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Mycobiota and occurrence of Fumonisin B_1 in wheat harvested in Southern Brazil

Micobiota e ocorrência de Fumonisina B_1 em trigo colhido no Sul do Brasil

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ABSTRACT

*Although fumonisin B_1 (FB_1) is a mycotoxin poorly studied in wheat, the **Fusarium** genus fungi have been frequently detected in this cereal, especially in subtropical regions such as southern Brazil, thus studies on their occurrence are required. This study evaluated the mycobiota, water activity and the presence of FB_1 in 11 wheat cultivars grown in the northeastern and northwestern of Rio Grande do Sul. The mycobiota was identified by morphological and molecular techniques and FB_1 was determined by liquid chromatography with fluorescence detection (HPLC-FL). Microbial counts lower than 10^3CFU were found in the samples, and the fungi genera **Phoma**, **Cladosporium** and **Fusarium** were the most frequent. Despite this, 54% of the samples were contaminated with FB_1 at levels ranging from 958 to 4,906µg FB_1 kg^{-1}.*

Key words: ***Fusarium***; *FB_1*, ***Triticum aestivum*** *L.*

RESUMO

*Embora Fumonisina B_1 (FB_1) seja uma micotoxina pouco estudada em trigo, os fungos do gênero **Fusarium** são frequentemente detectados neste cereal, principalmente em regiões subtropicais, como o Sul do Brasil, o que justifica a necessidade de avaliar sua ocorrência. Neste estudo, foram avaliadas a micobiota, atividade de água e a presença de FB_1 em 11 cultivares de trigo cultivados nas regiões nordeste e noroeste do Rio Grande do Sul. A micobiota foi identificada por técnicas morfológicas e moleculares e a FB_1 foi determinada por cromatografia líquida e detecção por fluorescência (HPLC-FL). Nas amostras analisadas, foi verificada uma contagem inferior a 10^3UFC, e os fungos dos gêneros **Phoma**, **Cladosporium** e **Fusarium** foram os mais frequentes. Apesar disso, 54% das amostras estavam contaminadas com FB_1 em níveis variando de 958 a 4906µg de FB_1 kg^{-1}.*

Palavras-chave: ***Fusarium***, *FB_1*, ***Triticum aestivum*** *L.*

INTRODUCTION

Cereals play an important role in both the human diet and economic development of various regions. Wheat is the second most produced cereal in the world, with significant weight in the global agricultural economy (MAPA, 2013). In Brazil, this cereal is grown in the South, Southeast, and Midwest regions. The estimate of the 2013/14 planting season shows a growth of 15.1% area and a production of about 4.8 million tons, 54.6% originating from Rio Grande do Sul, 36.0% from Paraná, and the rest from the other producing states (CONAB, 2013).

Wheat is very susceptible to fungal infection and mycotoxin contamination. The subtropical climate of southern Brazil associated with the non-adoption of direct planting systems can favor the onset of severe epidemics, such as scab, a major fungal disease that attacks wheat grain, leading to serious problems including production losses and low quality of grain (ASTOLFI et al., 2011). In addition to the economic impacts, fungi also produce mycotoxins, and the most frequently reported in wheat are deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEA) (CALORI-DOMINGUES et al., 2007; ASTOLFI et al., 2011; DEL PONTE et al., 2012).

Fumonisin B_1 (FB_1) is a mycotoxin mainly produced by the fungi ***Fusarium verticillioides*** and ***Fusarium proliferatum***, which are often found in maize and maize-based products (NELSON et

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al., 1992). The toxicity of FB_1 represents serious health problems, since this toxin can lead to equine leukoencephalomacia (MARASAS et al., 1988), porcine pulmonary edema (HASCHEK et al., 1992), neural tube deviations in rats (VOSS et al., 2007), and, in extreme cases, human esophageal cancer (RHEEDER et al., 1992). For these reasons, this mycotoxin has been classified by the IARC as a Group 2B carcinogen (1993).

Although fumonisins are widely distributed geographically, the quantification of their impacts has been evaluated mainly in corn. They have been also found in other grains such as sorghum (SILVA et al., 2000; REIS et al., 2010), wheat (CHEHRI et al., 2010; PALACIOS et al., 2011), and fruits (KARBANCIOGLU-GÜLER & HEPERKAN, 2009; PERRONE et al., 2013), but in Brazil little has been studied on the occurrence of FB_1 in wheat grains.

From 2011, Brazilian legislation has established maximum permitted limits for fumonisins ($B_1 + B_2$), whose values are defined only for corn and its derivatives (ANVISA, 2011). In maize grains for further processing, this limit is 5,000µg kg^{-1}, while for corn-based products these values vary from 200 to 2,500µg kg^{-1}. In addition, due to the higher number of initiatives to study the occurrence of mycotoxins in cereals produced in Brazil, the law may be more restrictive over the years.

