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# Uso de carragena para a redução de desoxinivalenol em grãos de trigo e cevada

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## INFO

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## ABSTRACT

*Use of carrageenan for the reduction of deoxynivalenol contamination in wheat and barley kernels.* Wheat and barley are among the more important staple foods for human and animal nutrition in the World. Nevertheless, these crops constantly suffer from the Fusarium Heat Blight disease, responsible for decrease in yields and the bioaccumulation of the trichothecene deoxynivalenol in kernels. Searching for alternatives to overcome these problems, a set of 14 trials involving wheat and barley cultivars over two consecutive harvest seasons (2018-2019) were carried out in the States of Santa Catarina and Rio Grande do Sul, Brazil. The tests consisted in the use of a carrageenan-rich product (Algomel Push®) produced from the red-algae *Solieria chordalis* in the rates of 1.0 and 2.0 L $ha^{-1}$ sprayed at the beginning of the tillering stage. Overall, the use of carrageenan decreased DON contamination up to 34.64% and 35.74% when 1.0 and 2.0 L $ha^{-1}$ of carrageenan was applied in wheat and barley, respectively. An increase in yield and kernels with better bromatological aspects were also seen after the use of carrageenan. Our findings suggest that the bioactive compounds found in the product, mostly carrageenan, have the ability of eliciting the plant's mechanisms of defence and growth, and can be considered a potential option/tool for farmers and industries to cope with the problem of food quality in their mycotoxicological and bromatological aspects.

## RESUMO

**Palavras-chaves**
DON
eliciação
giberela
Fusarium graminearum
Solieria chordalis

O trigo e a cevada estão entre os alimentos básicos mais importantes para a nutrição humana e animal do mundo. No entanto, essas culturas sofrem constantemente com a doença denominada giberela, responsável pela diminuição da produção e pela acumulação do *tricoteceno desoxinivalenol* nos grãos. Buscando alternativas para superar esses problemas, um total de 14 ensaios envolvendo cultivares de trigo e cevada em duas safras consecutivas (2018 – 2019) foram realizados nos estados de Santa Catarina e Rio Grande do Sul, Brasil. Os testes consistiram no uso de um produto rico em carragena (Algomel Push®) produzido a partir da alga vermelha *Solieria chordalis* nas doses de 1,0 e 2,0 L $ha^{-1}$ aplicados no início do perfilhamento das plantas. No geral, o uso de carragena diminuiu a contaminação por DON em 34,64% e 35,74% quando 1,0 e 2,0 L $ha^{-1}$ foram aplicados em trigo e cevada, respectivamente. Observou-se um aumento de produtividade e uma melhora nos aspectos bromatológicos dos grãos colhidos após o uso da carragena. Esses resultados sugerem que os compostos bioativos encontrados no produto, principalmente a carragena, têm a capacidade de eliciar mecanismos de defesa e crescimento da planta e pode ser considerado uma ferramenta potencial para agricultores e indústrias lidar com o problema da qualidade dos alimentos em seus aspectos micotoxicologicos e bromatológicos.

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## INTRODUCTION

Wheat (Triticum aestivum L.) has been considered the World's major staple food for human nutrition for over eight thousand years (Dixon et al., 2009). However, wheat and other winter crops constantly suffer from the incidence of mycotoxins such as zearalenone (ZEA), fumonisin, aflatoxin and, in special, deoxynivalenol (DON) (Wegulo, 2012). These toxins, invisible to the naked eyes, are secondary toxic bio-compounds naturally synthetized by different species of filamentous phytopathogenic fungi when under stress (Berthiller et al., 2013; Wu et al., 2017; Olmix Group, 2018).

In wheat and other winter cereals, DON has been considered the most problematic mycotoxin (Biomin, 2019) as it is highly produced and capable of causing many health problems to humans and animals, such as abdominal pain, headaches, diarrhoea, nausea, vomiting, throat irritation, dizziness, loss of appetite and anorexia, reduction of thyroid size, among others (Wegulo, 2012; Marin et al., 2013).

Barley (Hordeum vulgare), on the other hand, is largely used by malting industries for beer production and also suffers from the incidence of DON, which besides causing yields to drop also harms the industry due to the "gushing" problem, an excessive foaming of the beer as bottles/cans are opened (Piacentini et al., 2015).

