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# Activity of rice bran proteic extracts against *Fusarium graminearum*

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**The application of natural antifungal substances is motivated by the need for alternatives to existing methods that are not always applicable, efficient, or that do not pose risk to consumers or the environment. Furthermore, studies on the behaviour of toxigenic species in the presence of natural fungicides have enabled their safe application in the food chain. This study aimed to identify the fraction of the rice grain with greater inhibitory activity of amylase and related to its antifungal and antimycotoxigenic potential against *Fusarium graminearum* CQ 244 biomass. The greatest inhibitory effect was observed in extracts of bran, which inhibited by 90% the fungal amylase activity. The primary fractionation of the rice bran extract was more efficient when ethanolic extracts was precipitated by acetone, resulting in a specific inhibition estimated at 20 $\mu g_{hydrolysed\ starch}$ $min^{-1}$mg $protein^{1}$, PF 45 and recovery 61%. The rice bran protein extracts showed fungistatic activity against *F. graminearum*, with $MIC_{50}$ of 419 µg $ml^{-1}$ and 168 mg $ml^{-1}$ estimated from glucosamine and amylase inhibition, respectively, which cause 63% biomass inhibition and 40% of the nivalenol (NIV) production.**

**Key words:** Rice, *Fusarium graminearum*, glucosamine, amylase, nivalenol (NIV).

## INTRODUCTION

The presence of alpha-amylase inhibitors in beans, corn, rice, rye and other grains is related to the mechanism of germination cycle regulation and defense against contamination by fungi or other pests (Mosolov et al., 2001; Figueira et al., 2003; Marsaro-Júnior et al., 2005; Mosca et al., 2008). These amylase inhibitors present proteic character (Pagnussatt et al., 2011; Pagnussatt et al., 2012) and its extraction and purification based on proteic properties like salt solubility, adsorption by solvents, organic polymers and pH variation associated to separation operations, the make primary purification possible and allowed the study of the specific effect against fungal growth related to amylase activity (Iulek et al., 2001).

Reports of grain losses by contamination by toxigenic fungal species such as *Fusarium graminearum* are frequent in different production regions (Del Ponte et al., 2012, Kokkonen and Laitila, 2012; Abia et al., 2013; Morcia et al., 20013), despite the plants having natural defenses. Unlike other grains, rice is not considered a preferential target of this specie, because crop damage are not very frequent although the mycotoxins they produce have been found even with the use of fungicides (Dors et al., 2011; Heidtmann-Bemvenuti et al., 2012).

The fungal contamination may occur in any part of the plant, but the grains, are the most susceptibilities part (Usha et al., 1993). In rice, the external grain portion, compound by lignocelulosic and proteic material, is related to the fungal resistance associated to physical barriers and enzymatic inhibition. The mycotoxin production is a stress consequence promoted by the resistant cultivars and fungicide sprays during

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flowering, but losses in both the yield and quality of grain cannot be prevented under environmental conditions favorable for epidemics (Keller et al., 2013). During rice milling, the endosperm, bran and husk are separated with the bran accounting for 8 to 10% of the grain (Liu et al., 2009). The endosperm is widely consumed in the world diet, but the bran and husk are underutilized, this has demanded the search for innovative solutions to use these portions efficiently in a nutritional, functional and economical manner (Singh, et al., 2000). Thus, we seek the presence of enzyme inhibitors in fractions of rice milling tocontribute as an alternative to the valuation of co-products and also to reduce the mycotoxin contamination, since the inhibitors are natural substances found in cereals and do not cause stress to the fungus.

This study aimed to identify the fraction of the rice grain with greater inhibitory activity of amylase and related to their properties against on *F. graminearum*.

## MATERIALS AND METHODS

### Samples

BR-IRGA 417 rice (*Oryza sativa* L.) was grown in experimental fields of the Riograndense Rice Institute, (IRGA-Brazil), 2010 crop. After harvesting, the grains were milled in a Suzuki mini laboratory mill, separating the husk, bran and endosperm fractions. These were ground in slicer (Tecnal, model TE-631) and sieved to obtain uniform particle size (32 mesh). The bran was defatted with petroleum ether, on cold by stirring.

