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Incidence of storage fungi according to topdressing nitrogen and maize varieties

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ABSTRACT

Maize is the main cereal grown in Brazil and is used mainly for animal feed and food consumption of its derivates. Under favorable environmental conditions, toxigenic fungi such as *Fusarium*, *Aspergillus,* and *Penicillium* spp. could produce mycotoxins in maize grain during plant growth or storage. This study aimed to evaluate the incidence of toxigenic fungi and its relationship with the grain weight of maize varieties grown under high and low topdressing nitrogen. The first factor consisted of 11 open-pollinated varieties, and the second factor of two topdressing nitrogen rates (60 and 180 kg ha^{-1}). After harvesting the experimental units, the following variables were evaluated: 100-grain weight (HGW) and incidence of *A. flavus*, *Penicillium* spp., and *Fusarium* spp. There were differences ($p<0.05$) among maize varieties for all evaluated variables. There was a significant interaction between varieties and nitrogen for the percentage of seeds infected by *A. flavus, Fusarium* spp., and HGW. The incidence of fungi and HGW of maize depends on the variety and the topdressing nitrogen used.

Keywords: *Aspergillus flavus, Penicillium* spp., *Fusarium* spp., Grain quality, *Zea mays* L.

Incidência de fungos de armazenamento em função de nitrogênio em cobertura e variedades de milho

RESUMO

O milho é o principal cereal cultivado no Brasil e é utilizado principalmente para alimentação animal e consumo alimentar de seus derivados. Em condições ambientais favoráveis, fungos toxigênicos como *Fusarium*, *Aspergillus* e *Penicillium* spp. poderiam produzir micotoxinas nos grãos de milho durante o crescimento da planta ou no armazenamento. O objetivo deste trabalho foi avaliar a incidência de fungos toxigênicos e sua relação com o peso de grãos de variedades de milho cultivadas sob alta e baixa adubação de nitrogênio. O primeiro fator consistiu de 11 variedades de polinização aberta e o segundo fator de duas doses de nitrogênio em cobertura (60 e 180 kg ha^{-1}). Após a colheita das unidades experimentais, foram avaliadas as seguintes variáveis: massa de cem grãos (MCG) e incidência de *A. flavus*, *Penicillium* spp. e *Fusarium* spp. Houve diferenças ($p<0,05$) entre as variedades de milho para todas as variáveis avaliadas. Houve interação significativa entre variedades x nitrogênio para a porcentagem de sementes infectadas por *A. flavus*, *Fusarium* spp. e MCG. A incidência de fungos e o MCG do milho dependem da variedade e do nitrogênio de cobertura usado.

Palavras-chave: *Aspergillus flavus, Penicillium* spp., *Fusarium* spp., Qualidade de grãos, *Zea mays* L..

1. Introduction

Phytopathogenic agents pose severe risks to human and animal health and remain a major concern for food safety. A problem encountered in the harvest is the vulnerability to contamination with mycotoxins, aflatoxin produced by the pathogen *Aspergillus flavus*, and fumonisin by *Fusarium* spp. (Konca and Tunc, 2020). Grains contaminated with aflatoxins worldwide are destroyed before entering the food flow. However, inspection is not allowed in some countries, and infected maize is consumed by the farmer who produces it. In addition to causing considerable losses in maize crops, these fungi cause various diseases, such as stem, cob, and root rot, and even death of the seedlings (Manoza et al., 2017).

The interaction between plants and pathogenic microorganisms can be influenced by environmental factors, such as temperature, humidity, light, and nutrients (Qin et al., 2017). Pest and weed control, deficit irrigation, and adequate fertilization may inhibit toxigenic fungi (Zhang et al., 2019). Nitrogen is the main nutrient demanded by maize. The supply of this mineral in the crops is done by applying fertilizers to the soil or leaf (Khattak and Khalil, 2009).

In maize, nitrogen fertilizing is carried out mainly via soil, and the rates are split to avoid losses due to volatilization. When applied to soil, nitrogen is converted to mineral form (nitrate) so plants can take it up (Khattak and Khalil, 2009). The mineral N fertilizers provide one or both of the primary N forms taken up by plants, namely, ammonium (NH^{4+}) and nitrate (NO^{3-}). Thus, sufficient nitrogen rates increase the growth rate of maize roots, resulting in healthier and more resistant plants against damage caused by the pathogen by creating strong cell walls that function as a mechanical barrier in the entry of pathogens (Li et al., 2020). Several studies have shown that nitrogen can alter the plant's resilience through growth patterns associated with nitrogen-mediated signaling effects and transduction (Guo et al., 2019).