DON, ZEA and fumonisins are mycotoxins produced by the Genus of fungus *Fusarium* (Olmix Group, 2018), and their presence in wheat and barley is associated mainly with the *Fusarium* Head Blight (FHB) disease (Wegulo, 2012; Piacentini et al., 2015; Wu et al., 2017). The biosynthesis of these different mycotoxins is the consequence of different condition of environmental stresses (variation in temperatures, humidity, oxygenation, pH, bacterial/viral/fungal attack, the use of inefficient pesticides, such as fungicides, etc.), therefore, the same species of a fungus can produce different mycotoxins as the same mycotoxin can be produced by different species of fungi (Olmix Group, 2018). DON for instance, also known as the vomitoxin, is a trichothecene mainly produced by *Fusarium graminearum* when pH is acidic and temperatures are, on average, around 23.7 °C (Wu et al., 2017).

It is estimated that around 25% of all the harvested crops around the World are contaminated by mycotoxins and, besides being a threat to human and animal health and life are the cause to losses of billions of dollars around the World every year (Marin et al., 2013). According to the "World Mycotoxin Survey 2019 report" launched by Biomin® (2019), 6.1 out of 10 samples tested around the globe had amounts of mycotoxins above the threshold levels.

Alternatives to diminish the incidence of *Fusarium* in plants and the problem of mycotoxin contamination in agricultural products have been intensively sought (Wegulo, 2012). The use of seaweed extracts capable of increasing tolerance of plants to abiotic stresses such as freezing temperatures, sensitivity to salinity, water stress (drought or flooding), high temperature stress, and heavy metal concentration (Sangha et al., 2010, Chi et al., 2019) are potential candidates to diminish mycotoxin presence in cereals. Also, these extracts increase the tolerance to biotic stresses in plants (Mercier et al., 2001; Sangha et al., 2011; Gonzáles et al., 2013; Fesel & Zuccaro, 2016).

Carrageenans, exclusively found in red seaweed, are hydrophilic sulphated galactoses that can be divided into six types, iota(ι)-, kappa(κ)-, lambda(λ)-, mu(μ)-, nu(υ)-, and theta(Θ) (Shukla et al., 2016). The species of red seaweed, *Solieria chordalis*, contains much of its carrageenan content composed of ι-carrageenan, with a degree of sulphation of 33%, making it very soluble and capable of strongly elicit plants (Shukla et al., 2016).

In light of the above information and, considering the high infestation of mycotoxins, especially DON, in Brazilian wheat and barley fields, this study aimed at using a high concentrated carrageenan product to reduce the contamination of DON in wheat and barley while helping at increasing yields and the bromatological quality of the kernels.

## MATERIAL AND METHODS

The experiment was conducted over two harvest seasons, 2018 and 2019, with different cultivars and in different locations in the states of Santa Catarina (SC) and Rio Grande do Sul (RS), Brazil (Table 1).

All tests, either in conventional or organic cultivation systems, were carried out with the use of different amounts of seed density and cultivars, different rates and kinds of fertilizers (mineral or organic), and different chemical treatments (in the case of conventional systems). The main goal in this case was to check whether the highly concentrated carrageenan product (Algomel Push®) would be efficient for the reduction of mycotoxin biosynthesis by *Fusarium graminearum* in wheat and barley cultivars. To check this, the only variable that varied within each of the studied areas was the use or not of carrageenan in the fields,


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which explains why the variables: cultivar, fertilizer rates and kinds, chemical control products and rates, soil and climatic conditions, etc. were not considered in this report.

