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Short communication: effects of foliar fertilisation with microalgal extracts, micronutrients, growth regulators and biofertilisers on wheat productivity

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ABSTRACT

Wheat (*Triticum aestivum*) is an important crop produced worldwide. Natural or synthetic products including phytostimulants, biofertilisers, biostimulants, bioregulators or phytoprotectors have been developed over the years. The objective of this study was to evaluate the effects of foliar application of micronutrients (essential minerals), growth regulators, extracts of the microalga *Porphyridium cruentum* and their mixtures on variables related to the growth and production of wheat (height of the plants at harvest, number of tillers per plant, weight of 1000 grains, pod length and yield). The experiment consisted of randomised blocks with four replications, consisting of 16 treatments (control, Zn, B, Cu, Mn, Zn+Cu+B+Mn, indolebutyric acid (AIB), 6-benzylaminopurine (BAP), Zn+Cu+B+Mn+AIB+BAP, *P. cruentum* freeze-dried polysaccharide (PS), *P. cruentum* freeze-dried material (FM), Zn+Cu+B+Mn+PS+FM, AIB+BAP+FM+gibberellic acid (GA), commercial-B, commercial-A and commercial-E). The single foliar application of micronutrients, commercial products containing micronutrients, growth regulators (indolbutyric acid, 6-bezylaminopurine, gibberellic acid and their mixtures), freeze-dried polysaccharide and biomass of *P. cruentum*, and mixtures of micronutrients and growth regulators did not potentiate the development and productivity of the wheat crop. The average height of wheat plants at harvest was 77.81 cm and ranged from 80.05 cm when the comercial-E product was applied to 74.43 cm with the application of *P. cruentum* polysaccharide extract at a dose of 62 g ha^{-1}, which resulted in lower crop yield, although the difference was not significant. Additionally, the individual application of the *P. cruentum* polysaccharide extract did not significantly influence on plant growth.

Keywords: Biostimulants, Foliar fertilisation, Growth regulators, *Porphyridium cruentum, Triticum aestivum*

Comunicação curta: efeitos da fertilização foliar com extratos de microalgas, micronutrientes, reguladores de crescimento e biofertilizantes na produtividade do trigo

RESUMO

O trigo (*Triticum aestivum*) é uma das culturas mais importantes produzidas no mundo. Ao longo dos anos, produtos (naturais ou sintéticos) chamados fitoestimulantes, biofertilizantes, bioestimulantes, biorreguladores ou fitoprotetores foram desenvolvidos. O objetivo deste trabalho foi avaliar os efeitos da aplicação foliar de micronutrientes (minerais essenciais), reguladores de crescimento, extratos da microalga *Porphyridium cruentum* e suas misturas sobre variáveis relacionadas ao crescimento e produção da cultura do trigo (altura das plantas na colheita, número de perfilhos por planta, peso de 1000 grãos, comprimento da vagem e produtividade). O experimento foi constituído de blocos casualizados com quatro repetições, constituído por 16 tratamentos (controle, Zn, B, Cu, Mn, Zn+Cu+B+Mn, ácido indolbutírico (AIB), 6-benzilaminopurina (BAP), Zn+Cu+B+Mn+AIB+BAP, Polissacarídeo liofilizado de *P. cruentum* (os), *P. cruentum* material liofilizado (FM), Zn+Cu+B+Mn+PS+FM, AIB+BAP+FM+ácido giberélico (GA), comercial-B, comercial-A e comercial-E). A aplicação foliar única de micronutrientes, produtos comerciais contendo micronutrientes, reguladores de crescimento ácido indolbutírico, 6-bezilaminopurina, ácido giberélico e suas misturas, polissacarídeo liofilizado e biomassa de *P. cruentum*, e misturas de micronutrientes e reguladores de crescimento não potencializaram o desenvolvimento e a produtividade da cultura do trigo. A altura média das plantas de trigo na colheita foi de 77,81 cm e variou de 80,05 cm quando o produto comercial-E foi aplicado, até 74,43 cm com a aplicação do extrato polissacarídico de *P. cruentum* na dose de 62 g ha^{-1}, cujo resultou em menor produtividade da cultura, embora a diferença não tenha sido significativa. Além disso, o extrato polissacarídeo isolado de *P. cruentum* não teve impacto significativo no crescimento da planta quando aplicado individualmente.