Thus, nitrogen availability can limit the growth of the pathogen in defense of the plant. However, excess nitrogen causes greater vegetative growth and leaf expansion, resulting in thinner cell walls that facilitate fungal penetration (Hammad et al., 2020). When using slow-release fertilizers, the vegetative phase is prolonged in maize plants, which provides more appropriate conditions for the development of pathogens (Wang et al., 2021). When urea is applied, the incidence of toxins is lower, and the nitrogen source influences the maize contamination by mycotoxin (Bueno et al., 2020).

According to Manoza et al. (2017), a higher nitrogen application rate caused a decreased aflatoxin, even with an insignificant effect. These rates can be adjusted to mitigate the toxin contamination problem in maize grains. Thus, it is considered that nitrogen fertilization is one of the alternatives to reduce the spread of toxins since it is related to the biosynthesis of these mycotoxins. This study hypothesizes that topdressing nitrogen increases the grain mass of maize varieties and reduces the incidence of *A. flavus*, *Penicillium* spp., and *Fusarium* spp. in their grains. Therefore, this study aimed to evaluate the incidence of toxigenic fungi and its relationship with the grain mass of maize varieties grown under high and low topdressing nitrogen.

2. Material and Methods

The experiment was conducted at the Chapadão Foundation for Agricultural Research Support (Fundação Chapadão), located in Chapadão do Sul (18°41'33"S, 52°40'45"W, and 810 m of altitude), Mato Grosso do Sul. The region's climate is Savana Tropical (Aw-type), and the soil was classified as clay dystrophic Oxisol. The experiment was implemented in a randomized complete block design with four replications in a factorial scheme. The first factor consisted of eleven open-pollinated varieties (Table 1), and the second factor consisted of two topdressing nitrogen (60 and 180 kg ha^{-1}).

Conventional soil preparation was conducted. Sowing was conducted manually in each plot. Sowing occurred in 0.45 m row spacing and a density of 2.5 plants m^{-1}. Each experimental unit was composed of five rows with 5.5 m in length. The choice of varieties was based on availability in the region. In the implantation of the experiment, 300 kg ha^{-1} of NPK (04-20-20) was used. In the dose of 60 kg ha^{-1}, the application was conducted at V4 (15 days after emergence). For the dose of 180 kg ha^{-1}, applications were divided into 60 kg ha^{-1} in V4, V6, and V8 (15, 25, and 40 days after emergence, respectively). Urea was the N source (45%) in all treatments.

Five plants were randomly chosen in central rows of each experimental unit to harvest the ears. The harvest was conducted when the maize grains had moisture content between 19.2% and 14.8%. After harvesting, the following variables were evaluated: 100-grain weight (HGW) and incidence of *Aspergillus flavus* (ASP), *Penicillium* spp. (PEN), and *Fusarium* spp. (FUS). The identification and evaluation of fungi present in maize seed samples was conducted at the phytopathology laboratory of the Federal University of Mato Grosso do Sul, Brazil. Twenty-five seeds of each variety were collected for each replicate. The method used was the sanity Bottler test which consisted of depositing 25 seeds on two pieces of filter paper moistened with distilled water placed in a germinating box (11 cm x 11 cm x 3.5 cm) for each experimental unit.

Table 1. Open pollinated varieties (*Zea mays* L.) used and company responsible for the commercialization.

Commercial variety	Company
AL Alvaré	Cati Sementes
AL Diratininga	Cati Sementes
BRS 106	Embrapa Milho e Sorgo
BRS 4103	Embrapa Milho e Sorgo
BRS Caimbé	Embrapa Milho e Sorgo
BRS Gorotuba	Embrapa Milho e Sorgo
Cativerde	Cati Sementes
SC 5154	Epagri
SC 5155	Epagri
SC 5156	Epagri

The seeds were then subjected to a heat shock to inhibit germination after 24 hours at room temperature and 24 hours in a freezer. The seeds were then kept for seven days in a B.O.D (Climatic Chamber, Brand Eletrolab, Model EL101/4, 220V) at 22ºC under a photoperiod of 12 hours to promote fungal growth. The genera of fungi growing on seeds were identified by morphological structures using optical and stereoscopic microscopes (Barnett and Hunter, 1998). *Aspergillus flavus* was identified according to the method of Klich (2002).

