillering, often attributed to bioactive compounds such as cytokinins, auxins, and polysaccharides that enhance cell division, nutrient mobilization and root architecture (Trivedi et al. 2023; Fatima and Al-Yasari 2024). Furthermore, the use of a single NPK regime (10:10:10 at 5 g per pot) precludes assessment of the extract's performance under reduced or variable fertilization inputs, a scenario increasingly relevant to sustainable barley production and one in which seaweed-based biostimulants have shown stronger additive effects (Cozzolino et al. 2021). Future experiments should test this extract under a gradient of nitrogen availability to disentangle nutritional interactions from direct biostimulant effects.

An additional limitation related to the experimental setup concerns the use of 10-L pots, whose restricted soil volume may have constrained root expansion and limited the expression of root-promoting effects commonly reported for *K. alvarezii* extracts, such as enhanced root length, lateral root density, and root biomass (Trivedi et al. 2018; Kumar et al.

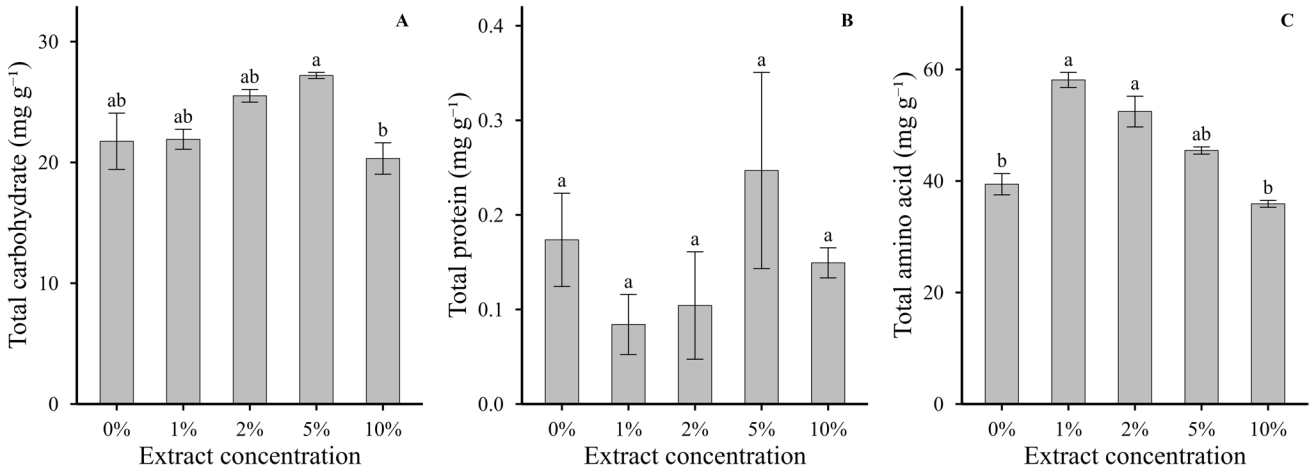



**Fig. 4** Biochemical parameters (mg $g^{-1}$) of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses diluted in water (0, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – total carbohydrates (TC); B – total protein (TP); C – total amino acid (TAA). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