Considering the health risks associated with the consumption of cereal products contaminated with mycotoxins, it is necessary to investigate the presence of FB_1 in other cereals such as wheat, to verify the quality of agricultural products in relation to mycotoxins contamination, and thus to provide data as inputs to strengthen existing legislation. Therefore, the aim of this study was to identify the mycobiota and the incidence of FB_1 in wheat grains grown in southern Brazil.

MATERIAL AND METHODS

Wheat grains sample

Wheat grains were randomly collected on private properties in northeastern and northwestern of Rio Grande do Sul State, Brazil. After 2010/2011 cropping seasons, the grains were harvested and selected manually to eliminate symptomatic grains, remnants of culture, and insects. The cultivars used in this study were OR Marfim, Abalone, Fundacep Raízes, BRS Tarumã, Horizonte, Mirante, Fundacep 50, Fundacep Cristalino, BRS Guamirim, Quartzo and Codetec 114.

Isolation and Identification of mycobiota

For the isolation and identification of the mycobiota, 10 g sample were mechanically crushed, and mixed with 90 mL sterile distilled water. Successive decimal dilutions were performed in the same diluent to 10^{-4}, and 0.1 ml of each sample was inoculated onto DG18 culture medium, according to the methodology described by PITT & HOCKING (2009). Grain asepsis was not performed in order to isolate the total mycobiota from the wheat grain. Then, the plates were incubated at 25 °C for 7 days, followed by enumeration of the colonies formed. The number of colonies was corrected by the dilution factor to give colony-forming units (CFU).

For all isolates, the identification was performed by classical taxonomy, by comparing the macroscopic and microscopic morphological characteristics of the colonies with literature (PITT & HOCKING, 2009). The identification of the isolates was confirmed by ITS ribosomal DNA sequencing analysis (WHITE et al., 1990). In total, 56 strains were isolated and their ITS region was sequenced; however, only the ***Fusarium*** isolates were identified by sequencing parts of translation elongation factor (TEF-α), which is suitable for identification of these species (CARBONE & KHON, 1999). The sequences were compared with specific sequence databases, using NBLAST, with over 99% sequence identity.

Determination of water activity

Water activity of the wheat grains was determined using the apparatus AQUALAB CX-2 (Decagon Devices Inc.).

FB_1 quantification

Extraction procedure

For extraction of FB_1, 10 g sample previously ground and sieved (24 mesh) were vortexed for 1 minute with 20 mL of 50% Acetonitrile (2%, acidified with acetic acid). After addition of NaCl and Na_2SO_4, the mixture was stirred under the same conditions, and centrifuged at 3,220 *x g* for 5 min. The clean-up step consisted of salt addition (Na_2SO_4, and Celite) to the extract, followed by vortexing for 30 seconds and centrifugation at 3,220 *x g* for 5 minutes. The supernatant was filtered through Millex 0.22 mM membrane, and stored under refrigeration for subsequent quantification (PETRARCA, 2014).

Derivatization was performed according to AOAC (2005). A 25 µL extract or standard was mixed with 225 µL derivatization reagent (40 mg OPA in 1 mL methanol, 5 mL 0.1 M sodium tetraborate (pH 8.9 to 9.1), and 50 µL 2-mercaptoethanol - OPA-MCE).

The mixture was homogenized for 30 seconds in ultrasonic bath at 25°C under protection from light, and an aliquot was injected into the chromatograph.

Instrumentation and chromatographic analysis

High performance liquid chromatography was performed on a Shimadzu HPLC system comprising a high-pressure pump (LC-TA), mobile phase degasser (DGU), controller (CBM-20A), with manual sample injector (7725i) coupled to a fluorescence detector (FL-10AXL). The equipment control and data acquisition was provided by LC Solution software. A chromatographic column Kromasil C-18 (5µm, 150 x 4.6mm) maintained at 30°C was used, and acetonitrile: water (1:1 v/v), with pH adjusted to 2.45 was used as mobile phase, in isocratic elution mode, at a flow rate of 1mL min^{-1}. FB_1 detection was performed by comparing the retention times of the sample and standard at 6.3 ± 0.3min, and quantification was carried out by external standard method, using the areas of the chromatographic peaks (BECKER- ALGERI et al., 2013). A calibration curve using the wheat extract in the range from 680 to 6,000 µg kg^{-1} was also used to estimate linearity and matrix effects. The parameters of merit were the limit of detection (LOD) and quantification (LOQ), linearity, recovery and matrix effect, as recommended by ANVISA (2005) and INMETRO (2003).