The percentage quantification of the fungi incidence was performed according to the procedures recommended by Brazil (2009). The HGW was evaluated after correcting the humidity to 13%, expressed in grams. After verifying the data normality, homogeneity, and homoscedasticity, an analysis of variance was performed, and the means were grouped by the Scott-Knott test at 5% probability. After verifying the effect of the interaction between maize varieties and topdressing nitrogen, principal component analysis was applied to verify the interrelationship between variables and treatments (variety-nitrogen combination).

A biplot was built with the first two principal components due to the ease of interpreting these results. In this biplot, three clusters were defined to use the k-means algorithm, which groups treatments whose centroids are closest until there is no significant variation in the minimum distance of each observation to each centroid (Bhering, 2017). These analyses were performed using the “ggfortify” package of the free software R and followed the procedures recommended by Naldi et al. (2011).

3. Results and Discussion

There are significant differences (p<0.05) among maize varieties for all variables. Table 2 shows the analysis of variance for the percentage of seeds infected by *A. flavus* (ASP), *Penicillium* spp. (PEN), *Fusarium* spp. (FUS) and 100-grain weight (HGW). Topdressing nitrogen affected the incidence of *A. flavus* and HGW. There was a significant interaction between varieties x nitrogen for the percentage of seeds infected by *A. flavus*, *Fusarium* spp. and HGW. These results indicate that the incidence of these fungi depends on the maize variety used and the rate of topdressing nitrogen.

It is possible to verify that the lower rate of topdressing nitrogen provided a higher incidence of this fungus in AL Alvaré, BRS 4103, and Cativerde (Table 3). The other varieties did not differ among them as the nitrogen rates for this variable. The varieties AL Diratininga, BRS 106, BRS 4104, BRS Caimbé, and SC 5156 presented low percentages of infected seeds by *A. flavus* regardless of the topdressing nitrogen condition. However, it is interesting to note that there were changes in the ranking of varieties according to the topdressing nitrogen. The AL Alvaré and BRS 4103 varieties presented a low incidence of this fungus when cultivated with high topdressing nitrogen rates. However, this situation was reversed at a low rate, and these varieties showed the highest infection percentages.

For the percentage of seeds infected by *Penicillium* spp., there were differences (p<0.05) only among maize varieties (Table 4). The varieties BRS Caimbé and SC 5156 presented the highest infection percentages by this fungus. These varieties are among those that presented the lowest percentages of *A. flavus* infected seeds regardless of the nitrogen topdressing condition. This finding indicates a negative correlation (r = -0.61) between the incidence of *Penicillium* spp. and *A. flavus* on seeds of maize varieties. The lower rate of topdressing nitrogen provided a higher incidence of this fungus in BRS Caimbé, SC 5154, and SC 5155. The other varieties did not differ among them as the nitrogen rates for this variable.

Table 2. Summary of the analysis of variance (Mean square) for percentage of seeds infected by *A. flavus* (ASP), *Penicillium* spp. (PEN), *Fusarium* spp. (FUS) and hundred-grain weight (HGW) evaluated in 11 maize varieties growth under high and low topdressing nitrogen

Source of variation	DF	ASP	PEN	FUS	HGW
		Mean Square			
Variety (V)	10	210.13*	642.28*	587.78*	238.44*
Nitrogen (N)	1	680.97*	11.88^{ns}	190.06^{ns}	113.56*
VxN	10	258.04*	193.21^{ns}	1146.33*	89.97*
Error	44	36.36	216.97	256.48	28.45
Coefficient of variation (%)		19.58	21.38	26.91	18.45

^{ns} and *: not significant and significant at 5% probability by the F test, respectively; DF: degrees of freedom.