Table 1 - Details of different tests carried out in the states of SC and RS (Brazil) aiming at reducing the amount of DON in Wheat and Barley kernels.

| ID | Harvest Season | City (State) | Crop | Cultivar | Last crop[1] | Cultivation system[2,3(*)] | Treatments | Variables analyzed[4] |
|---|---|---|---|---|---|---|---|---|
| 01 | 2018 | Chapecó (SC) | Wheat | TBio Sinuelo | Soy-beans | Organic | • Control<br>• 1.0 L ha$^{-1}$ *S. chordalis*<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON contamination<br>• Yield** |
| 02 | 2019 | Cunha Porã (SC) | Wheat | TBio Audaz | Soy-beans | Conventional | • Control<br>• 1.0 L ha$^{-1}$ *S. chordalis*<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON contamination<br>• Yield***<br>• Bromatological analysis |
| 03 | 2019 | Campo Novo (RS) | Wheat | TBio Audaz | Soy-beans | Conventional | • Control<br>• 1.0 L ha$^{-1}$ *S. chordalis*<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON contamination<br>• Yield***<br>• Bromatological analysis |
| 04 | 2019 | Chapecó (SC) | Wheat | BRS374 | Soy-beans | Conventional | • Control<br>• 1.0 L ha$^{-1}$ *S. chordalis*<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON contamination<br>• Yield**<br>• Bromatological analysis |
| 05 | 2019 | Panambi (RS) | Wheat | TBio Ponteiro | Soy-beans | Conventional | • Control<br>• 1.0 L ha$^{-1}$ *S. chordalis*<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON contamination<br>• Yield*** |
| 06 | 2019 | Estação (RS) | Wheat | FPS Amplitude | Soy-beans | Conventional* | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON |
| 07 | 2019 | Panambi (RS) | Wheat | TBio Toruk | Soy-beans | Conventional* | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON |
| 08 | 2019 | Tenente Portela (RS) | Wheat | TBio Sossego | Soy-beans | Conventional* | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON |
| 09 | 2019 | Rondinha (RS) | Wheat | FPS Certero | Soy-beans | Conventional* | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON |
| 10 | 2019 | Condor (RS) | Wheat | TBio Audaz | Soy-beans | Conventional* | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON |
| 11 | 2019 | Erebango (RS) | Barley | BRS Cauê | Soy-beans | Conventional | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON<br>• Bromatological analysis |
| 12 | 2019 | Tapera (RS) | Barley | BRS Cauê | Soy-beans | Conventional | • Control<br>• 1.0 L ha$^{-1}$ *S. chordalis*<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON<br>• Bromatological analysis |
| 13 | 2019 | Ibirubá (RS) | Barley | BRS Cauê | Soy-beans | Conventional* | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON<br>• Bromatological analysis |
| 14 | 2019 | Curitibanos (SC) | Barley | BRS Cauê | Soy-beans | Conventional* | • Control<br>• 2.0 L ha$^{-1}$ *S. chordalis* | • DON |

[1]Previous cultivated crop before the sowing of wheat or barley.
[2]Organic systems (control and treated areas) made use of no chemical treatments (herbicides, fungicides, insecticides). Conventional systems (control and treated areas) made use of chemical treatments (herbicides, fungicides, insecticides) and in some cases of foliar fertilizers.
[3]When commercial tests were carried out, a unique dosage of 2 L ha$^{-1}$ was used (yield evaluations were not carried out for commercial field tests). Commercial tests are represented by the (*) symbol.
[4]Variables were analysed considering the importance of the test. Deoxynivalenol (DON) and bromatological analyses were performed by Near-Infrared Spectrometry (NIRS), except for test number 01, which had DON analysed by Enzyme Linked Immuno-Sorbent Assay (ELISA). Yield evaluation was carried out in two different ways, with replicates (**) and without replicates (***) (no statistical evaluation).

Experiments were carried out in two different situations, totally randomized scientific tests with replicates, and commercial field tests, in which a pre-defined area of 2.5 hectares was treated with carrageenan in association with other products (either herbicides or fungicides). For commercial


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tests, the yield was not assessed. For the scientific tests, data for DON (test ID 01, 02, 03, 04, 05, 11, 12) and bromatological analyses (test ID 02, 03, 04, 11, 12, 13) were submitted to analysis of variance (ANOVA) and when the means were significantly different, they were compared using Tukey's HSD statistical test. Data for DON, yield and bromatological analyses for tests without replicates were also presented in this report. All data submitted to ANOVA and Tukey's HSD test were analysed using the statistical program RStudio© version 1.0.136.