For construction of the analytical curve, solutions at concentrations ranging from 0.03 to 0.3 µg L^{-1} were prepared. Each concentration was injected in triplicate, and data were obtained from linear regression using the software provided by the equipment. Curves with r^2 values > 0.90 were considered with good linearity (INMETRO, 2003). For determination of LOD and LOQ, successive injections of FB_1 standard solution were performed in order of decreasing concentration until the peak signal-to-noise ratio was more than three times the FB_1 retention time. LOQ is the lowest concentration of FB_1 that can be measured. LOD and LOQ were established as the amount of analyte, which produces 3:1 and 10:1 signal-to-noise ratio, respectively (INMETRO, 2003).

The recovery tests for FB_1 were performed with samples grains with 3 concentration levels of FB_1: 1800, 2400, and 3600 µg kg^{-1}, and the recovery was calculated and extracted: R = [(C1-C2)/C3] x 100, where R = method recovery (%); C1 = concentration determined in the grains sample; C2 = concentration determined in unfortified sample; C3 = concentration of the standard used for fortification. The contamination of the samples was performed 12 h before the beginning of the measurements to provide higher interaction between the matrix and the mycotoxin.

The matrix effect was evaluated by comparing the ratio of the solvent curve to the matrix curve slope. Solutions at seven FB_1 levels were prepared in solvent and matrix, and triplicate injections were performed for each concentration level. The matrix effect was calculated as: EM = 100 x [1-(Dm/Ds)], in which MS = Matrix Effect (%); Dm = Slope of matrix curve; Ds = Slope of solvent curve.

Statistical analysis

The results obtained in the quantification of FB_1 were analyzed using analysis of variance (ANOVA), and *t* test for comparison between means, at 5% significance level.

RESULTS AND DISCUSSION

Several factors affect fungal diversity in crops increasing susceptibility or resistance to infections, such as geographical location, climate, cultural practices, relationship between microorganisms, and environmental conditions (VUJANOVIC et al., 2012). According to OMINSKI et al. (1994), insects and fungi represent a major cause of deterioration and losses of stored grains and seeds.

Ascomycota fungi belonging to the order ***Pleosporales*** (50%), ***Hypocreales*** (12.5%), ***Capnodiales*** (12.5%) and ***Eurotiales*** (12.5%), and undefined taxonomic order (12.5%) were isolated in 11 wheat grain. Figure 1 shows the percentage of fungi genera, in which there is a predominance of the genus ***Phoma*** (29%) and ***Cladosporium*** (25%). The genus ***Fusarium*** was the third most frequent, with 19% infection in the wheat cultivars studied. Other authors reported ***Fusarium***, ***Cladosporium*** and ***Pyrenophora*** genera as the main contaminants of wheat grains (VUJANOVIC et al., 2012).

The profile of the incidence of fungal species evidenced contamination both in the field (***Alternaria*** sp., ***Fusarium*** sp., ***Epicoccum*** sp., ***Cladosporium*** sp. and ***Phoma*** sp.) and during storage (***Aspergillus*** sp.). In addition, toxigenic species such as ***Fusarium verticillioides***, ***Cladosporium cladosporioides***, ***Phoma herbarum***, ***Phoma putatium***, ***Epicoccum nigrum***, ***Epicoccum sorghi***, ***Alternaria alternata***, ***Aspergillus restrictus*** and ***Pyrenophora tritici- repentis*** were also found, demonstrating the importance of improving good farming practices to

minimize the problem of fungal contamination of grains, especially those with toxigenic potential.

Table 1 shows the results of enumeration and identification of mycobiota, water activity, and FB_1 production. In many practical situations, the water activity is a dominant intrinsic factor for maintenance or degradation of cereal, thus better knowledge on the relationships between mycobiota and water can provide subsidies to predict the period of food preservation and the potential of spoilage fungi (PITT & HOCKING, 2009). The water activity values ranged from 0.55 to 0.65, averaging 0.58, which is below the optimal value for growth of most fungal species, which is 0.8 (SMITH & MOSS 1985), as shown by the microbial count below 10^3 CFU found in the present study. In addition, other factors may have contributed to the low counts, such as humidity, gas concentration, and good storage conditions.

Sporadic reports on contamination of wheat grains with FB_1 are found in literature (STANKOVIC et al., 2012). According to MARIN et al. (1999), the reason for this low occurrence in barley and wheat may be due to the presence of competing mycobiota (which are quite different from maize), which may inhibit the synthesis of fumonisins by ***Fusarium*** sp., or degrade the mycotoxin soon after being produced. Furthermore, according to the authors, several nutritional components in wheat could act as inhibitors of the biosynthesis by fumonisins.

