Communications in PLANT SCIENCES

http://cpsjournal.org
e-ISSN 2237-4027
Volume 11 (2021)

SHORT RESEARCH ARTICLE

An algal sulphated polysaccharide capable of reducing mycotoxin biosynthesis by *Fusarium*

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OPEN ACCESS

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Submitted on May 06, 2021
Accepted on August 27, 2021
Early View on August 30, 2021
Final Publication on August 30, 2021

Author declares no conflict of interest

KEYWORDS:
Deoxynivalenol
Fumonisine
Fusarium graminearum
Fusarium verticillioides
Fusarium Ear Rot
Gibberella Ear Rot

ABSTRACT

The incidence of *Fusarium* sp. in maize, besides causing drops in yields also causes the bromatological quality of the final product to decrease and mycotoxin accumulation to increase. Nineteen field trials have been performed in 4 different states of Brazil during three consecutive harvest seasons using 17 different maize cultivars aiming at assessing the use of carrageenan as a biological compound to decrease mycotoxin biosynthesis and accumulation and, increase maize silage yields and quality. Overall, carrageenan has proven to raise the bromatological quality of the silage, increase yields and decrease fumonisine and deoxynivalenol contamination by 50.4% and 45.7%, respectively.

Highlighted Conclusion

Carrageenan has the capability to help on the suppression of DON and FUMO biosynthesis/accumulation and to help on the increase of the bromatological quality of maize silage.

INTRODUCTION AND METODOLOGY

Maize is the most produced cereal around the World (FAO 2021), and the second most important annual crop in Brazil (IBGE 2021). As Brazil is among the World's biggest producers of milk and beef, maize silage is highly used in the country as it represents around 50% of the dry matter intake daily consumed by cows. It is estimated that between 9.7 to 22.6% of the area occupied by maize in Brazil is used for silage production (IBGE 2021).

Although Brazil produces large amounts of maize kernels and silage, more than 70% of this production comes from the second summer harvest of each year (IBGE 2021), which presents abiotic conditions that favor higher infestations of maize ears by *Fusarium*.

Fusarium species, mainly *F. graminearum* and *F. verticillioides*, are responsible for causing the *Fusarium* Ear Rot in maize plants (Presello et al. 2007; Ponts 2015; Nerbass et al. 2016; Miedaner et al. 2017; Galic et al. 2019). Besides causing yields to drop considerably (Presello et al. 2007; Galic et al. 2019), the presence of *Fusarium* in the plant aligned with stressful conditions during the development of the fungi (variations in temperature, humidity, pH, competition between fungi, application of inefficient fungicides, etc.) create perfect conditions for the biosynthesis of mycotoxins (Ponts 2015; Miedaner et al. 2017; Ducatti et al. 2021).

Fumonisine (FUMO), Zearalenone (ZEA) and Trichothecenes (especially Deoxynivalenol – DON) are the main mycotoxins produced by *Fusarium* species. These mycotoxins are secondary metabolites, invisible to the naked eye, produced as a defense mechanism to cope with stressful situations (Wegulo 2012; Ponts 2015). These toxins are capable of causing weight loss, decrease milk quality and yield, affect the immunologic system of animals, cause vomiting disorders, cancer, reduction in the assimilation of nutrients present in the feed, reproductive problems, among others (Marin et al. 2013).

FUMO, ZEA and DON are mainly produced under field conditions and after plants/kernels have been contaminated, few are the treatments that can be put into practice to decrease their contamination in food and feed. One of the most used alternatives to cope with mycotoxin contamination in animal feed is the use of mycotoxin binders (Kolossova et al. 2009), which in most of the cases present a low spectrum of control and make feed prices to increase.

A possible solution to this problem might be the use of biological/synthetic compounds capable of eliciting the plants' mechanisms of defense, hence making them less susceptible to fungi attack/development and mycotoxin biosynthesis (Wiesel et al. 2014; Ducatti et al. 2021). The search for a compound to be used in the fields to help in the suppression of mycotoxin biosynthesis is a must for agriculture.