Palavras-chave: Bioestimulantes, Fertilização foliar, Reguladores de crescimento, *Porphyridium cruentum, Triticum aestivum.*

1. Introduction

Wheat (*Triticum aestivum* L.), together with corn, is one of the most important and productive crops in the world and is the main source of cereal-based products (Shaheen et al., 2024). Apart from using highly improved cultivars, high-tech machinery and macronutrients, wheat productivity can be increased by fertilisation with micronutrients (Kohli et al., 2023; Kabir et al., 2024; Shaheen et al., 2024; Wu et al., 2024). Thus, over the years, natural or synthetic products called phytostimulants, biofertilisers, biostimulants, bioregulators or phytoprotectors have been developed, which, in small amounts, can stimulate the growth and development of plants (Bano et al., 2022). These compounds can contain a mixture of amino acids, vitamins, micronutrients and plant growth regulators and can be applied to the soil, directly to the plants or via seed treatment (Arif et al., 2023). Additionally, indolebutyric acid (AIB), 6-benzylaminopurine (BAP), and gibberellic acid (GA) are phytohormones that act as plant growth regulators, influencing various physiological processes in plants. For example, AIB promotes root growth, BAP stimulates cell division and shoot development, and GA stimulates stem elongation, germination and sprouting, among other functions (Islam and Mohammad, 2022).

Recently, the use of microalgae extracts for this purpose has been considered (Colla and Rouphael, 2020; Refaay et al., 2021) because these microorganisms present a simple cell structure, rapid growth and the ability to synthesise many metabolites of interest (Kumar and Singh, 2020). Although the red microalga *Porphyridium cruentum* (Rhodophyta) is still poorly studied for this purpose, depending on the cultivation conditions, its biomass can contain high levels of carbohydrates, crude protein, lipids and minerals (Vasileva and Ivanova, 2019), indicating its great potential for application in increasing productivity. *P. cruentum* can also synthesise bioactive compounds, such as pigments (phycoerythrin) and polyunsaturated fatty acids, such as arachidonic and eicosapentaenoic acids as well as sulfated polysaccharides (Gallego et al., 2019; Medina-Cabrera et al., 2020). These polysaccharides are mainly composed of sugars such as xylose, galactose, glucose, glucuronic acid and 3-O-methyl-xylol (Chanda et al., 2019), which have biostimulant potential and can also improve plant defence mechanisms (Rachidi et al., 2020; Rachidi et al., 2021).

Wheat productivity is directly or indirectly affected by frequent extreme weather events, which reduce its supply as a raw material for food production, therefore, optimising its production is necessary (Li et al., 2021). Consequently, to further enhance our understanding of the benefits of microalgae extracts in agriculture, the objective of this work was to evaluate the effects of foliar application of micronutrients, growth regulators, extracts of the microalga *P. cruentum* and their mixtures on variables related to the growth and yield of wheat.

2. Material and Methods

The experiment was carried out at Fazenda Escola Capão da Onça of the State University of Ponta Grossa (UEPG), in an area with an average altitude of 1002 m above sea level. The climate of the region is classified as Cfb mesothermal humid subtropical, according to the Köppen classification (Peel et al., 2007). The soil in the area is classified as Haplic Dystrophic Cambisol of medium texture (Santos et al., 2018). The chemical characterisation of the soil in the experimental area at depths ranging from 0 to 20 cm is shown in Table 1.

On May 11, 2021, the experimental area was sprayed with the herbicide glyphosate at a dose of 4 L b.w. ha^{-1}, graminicide herbicide at a 0.7 L b.c. ha^{-1}, herbicide 400 EC at a 70 ml b.w. ha^{-1} and U46 at a 1.2 L b.w. ha^{-1} with the addition of the adjuvant at 0.5%. The experimental design adopted was randomised blocks, with 16 treatments repeated four times, giving a total of 64 experimental units. The treatments are described in Table 2.