Furthermore in maize, high levels of FB_1 have been also detected in other cereals, but there are few reports on this subject. MASHININI & DUTTON (2007) evaluated wheat grain and wheat-based products in South Africa, and reported the occurrence of FB_1 in 2% samples of wheat-based products (breakfast cereals), and 4% samples of grain stored in storage tanks, at levels of 1-30 mg kg^{-1} in storage tanks, and 1-2 mg kg^{-1} at the retail market. In some cases, the FB_1 levels are higher, once a study on wheat grains in Serbia demonstrated that the occurrence ranged from 82.1 to 92.0%, with levels from 750 to 5400 µg kg^{-1} (STANKOVIC et al., 2012).

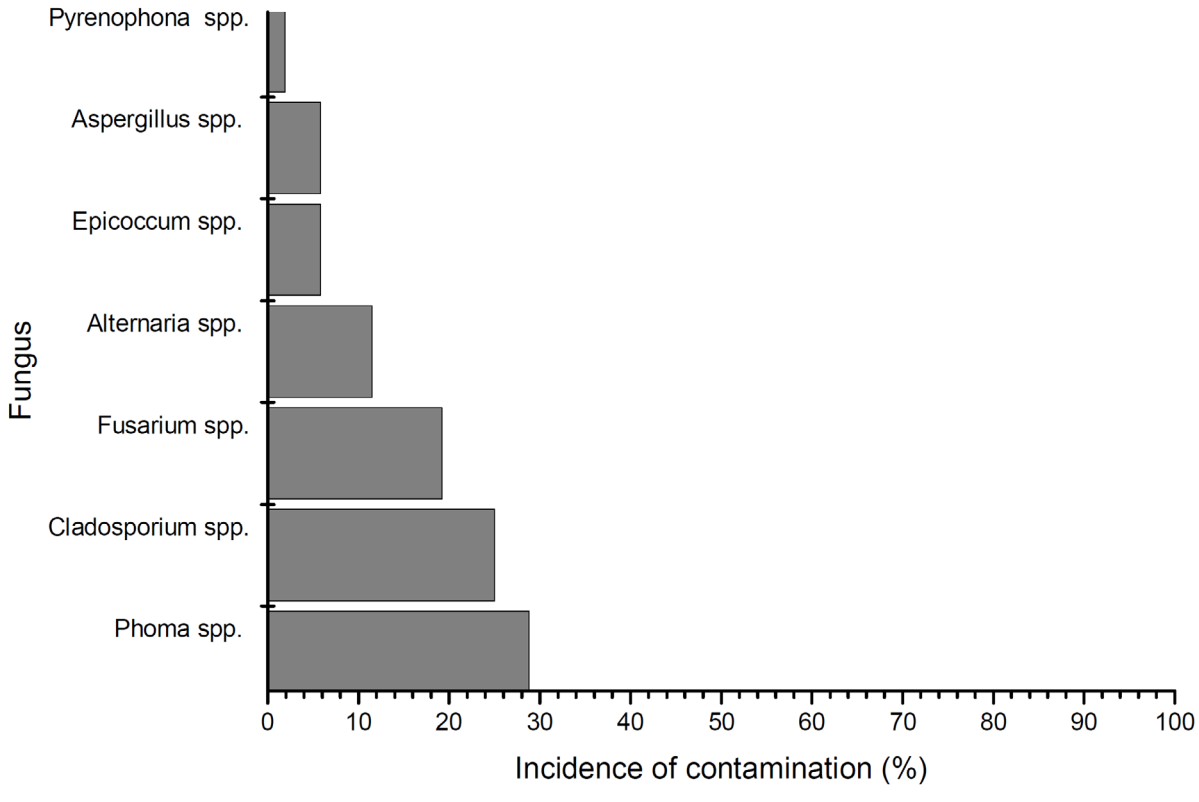

Figure 1 – Frequency of genera isolated from wheat grains.

Table 1 - Enumeration and identification of mycobiota, water activity, and FB_1 in wheat grains.