The application of the carrageenan rich product (Algomel Push®), in all cases, was carried out through the use of sprayers in the beginning of the tillering stage for wheat and barley cultivars (water volume varied among the different tested areas). All tested areas received a single application of the product, either in the rate of 1.0 or 2.0 L $ha^{-1}$. Algomel Push® is composed 43% from a red-algae named *Solieria chordalis* and besides having carrageenan (mostly ι-carrageenan) also brings other essential components for plant development and strength such as amino acids, macro- and micro-nutrients, vitamins, carbohydrates, fatty-acids, phytohormones, among others.

## RESULTS AND DISCUSSION

After the application of a unique dose of Algomel Push® on wheat and barley plants, at the beginning of the tillering growth stage, we could see that the content of DON in harvested kernels decreased on average by 34.64% and 26.74% when 1.0 and 2.0 L $ha^{-1}$ were applied on wheat plants, respectively. For barley, the average reduction of DON content was of 11.83% and 35.74% when 1.0 and 2.0 L $ha^{-1}$ were applied, respectively (Table 2). By checking Table 2 it is possible to see that the highest decrease in DON content was encountered in the year of 2018 for wheat, probably because it was a very humid year with many adverse conditions for plant growth and production.

Table 2 - Deoxynivalenol quantification for the tested areas.

| Harvest year | Crop | Test ID | Deoxynivalenol quantification (ppb) ± Standard Error | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 L/ha | Difference to Control (%) | 2 L/ha | Difference to Control (%) | *p*- stat. |
| 2018 | Wheat | 01 | 2665±257.5 $^{b}$ | 845±42.5 $^{a}$ | -68.2 | 565±107.5 $^{a}$ | -78.7 | 0.0004 |
| 2019 | | 02 | 1241±29.5 $^{b}$ | 929±13.5 $^{a}$ | -25.1 | 1012±2.5 $^{a}$ | -18.4 | 0.0001 |
| | | 03 | 821±21.3 $^{c}$ | 350±0.25 $^{*a}$ | -57.3 | 746±9.1 $^{b}$ | -9.13 | $2.78e^{-9}$ |
| | | 04 | 409±59.0 $^{a}$ | 350±0.25 $^{*a}$ | -14.3 | 396±34.7 $^{a}$ | -3.05 | 0.56 |
| | | 05 | 1269±60.8 $^{a}$ | 1167±34.4 $^{a}$ | -8.03 | 1359±86.4 $^{a}$ | +7.01 | 0.22 |
| | | 06 | 1329 | - | - | 570 | -57.1 | |
| | | 07 | 5783 | - | - | 2521 | -56.4 | |
| | | 08 | 1281 | - | - | 1020 | -20.3 | |
| | | 09 | 1194 | - | - | 933 | -21.8 | |
| | | 10 | 1371 | - | - | 1244 | -9.26 | |
| | Barley | 11 | 739±4.5 $^{b}$ | 749±3.7 $^{b}$ | +1.35 | 711±6.9 $^{a}$ | -3.78 | 0.01 |
| | | 12 | 3823±139.0 $^{c}$ | 2866±221.5 $^{b}$ | -25.0 | 1912±64.0 $^{a}$ | -49.9 | $4.18e^{-5}$ |
| | | 13 | 1820 | - | - | 431 | -76.3 | |
| | | 14 | 2031 | - | - | 1769 | -12.9 | |

*Limit of quantification for the test via NIRS.
Means with different letters on the same line are statistically different according to the Tukey's HSD statistical method at a confidence level of 5%.

The use of different biomolecules isolated and concentrated from different species of macroalgae, either brown, green or red, with the aim at increasing plant resistance to biotic and abiotic stresses, eliciting plants' mechanisms of defence such as the production of secondary metabolites and consequently increasing yields is rapidly increasing in agriculture (Stadnik & Freitas 2014).

Sangha et al. (2014), testing the effects of ι- and κ-carrageenan on *Arabidopsis thaliana* being attacked by *Trichoplusia ni*, have shown that leaf damage, larval weight and larval attraction to *A. thaliana* was reduced when plants received these types of carrageenans. Nevertheless, not only mechanisms of defence are triggered in plants, the application of carrageenan has also the ability to better the physiological aspects of the plants, thus increasing its development and yields.