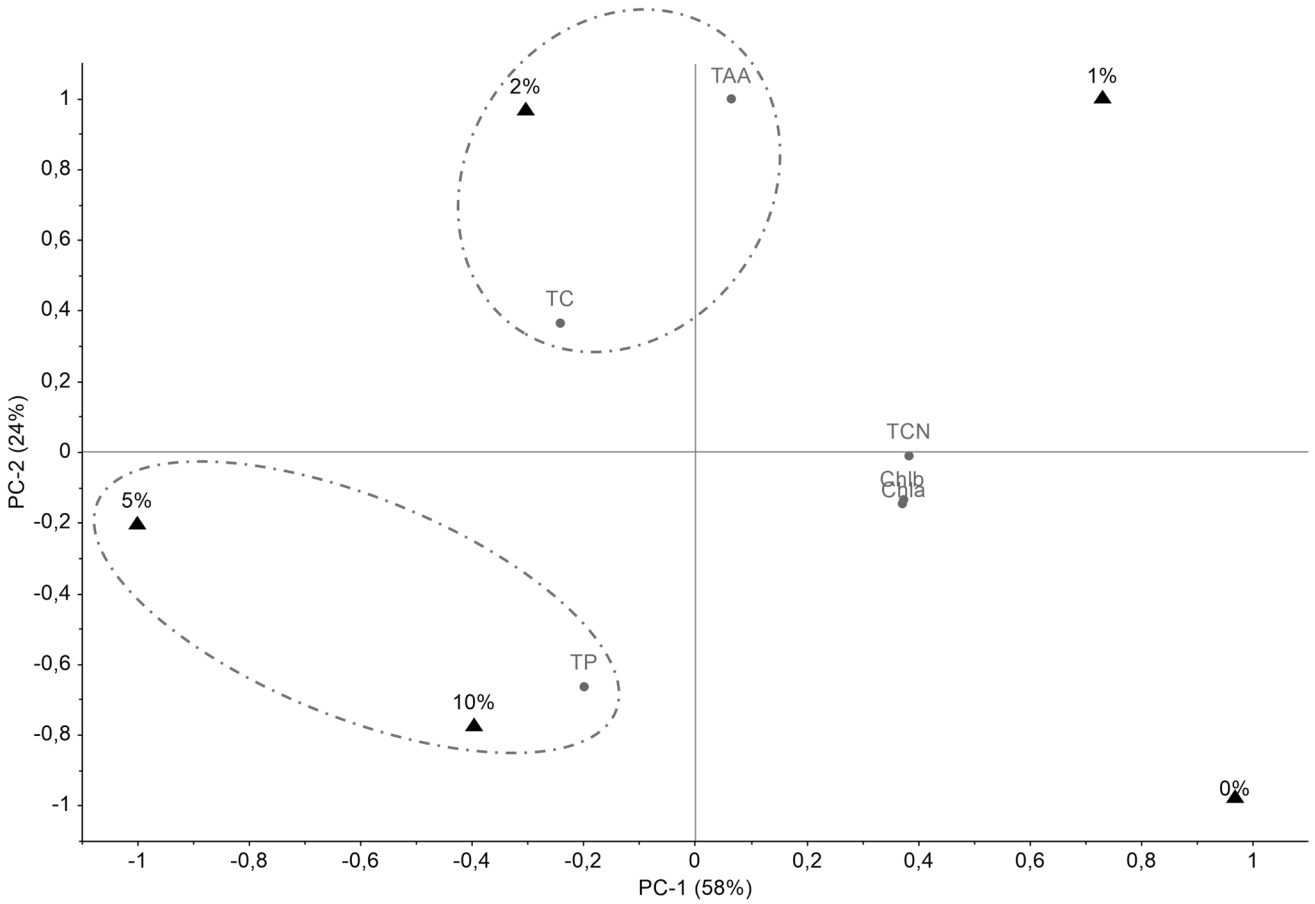



**Fig. 5** Principal component analysis (PC1 and PC2) calculated from the dataset of plant biochemical components chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total carotenoids (TCN), total carbohydrates (TC), total proteins (TP), and total amino acids (TAA), of barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil

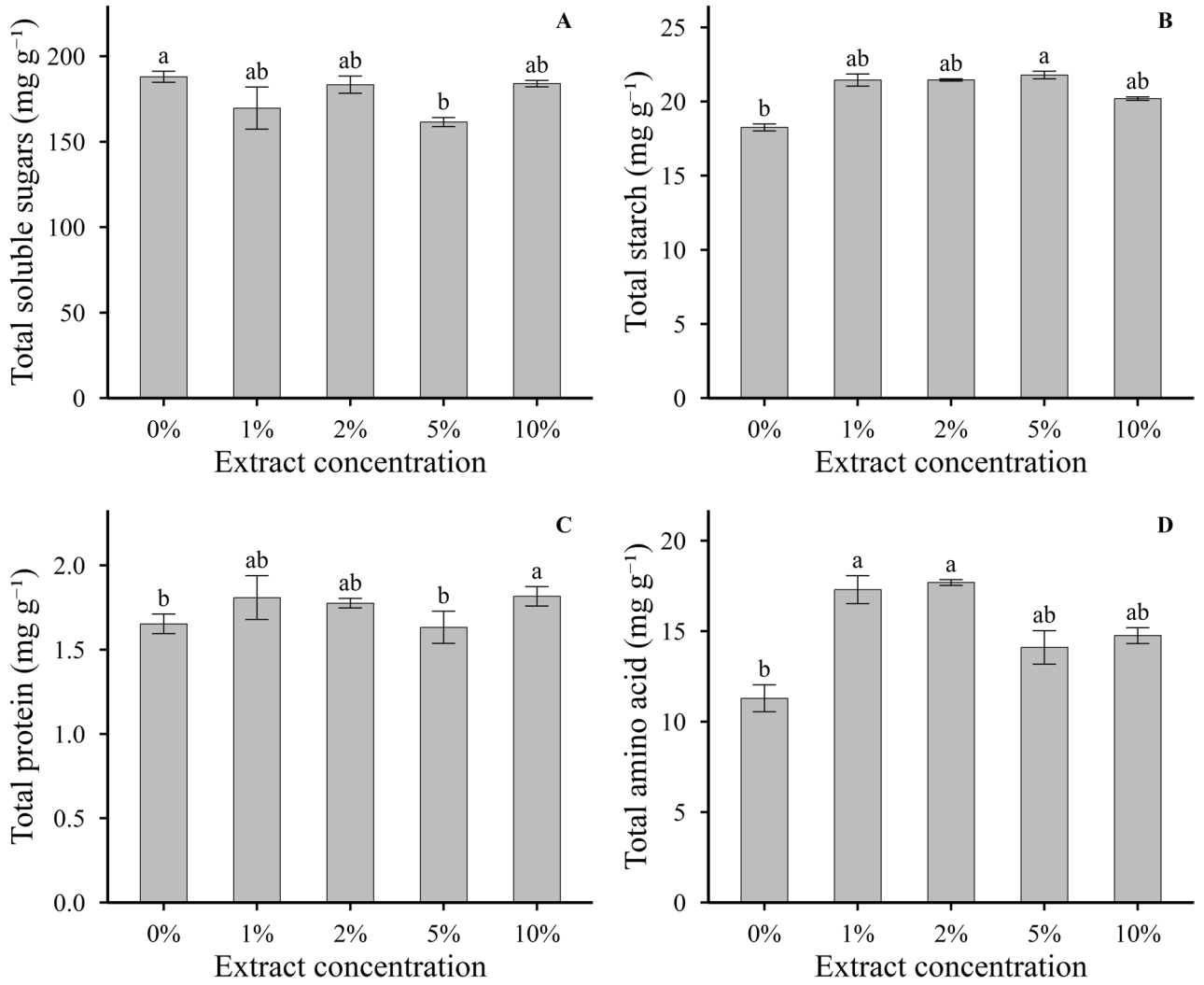



**Fig. 6** Biochemical parameters (mg $g^{-1}$) of kernels produced by barley plants treated with foliar application of aqueous extract of *Kappaphycus alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil. A – total soluble sugars (TSS); B – total starch (TS); C – total protein (TP); D – total amino acid (TAA). Values followed by different letters differ significantly for the Dunn's test with Holm-Bonferroni adjustment ($p < 0.05$)

2020). Root morphology was not assessed in the current study, and this represents both a limitation and an important avenue for future greenhouse and field investigations.

A review of the literature specific to barley revealed highly inconsistent effects of seaweed-based biostimulants on morphology, likely due to the wide variation in experimental conditions, extract formulations, and application protocols among the studies (Table 2). Multi-year field trials have documented subtle improvements in grain yield, even in the absence of significant alterations to plant height, tiller density, or spike formation (Taylor et al. 1990; Ashworth et al. 2025). When morphological changes do occur, they are context-dependent, modulated by variables such as formulation (e.g., extract purity and concentration), application timing (e.g., tillering vs. heading stages), environmental factors (e.g., soil type, climate variability), and historical agronomic practices (e.g., prior fertilizer regimes) (Szczepanek 2017; Hřivna et al. 2024).

For example, plant height in barley often remains stable across foliar applications of various seaweed extracts in non-stressed, well-fertilized settings, with increases primarily documented under abiotic stressors such as drought or salinity, or when the extracts were applied via soil drenching to target root zones more effectively (Szczepanek 2017). Tiller and spike numbers are similarly variable, frequently influenced by weather patterns that overshadow foliar-induced responses; in some cases, enhancements require synergistic practices, such as combining seaweed extracts with reduced nitrogen applications, or seed priming to optimize resource partitioning (Cozzolino et al. 2021; Hřivna et al. 2024).