Cultivar	Identified of mycobiota and enumeration (10^2CFU)	Aa	FB_1 (µg kg^{-1})
'Fundacep 50'	3 x 10^2 ***Fusarium verticillioides*** 1 x 10^2 ***Phoma rubrum*** 14 x 10^2 ***Phoma herbarum***	0.56	958 ± 7.0[b]
'Fundacep Cristalino'	3 x 10^2 ***Epicoccum sorghi*** 1 x 10^2 ***Phoma paspali*** 15 x 10^2 ***Phoma herbarum***	0.56	<LOQ
'Horizonte'	4 x 10^2 ***Cladosporium cladosporioides*** 1 x 10^2 ***Epicoccum nigrum*** 1 x 10^2 ***Phoma putatium***	0.63	963 ± 5.0[b]
'OR Marfim'	1 x 10^2 ***Cladosporium cladosporioides*** 1 x 10^2 ***Phoma herbarum*** 3 x 10^2 ***Fusarium verticillioides***	0.62	3620 ± 239[a]
'Abalone'	25 x 10^2 ***Fusarium verticillioides*** 12 x 10^2 ***Cladosporium. cladosporioides*** 1 x 10^2 ***Epicoccum nigrum***	0.57	5319 ± 27[c]
'Quartzo'	1 x 10^2 ***Fusarium verticillioides*** 2 x 10^2 ***Cladosporium cladosporioides*** 1 x 10^2 ***Aspergillus restrictus***	0.63	4906 ± 190[a]
'BRS Guamirim'	1 x 10^2 ***Alternaria alternata*** 2 x 10^2 ***Pyrenophora tritici-repentis*** 3 x 10^2 ***Fusarium verticillioides*** 1 x 10^2 ***Aspergillus rubrum***	0.65	<LOQ
'Codetec 114'	8 x 10^2 ***Cladosporium cladosporioides*** 26 x 10^2 ***Phoma herbarum***	0.56	1120 ± 37[b]
'Fundacep Raízes'	2 x 10^2 ***Fusarium verticillioides*** 1 x 10^2 ***Cladosporium cladosporioides***	0.55	<LOQ
'BRS Tarumã'	20 x 10^2 ***Fusarium verticillioides*** 20 x 10^2 ***Cladosporium cladosporioides***	0.55	<LOQ
'Mirante'	1x 10^2 ***Fusarium verticillioides*** 2x 10^2 ***Alternaria alternata***	0.56	<LOQ

Aa= water activity; FB_1 = Fumonisin B_1, and CFU= colony forming units.

MALLMANN et al. (2001) studied the occurrence of FB_1 in cereals and feed, and found a contamination of up to 24,350 µg kg^{-1}. These data guided the interest in quantifying this mycotoxin in other cereals.

The method for determination of FB_1 had the following efficiency indicators: LOD = 200 µg kg^{-1}, LOQ = 680 µg kg^{-1}, linearity from 0.075 to 0.75 µg mL^{-1}, analytical curve in a linearity range, correlation coefficient (r^2) = 0.997, recovery = 91% at 1, 2, and 3 times the LOD, and matrix effect = 84%. Therefore, this method is suitable for determining FB_1 in the wheat samples according to the parameters suggested by ANVISA (2005) and INMETRO (2003).

In this study, natural fumonisin contamination was present 54% of all the wheat

cultivars (11/6) analyzed, in other cases the level of toxin was below the LOQ. According to the t-test, the cultivars Quartzo and OR Marfim presented the highest contamination, with values of 4,906 and 3,620 µg FB_1 kg^{-1}, respectively.

Although fumonisins are produced mainly by ***Fusarium verticillioides*** and ***Fusarium proliferatum,*** some authors have reported that other ***Fusarium*** species also have the ability to synthesize the toxin, including ***F. dlaminii, F. napiforme, F. nygamai*** and ***F. oxysporum*** (MOGENSEN et al., 2009). Other researchers have reported the production of fumonisin by different fungi species such as ***Alternata alternaria*** (CHEN et al., 1992) and ***Aspergillus niger*** (FRISVAD et al., 2011). The use of molecular techniques to monitor gene expression profiling responsible for the synthesis of fumonisins by other species is a current trend. Thus, it is possible to identify possible targets for the control of contamination by these mycotoxins in various commodities (MYUNG et al., 2009).

An atypical behavior was observed, since some cultivars were not contaminated by ***Fusarium*** species, despite the FB_1 production. In other cases, the contamination was below the limit of quantification (LOQ), as reported by other authors. PALACIOS et al. (2011) found that although many wheat samples were not contaminated by ***Fusarium*** species, the production of FB_1 occurred, probably due to the presence of ***Gibberella fujikuroi*** species complex, which was not identified by the morphologic analysis, but may have produced the mycotoxin.

The fungal deterioration potential and the lower grain quality and yield has guided the search of new techniques to control and reduce infestations, including the adoption of good practices on farming and during drying and storage of grains (CARVALHO et al., 2010), planting more resistant cultivars (CHEN et al., 2012), and use of new pesticides (CHEN et al., 2012). In addition, it is necessary to focus on acceptable levels for the occurrence of FB1, not only by the requirement of importing countries but also to minimize the risks to consumers.

The incidence of FB_1 in this preliminary study suggests that the risk exists regardless of the failure to identify the producing species in the matrix. Therefore, more representative sampling is needed to prove whether it is a continuous risk factor, or it was a random occurrence of FB_1 in the collection period. Based on these findings, programs which aimed to prevent and control pollution can be established, as well as studies on human exposure to mycotoxins and maximum acceptable mycotoxins levels in wheat grains.