Gonzáles et al. (2013) have shown that different types of carrageenans can favour the development of roots and shoots, increase plant biomass and height, net photosynthesis, rubisco activity, $CO_2$ fixation, biosynthesis of bio-regulators, essential oil biosynthesis, polyphenolic compounds and nitrogen assimilation (also demonstrated in Figure 1), This helps explain the yield increase observed in Figure 2. On average, yield increase for wheat was of 15.06% and 14.38% when 1.0 and 2.0 L $ha^{-1}$ of carrageenan were applied on the fields (Figure 2).

The presence of amino acids, fatty acids, vitamins, macro- and micro-nutrients, carbohydrate, phytohormones, betaines, osmo-protectants,


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among others, present in products produced from any kind of plant extract have the potential of eliciting plants to synthetize phytoalexins and other metabolites of defence (Dixon & Harrison, 1994; Sangha et al., 2014). However, the biomolecules found exclusively on algal extracts are also able to elicit plants by either mimicking the attack of pathogens and/or activating different signalling pathways (Figure 1) (Fesel & Zuccaro, 2016; Shukla et al., 2016).

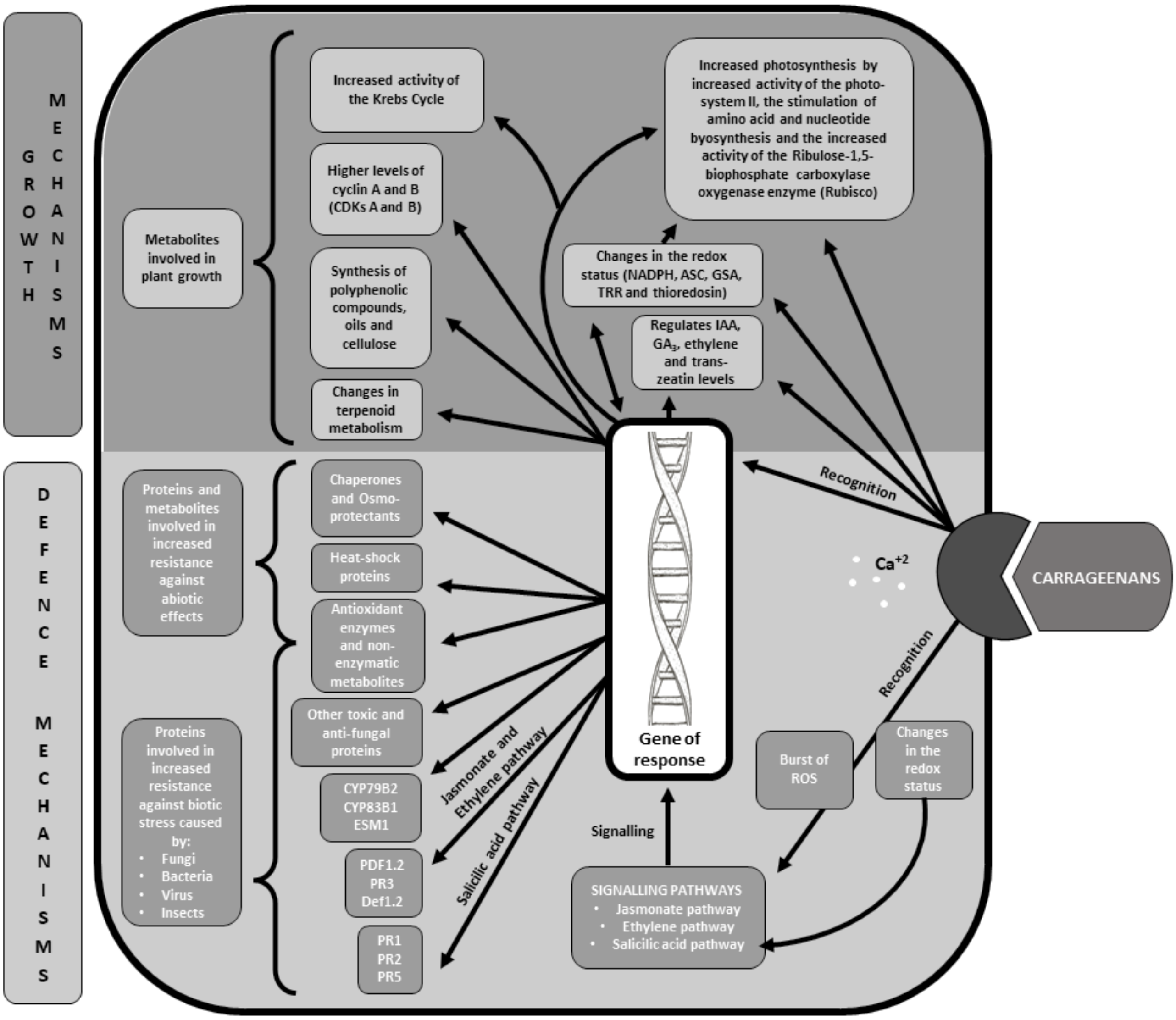


Figure 1 - Cellular responses and the production of defence/growth metabolites after elicitation by the use of Carrageenans. Calcium ions work as biochemical signalers within the cell. Complied and adapted from Shukla et al. (2016) and Chi et al. (2019).

Fungi and bacteria are beta-glucan and chitin/chitosan rich organisms (Dixon & Harrison, 1994; Fesel & Zuccaro, 2016). When invading plants, these organisms are recognized by PR-proteins (glucanases and chitinases) (Dixon & Harrison, 1994; Mercier et al., 2001), which trigger many signals