### Identification of rice fraction with inhibitory activity

The enzyme inhibitors were extracted under orbital shaking (200 rpm) with 95% ethanol, a ratio of 1:7 (w/v) for 7 h and the crude extract was separated by centrifugation and filtration (Pagnussatt et al., 2011). Crude extracts of rice fractions were assessed for their ability to inhibit the action of commercial fungal amylase from *Aspergillus oryzae* (Fungamyl®), provided by Novozymes®, Brazil, containing 0.05 mg protein $mL^{-1}$. Enzyme activity was determined by the iodometric method (Baraj et al., 2010, Pagnussatt et al., 2012) in experiments and in control.

The extracts containing the amylase inhibitor were incubated with commercial fungal α--amylase and sodium acetate buffer (pH 7.0) for 30 min at 25°C. After a soluble starch solution 0.5% (w/v) was added maintaining the reaction for 30 min at 25°C. The reaction was interrupted by hydrochloric acid 0.1 M addition. The residual starch was determined by iodometry, and the absorbance of the formed complex measured at 620 nm (quadruplicate). The amylase activity was expressed as µg starch $mL^{-1}$ $min^{-1}$. One unit of amylase was defined as the amount of enzyme required to hydrolyze 0.06 mg starch per min (Pagnussatt et al., 2011). The starch hydrolyzed by the enzyme in the control experiment (without inhibition extraction) was considered the maximum velocity reaction (v).

### Primary purification of the extract inhibitor

The rice bran composition was determined by the AOAC (2000) methods: Humidity (No. 935.29), ash (No. 923.03), protein (No. 920.87), lipids (No. 920.85) and crude fiber (No. 991.43). Carbohydrates were estimated by difference. The crude extract containing the bran enzyme inhibitors was obtained with 95% ethanol as described above and also with water in the ratio 1:3 (w/v) (Pereira et al., 2010). The protein extracted was precipitate by decreasing extract dielectric constant with organic solvents and a change in pH was also tested about their efficiency to protein recuperation. The total protein in the purified extracts was carried out by the Lowry (1951) method. The specific inhibitory activity (µg hydrolyzed starch $min^{-1}$ mg $protein^{-1}$) was estimated through the inhibition of enzyme activity per mg of soluble protein.

### Protein precipitation by adding organic solvents

In the ethanol extract (F1) of the proteins precipitation was accomplished adding acetone, 1:3 (v / v) standing 12 h at 4°C and after being centrifuged at 2250 x g, 20 min at 4°C, the precipitate was allowed to stand for 30 min at room temperature and then resuspended with 5 ml of water (F2). In the aqueous extract (F1'), the precipitation was performed with acetone or ethanol in proportion 1:3 (v / v) standing 12 h at 4°C and after, centrifuging at 2250 *x g*, 20 min at 4°C the precipitate was allowed to stand for 30 min and the precipitated from acetone (F2'a) or ethanol (F2'b) were resuspended with 5 mL of water (Figure 1).

### Protein precipitation by pH change

In the ethanol extract (F1), the fractioning was performed by acidification. The protein contained in this extract were precipitated with HCl 6M (F2a), in a 1:1 ratio (v/v) and allowed to stand and centrifuged as described above. The precipitate was resuspended in water to protein and enzyme inhibition activity determination. In the aqueous extract (F1') with initial pH 6.0 (F2'd) two precipitations were performed, one with adjustment to pH 5 (F2'c) and the other to pH 7 (F2'e) using HCl 1M and NaOH 2.5 M, followed by standing for 12 h at 4°C and centrifugation at 2250 *x g*, 20 min at the same temperature. In this case, the precipitated proteins were resuspended with 5 mL of 0.02 M NaOH (Figure 2).

The efficiency of the extraction process was evaluated by calculating the purification factor (PF), comparing the specific inhibition of alpha-amylase in the fraction from the purification with the specific inhibition of alpha-amylase in the crude extract, FP = (final specific enzyme inhibition / initial specific enzyme inhibition). Protein inhibitor recovery (PR) was calculated by the equation: PR = (final enzyme inhibition) / initial enzyme inhibition) x 100.

