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THE INFLUENCE OF STORAGE CONDITION ON MYCOTOXIN LOAD ON MAIN FEED INPUTS IN SOUTHEAST ROMANIA

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Abstract

Mycotoxins are toxic compounds for animals and humans, naturally produced by different types of fungi. Exposure to mycotoxins through the consumption of contaminated food or feed leads to gastrointestinal and renal disorders to the point of immune deficiency and cancer. Most mycotoxins are chemically stable and persist in food processing. Fusarium graminearum is a dangerous pathogen of the cereals producing mycotoxins (trichothecene and zearalenone) harmful for human and animal health. Given the implementation of the requirements of the European Green Deal, especially those related to organic products, and in the context of climate change, especially the fluctuations in temperature and humidity, the increase of the presence of mycotoxins in food and feed is expected. In these conditions, the evaluation of the degree of mycotoxin contamination in different steps on the food chain, with fast and accurate methods, is very important. The aim of this work was to investigate the dynamics of fungi and related mycotoxins on wheat, maize and sunflower during cereal storage in vertical and classic silos.

***Key words**: Ochratoxin A, Fusarium graminearum, wheat, storage.*

INTRODUCTION

Cereals play a critical and irreplaceable role in both agricultural production and nourishing the global population (Tremmel-Bede et al., 2022). In developed nations where cereal grain production is significant for both food and feed purposes, substantial quantities of grains are retained in storage for extended periods, often exceeding a year, to meet the demands of domestic cereal industries and fulfill import and export requirements. As defined by Dunkel (1992), the concept of stored grain ecosystems revolves around dormant autotrophs, which are the seeds themselves. These seeds serve a dual purpose, functioning as both an energy source and a habitat for a diverse array of heterotrophic organisms, including fungi, bacteria, insects, and mites (Fleurat-Lessard, 2017).

In adverse field conditions that pose stress to crops, certain fungi have the capability to produce mycotoxins. These mycotoxins are secondary metabolites that have the potential to taint a variety of agricultural products, both in the field and during storage (Yao et al., 2015). Among the crops commonly vulnerable to contamination by aflatoxins, we find cereals such as maize, rice, and wheat (Shephard et al., 2013). Additionally, it's worth noting that there are fungi that infect seeds and serve as sources of specific mycotoxins (Chulze, 2010; Ittu, 2006). These mycotoxins can pose significant risks to consumers within the food chain.

The interconnection between well-being in individuals, the well-being of societies, and the well-being of the planet underscores the significance of sustainable food systems within the framework of the European Green Deal, the European Union's strategy for sustainable and equitable development. This strategy is crafted to invigorate the economy, enhance the health and well-being of individuals, and uphold the preservation of nature. The European agricultural and food system, underpinned by the Common Agricultural Policy, already serves as a global benchmark for its commitment to safety, supply reliability, nutritional value, and overall quality (CAP strategic plans, European Commission, europa.eu, 2023). Mycotoxins are toxic compounds generated by specific fungal species that thrive on grain and feed products when stored under unfavorable moisture conditions. These mycotoxins possess the potential to yield severe consequences for both human and animal well-being. To mitigate the risk of introducing mycotoxin-contaminated food and feed into the grain processing chains, the European Union has implemented several regulations (European Commission, 2006, 2007, 2010; Meucci et al., 2021; Codex Alimentarius Commission, 2014). The practice of storing cereals dates back to ancient times, primarily due to the seasonal nature of cereal crops that are consumed year-round. For strategic purposes and to minimize transportation needs, it is advisable to decentralize and distribute grain storage across a broad geographic area, utilizing the grain close to its point of production (De Martini et al., 2009). Typically, cereal storage takes place in round (or occasionally square) bins or silos constructed from materials such as metal or cement. These storage facilities come in various capacities and are equipped with sophisticated infrastructure (Silva, 2002; Silva et al., 2012). Stored maize represents a constructed ecosystem in which alterations in quality and nutritional content take place due to the intricate interplay among physical, chemical, and biological factors. A significant concern within this context revolves around fungal spoilage and the potential contamination of mycotoxins. Fungal infections can lead to mycotoxin contamination at various stages, including growth, harvest, storage, transportation, and processing (Chulze, 2010).

Metal silos, characterized by their cylindrical shape and hermetic sealing, are constructed using galvanized iron sheets. This technology has proven highly effective in safeguarding harvested grains against infestations by storage insects and rodent pests. The hermetic nature of metal silos ensures the absence of oxygen inside, which leads to the demise of any internal insect pests. Furthermore, it serves as a complete barrier against any pests or pathogens that might attempt to infiltrate the grain (Terefa et al., 2011). To address concerns related to animal and human health, maximum allowable limits for *Fusarium* mycotoxins in wheat, maize grain, and their by-products have been established globally (Machado et al., 2021).