within plants for the production of secondary metabolites of defence/growth, such as antioxidant enzymes, anti-fungal proteins, reactive oxygen species (ROS), bio-regulators, fitoalexins, etc. (Figure 1) (Mercier et al., 2001; Sangha et al., 2014; Fesel & Zuccaro, 2016; Chi et al., 2019). Carrageenans are able to activate these mechanisms without the plant being attacked by a fungus/bacteria.

Mercier et al. (2001), comparing the effect of laminarins (β-Glucan-rich polysaccharides) vs. carrageenans used as elicitors of plant defence on tobacco trees, have shown that carrageenans were proven to be more efficient than laminarins, as they


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were able to elicit defence-related genes without compromising the primary metabolism of the plants.

According to Mercier et al. (2001), carrageenans have a strong and delayed potential for eliciting plants to produce secondary metabolites of defence. Salicylic acid (SA), for instance, was highly produced when tobacco plants were treated with carrageenan. In their study, assessments were carried out at 0, 8, 18, 24, 96 and 168 (highest SA content found) hours after the application of the carrageenan.

Although not tested, we believe that carrageenan eliciting effects on plants last much longer than that, contributing for the high decrease in DON biosynthesis by *Fusarium graminearum* in wheat and barley cultivars.

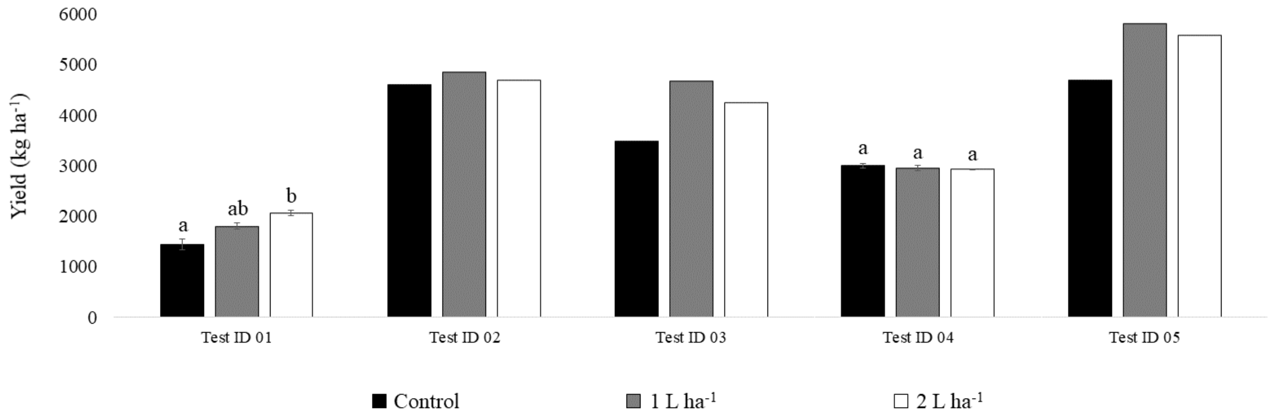


Figure 2 - Yield of wheat for the different tested areas. Columns containing different letters, for each tested area, are statistically different according to the Tukey's HSD statistical method at a confidence level of 5%. Columns that contain no letters have no replicates, therefore no statistical analyses have been performed.