The *P. cruentum* strain was obtained from the Laboratory of Algae Cultivation at the Federal University of Santa Catarina (UFSC), Brazil. It was grown in a modified f/2-enriched seawater medium and maintained until reaching the stationary phase (7–10 days). The microalga biomass came from a culture of *Porphyridium cruentum* that was separated and lyophilized. The polysaccharide (PS) crude extract of the microalgae *P. cruentum* was extracted following the method described by Farias et al. (2000) and Ozório et al. (2018). The crude polysaccharide was extracted using a crude papain solution (30 mg mL^{-1}) in 250 mL of 0.1 M sodium acetate buffer (pH 5.0) with 5 mM EDTA and 5 mM cysteine from 5 g of biomass.

Table 1. Chemical characteristics of the soil in the experimental area, classified as Haplic Dystrophic Cambisol (Santos et al., 2018).

Attributes	Units	Depth
		0-20 cm
pH in $CaCl_2$		4.90
H + Al	cmolc dm^{-3}	4.28
exchangeable Al	cmolc dm^{-3}	0.0
exchangeable Ca	cmolc dm^{-3}	1.9
exchangeable Mg	cmolc dm^{-3}	1.0
exchangeable K	cmolc dm^{-3}	0.28
P	mg dm^{-3}	27.0
Organic-C	g dm^{-3}	16.0
CTC in pH 7.0	cmolc dm^{-3}	7.46
Effective CTC	cmolc dm^{-3}	3.18
Base Sat. (V)	%	42.6
Al Sat (m)	%	0.0
Ca Sat.	%	25.5
Mg Sat.	%	13.4
K Sat.	%	3.8
Ca/Mg Relation		1.9
Ca + Mg/K Relation		10.4

H + Al = Buffer Solution SMP; Exchangeable Al, Ca e Mg = KCl 1 mol L^{-1}; P and K = $Mehlich^{-1}$ and organic-C = Walkley-Black. Source: Prepared by the ABC Foundation Laboratory.

Table 2. Micronutrients, growth regulators, phytostimulants and microalga (*Porphyridium cruentum*) extracts used via foliar fertilization in wheat.

Treatments	Source	Dose-composition
1- CONTROL	-	-
2- Zn	$ZnSO_4.7H_2O$	300 g of Zn ha^{-1}
3- Cu	ANHYDROUS CuSO4	150 g of Cu ha^{-1}
4- B	H_3BO_3	300 g of B ha^{-1}
5- Mn	$MnSO_4.H_2O$	300 g of Mn ha^{-1}
6-Zn+Cu+B+Mn	Respective sources	Respective doses
7-AIB	Indolebutyric acid	0.0375 g ha^{-1}
8- BAP	6-benzylaminopurine	0.0675 g ha^{-1}
9- Zn+Cu+B+Mn+AIB+BAP	Respective sources	Respective doses
10- *P. cruentum*	Freeze-dried polysaccharide (PS)	62 g ha^{-1} (0.499 g 80 m^2)
11- *P. cruentum*	Freeze-dried material (FM)	415 g ha^1 (3.32 g 80 m^2)
12- Zn+Cu+B+Mn+PS+FM	Respective sources	Respective doses
13-AIB+BAP+FM+GA	(GA=Gibberellic acid)	GA= 0.0375 g ha^{-1}
14- Commercial-B	Commercial	20% of Zn; 3% of Mo
15- Commercial-A	Commercial	12% of N; 3% of K_2O; 0,15% of Cu; 0,1% of Fe; 0,1% of Mn; 3,5% of Zn
16- Commercial-E	Commercial	5% of N, 0,02% of Co and 8,5% of Zn

The resulting product was filtered and centrifuged at 7965 × *g* for 20 min at 10 °C. The crude PS extract in the supernatant was concentrated by precipitation with 16 mL of 10% cetylpyridinium chloride (CPC), washed with 200 mL of 0.05% CPC, and dissolved in 174 mL of 2 M NaCl:ethanol (100:15 v/v). Following this, another precipitation was conducted using absolute ethanol for 24 h at 4 °C. The resulting product was rinsed twice with 200 mL of 80% ethanol, once with 200 mL of absolute ethanol, and subsequently dried in an oven at 60 °C for 24 h. The microalgae products were used at a dosage of 250 mg ha^{-1}.