CONCLUSION

In the samples analyzed in the present study, the predominant fungi genera were ***Phoma, Cladosporium,*** and ***Fusarium***, comprising 29, 25, and 19%, respectively. FB_1 production was observed in 54% of the samples at levels ranging from 958 to 4,906 µg FB_1 kg^{-1}. It is evident that further studies on mycotoxicology quality of grains produced in Brazil are needed to prevent the risks associated with ingestion of contaminated food.

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REFERENCES

ANVISA (AGÊNCIA NACIONAL DA VIGILÂNCIA SANITÁRIA). Ministério da Saúde. Portaria n.37, 22 de fevereiro de 2011. **Limites Máximos Tolerados (LMT) para micotoxinas**. Available from: <ftp.saude.sp.gov.br/ftpsessp/bibliote/informe_eletronico/2011/iels.fev.11/Iels35/U_RS-MS-ANVISA-RDC-7_180211.pdf>. Accessed: Jan 2013.

ANVISA (AGÊNCIA NACIONAL DA VIGILÂNCIA SANITÁRIA). Ministério da Saúde. **Métodos físico-químicos para análise de alimentos**. 4.ed. Brasília, 2005. 1020p.

AOAC (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS). **Analytical chemists**. 18.ed. Gaithersburg, 2005.

ASTOLFI, P. et al. Molecular survey of trichothecene genotypes of ***Fusarium graminearum*** species complex from barley in Southern Brazil. **International of Food Microbiology**, v.148, p.197-201, 2011. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21665312>. Accessed: Nov. 23, 2013. doi: 10.1016/j.ijfoodmicro.2011.05.019.

BECKER-ALGERI, T.A. et al. Thermal treatments and their effects on the fumonisin B_1 level in rice. **Food Control**, v.34, p.488-493, 2013. Available from: <http://www.sciencedirect.com/science/article/pii/S0956713513002570>. Accessed: Nov. 20, 2013. doi: 10.1016/j.foodcont.2013.05.016.

CALORI-DOMINGUES, M.A et al. Ocorrência de desoxinivalenol em trigo nacional e importado utilizado no Brasil. **Ciência e Tecnologia de Alimentos**, v.27, n.1, p.181-185, 2007. Available from: <http://www.scielo.br/pdf/cta/v27n1/31.pdf>. Accessed: Nov. 29, 2013. doi: 10.1590/S0101-20612007000100032.

CARBONE, I.; KHON, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. **Mycologia**, v.91, p.553-556, 1999. Available from: <http://www.jstor.org/discover/10.2307/3761358?uid=14651344&uid=3737664&uid=2129&uid=37603&uid=5909624&uid=2&uid=70&uid=3&uid=67&uid=62&sid=21103130626417>. Accessed: Jul. 29, 2013. doi: 10.2307/3761358.

CARVALHO, R.A. et al. Incidência de fungos toxigênicos e aflatoxinas em arroz. **Ciência Agrotécnica**, v.34, n.4, p.946-952, 2010. Available from: <http://dx.doi.org/10.1590/S1413-70542010000400021>. Accessed: Nov. 02, 2013. doi: 10.1590/S1413-70542010000400021.

CHEHRI, K. et al. Occurrence of ***Fusarium*** spp. Fumonisins in stored wheat grains marketed in Iran. **Toxins**, v.2, n.12, p.2816-2823, 2010. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22069576>. Accessed: Jul. 15, 2014. doi: 10.3390/toxins2122816.

CHEN, J. et al. Detection and verification of quantitative trait loci for resistance to Fusarium ear rot in maize. **Molecular Breeding**, v.30, p.1649-1656, 2012. Available from: <http://link.springer.com/article/10.1007%2Fs11032-012-9748-1#page-1>. Accessed: Oct. 28, 2013. doi: 10.1007/s11032-012-9748-1.

CHEN, J. et al. Production of the Mycotoxin Fumonisin B_1 by ***Alternaria alternata*** f. sp. ***lycopersici***. **Applied And Environmental Microbiology**, v.58. n.12, p.3928-3931, 1992. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC183206/>. Accessed: Jun. 13, 2014.

CHEN, Y. et al. Sensitivity of ***Fusarium verticillioides*** isolates from rice to a novel cyanoacrylate fungicide. **Crop Protection**, v.39, p.106-109, 2012. Available from: <http://www.sciencedirect.com/science/article/pii/S0261219412000774>. Accessed: Nov. 02, 2013.

CONAB (COMPANHIA NACIONAL DE ABASTECIMENTO). **Levantamento de safra**. Available from: <http://www.conab.gov.br/OlalaCMS/uploads/arquivos/13_10_23_13_46_38_boletim_portugues_outubro_2013.pdf>. Accessed: Nov. 25, 2013.