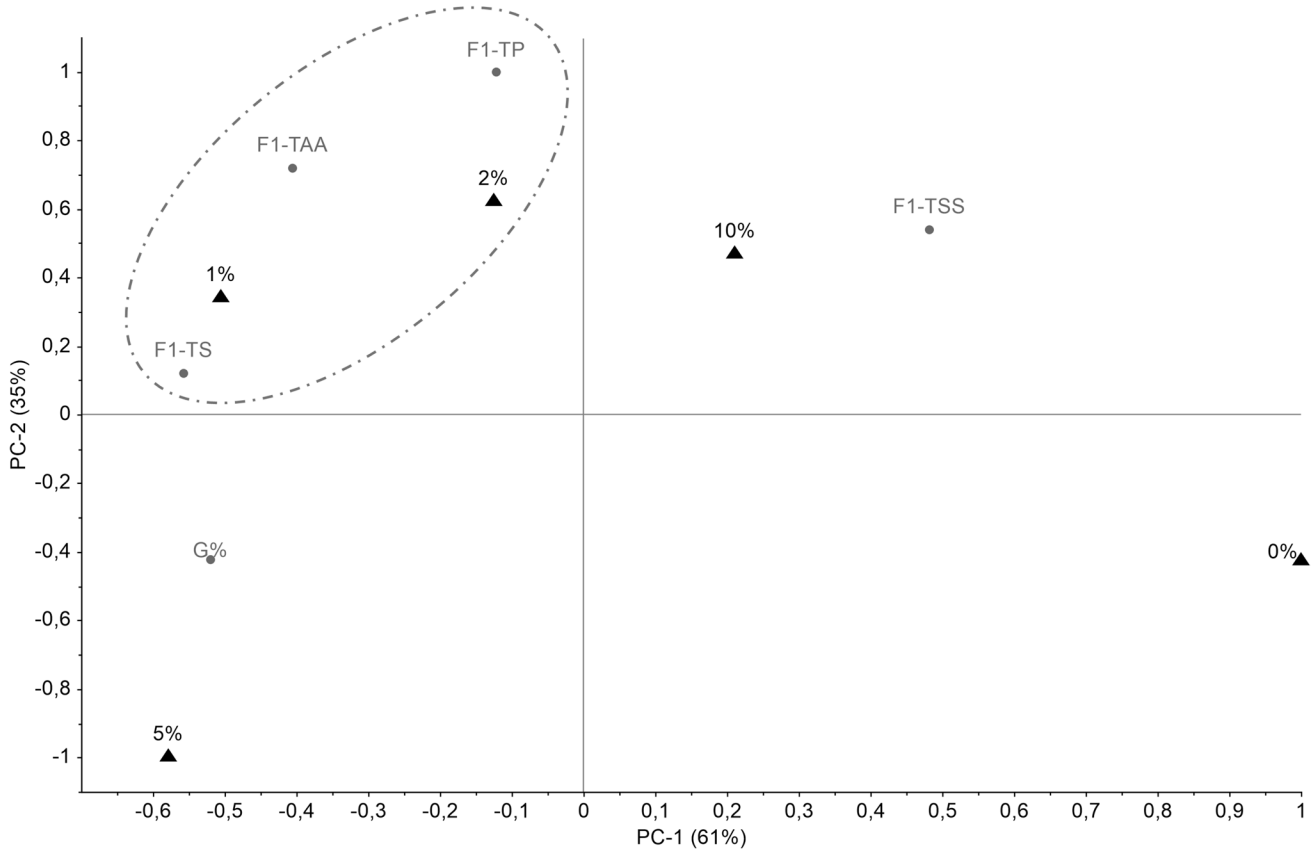



**Fig. 7** Principal component analysis (PC1 and PC2) calculated from the dataset of kernel germination (G%) and biochemical components: total starch (F1-TS), total soluble sugars (TSS) total protein (TP), and total amino acids (TAA), of barley plants treated with foliar application of aqueous extract of *K. alvarezii* at different doses (control, 1, 2, 5, and 10% v/v), from raw materials cultivated at Santa Catarina, Brazil

Under conditions of high soil fertility, as simulated here with NPK supplementation, vegetative and partitioning traits may reach a physiological plateau, limiting the ability of the *Kappaphycus* extract to elicit measurable changes (Ashworth et al. 2025). This could also stem from the inherent genetic traits of specific varieties of barley, which may confer less responsiveness to exogenous signals as compared to other cereals, or the experimental design's absence of imposed stressors. It is also important to note that the observational nature of this study does not allow resolution of the molecular and physiological mechanisms underlying the responses observed, including the potential roles of bioactive compounds such as cytokinins, betaines, and sulfated polysaccharides. Elucidating these mechanistic pathways in barley represents a priority for future research. Future research should aim to decouple these effects by testing particular extracts under varying nutrient regimes (e.g., reduced NPK), or in conjunction with abiotic stressors, to clarify whether its primary role is corrective under stress or optimizing under non-limiting conditions. Regarding the application regime adopted here (five foliar sprays at 14-day intervals from 14 to 74 DAS), it should be acknowledged that timing, frequency, and phenological stage of application can strongly modulate biostimulant performance (Szczepanek 2017; Hřivna et al. 2024). Applications initiated closer to the reproductive stage, such as at flag leaf emergence or anthesis, may produce different or complementary effects on yield components and should be explored in future studies.