### Antifungal activity of protein extracts

The toxigenic species of *F. graminearum* CQ 244 given by the Laboratory of Plant Epidemiology of the Federal University of Rio Grande do Sul (UFRGS) was maintained on potato dextrose agar (PDA) during 14 days and the spores recovered from the agar surface with sterile solution Tween 80 (1%, v/v) following the direct enumeration in a Neubauer chamber. Fungal growth was tested by the agar dilution method, in PDA medium. The proteic compounds (100 mg of soluble protein, corresponding to 4 ml of extract in 26 ml of medium) was added to the culture medium at a temperature of 35 to 40°C and poured into Petri dishes. After solidification of the media, a spore solution containing $4 \times 10^6$ spores *F. graminearum* $ml^{-1}$ was added at the Petri dishes (Pagnussatt et al., 2013). The experiment was conducted at 30°C for 35 days in dark condition, with samples taken from the culture every 7 days. The inhibition percentage was obtained by the equation (%I) = (C - T) / C × 100, where C is the development indicator (glucosamine or amylase) in the control (absence of the rice bran extract, sterile water was added in place of the extract) and T was the treated with rice bran extract (RBE) (Souza et al., 2011). The median inhibitory

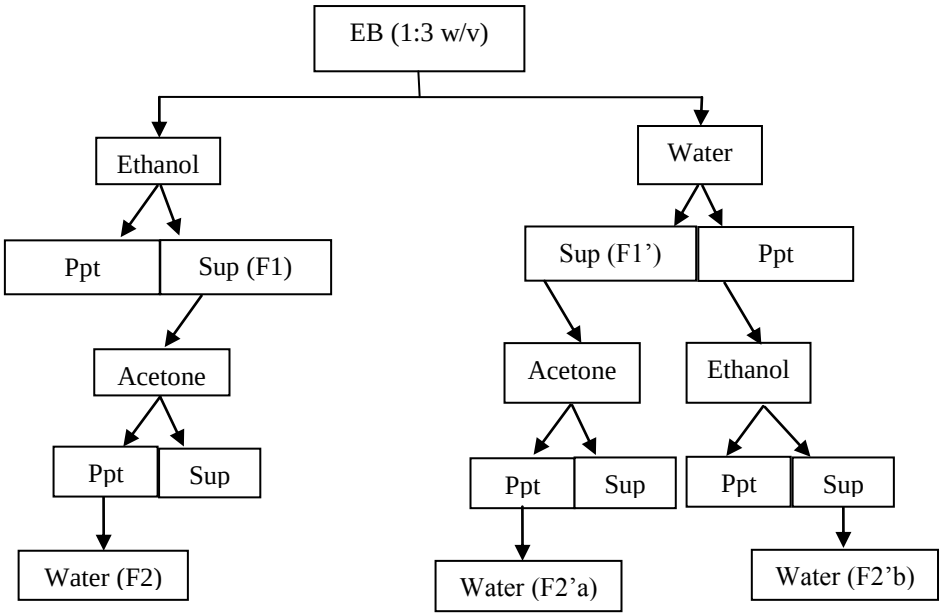


**Figure 1.** Protein fractionation by adding organic solvents. Sup, Supernatant; Ppt, precipitate.

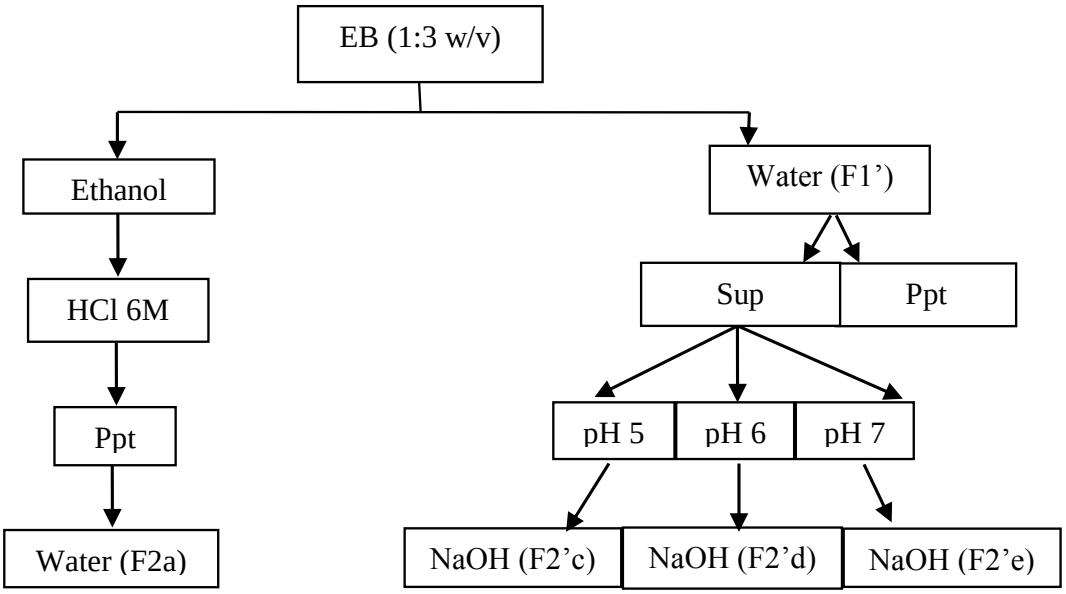


**Figure 2.** Protein fractionation by pH variation. Sup, Supernatant; Ppt, precipitate.

concentration ($MIC_{50}$) was considered as the RBE concentration that resulted in 50% inhibition of the fungal growth when compared to the control groups.