Table 3. Deployment of the significant interaction between maize varieties and topdressing nitrogen for the percentage (%) of seeds infected by *Aspergillus flavus.*

Variety	Topdressing nitrogen	
	180 kg ha^{-1} de N	60 kg ha^{-1} de N
AL Alvaré	1.33 bB	36.00 aA
AL Diratininga	5.33 bA	5.33 cA
BRS 106	5.33 bA	5.33 cA
BRS 4103	0.00 bB	26.67 aA
BRS 4104	6.67 bA	4.00 cA
BRS Caimbé	2.67 bA	4.00 cA
BRS Gorotuba	17.33 aA	16.00 bA
Cativerde	6.67 bB	20.00 bA
SC 5154	4.00 bA	10.67 cA
SC 5155	4.00 bA	9.33 cA
SC 5156	0.00 bA	0.00 dA
Standard error	1.53	3.44
Mean	5.45	11.88

Means followed by lower case letters in the same column and upper case in the same line belong to the same group by the Skott-Knott test at 5% probability.

Table 4. Clustering means for the variable percentage of seeds infected by *Penicillium* spp. evaluated in 11 maize varieties

Variety	*Penicillium* spp. (%)
AL Alvaré	60.00 b
AL Diratininga	68.67 b
BRS 106	66.00 b
BRS 4103	68.00 b
BRS 4104	73.33 b
BRS Caimbé	89.33 a
BRS Gorotuba	57.33 b
Cativerde	60.67 b
SC 5154	59.33 b
SC 5155	70.67 b
SC 5156	84.67 a
Standard error	2.46
Mean	68.91

Means followed by equal letters in the same column belong to the same group by the Skott-Knott test at 5% probability.

Table 5 shows the breakdown of the significant interaction between corn varieties and nitrogen in coverage. Table 6 shows the significant interaction between maize and topdressing nitrogen varieties for HGW. Except for the varieties BRS 4104, Cativerde, SC 5155, and SC 5156, whose HGW did not differ regarding the rates of topdressing nitrogen, all the other varieties showed higher means under high topdressing nitrogen. Under low topdressing nitrogen, BRS 4103, BRS 4104, BRS Caimbé, BRS Gorotuba, and SC 5154 obtained the highest HGW. When high topdressing N is applied, BRS 4104, BRS Caimbé, Cativerde, SC 5154, and SC 5155 presented the highest means for HGW. However, under these conditions, the fungus that most influenced the HGW was *Aspergillus flavus*.

The findings obtained here are similar to those of Jiang et al. (2014), in which decreased fertilization contributed to increased aflatoxin contamination. According to the authors, certain amino acids, especially proline, support higher levels of aflatoxin synthesis by *A. flavus.* On the other hand, Krnjaja et al. (2021) reported that the fungi incidence is unrelated to the amount or source of nitrogen. Physiologists and pathologists poorly understand this incoherence of results because nitrogen is inconsistent and contradictory in disease development (Krnjaja et al., 2021).

Table 5. Deployment of the significant interaction between maize varieties and topdressing nitrogen for the percentage (%) of seeds infected by *Fusarium* spp

Variety	Topdressing nitrogen	
	180 kg ha^{-1} de N	60 kg ha^{-1} de N
AL Alvaré	53.33 bA	49.33 bA
AL Diratininga	53.33 bA	73.33 aA
BRS 106	46.67 bA	50.67 bA
BRS 4103	53.33 bA	74.67 aA
BRS 4104	48.00 bA	45.33 bA
BRS Caimbé	56.00 bB	89.33 aA
BRS Gorotuba	54.67 bA	68.00 bA
Cativerde	62.67 aA	64.00 bA
SC 5154	26.67 bB	77.33 aA
SC 5155	26.67 bB	81.33 aA
SC 5156	74.67 aA	80.00 aA
Standard error	5.33	4.91
Mean	61.21	57.82

Means followed by lower case letters in the same column and upper case on the same line belong to the same group by the Scott-Knott test at 5% probability.

Table 6. Deployment of the significant interaction between maize varieties and topdressing nitrogen for the hundred-grain weight (g).