The influence of concentrations of the specific *Kappaphycus* extract tested here was selective, enhancing the quality of kernel production rather than its quantity. While it did not affect the number of kernels per pot, or per spike, it significantly improved grain set and thousand-kernel weight (TKW). The increase in grain set at low concentrations (1–2%) suggested a promotion of reproductive efficiency and pollination success, likely through improved resource allocation during flowering. The significant increase in grain set in barley plants can be interpreted through the physiological critical window of anthesis. This period, encompassing flowering and pollination, is decisive for yield determination in barley, as abiotic stresses often lead to floret abortion and directly limit the final number of grains (Dolferus

**Table 2** Comparative overview of reported effects of seaweed-derived extracts on barley (*Hordeum vulgare L.*)

| Parameters | Taylor et al. (1990) | El-Sharkawy et al. (2017) | Szczepanek et al. (2018) | Cozzolino et al. (2021) | Goñi et al. (2021) | Hřivna et al. (2024) | Al-Musawi et al. (2025) | Present work |
|---|---|---|---|---|---|---|---|---|
| Seaweed | Unspecified marine seaweed (likely *Ascophyllum nodosum* or similar, containing cytokinins) | *Ascophyllum nodosum* | *Ecklonia maxima* (Kelpak) | *Ecklonia maxima* | *Ascophyllum nodosum* | *Ascophyllum nodosum* | *Ascophyllum nodosum* | *Kappaphycus alvarezii* |
| Extract type | Liquid extract with cytokinins | Commercial liquid extract | Seaweed extract (aqueous) | Seaweed extract (cold cell burst, high auxin:cytokinin ratio) | ANE biostimulant PSI-362 | Liquid extract (1% *A. nodosum*) | ANE Goëmar GA142 | Aqueous extract |
| Application method | Foliar spray at 4–5 leaf stage (1 L $ha^{-1}$ or 2.5 × rate) | Foliar (4 kg $ha^{-1}$, once) | Foliar (2 L $ha^{-1}$ or sequential 1.5 L $ha^{-1}$) | Foliar (3 applications at 3.0 mL $L^{-1}$) | Coated on fertilizer (CAN + S) | Foliar (1 L $há^{-1}$) | Foliar (1 L $ha^{-1}$, 1:50 dilution) | Foliar (1–10% v/v) |
| Morphological Results | No significant changes in plant height, tiller number, head length, or maturity; no promotion under optimal or stress conditions | Increased shoot/root dry weight (up to 4.19/3.89 g), tillers (up to 7.44), root length (up to 18.22 cm) under salt (10–15 dS $m^{-1}$) | Root weight + 15–17%; grain/spike + 4%; TGW + 4.3% (sequential application superior; better root development under varied weather) | Height + 4.2%; spikes + 9%; biomass + 15.7%; TKW + 3.9%; kernel diameter + 2.3% (higher at N40/N60, plateau at N40 in warmer year; extract amplifies at mid-N doses) | Yield + 5.57%; biomass + 13.88–16.67% (coating enhances under N/S fertilization; no stress-specific morph changes) | Increased root size (length/volume up to 20%); no change in shoot height; better under dry conditions | Aboveground biomass + 76.82%; spikelets $m^{-2}$ + 75%; TGW + 27%; grains/spike + 15.22% (UI + B superior; genotype-specific, e.g., late-maturing more responsive early-stage) | No significant changes in plant height, total tillers, and total spikes |
| Biochemical Results | No changes in nutrient uptake or stress-related markers; cytokinin levels did not enhance metabolism | Reduced $Na^+/K^+$ ratio (to 5.54–7.15); increased proline (up to 0.48 µmol $g^{-1}$ FW), catalase (up to 450 mmol $min^{-1}$ $g^{-1}$ FW); decreased EL (to 72.39%) | P content +; N/P uptake + (enhanced nutrient efficiency) | Grain protein + 24.2% (11.8% vs. 9.5%; exceeds 11.5% at N60; NUE + 17.9%; inverse with N dose) | Nitrate + 17.9–72.2%; NUE + 29.85–60.26%; NR + 24.82%; GS + 30.63% (higher under coated fertilizer) | Enhanced N uptake (up to 15%); improved nutrient efficiency | Grain protein 9.5–11.5% (optimal malting; stable under reduced N) | Leaf amino acids (+ 47,44% at 1%), kernel starch (+ 19.3% at 5%); kernel protein (+ 10.3% at 10%); kernel amino acids (+ 56.6% at 1–2%) |