**Determination of glucosamine production and amylase activity in the *F. graminearum***

For the quantification of the glucosamine content, each 1 g of biomass was dried at 60°C for 4 h, 5 ml of 6 M HCl 6 mol L-1 was added and the mixture was autoclaved at 121°C for 20 min. The hydrolyzed material was neutralized with NaOH 3 M, and reverse titration was carried out with $KHSO_4$ (1 g 100 $ml^{-1}$). Finally, the colorimetric method was used for the determination of glucosamine (Souza et al., 2011). The absorbance units were obtained by spectrophotometry (Varian, Cary 100, California, EUA) at 530 nm, and the concentrations were established using a standard curve for glucosamine (0.01 to 0.2 g $L^{-1}$). The measurements were carried out in triplicate, and the results were expressed as glucosamine per mg sample.

The enzyme extract was obtained from the fungal biomass with 20 mL of NaCl 0.9% in an ultrasonic bath (Unique, USC-800A, São Paulo, Brasil) for 40 min, centrifuged (Cientec, CT-5000R, São Paulo, Brasil), and filtered. The α-amylase activity was determined

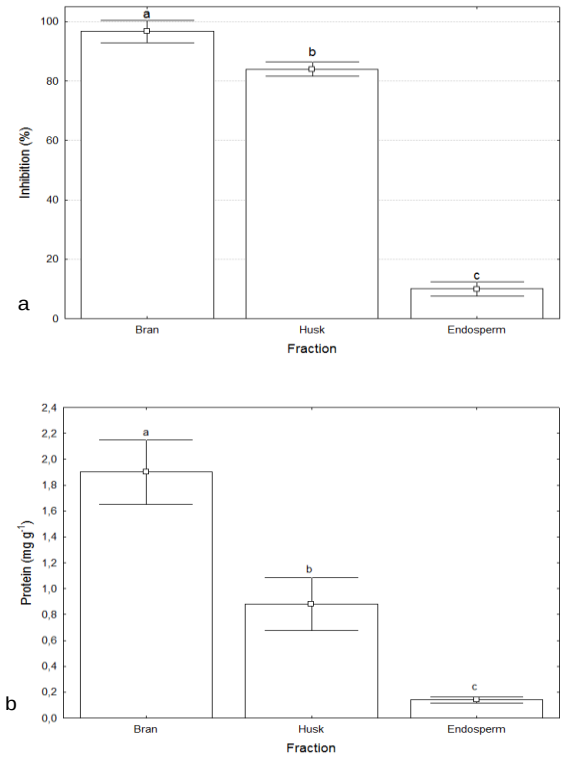


**Figure 3.** Inhibition (a) and soluble protein (b) of commercial fungal amylase in the presence of extracts of rice bran, husk and endosperm.* Same lower case letters mean non-significant differences between the means at 0.05 probability by Tukey test.

by starch degradation estimated quantitatively by iodometric titration.

### Determination of mycotoxins in biomass

The chromatographic patterns for the determination of deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) purchased from Sigma-Aldrich ® were used to prepare stock solutions. From the stock solutions dissolved in benzene: acetonitrile (95:5, v/v) working solutions were prepared, which was quantified spectrophotometrically (AOAC, 2000).