DEL PONTE, E.M. et al. Deoxynivalenol and nivalenol in comercial wheat grain related to Fusarium head blight epidemics in Southern Brazil. **Food Chemistry**, v.132, p.1087-1091, 2012. Available from: <http://www.sciencedirect.com/science/article/pii/S0308814611016177>. Accessed: Nov. 26, 2013. doi: 10.1016/j.foodchem.2011.10.108.

FRISVAD, J.C. et al. Fumonisin and Ochratoxin production in Industrial ***Aspergillus niger*** strains. **PLoS ONE**, v.6, n.8, p.1-12, 2011. Avaliable from: <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0023496>. Accessed: Jun. 08, 2014. doi: 10.1371/journal.pone.0023496.

HASCHEK, W.M. et al. Characterization of fumonisin toxicity in orally and intravenously dosed swine. **Mycopathologia**, v.117, p.83-96, 1992. Avaliable from: <http://link.springer.com/article/10.1007/BF00497283#page-1>. Accessed: Jul. 02, 2014.

IARC (INTERNATIONAL AGENCY FOR RESEARCH ON CANCER). Toxins derived from ***Fusarium moniliforme***. Fumonisins B_1 and B_2 and Fusarin C. p. 445-466. In: IARC. **Monographs on the Evaluation of Carcinogenic Risks to Humans**. Lyon, France: IARC, 1993. V.56. Avaliable from: <http://monographs.iarc.fr/ENG/Monographs/vol82/mono82-5.pdf>. Accessed: Jul. 02, 2014.

INMETRO (INSTITUTO NACIONAL DE METROLOGIA). Normalização e Qualidade Industrial. **Orientações sobre validação de métodos de ensaios químicos**. Rio de Janeiro, DOQ-CGCRE-008, 35p., 2003.

KARBANCIOGLU-GÜLER, F.; HEPERKAN, D. Natural occurrence of fumonisina B_1 in dried figs as an unexpected hazard. **Food and Chemical Toxicology**, v.47, p.289-292, 2009. Avaliable from: <http://www.researchgate.net/publication/23496850_Natural_occurrence_of_fumonisin_B1_in_dried_figs_as_an_unexpected_hazard/file/72e7e51b1b8006eb23.pdf.>. Accessed: Jul. 02, 2014. doi: 10.1016/j.fct.2008.11.003.

MALLMANN, C.A. et al. Fumonisin B_1 levels in cereals and feeds from Southern Brazil. **Arquivos do Instituto Biológico**, v.68, n.1, p.41-45, 2001. Avaliable from: <http://www.lamic.ufsm.br/papers/6b.pdf>. Accessed: Oct. 28, 2013.

MAPA (MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO). **Culturas - Trigo**. Disponível em: <http://www.agricultura.gov.br/vegetal/culturas/trigo>. Accessed: Jun. 02, 2013.

MARASAS, W.F.O. et al. Leukoencephalomalacia in horse induced by Fumonisin B_1 isolated from ***Fusarium moniliforme***. **Onderstepoort Journal of Veterinary Research**, v.55, p.197-203, 1988. Avaliable from: <http://www.ncbi.nlm.nih.gov/pubmed/3217091>. Accessed: Jul. 02, 2014.

MARIN, S. et al. Fumonisin B_1 production and growth of ***Fusarium moniliforme*** and ***Fusarium proliferatum*** on maize, wheat, and barley grain. **Journal of Food Science**, v.64, p.921-924, 1999. Avaliable from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1999.tb15941.x/abstract>. Accessed: Nov. 28, 2013. doi: 10.1111/j.1365-2621.1999.tb15941.x.

MASHININI, K.; DUTTON, M. The incidence of Fungi and Mycotoxins is South Africa wheat and wheat-based products**. Journal of Environmental Science and Health, Part B**: Pesticides, Food Contaminants, and Agricultural Wastes, v.41, n.3, p.285-296, 2007. Avaliable from: <http://dx.doi.org/10.1080/03601230500357348>. Accessed: Jul. 02, 2014. doi: 10.1080/03601230500357348.

MOGENSEN, J.M. et al. Effect of temperature and water activity on the production offumonisins by ***Aspergillus niger*** and different ***Fusarium*** species. **BMC Microbiology**, v.9, n.281, p.1-12, 2009. Avaliable from: <http://www.biomedcentral.com/1471-2180/9/281>. Accessed: Jun. 15, 2014. doi:10.1186/1471-2180-9-281.

MYUNG, K. et al. FvVE1 regulates biosynthesis of Fumonisins and Fusarins in ***Fusarium verticillioides***. **Journal of Agricultural and Food Chemistry**, v.57, n.11, p.5089-5094, 2009. Avaliable from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2692565/>. Accessed: Jun. 13, 2014. doi:10.1021/jf900783u.