**Table 2** (continued)

| Parameters | Taylor et al. (1990) | El-Sharkawy et al. (2017) | Szczepanek et al. (2018) | Cozzolino et al. (2021) | Goñi et al. (2021) | Hřivna et al. (2024) | Al-Musawi et al. (2025) | Present work |
|---|---|---|---|---|---|---|---|---|
| Yield Results | No increase in grain yield (field trials over 4 years); similar to kinetin application; no edge cases like drought benefits observed | Increased relative yield (up to 75.23%); better under salt stress; no non-stress benefits | Yield + 4% (sequential better; grain quality improved via TGW) | Grain yield + 17.0%; hectolitre mass + 3.8% (higher in warmer year; N40 sufficient with extract, reducing N by 35%; harvest index unaffected) | Yield + 5.57% (via biomass/NUE; no detailed components) | Increased grain yield (up to 10%) and components; better in low-N soils | Grain yield + 71%; maltable fraction + 22% (UI + B; retention > 90%; environment/genotype interactions; GPC optimal) | Increased GS (+ 18.24% at 1%) and TKW (+ 4.2% at 5–10%). No significant changes in kernels per pot and kernels per spike |

et al. 2011; Boussora et al. 2019; Kamal et al. 2022). The enhanced grain set suggests that this extract positively influenced this sensitive stage, potentially contributing to patterns observed in yield variables such as NKS (grouped in the PCA with GS), although without altering the total kernel (NKP). This may be mediated by improvements in the plant's nutritional status (Askarnejad et al. 2021), a more favorable hormonal balance for floret fertility (Boden 2016; Marzec and Alqudah 2018), or enhanced resilience to a transient environmental stress during flowering such as a high night temperature (García et al. 2015; supplementary material Fig. S3). Consequently, the application of *K. alvarezii* extract did not increase the potential number of florets/kernels but rather secured a higher success rate in their development into viable grains, highlighting a role in optimizing reproductive efficiency.

In contrast, the higher TKW at moderate-to-high concentrations (5–10%) points to a direct role in enhancing grain filling and reserve accumulation. These findings align with barley-specific studies where the foliar application of *E. maxima* extract (large brown seaweed species native to the cold, nutrient-rich waters) boosted grain number per spike, thousand-grain weight, and overall yield by 8–20%, attributed to enhanced root development, nutrient uptake (N and P), and hormonal signaling (Szczepanek et al. 2018). In broader cereals like maize, *K. alvarezii* extracts have demonstrated similar yield enhancements under non-stress conditions, with optimal responses at concentrations of 5–10% (e.g., 19–36% yield increases), attributed to bioactive compounds including cytokinins, auxins, and polysaccharides that promote cell division, expansion, and stress tolerance (Rabhi et al. 2025). The dose-dependent patterns observed here, favoring low doses for grain set and higher for TKW, echo context-specific responses in field trials, where seaweed extracts often optimize partitioning under non-stress environments by modulating gene expression for nutrient efficiency (Trivedi et al. 2018; Kumar et al. 2020).

Biochemically, chlorophyll is primarily responsible for capturing light energy, which is then converted into chemical energy through photosynthesis. In contrast, carotenoids assist in light absorption and provide essential photoprotection by dissipating excess energy, thus preventing damage to the photosynthetic apparatus (Chen 2015; Nguyễn and Sung 2024). The observed pigment reduction in 5% concentration diverges from typical *K. alvarezii* extract, which generally enhances chlorophyll and carotenoid levels on plants, even under non-stress conditions (e.g., Nivetha et al. 2024; Nunes et al. 2025b).

In contrast, the analysis of total carbohydrate (TC) levels revealed a peak at the 5% concentration of the aqueous extract. Although this value did not significantly differ from the control, it was grouped only with the 2% treatment in the PCA (Fig. 5). TC in plant leaves is essential for energy

storage and metabolic processes (Lal and Bhatla 2023). The accumulation of carbohydrates can modulate photosynthetic rates, where excess may lead to feedback inhibition, underscoring their role in maintaining plant homeostasis (Araya et al. 2006; Khornti 2023), and serving as reserves under stress conditions, such as cold stress (Babazadeh et al. 2023).