Our hypothesis for DON reduction, yet to be investigated, is that carrageenan increases the content of, among others, mainly SA in plants, and that by feeding on these compounds, fungi and bacteria have their metabolism decelerated and therefore are less prone to be stressed by environmental conditions, thus reducing the amount of mycotoxin biosynthesis and the consumption of organic carbon from the plant.

Table 3 - Mean increase (%) of bromatological characteristics of wheat and barley kernels harvested in SC and RS, Brazil, during two harvested seasons (2018 and 2019) compared to the control.

| Crop | Dose ($L\ ha^{-1}$) | Bromatological characteristics | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Crude Protein (%) | Ether extract (%) | Dry Matter (%) | Crude Fiber (%) | Ashes (%) | NDF (%)[1] | ADF (%)[2] |
| Wheat | 1.0 | +4.21 | +0.09 | +0.04 | +6.70 | +0.63 | +4.30 | +10.85 |
| | 2.0 | +0.74 | -2.69 | +0.51 | +1.59 | +2.67 | +1.01 | +5.17 |
| Barley | 1.0 | -3.40 | +0.35 | -0.16 | +5.00 | -0.85 | - | - |
| | 2.0 | +9.46 | +0.92 | +0.93 | +13.93 | +6.45 | - | - |

[1,2]These parameters have not been measured for barley cultivars.

The incidence of the FHB disease in wheat and barley was not assessed in this study, however, considering the study review performed by Wegulo (2012), which presents a positive linear correlation ($r^2$=0.53) between the incidence and intensity of FHB vs. DON concentration in kernels of wheat, we believe that carrageenans have a positive indirect impact on the presence of *Fusarium* Head Blight disease on plants, which in the case of our experiments might have been reduced, on average, up to 18.35% and 14.17% when 1.0 and 2.0 L $ha^{-1}$ of carrageenans were applied, respectively.

For barley, Buerstmayr et al. (2004), have reported a positive linear correlation between FHB incidence and intensity vs. DON accumulation of 0.87. Therefore, we believe FHB might have been reduced, on average, up to 10.30% and 31.10% when 1.0 and 2.0 L $ha^{-1}$ of carrageenans were applied in the tested areas.

Many are the factors that influence the quality of harvested products, such as the fertilization level of the field, chemical treatments, weather conditions, cultivars, insects, diseases, etc. In our case, and as all variables, but the application of carrageenan,


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didn't vary within each tested area we have linked this increase in the bromatological quality (Figure 3 and Table 3) of the kernels due to the application of Algomel Push®.

Besides supplying extra nutritional elements (minerals, vitamins, macro- and micro-nutrients, etc.) necessary to the development of the plants, this product also brings bio-active molecules capable of eliciting the plant's ability to increase its natural defences against biotic and abiotic stresses.

As shown in table 3, there was an increment in all the bromatological aspects for wheat kernels when 1.0 L ha$^{-1}$ of carrageenan was used to elicit the mechanisms of defence/growth of this crop. In the same way, this was the dosage that allowed the crop to reduce DON contamination and increase yields at most (Table 2 and Figure 2). On the other hand, for barley the best dosage encountered was of 2.0 L ha$^{-1}$, allowing for better bromatological aspects and DON reduction (Table 2 and Table 3).