Variety	Topdressing nitrogen	
	180 kg ha^{-1} de N	60 kg ha^{-1} de N
AL Alvaré	30.11 bA	27.87 bB
AL Diratininga	28.90 bA	27.76 bA
BRS 106	30.56 bA	26.44 bB
BRS 4103	31.40 aA	27.29 bB
BRS 4104	32.93 aA	29.48 aA
BRS Caimbé	35.62 aA	30.17 aB
BRS Gorotuba	34.99 aA	26.04 bB
Cativerde	29.07 bA	29.04 aA
SC 5154	33.57 aA	28.64 aB
SC 5155	29.22 bA	28.85 aA
SC 5156	27.89 bA	26.58 bA
Standard error	0.79	0.40
Mean	31.30	28.01

Means followed by lower case letters in the same column and upper case on the same line belong to the same group by the Scott-Knott test at 5% probability.

Other factors that affect the fungus spread should be considered, such as the density of plants, genotype, microclimate, and interaction with other nutrients (Macholdt et al., 2020). According to Pena et al. (2020), climate is a significant factor affecting fungus incidence. When evaluating the propagation of *A. flavus* and *Penicillium* spp. in maize, these authors verified that the incidence was very low in the rainy season, and hence, there was less competition with *Fusarium* spp. Under drought conditions, Garbaba et al. (2018) observed an increased occurrence of *A. flavus* in maize and greater competitiveness among microorganisms.

The reduction of the *A. flavus* pathogen may be due to competition with other microorganisms, such as *Penicillium* (Table 4). As reported in previous studies (Pena et al., 2020; Macholdt et al., 2020), *Fusarium* is the genus that competes with the pathogen *Aspergillus* spp. in most cases. Studies addressing the competition between microorganisms under biological control in agricultural products (Hamdi et al., 2018) also show variation in the incidence of the *Penicillium*, *Aspergillus*, and *Fusarium* in the presence of other microorganisms, which supports the occurrence of competition by the culture medium. Thus, when applying low nitrogen rates, the plant undergoes abiotic stress, which makes possible a greater susceptibility to the attack of pests and diseases (Feng et al., 2021).

Thus, plants suffering from abiotic stress tend to lose grain yield and quality and are predisposed to contamination by mycotoxins (Zhou et al., 2017). Similarly, high doses of nitrogen may increase fumonisin contamination since excess nitrogen potentiates the incidence of the pathogen, and the nutrient becomes toxic to the plant. These authors report not only problems due to low rates but also due to excess fertilizers. High nitrogen rates cause a delay in physiological maturity, so the fungi have more time for colonization (Tran et al., 2021).

It is essential to highlight the effect of topdressing nitrogen on the incidence of *A. flavus*. The varieties AL Diratininga, BRS 4103, BRS Caimbé, SC 5154, and SC 5155 presented the lowest incidence of this fungus when cultivated under a high rate of topdressing nitrogen. However, when the topdressing nitrogen rate was low, these varieties had the highest percentages of seeds infected by *A. flavus*. In the literature, the results for HGW are variable. When evaluating a single variety, some authors observed a positive correlation between nitrogen and HGW (Lange et al., 2014). When comparing maize varieties, the authors observed the same behavior concerning the nitrogen application, but one stood out with higher means due to its rusticity and tolerance.

Similarly, Sichocki et al. (2014) observed the same behavior in the increased grain weight according to the topdressing fertilization. However, when nitrogen rates are raised excessively, the absorption and utilization of nutrients are restricted and cause low economic returns. For other authors, the 100-grain weight is not altered when increasing the nitrogen rate or source (Cheng et al., 2015). Ohland et al. (2005) reported that this variable is conditioned by genetic factors and not by nitrogen fertilization. Principal component analysis associated with the k-means algorithm for delimiting three groups (Figure 2) grouped 69.4% of the total variation in the first two axes.