Mycotoxins were extracted using the method adapted from Tanaka (2000). The extracts were resuspended in benzene (200 µL) and applied to plates for high performance thin layer chromatography plates (HPTLC) (nano-adamant, specific surface area ~ $500m^2g^{-1}$, average pore diameter of 60A, specific pore volume of 0.75 mL $g^{-1}$ particles 2-10µm Macherey-Nangel®, Germany) for their detection and quantification in the resuspended extracts. The mycotoxins were eluated with toluene: ethyl acetate: formic acid in the proportions (6:4:1, v/v /v) and quantified by comparison with fluorescence intensity of standards after derivatization with aluminum chloride solution 15% (w/v). The detection limits, quantification and retardation factor ($R_f$) of the method (Anvisa, 2003) were evaluated.

### Statistical analysis

The significance of each treatment was determined by analysis of variance (ANOVA) and the means were compared with each other by Tukey's test at the 5% level considering the amylase and fungal growth.

## RESULTS AND DISCUSSION

### Inhibitory activity of rice derivatives

In our previous studies, the soluble proteins of rice grain had been characterized and the class of prolamine (18.5%, v/v) was the predominant one (Pagnussatt et al., 2012). In view of this, it was decided to verify the presence of prolamines in different portions of the grain of rice separated during processing. Moreover, this class of proteins is that whose peptide chains possess inhibitory activity of amylolytic enzymes, that is, part of the grain defense mechanisms against the attack by pests and germination control (Figueira et al., 2003). The inhibitory activities of fractions of rice milling had their crude extracts tested against the commercial alpha-amylase activity (Figure 3), a variation directly proportional to the increase in protein content present in them being shown, confirming the presence of inhibitors and their proteic character.

The total soluble protein content and inhibitory effect

**Table 1.** Specific inhibition profile of alpha-amylase by fractionated extracts *

| Fraction† | Activity inhibited‡ | Proteins§ | Specific inhibition# | PF†† | RP‡‡ (%) |
|---|---|---|---|---|---|
| EB‡‡‡ ET (F1) | 13.47 | 30.50 | 0.44 ± 0.00f | 1 | |
| ET: AC: AG (F2) | 16.35 | 0.83 | 19.90 ± 0.03 a | 44.99 | 61 |
| ET: HCl 6M: AG (F2a) | 3.54 | 1.53 | 2.32 ± 0.55 e.f | 5.26 | 26 |
| EB‡‡‡AG (F1') | 11.63 | 32.37 | 0.36 ± 0.15 f | 1 | |
| AG: AC: AG (F2'a) | 20.59 | 3.15 | 6.54 ± 0.91 c | 18.17 | 89 |
| AG: ET:AG(F2'b) | 25.18 | 3.13 | 8.05 ± 0.44 b.c | 22.36 | 108 |
| AG: pH 5:NaOH (F2'c) | 20.45 | 34.50 | 0.59 ± 0.03 e.f | 1.65 | 176 |
| AG: pH 6:NaOH (F2'd) | 8.53 | 3.46 | 2.47 ± 0.08 d.e | 6.86 | 37 |
| AG: pH 7:NaOH (F2'e) | 33.50 | 11.90 | 2.82 ± 0.69 d | 7.83 | 173 |

*Significant at 0.05 probability by Tukey test. †The proteins were extracted, precipitated and resuspended in: ET: Ethanol; AG: water; AC: acetone. ‡ (mg starch hydrolyzate g sample min $^{-1}$); § (mg g $^{-1}$); # (starch hydrolyzate mg min $^{-1}$ mg protein $^{-1}$). ††PF, purification factor, within a column differences in specific inhibition were significant at p< 0.05 on the bases of Tukey's Test . ‡‡RP, recovery; ‡‡‡EB, crude extract.

were higher in the bran extracts than the extracts from rice husk and endosperm rice bran inhibitory was around 90% (w/v) against fungal alpha-amylase, while the husk inhibited by 80% (w/v). These results demonstrated that the inhibitors are distributed in the outer portions of the grain, which is consistent with the fact that these fractions are most affected by physical damage during cultivation and processing, therefore where the risk of fungal contamination is greater and the defense mechanisms are necessary (Sospedra et al., 2010). From this evidence and depending on the availability in the supply chain, the rice bran was selected as the source of enzyme inhibitors for use in fractionation and in *in vitro* tests against the *F. graminearum* growth and the production of mycotoxins.