Based on the exposed above, this work aimed at testing the efficacy of an exclusive sulphated algal polysaccharide, carrageenan, for the reduction of mycotoxin biosynthesis in maize silage, while in exchange, helping for the improvement of silage quality and yield.

A total of 19 trials with 17 different cultivars were performed during the maize harvest seasons of 2019/2020, 2020 and 2020/2021 in the states of Goiás, Paraná, Santa Catarina and Rio Grande do Sul, Brazil. For all the trials, the only variable that varied between the treated and the control areas was the application or not of carrageenan (2L/ha of Algomel PUSH®). Carrageenan was applied in a single application between the phenological stages V4 and V8 (chosen based on the application of other products). For most of the trials, carrageenan was applied at V4 combined with herbicides. Fertilizer rates and kinds, chemical controls and dosages, sowing densities, cultivars, and sowing/harvest dates did not vary for the control and the treated areas within each trial.

RESULTS AND DISCUSSION

It was noticed that the sooner the product was applied the better were the results obtained. These observations might be correlated to a long-lasting effect of carrageenan on plants. It's believed that, after carrageenan is applied, and because it possesses different degrees of solubility, its effects last for a long time as plants slowly absorb different amounts of carrageenan over time.

The higher the degree of sulphation of the carrageenan, the more powerful it is, i.e. faster and stronger it starts to elicit the mechanisms of defense within plants (Shukla et al. 2016). Ducatti et al. (2021) has shown the effect of carrageenan on wheat and barley plants during three harvest seasons, with reductions of DON accumulation of 34.6% and 35.7% on average, respectively. On Table 1, we can find the mean results of yield and bromatological characteristics of maize silage treated with carrageenan vs. the control for the 19 trials performed.

Table 1. Mean results for the yield and bromatological variables analyzed for maize silage during the maize harvest seasons of 2019/2020, 2020 and 2020/2021 in the states of Goiás, Paraná, Santa Catarina and Rio Grande do Sul, Brazil.

Variable	Control	Carrageenan	Variation (%)
Yield (DM – t/ha) ± SE[1]	25.16 ± 8.32	26.68 ± 7.76	+6.03
Crude Protein (%DM) ± SE	7.79 ± 0.17	7.81 ± 0.18	+0.31
Ether Extract (%DM) ± SE	2.75 ± 0.08	2.79 ± 0.07	+1.59
Starch (%DM) ± SE	26.39 ± 2.14	26.74 ± 2.16	+1.32
Lignin (%DM) ± SE	3.16 ± 0.38	3.12 ± 0.40	-1.12
TDN (%DM) ± SE[2]	68.49 ± 1.23	69.11 ± 1.06	+0.89
NLE (Mcal/kg) ± SE[3]	1.45 ± 0.03	1.47 ± 0.03	+1.17
Milk Yield ± SE[4]	1435.76 ± 43.58	1461.20 ± 37.29	+1.77

The cultivars used for the trials were: 3 x Pioneer3707, Pioneer3016, Pioneer3565, Brevant2B688, Brevant2433, Brevant2688, Forseed533, SHS7970, DKB277, DKB177, DKB255, AG8088, AG9025, Biomatrix709, Syngenta488, NK520 and LG36790. Crude protein, ether extract, starch and lignin were performed via Near-Infrared Spectrometry after 60 days from silage production. TDN, NLE and Milk estimation were performed via Milk 2006. SE = standard error.

[1]Corrected to a standardized dry matter of 35%; [2] Total digestible nutrients; [3] Net lactation energy; [4] kg of milk per ton of dry matter.