NELSON, P.E. et al. Fumonisin B_1 production by *Fusarium* species other than ***F. moniliforme*** in Section ***Liseola*** and by some related species. **Applied and Environmental Microbiology**, v.58, n.3, p.984-989, 1992. Avaliable from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC195366/>. Accessed: Jun. 28, 2014.

OMINSKI, K.H. et al. Ecological aspects of growth and mycotoxin production by storage fungi. In: MILLER, J.D.; TRENHOLM, H.L. **Mycotoxins in grains**: compounds other than aflatoxin. St Paul: Eagan1994. p.287-312.

PALACIOS, S.A. et al. Occurrence of ***Fusarium*** spp. and Fumonisin in durum wheat grains**. Journal of Agricultural and Food Chemistry**, v.59, p.12264-12269, 2011. Avaliable from: <http://pubs.acs.org/doi/abs/10.1021/jf204035y>. Accessed: Nov. 25, 2013. doi: 10.1021/jf204035y.

PERRONE,G. et al. Occurrence of ochratoxin A, fumonisin B_2 and black *aspergilli* in raisins from Western Greece regions in relation to environmental and geographical factors. **Food additives & contaminants. Part A**, **Chemistry, analysis, control, exposure & risk assessment**, v.30, n.7, p.1339-1347, 2013. Avaliable from: <http://www.ncbi.nlm.nih.gov/pubmed/23767884>. Accessed: Jul. 10, 2014. doi:10.1080/19440049.2013.796594.

PETRARCA, M. H. et al. Optimisation of a sample preparation method for the determination of fumonisin B(1) in rice. **Food Chemistry**, v.158, p.270-277, 2014. Avaliable from: <http://www.sciencedirect.com/science/article/pii/S0308814614003112>. Accessed: Jul. 15, 2014. doi: 10.1016/j.foodchem.2014.02.126.

PITT, J.L.; HOCKING, A.D. **Fungi and food spoilage**. London: Blackie Academic & Professional, 2009. 519p.

REIS, T.A et al. Mycoflora and fumonisin contamination in Brazilian sorghum from sowing to harvest. **Journal of the Science of Food and Agriculture**, v.90, p.1445-1451, 2010. Avaliable from: <http://onlinelibrary.wiley.com/doi/10.1002/jsfa.3962/pdf>. Acessed: Jul. 10, 2014. doi: 10.1002/jsfa.3962.

RHEEDER, J.P. et al. ***Fusarium moniliforme*** and Fumonisins in corn in relation to human esophageal cancer in Transkey. **Phytopathology**, v.82, p.353-357. 1992. Avaliable from: <http://www.apsnet.org/publications/phytopathology/backissues/Documents/1992Abstracts/Phyto82_353.htm>. Accessed: Jul. 02, 2014. doi: 10.1094/Phyto-82-353.

SILVA, J.B. et al. Mycoflora and occurrence of Aflatoxin B_1 and Fumonisin B_1 during storage of Brazilian sorghum. **Journal of Agricultural and Food Chemistry**, v.48, p.4352-4356, 2000. Avaliable from: <http://www.ncbi.nlm.nih.gov/pubmed/10995362>. Accessed: Jul. 10, 2014. doi: 10.1021/jf990054w.

SMITH, J.E.; MOSS, M.O. **Mycotoxins**: formation, analysis and significance. New York: J. Wiley & Sons, 1985. 148p.

STANKOVIC, S. et al. Fumonisin B_1 and its co-occurrence with other fusariotoxins in naturally-contaminated wheat grain. **Food Control**, v.23, p.384-388, 2012. Avaliable from: <http://www.sciencedirect.com/science/article/pii/S0956713511003112>. Acessed: Oct. 28, 2013. doi: 10.1016/j.foodcont.2011.08.00.

VOSS, K.A. et al. Fumonisins: toxicokinetics, mechanism of action and toxicity. **Animal Feed Science and Technology**, v.137, p.299-325, 2007. Avaliable from: <http://en.liferainbow.com.tw/ktmllite/files/uploads/Fumonisin%20toxicokinetics.pdf>. Acessed: Jul. 02, 2014. doi: 10.1016/j.anifeedsci.2007.06.007.

VUJANOVIC, V. et al. Fungal communities associated with durum wheat production system: A characterization by growth stage, plant organ and preceding crop. **Crop Protection**, v.37, p.26-34, 2012. Avaliable from: <http://www.sciencedirect.com/science/article/pii/S0261219412000324>. Accessed: Oct. 20, 2013. doi: 10.1016/j.cropro.2012.02.006.

WHITE, T.J. et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS, M.A. **PCR protocols**: a guide to the methods and applications. New York: Academic, 1990. p.315-322.