Some works demonstrate increases in total or non-structural carbohydrates in barley leaves treated with seaweed extracts. For instance, seaweed extracts enhance non-structural carbohydrates in barley, promoting freezing tolerance by facilitating energy storage and water adjustments under cold stress, with applications leading to multifaceted phytostimulatory effects that boost overall crop resilience and yield (Ali et al. 2021a). In brewing barley subjected to varying nitrogen levels, *A. nodosum* extract increases grain protein and metabolic profiles, indirectly supporting leaf carbohydrate dynamics through reduced nitrogen dependency and enhanced biomass, though direct leaf TC measurements are not always specified (Cozzolino et al. 2021).

Studies with other cereals treated with other extracts of *K. alvarezii* showed notable TC elevations, particularly in maize leaves, where foliar applications of the AgroGain® (commercial biostimulant) at 1 mL $L^{-1}$ increased total soluble sugars by up to ninefold, linked to heightened photosynthetic activity, chlorophyll content, and rhizosphere microbial priming that enhances nutrient utilization and plant vigor (Nivetha et al. 2024). In non-cereal plants, such as basil (*Ocimum basilicum*), the application of a similarly prepared aqueous extract of *K. alvarezii* from southern Brazil also increased plant TC, with a peak at 5–7% concentrations (Nunes et al. 2025b). These patterns suggested that 5% peak in TC may reflect an optimal dose for carbon assimilation in barley, potentially aiding stress resilience and yield, consistent with seaweed's role in sustainable crop production. It is important to note that while both extracts are aqueous preparations of *K. alvarezii* from Santa Catarina, they differ in production scale: the extract used in the present study was produced at an industrial scale (provided by VerdeMar®), whereas the extract in Nunes et al. (2025b) was prepared at a laboratory scale. These differences in processing scale may result in variations in their specific biochemical profiles and, consequently, their bioactivity. Such variations underscore that the bioactivity of algal extracts is not only species-dependent but also influenced by biogeographic factors (Nunes et al. 2025a), seasonal and processing differences (even if the same seaweed), and these are points that need explicit investigation in future comparative studies. It should therefore be emphasized that the effects reported here pertain specifically to an autumn-harvested biomass from Palhoça, Santa Catarina, Brazil, processed at an industrial scale, and may not fully represent the agronomic performance of *K. alvarezii* extracts derived from different origins, seasons, or processing methods.

The application of the *K. alvarezii* extract in this study influenced the levels of total proteins (TP) and total amino acids (TAA) in leaves. While foliar TP showed no significant changes (overall average: $0.15 \pm 0.08$ mg $g^{-1}$), it was grouped with the 5% and 10% treatments in the PCA. In contrast, the TAA content in leaves was significantly increased by the 1%, 2%, and 5% concentrations, representing increases of 47.4%, 33.1%, and 15.3%, respectively, over the control ($39.42 \pm 1.91$ mg $g^{-1}$). Although foliar TP was not significantly influenced by this *K. alvarezii* aqueous extract, the marked increase in TAA corroborates the findings of Goñi et al. (2021), who reported significant increases of approximately 23% in total soluble proteins and free amino acids in barley plants treated with *A. nodosum* extract. This modulation is significant, as amino acids are not only the building blocks of proteins but also act as crucial signaling molecules for nitrogen distribution, influencing plant architecture and stress responses (Guo et al. 2021).

In turn, kernels exhibited significant biochemical alterations following foliar application of *K. alvarezii* aqueous extract, with implications for grain quality and malting performance. The elevation in total starch content (F1-TS) at 5% concentration, representing a 19.3% increase over the control, underscores enhanced reserve accumulation, as starch constitutes the predominant fermentable substrate in malting processes (Schepper and Courtin 2024). This augmentation predicts improved extract yields during mashing, a paramount criterion for malt economic viability and quality (Evans et al. 2005; Nehra et al. 2024). Furthermore, the observed reduction in total soluble sugars (F1-TSS) at the same concentration (14.1% decrease) reflects a metabolic pivot favoring starch polymerization over transient sugar retention during grain maturation (Shewry et al. 2002). Such a profile confers kernel stability, facilitating controlled saccharide release in malting rather than premature availability (Briggs 1998).