## Purification primary of rice bran inhibitors

The rice bran composition is influence by the origin of raw materials and milling process conditions. The levels recommended by industrial grain applications are (w/v): 16% minimum lipids, 13% minimum protein, 9% maximum fiber, 12% maximum humidity and maximum ash of 10%[11]. Brazilian law does not set specific values for the rice bran composition. The rice bran employed is this work presented 21.3 ± 0.3% lipids, 10% ± 0.1 humidity, 6.9 ± 0.8% crude fiber, 10.5 ± 0.4% ash and 11.6 ± 2.0% protein (w/v), similar to that frequently found by other authors (Silveira et al., 2007; Kupski et al., 2012).

The alpha-amylase inhibitor parcial purification by ethanol and acetone precipitation were adopted considering that these solvents, when decreasing the extracts dielectric constant, favor proteins separation from other compounds, and allows the concentration of the fraction containing the inhibitory activity (Pereira et al., 2010). The fungal amylase inhibition by the crude extracts and subjection to primary purification with the organic solvents did not cause the loss of inhibitory activity, which remained around 56% (w/v). The highest specific inhibition values were obtained after precipitation using ethanol and acetone (19.9 μg $_{\text{hydrolysed starch}}$ min $^{-1}$ mg $_{\text{protein}}$ $^{-1}$), among other purification conditions (Table 1).

The lower amylase inhibitory effects of the extracts obtained by pH precipitation suggest that this properties determined the molecules hydrophobic regions which exposure was determined by the pH variation. The highest PF was obtained when using ethanol in extraction step and acetone in the precipitation, with a value of 45, reinforce this and also showing a specific inhibition higher than the crude extract. The protein recovery under this condition was 61%. Higher protein recovery (PR) was observed in the tests with water extraction and ethanol precipitation (108%, w/v), indicating that this condition allowed other proteins extraction whose inhibitor character was lower than the further.

The best conditions to obtain the fungal alpha-amylase inhibition from bran rice, demonstrated by the higher purification factor were adopted to follow the study of effect against *F. graminearum* CQ 244. Since precipitation is considered a low resolution stage, depending on the type of application. In this work, the interest was to verify the antifungal properties in the rice and understanding better the effect on *F. graminearum* before to continue the inhibitor purification.

## Antifungal effect

The choice of *F. graminearum* for fungal inhibition model is justified because it is a contaminant of grains with the highest susceptibilities between the flowering -to-early stages of grain (Scotti et al., 2001), when there is greater susceptibility of the plant structure, instead of it the low occurrence in rice. The inhibition of growth by measuring

**Table 2.** Minim Inhibitory concentration ($IC_{50}$) and inhibition of fungal growth (% I) of the rice bran against *Fusarium graminearum*†.

| Days | $IC_{50}$ mg $mL^{-1}$† | | $I^2$ (%) | |
|---|---|---|---|---|
| | Glucosamine* | Amylase* | Glucosamine | Amylase |
| 7º | 1652±12,3$^{f}$ | 64±0,88$^{A}$ | 2 | 79 |
| 14º | 166±8,70$^{e}$ | 336±64,1$^{E}$ | 84 | 85 |
| 21º | 95±3,76$^{c}$ | 178±16,5$^{D}$ | 62 | 72 |
| 28º | 63±0,66$^{ab}$ | 84±5,64$^{AB}$ | 44 | 39 |
| 35º | 119±20,1$^{d}$ | 180±6,51$^{D}$ | 88 | 82 |
| Average over time | 419 | 168 | 56 | 72 |

*Significant at 0.05 probability by Tukey's test. †Results represent the mean of three determinations ± standard deviation. [2]Dados standardized. C (control) = 100%.

halos was not considered appropriate to monitor fungal growth, an uneven distribution of colonies in culture media was observed, especially those containing extract inhibitors that modified morphological hyphae characteristics. In this work, it was possible to visualize changes in the morphological characteristics of the colonies which did not present cottony hyphae and color characteristic of the species. In view of this, levels of glucosamine and amylase activity in fungal biomass were adopted as indicators of *F. graminearum* growth, since the inhibition of amylase had already been verified during the protein inhibitors screening.