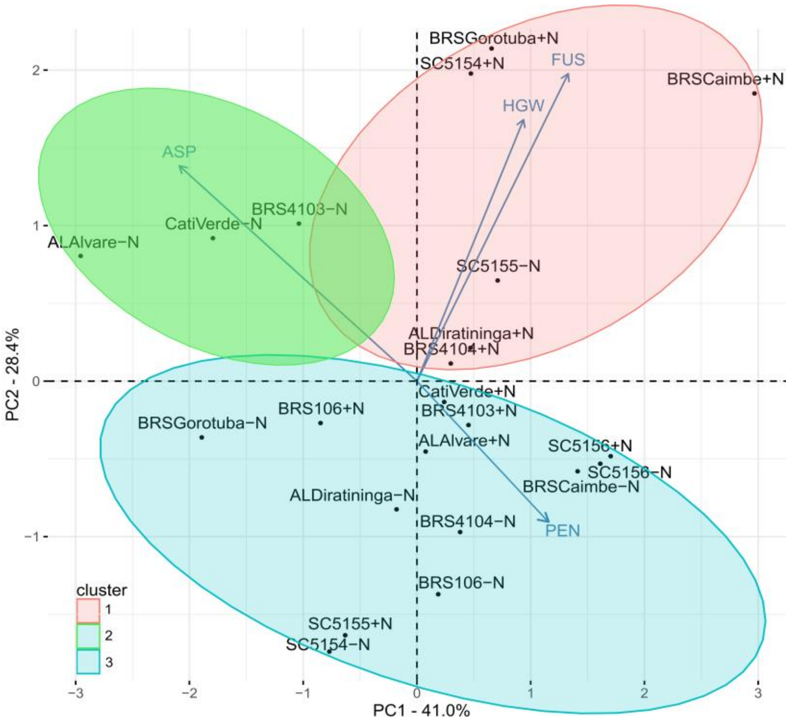

Figure 2. Principal component analysis for the variables *A. flavus* (ASP), *Penicillium* spp. (PEN), *Fusarium* spp. (FUS) and hundred-grain weight (HGW) evaluated in 11 maize varieties grown under high (+N) and low (-N) topdressing nitrogen.

The Figure 2 shows that three cultivars (AL Alvare, Cativerde, and BRS 4103) showed a higher occurrence of *Aspergullus* spp. when supplemented with 60 kg N. There may be greater susceptibility of the cultivars to this fungus, with insignificant effect of nitrogen fertilization on it. In the *Penicillium* cluster, the same cultivars were observed with both fertilizations, indicating a slight effect of nitrogen on the incidence of the fungus. On the other hand, we observed that there is an effect of N on grain mass but that there was *Fusarium* spp. infection in cultivars with higher grain masses.

Apparently, there is competition between *Penicillium* spp. and *Aspergillus* spp., there is a 180º angle between the vectors, indicating negative correction (r=-0.61), and there is no correlation between the incidence of *Aspergillus favus* and *Penicillium* spp. with *Fusarium* spp. forming an angle of approximately 90º. The largest 100-grain weight was observed when a higher incidence of *Fusarium* spp. occurred. The principal component analysis results demonstrated specific interactions among varieties *versus* topdressing nitrogen on fungi incidence in maize grains.

Overall, some varieties benefit considerably from a high rate of topdressing nitrogen. For example, AL Alvaré, BRS 4103, and Cativerde reduce the incidence of *A. flavus*, while BRS Caimbé and SC 5154 reduce the incidence of *Fusarium* spp. Some studies have reported the negative impact of fungi on stored maize grains (Tran et al., 2021). The findings of this research are important, as they demonstrate that adequate cultural practices such as topdressing nitrogen management impact the incidence of these fungi that affect the storage of maize grains. However, for future research, it is important to investigate the effect of topdressing nitrogen fertilization on the physical-chemical quality of maize grains.

4. Conclusions

Our findings demonstrate a significant interaction between maize varieties and topdressing nitrogen regarding the 100-grain weight and incidence of *A. flavus* and *Fusarium* spp. The varieties AL Alvaré, BRS 4103, and Cativerde have reduced *A. flavus* incidence when a high rate of topdressing nitrogen is used. Under the high rate of topdressing nitrogen, the varieties BRS Caimbé and SC 5154 have reduced incidence of *Fusarium* spp. Topdressing nitrogen fertilization does not affect the incidence of *Penicillium* spp. in maize grains. The occurrence of this pathogen is associated with the variety used.

Authors' Contribution

Tays Batista Silva, Marcela Silva Flores, Mariana Vale dos Santos, and Bruno Fernando Bertequine have done the collection of data, data analysis and interpretation, writing the article and final version of the article, Paulo Carteri Coradi, Maria Luiza Nunes Costa, Larissa Pereira Ribeiro Teodoro, and Paulo Eduardo Teodoro have done the research concept and design, data analysis and interpretation, writing the article, critical revision of the article and final version of the article.

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