The total protein (F1-TP) increased 10.3% in kernels at 10% treatment. While this rise is moderate, it could enhance attributes like foam retention and yeast nutrition but must be balanced to prevent problems in processing (Nehra et al. 2024). In turn, total amino acids (F1-TAA) had a substantial increment of 54.87% over the control at 1% and 2%. It represents the extract efficacy, as free amino nitrogen (FAN) is vital for yeast assimilability, ensuring fermentation integrity and mitigating off-flavor risks like hydrogen sulfide (Lekkas et al. 2005). It is important to highlight that these significant improvements in grain biochemistry occurred without any negative impact on the grain's viability. The germination index (G%) remained consistently high (98.5–100%) across all treatments, indicating that the extract did not induce seed dormancy or damage. This is a critical practical advantage, as high and uniform germination is a non-negotiable prerequisite for the malting process itself. Despite the scarcity of

literature on the effects of seaweed extracts on barley grain biochemistry, the available evidence points to significant yet species-specific improvements. For example, as summarized in Table 2, a study with *A. nodosum* extract on brewing barley under varying nitrogen regimes demonstrated a substantial 24.2% increase in grain protein (Cozzolino et al. 2021). While direct studies with *K. alvarezii* on barley grains are lacking, parallel research in wheat suggests this species can also modulate key grain components like starch and protein (Layek et al. 2015). These findings, together with our results on protein and amino acids, underscore that biochemical enhancements are possible, but their magnitude and nature are likely mediated by the distinct bioactive profiles of different algal extracts.

These quality enhancements carry significant potential value for the malting barley industry. The observed increases in thousand-kernel weight (+4.2%) and grain protein (+10.3%) directly contribute to improved malt extract potential and brewing quality, while the substantial rise in free amino acids (+56.6%) enhances free amino nitrogen (FAN) availability, a critical factor for efficient yeast fermentation and consistent beer production. Although a full cost–benefit analysis was beyond the scope of this study, these improvements suggest that strategic application of this extract could translate into higher-quality grain with premium market value. Future field-scale studies should include economic assessments to quantify the return on investment for growers and the malting industry.

## Conclusion

This study demonstrated that the primary agronomic value of the aqueous extract of *K. alvarezii* for barley lies in its specific capacity to enhance grain quality for the brewing industry, rather than in promoting vegetative growth, a pattern that contrasts with the more consistent growth stimulation reported in cereals like maize and wheat. It is important to note, however, that the absence of vegetative growth stimulation observed under these conditions (well-fertilized, pot-grown, and greenhouse-controlled) should not be interpreted as evidence of a general lack of vegetative responsiveness in barley to *K. alvarezii* extracts. This outcome is condition-specific and may differ under reduced fertility, field conditions, or abiotic stress. These findings suggest the extract's potential for complementary use, targeting quality traits specifically. The significant increases in kernel starch, protein, free amino acids, and thousand-kernel weight, achieved without compromising germination, represent a direct strategy to improve key malting parameters.

However, the context-dependent nature of the responses, particularly the selective effects on reproductive over vegetative traits, and the concentration-specific reduction in leaf pigments, underscores the importance of species-specific mechanistic investigation to fully interpret these patterns. Future research should focus on elucidating the molecular pathways triggered in barley, validating these quality enhancements under diverse field conditions, and exploring the synergistic use of this extract with reduced fertilizer regimes or in combination with other seaweed extracts to fully integrate it into sustainable malting barley production systems. Finally, these results highlight that different crops may respond distinctly to the same biostimulant input, reinforcing that the optimization of extract type, nutrient regime, and application strategy must be conducted on a crop-specific basis to fully unlock the potential of *K. alvarezii* as a sustainable agricultural input in diverse agroecosystems.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10811-026-03883-z.

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**Author's contributions** F.S.D., E.R.O., and M.M. contributed to the study conception and design. Material preparation and data collection were performed by F.S.D., E.R.O., G.Z.A., L.V.P.M., L.R.B.S., and I.F.A.. Analysis was performed by F.S.D., E.R.O., A.N., and A.R.S.. The first draft of the manuscript was written by F.S.D., and all authors commented on previous versions of the manuscript. Project administration and funding acquisition were performed by M.M. and S.M..

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**Data availability** The results of the analyses conducted for this manuscript are available and can be provided to researchers upon request. Interested parties are invited to contact the authors of the manuscript for access to the data.

## Declarations

**Competing interests** The authors declare no competing interests.

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