Through the content of glucosamine, maximal inhibition of 88% (w/v) was observed in dry biomass in the presence of the proteic extracts, on the 35$^{th}$ day of the experiment. Enzyme inhibitors from cereals inhibited 80% (w/v) average content of glucosamine against the same fungi studied in this work (Pagnussatt et al., 2012). Indeed very promising, because the cell wall is a good indication of the viability of fungal cultures (Scotti et al., 2001).

The determination of reduced amylase activity is another important variable when monitoring fungal inhibition, since these micro-organisms produce extracellular enzymes capable of hydrolyzing the starch medium. For amylase, $MIC_{50}$ was 168 mg $mL^{-1}$ culture medium (Table 2). On the 35$^{th}$ day the extracts showed of 82% (w/v) inhibition in the activity of amylase from fungal biomass compared to the control. Considering previously performed tests with commercial enzyme (Fungamyl®) (Pagnussatt et al., 2011), the results are consistent and confirm that inhibition of fungal growth occurs mainly by decreasing the availability of nutrients from carbohydrates, which is also reflected in the decreased production of glucosamine biomass.

The antifungal effect of the extracts was tested at the concentration required to inhibit 50% of glucosamine production and enzyme activity ($MIC_{50}$). The fungal cultivation in media containing rice bran extract showed $MIC_{50}$ of 419 mg $mL^{-1}$ culture medium. Considering the content of glucosamine, this value was higher than the $MIC_{50}$ 199 and 207 µg $ml^{-1}$ in extracts of wheat applied to *F. culmorum* and *F. graminearum*, respectively (Chilosi et al., 2000). The protein efficiency of prolamine fraction from rice as antifungal agent were also demonstrated before against *F. oxysporum*; *Fusaruim solani*; *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus parasiticus* with $MIC_{50}$ from 0.6 to 20 µg $mL^{-1}$ (Lee et al., 2007).

The fungal growth indicators studied in this research reinforce the idea that there are natural fungicides in controlling microbial growth in rice grain seeds which is located in the bran, since the extracts studied showed fungistatic effect. They still suggest that the recovery of these compounds from rice bran protein may be more feasible to establish a strategy for the control of fungal contamination losses. After the demonstration of the action of protein extracts of rice bran as a natural antifungal agent, its effect on the inhibition of the *Fusarium* toxins production was also evaluated. HPTLC was adopted due to the speed, low cost and good analytical like: limit of detection (LOD) = 30 ng, 20 ng and 60 ng, limit of quantification (LOQ) = 2.4 µg $kg^{-1}$, 0.07 µg $kg^{-1}$ and 0.12 µg $kg^{-1}$; retardation factor Rf = 0.1 cm, 0.3 cm and 0.8 cm for NIV, DON and ZEA, respectively. These parameters were very similar to others reported by gas chromatography methods (Garda-Buffon and Badiale-Furlong, 2004).

*F. graminearum* produced nivalenol, detected at day 28, whereas in the control culture produced 3.2 µg $g^{-1}$ the treated by the inhibition extracts, the production was 1.92 µg $g^{-1}$ (40% inhibition, w/v). deoxynivalenol (DON) and zearalenone (ZEA) were not produced by the fungus under the conditions of the study. The synthesis of the trichothecenes comprises of cyclized sesquiterpene ring, catalyzed by the tricodiene synthase enzyme, followed by four oxygenations and eight esterifications. This sequence leads to the formation of basic structures such as DON, NIV and its acetylates (Garda-Buffon et al., 2010). Each metabolic pathway requires the expression of a carrier protein and a network of regulatory genes and each chemotype encodes specific proteins. The strain used in this work, classified as nivalenol chemotype (Astolfi et al., 2010) was susceptible to the effect of the