An overall improve in the bromatological quality of the silage was observed for the 19 trials performed. This might be associated with a decrease in the fungi metabolism due to the presence of higher amounts of salicylic acid (SA) and other secondary metabolites of defense in the plant's sap (Hao et al. 2019), produced after elicitation with carrageenan (Wiesel et al. 2014; Shukla et al. 2016). Hao et al. (2019) has shown the importance of plant-SA for the reduction of *Fusarium* growth on agar plates. It is believed that when feeding on a plant that has a sap rich in secondary metabolites of defense, especially SA, *Fusarium* growth and its metabolism are considerably affected, allowing the plant to accumulate more carbon and re-direct it to its drains (leaves and grains), favoring the amount and the quality of the final product.

This reduction in the fungi metabolism may also influence the fungi to be less sensitive to stressful situations, hence diminishing the biosynthesis of mycotoxins. Fungicides, for example, are not fully effective in controlling *Fusarium* in the field, therefore, their application associated with *Fusarium* survival, provides high levels of stress to the fungi, which in turn favors mycotoxin biosynthesis and accumulation.

Fungi are sessile organisms that have as one of the main mechanisms of defense the production of secondary metabolites such as mycotoxins (Ponts 2015), therefore, carrageenan in this case, is used not to control the fungi,

but to elicit plants to produce compounds of defense (Shukla et al. 2016), which in turn, will affect the fungi development and sensitiveness to stressful conditions.

When the accumulation of DON and FUMO for the 19 experiments conducted during the three evaluated harvest seasons were analyzed, a reduction of 45.7% and 50.4% for DON and FUMO accumulation in maize silage was observed, respectively (Figure 1).

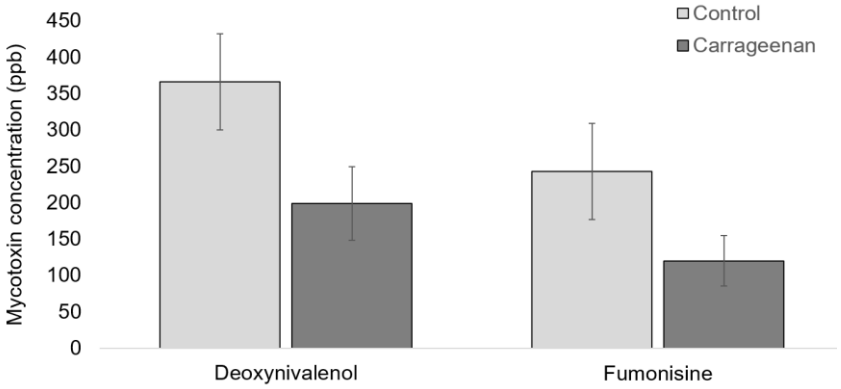

Figure 1. Mean deoxynivalenol and fumonisine concentrations (in ppb) for the 19 trials conducted during the maize harvest seasons, for silage production, of 2019/2020, 2020 and 2020/2021 in the states of Goiás, Paraná, Santa Catarina and Rio Grande do Sul, Brazil. Mycotoxin analyses were performed via Enzyme-Linked Immunosorbent Assay (ELISA) (AgraQuant® ELISA Test). Error bars show the standard error between the treatments.

As a consequence of the different hybrids used, harvest seasons and climatic conditions a large variance in the results from one area to the other was observed. Nevertheless, a constancy in the results was also observed, as yields increased, bromatological quality of maize silage increased and mycotoxin contaminations for DON and FUMO decreased in all the trials performed.

Our results go into accordance with one of the biggest concerns of governmental agencies around the World, the ever-increasing levels of mycotoxins in cereals (Marin et al. 2013). Following the results of Ducatti et al. (2021) for barley and wheat kernels, we could see a similar trend for maize silage. These promising results for mycotoxin decrease, associated with better quality of feed/food and yields, may contribute to make agriculture more profitable helping other sectors, such as the industry.

Future trials are necessary to assess the persistency of the eliciting effects of carrageenan in cereal plants as a function of time and its effects on fungi metabolism.

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