Wheat seeds were sown on May 25, 2021, using 140 kg ha^{-1} of seeds and 270 kg ha^{-1} of the 10-20-20 formula equally distributed in the area. Six seeds per linear meter were used, composing a population of 345,000 plants ha^{-1}. The experimental plots consisted of 23 sowing lines spaced 0.17 m apart, with each line having a length of 4 m, totalling an area of 16 m² (4 × 4 m), with a useful area of 3.6 m² in each plot (3 × 1.2 m). The emergence occurred between June 3 and 7, and the demarcation of the area was carried out on September 3, 2021, as was the drawing of treatments within each block.

Foliar application occurred on July 1 and 23, 2021, or 37 and 59 days after sowing (DAS). Foliar application was performed with a pressurized backpack sprayer (XR 110.02) with a 4-nozzle boom spaced 0.5 m apart and constant pressure maintained by compressed CO_2 at 0.3 bar.

After considering the number of experimental units and replicates, the quantity of each product (microalgal extracts, micronutrients, growth regulators, and biofertilisers) was calculated for a spray volume of 150 L ha^{-1}. Additionally, 100 kg ha^{-1} urea (45% N) was used for each application.

The evaluations were as follows: height of plants at harvest (m), number of tillers per plant on five plants in the central row of each plot, weight of 1000 grains (TGM), pod length (cm) and yield. The 1000-grain mass was obtained by weighing ten subsamples of 100 grains per experimental unit, which were extrapolated to a mass of 1000 grains, following a specific methodology (Brasil, 2009). The useful area was harvested on September 29 with a Zurn 150 Hege System experimental plot harvester, completing a cycle of 124 days, with the grains being subsequently weighed. The determination of grain moisture was performed before weighing via a portable grain moisture metre (Agrologic AL 101), which corrected the productivity values for moisture to 13% and extrapolated them to kg ha^{-1}.

The data obtained for each variable were subjected to analysis of variance via the SISVAR statistical programme (Ferreira, 2019). In cases of significance, Tukey's test was used to separate the means.

3. Results and Discussion

The analysis of variance revealed significance only for the variables number of tillers per plant (NTP) and productivity (PROD). The coefficients of variation (CVs) obtained for the variables plant height (PH), cob length (CL) and 1000-grain mass (TGM) showed good experimental precision, which demonstrated high homogeneity of the data and low random variation. However, for the variables NTP and PROD, the experimental precision was intermediate (Table 3).

The average height of wheat plants at harvest was 77.81 cm and ranged from 80.05 cm when the commercial-E product was applied to 74.43 cm with the application of *P. cruentum* polysaccharide extract at a dose of 62 g ha^{-1}. This treatment resulted in lower crop productivity, although the difference was not significant (Table 4). Similarly, Galindo et al. (2019) reported no difference in plant height (PH) with the foliar application of an extract of Ascophyllum nodosum. Importantly, the foliar application of 415 g ha^{-1} *P. cruentum* in the freeze-dried form did not reduce the development or yield potential of wheat (Table 4).

The highest number of tillers per wheat plant (NTP) was observed with the application of freeze-dried *P. cruentum*, with values of 26.76 and 25.35% greater than those presented when B was applied at a dose of 300 g ha^{-1} and from the AIB+BAP+ML+GA mixture (indolbutyric acid+6-benzylaminopurine+freeze-dried *P. cruentum*+gibberellic acid). The range between the deficiency and toxicity of B in plants is very narrow (Kohli et al., 2023); thus, the selected dose may have been excessive.

According to Landi et al. (2013), excess B promotes the formation of H2O2 on a large scale, inducing the plant to increase the content of glutathione in the leaf, aiming to attenuate the peroxidation of membrane phospholipids and diverting carbon skeletons from primary metabolism. The mixture of growth regulators with freeze-dried *P. cruentum* also had a suppressive effect on the NTP compared with that observed with the application of this material without any mixture; however, there was no significant difference in relation to the control (Table 4). Indeed, similar results were reported by Pacheco et al. (2021), who reported negative effects on the growth and root weight of kale (*Brassica oleracea*) when the biostimulating capacity of macroalgae was tested.