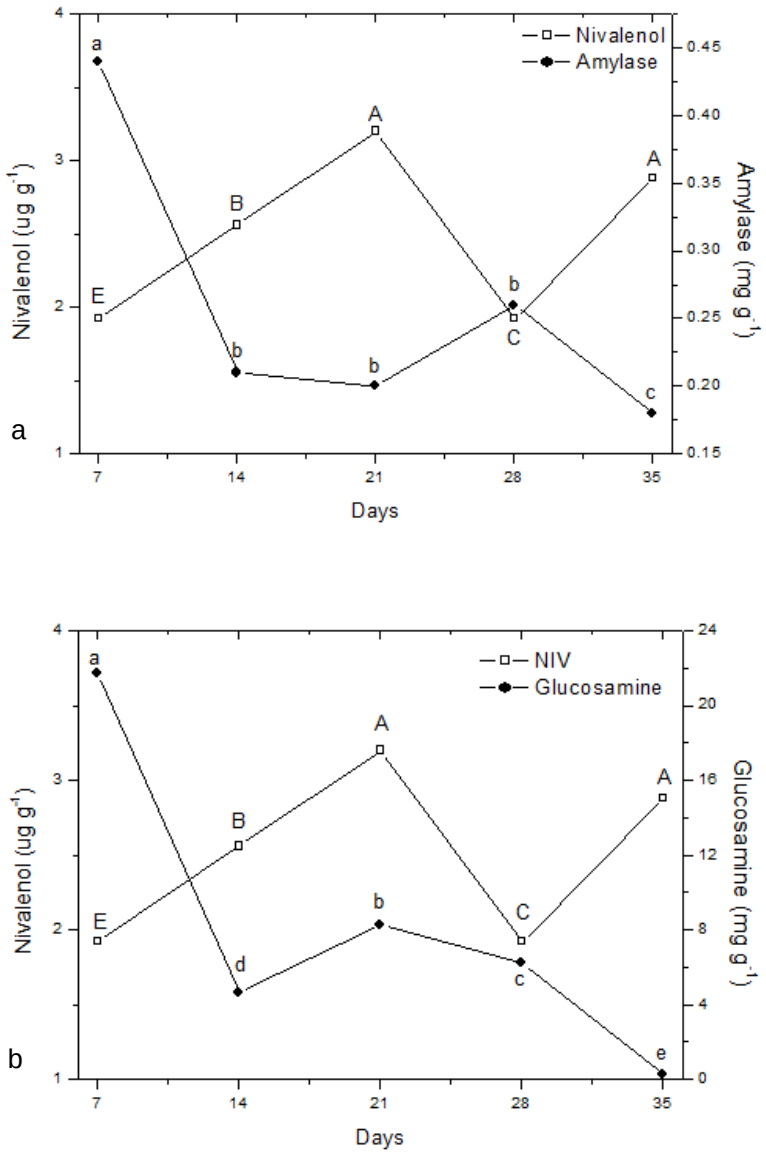


**Figure 4.** Production of nivalenol (µg $g^{-1}$), glucosamine (mg $g^{-1}$) and amylase (mg $g^{-1}$) in the presence of rice bran extracts. *Same lower case letters mean non-significant differences between the means at 0.05 probability by Tukey test. Same capital letters mean non-significant differences between the means at 0.05 probability by Tukey test.

inhibitors in some point of the route, possibly by complexing the carrier protein by the fungal multiplication was mostly affected by the bran extracts.

The lower production of fungal biomass in the presence of protein inhibitor extract of rice bran was on day 28 and 35, coincident with the inhibition of the production of NIV, relating the decreased amounts of glucosamine with the lowest levels of NIV detected (Figure 4b). At the point of greatest inhibition of amylase activity, higher production of NIV (Figure 4a) was also observed, indicating that the micro-organism could be trying to compensate for the stress caused by lack of nutrients.

However, these results were also promising and suggest that the proteic extract from bran rice might be efficient to be applied inhibiting others toxigenic species multiplication without the understanding the natural plant resistance mechanism are interesting to the economic, environmental and health point of view because it can prevent the indiscriminate use of pesticides. Moreover, it has shown that these inhibitory substances could be extracted from underused industrial fractions and reapplied during cultivation of cereals naturally during different stages of growth. The identification of inhibitors and their genetic coding can also be interesting for

genetic improvement of others cereal species not possessing these defenses, increasing their natural resistance to pathogen attack.

**Abbreviations: PF,** Purification factor; **PR,** protein inhibitor recovery; **T,** treated with rice bran extract; **RBE,** rice bran extract; **$MIC_{50}$,** median inhibitory concentration; **DON,** deoxynivalenol; **NIV,** nivalenol; **ZEA,** zearalenone; **HPTLC,** high performance thin layer chromatography plates; **LOD,** limit of detection; **LOQ,** limit of quantification; Rf, retardation factor; **PDA,** potato dextrose agar.

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