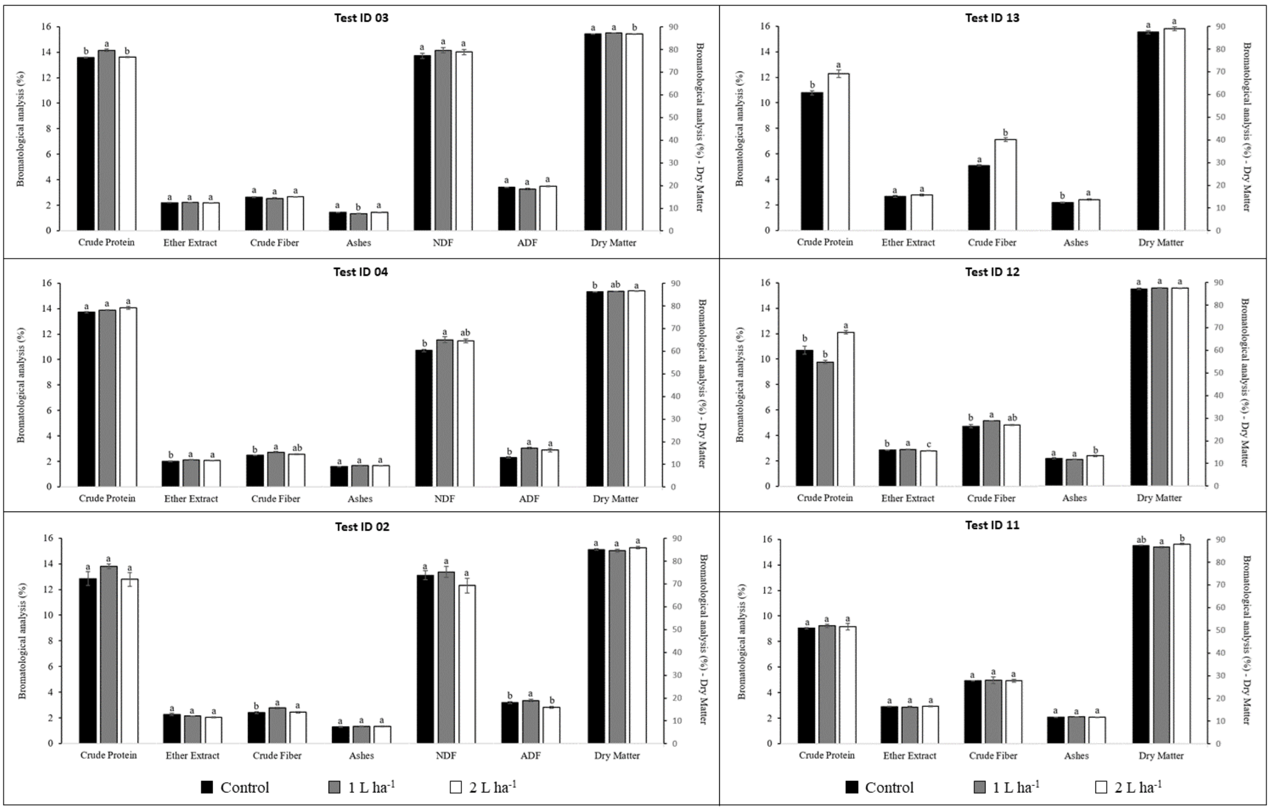


Figure 3 - Bromatological analyses (%) for different tested areas of wheat (test ID 02, 03 and 04) and barley (test ID 11, 12 and 13). Columns containing different letters, for each tested area, are statistically different according to the Tukey's HSD statistical method at a confidence level of 5%.

Large amounts of wheat and barley are sent to the production of animal feed every year in Brazil, mostly because they fall behind in quality associated to mycotoxin contamination (specially DON). By increasing not just the mycotoxicological quality of harvested crops (fewer mycotoxin), but also their bromatological aspects, we may favour the market in all aspects, helping the industry and farmers to thrive by supplying the industry with high-quality kernels while feeding animals with high-quality feeds.

## CONCLUSIONS

Carrageenans have the potential to increase the defence and growth mechanisms of wheat and barley plants, increasing their yields, helping in the reduction of deoxynivalenol contamination / accumulation and increasing the quality of the kernels (greater bromatological characteristics).

The use of carrageenan rich products during the development of winter cereals, such as wheat and barley, can contribute to the economic gains of farmers and industries. Farmers are able to have greater yields and supply the industry with better quality kernels, which in turns, makes the production of food, feed and beverages cheaper and easier (no need of extra additives such as mycotoxin binders).

Carrageenans may have a long-eliciting effect in plants, which may explain the results obtained in this study. To check weather this holds true or not,


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new studies aiming at checking the expression and activity of defence metabolites as a function of long periods of time, after the use of carrageenan, may be put into action (no data available).

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