The 1000-grain mass was not significantly influenced by the treatments; nonetheless, the application of *P. cruentum* in the freeze-dried form increased the mean value of the variable by 2.56% and 29.75% in comparison with those of the control and with the application of the growth regulator BAP (6-benzylaminopurine) at a dose of 0.0375 g ha^{-1} (Table 4). The mean value of the TGM variable was 29.67 g, which was much lower than that reported by Silva et al. (2019) for the wheat cultivar of different origin. The authors obtained an average PH lower than that achieved in this study (49.05 cm); however, the average productivity was greater (4677.94 kg ha^{-1}). Petterson et al. (2019) obtained mean values of 35.2 g and 3315.0 kg ha^{-1} for the wheat TGM and productivity indices, that is, higher and lower values than the average values obtained in this study (Table 4). TGM is a genetic trait that can be influenced in the field by climatic conditions during the maturation phase (Costa et al., 2013).

The foliar application of the micronutrients Zn, B, Cu and Mn did not maximize the metabolism of wheat plants at the doses and times at which they were used. Similarly, mixtures of micronutrients (Zn+Cu+B+Mn), micronutrients with growth regulators (Zn+Cu+B+Mn+AIB+BAP) and micronutrients with polysaccharide extracts of *P. cruentum* (Zn+Cu+B+Mn+PS+ML) did not benefit the wheat crop, which was supported by the variables AP, NPP, EC, MMG and PROD (Table 4).

These results corroborate the findings of Pacheco et al. (2021), who conducted a germination experiment to evaluate the biostimulating capacity of macroalgae polysaccharides and did not observe significant differences between treatments. Foliar applications of Mn+Zn increased pH and primary production components such as the number of grains per pod, number of pods per plant, and common bean yield.

Table 3. Summary of analysis of variance for the variables plant height at harvest (PH in cm), number of tillers per plant (NTP), cob length (CL in cm), thousand grain mass (TGM in g) and productivity (PROD kg ha^{-1}) as a function of foliar application of micronutrients, growth regulators, extracts of the microalgae *Porphyridium cruentum* and commercial biofertilizers.

Cause of Variation	DF	Mean Square (M.S.)				
		PH	NTP	CL	TGM	PROD
Treatments	15	7.4719ns	0.2733*	0.1990ns	16.2045ns	810082.96*
Blocks	03	61.2968	0.7300	0.6880	93.8378	450163.31
Residue	42	4.9248	0.1064	0.1244	8.3877	197722.67
CV (%)		2.85	10.97	5.12	9.76	11.33

ns= not significant; *significant at 5% probability.

Table 4. Plant height at harvest (PH), number of tillers per plant (NTP), cob length (CL), 1000 grain mass (TGM) and yield (PROD) as a function of foliar application of micronutrients, plant growth regulators growth, *Porphyridium cruentum* extracts and commercial biostimulant products.

Treatments	PH (cm)	NTP	CL (cm)	TGM (g)	PROD (kg ha^{-1})
CONTROL	78.45	3.30[ab]	7.18	31.53	4209.69[a]
Zn	76.95	2.85[ab]	6.89	32.05	4556.01[a]
Cu	78.95	2.75[ab]	7.20	28.78	3928.03[ab]
B	77.33	2.60[b]	6.74	28.97	3706.96[ab]
Mn	78.08	3.05[ab]	6.87	27.35	3491.62[ab]
Zn+Cu+B+Mn	76.13	3.15[ab]	6.70	29.46	4177.41[a]
AIB	77.75	2.75[ab]	6.96	30.82	4106.50[a]
BAP	78.15	3.00[ab]	6.56	24.94	3549.04[ab]
Zn+Cu+B+Mn+AIB+BAP	77.30	2.80[ab]	6.67	28.15	3461.81[ab]
P. cruentum (PS)	74.43	3.10[ab]	6.69	27.42	2920.17[b]
P. cruentum (FM)	76.75	3.55[a]	6.68	30.31	4164.10[a]
Zn+Cu+B+Mn+PS+FM	77.70	2.85[ab]	6.72	30.16	3574.91[ab]
AIB+BAP+ML+GA	78.85	2.65[b]	7.10	29.89	3858.79[ab]
Commercial-B	78.63	3.05[ab]	6.94	31.86	4384.60[a]
Commercial-A	79.45	2.85[ab]	7.06	32.36	4575.78[a]
Commercial-E	80.05	3.30[ab]	7.28	30.74	4130.26[a]
Mean	77.81	2.98	6.89	29.67	3924.73

[**]Means followed by the same lowercase letter in the column do not differ from each other by Tukey's test at 5%. Zn=zinc; B= boron; Cu=copper; Mn=manganese; AIB= indolebutyric acid; BAP=6-benzylaminopurine; POL= polypeptide; FM= freeze-dried material; GA=gibberellic acid; Commercial-B, Commercial-A, and Commercial-E = commercial biostimulant products.

The maximum technical efficiency was estimated at doses of 315 g ha^{-1} of Mn and 280 g ha^{-1} of Zn, resulting in a 60% higher yield than the control (Teixeira et al., 2004). For wheat, in the present study, foliar application at 18 days after emergence did not significantly increase any of the variables evaluated with the use of 300 g ha^{-1} of Mn or Zn. However, with the isolated and single application of Zn, 346.32 kg ha^{-1} more wheat was produced than the control, equivalent to 5.7 bags of 60 kg (Table 4).

According to Yu et al. (2020), several physicochemical parameters might affect both the biochemical composition and structure of microalgae and polysaccharides (represented here as FM and PS). For example, pH is a determining factor, and it can be affected by the polysaccharide concentration and temperature. Conductivity, a parameter that is directly related to salinity, can negatively affect plants if it is not within the ideal range (Kaya et al., 2006; Uçarli, 2021). Therefore, both of these parameters may have influenced the results of the present study.

Additionally, the commercial products, which are considered sources of micronutrients, did not significantly affect any variable, although the PROD was 366.09 kg ha^{-1} higher than that in the control using the second method (Table 4). A previous study on Commercial-A and Commercial-E products applied via seed treatment revealed a significant increase in the germination and vigour of 'Leader' snap bean seeds (Ohse et al., 2014a). Another study using Zn, B, Cu and their combinations, in addition to the commercial products Commercial-B and Commercial-A, did not reveal a positive effect on flaxseed germination, and a reduction in vigour was observed (Ohse et al., 2014b).

Fioreze and Rodrigues (2012) reported an increase in the percentage of viable tillers in the cultivars IAC 370 and IAC 375, with the former exhibiting the highest NTP and the later showing the highest viable ones after applying a plant regulator composed of 0.005% indolbutyric acid (IBA), 0.005% gibberellic acid and 0.009% kinetin. In the present study, foliar application of the growth regulators indolbutyric acid (0.0375 g ha^{-1}) and 6-benzylaminopurine (0.0675 g ha^{-1}) separately did not influence NTP or the other variables analysed. However, the application of a mixture of gibberellic acid (0.0375 g ha^{-1}) and freeze-dried *P. cruentum* (AIB+BAP+FM+GA) reduced the NPP by 19.7% in relation to that of the control (Table 4).

The findings of this study demonstrate that different responses to foliar application of growth regulators and biostimulants might occur depending on time of application, number of applications, dose and genetic variability, highlighting that the responses depend on factors such as nutritional and environmental conditions, and production system management. We suggest that, in the future, an adjuvant should be added to the mixture that will be applied, as it might reduce the surface tension of the droplets in the plants, leading to maximisation of nutrient and metabolite absorption.

4. Conclusions

The results obtained in this study revealed that, under the specific conditions used, the single foliar application of micronutrients and their mixtures, commercial products containing micronutrients, growth regulators and mixtures, and extracts of the microalga *P. cruentum* (freeze-dried and mixed with micronutrients and growth regulators) did not improve the development and yield of wheat (*Triticum aestivum*). Further investigation is suggested to examine the potential of aqueous extracts from different microalgae species in wheat farming.

Authors' Contribution

Validation, Investigation, Conceptualization, Methodology, Experimental run, Writing - original draft: Silvana Ohse, Vinícius Cordeiro de Moraes, and Lilyan Barbosa Ruivo. Validation, Investigation, Conceptualization, Methodology, Writing - original draft, Writing - review and editing: Camila Nader, Rafael Garcia Lopes, and Marco Shizuo Owatari. Funding acquisition, Project administration and Supervision: Roberto Bianchini Derner and Silvana Ohse.

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