The primary objective of this research endeavor was to conduct an in-depth examination into the ever-changing dynamics of fungal populations and their associated mycotoxins within the context of wheat, maize, and sunflower storage. Specifically, we sought to gain a comprehensive understanding of how these intricate processes unfold when these cereals are stored within both vertical and conventional silos.

MATERIALS AND METHODS

The biological material in this study consisted of five samples each of corn, wheat, barley, and sunflower seeds (Figure 1). These samples were collected from two distinct deposition sites within Ilfov County, specifically from classic silos.

Figure 1. Samples of sun flowers, barley and corn

To prepare the seeds for placement on the culture medium within Petri plates, a series of meticulous steps were followed. First, the grains underwent a thorough washing process with tap water. Subsequently, they were subjected to disinfection by immersion in 96% ethyl alcohol for a duration of one minute, followed by two consecutive washes with sterile water to remove any remnants of disinfectant. The disinfected grains were then carefully dried on sterile filter paper. Once the grains were placed on the culture medium within the Petri dishes, these dishes were incubated at a controlled temperature of 22°C for a period of nine days. Following the incubation period, a comprehensive analysis of the samples was conducted, both through visual inspection and using a stereomicroscope. To facilitate the proper identification of the fungi that had developed on the surfaces of the grains, microscope slides were prepared.

The data we acquired were subjected to an analysis that involved the computation of the fungal contamination index. This index, denoted as ICS%, was calculated utilizing the following formula: ICS% = (number of contaminated seeds/total number of seeds on the plates) x 100. Furthermore, the evaluation of mycelial growth on the grains was conducted under the scrutiny of a stereomicroscope, and this examination process was further complemented by a microscopic assessment. To determine the prevalence of grains affected by *Fusarium*, a comprehensive analysis was performed by considering the proportion of grains exhibiting disease symptoms in relation to the total number of corn grains subjected to analysis.

RESULTS AND DISCUSSIONS

It is firmly established within the scientific community that mycotoxin contamination can manifest in agricultural products both during their growth in the field and subsequent storage.

These analyses were conducted at the diagnostic laboratory dedicated to plant protection, within the Research Center for Studies of Food Quality and Agricultural Products.

To examine the microflora, present in the samples, we employed seeds from wheat, maize, and sunflower, which were placed on PDA culture media. The progression of fungal growth was observed over a span of nine days during the incubation period.

The year 2022 presented significant challenges for the maize crop, marked by instances of heat and water stress during critical phases such as pollination and ripening. The thermal and water conditions recorded during the months of July and August 2022 exerted a detrimental impact on corn development.

As a consequence, the corn harvest in the year 2022 experienced a significant reduction in yields. Regrettably, the limited harvests obtained were also discovered to be tainted with mycotoxigenic fungi. Several critical factors play a role in the development of storage fungi. These factors include the moisture content of the stored grain, the temperature maintained within the grain storage facility, and the duration for which the grain is kept in storage. Naturally, the longer the grain is stored, the higher the susceptibility it bears to fungal infestations during storage. However, it is important to note that the progression of this process is heavily influenced by the specific conditions of storage.

In summary, the contamination of grains by fungal pathogens can occur at multiple stages in the grain's journey from the field to storage. It can initiate during harvest due to improper drying or exposure to moisture, continue during transportation in less-than-ideal conditions, and persist in storage if the environment is conducive to fungal growth. It is crucial to implement proper agricultural and storage practices to minimize the risk of fungal contamination and mycotoxin production in grains, ensuring the safety and quality of the final products for consumption. These developments subsequently lead to grain spoilage. The emergence of storage fungi on seeds results in seed rot, a decline in quality and nutritional value, and the production of mycotoxins by certain fungi, which pose toxic risks to both humans and animals. Grains affected by storage fungi can exhibit discoloration and, in some cases, emit a musty odor. Various fungi have the potential to induce grain spoilage during storage, with the most prevalent species being *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp., and *Rhizopus stolonifera*. These pathogens naturally occur in the environment but can proliferate in seed lots due to factors such as contaminated equipment or storage bins, mechanical damage to the seeds, and insect infestations.

Table 1 provides data illustrating the microflora associated with wheat, as well as seeds from wheat, maize, barley, and sunflower.

In the process of examining the various pathogens that impact the seeds within our collected samples, we made several key observations. Specifically, we identified a diverse microflora present within these seeds, which included fungal species belonging to the following genera: *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. These fungi have the potential to harm the quality and viability of seeds, and some may also produce mycotoxins that can pose health risks if seeds are used for food or animal feed. Identifying and understanding the presence of these pathogens is essential for implementing effective management strategies to protect seed quality and agricultural productivity.

Table 1. Microflora detected on the studied seeds

Hybrids	The pathogens				
	Alternaria spp	*Penicillium* spp	*Rhizopus* spp	*Fusarium* spp	*Aspergillius* spp
Glossa	-	-	+	+	+
P9889	-	+	+	+	+
Experto	+	-	+	-	-
Carioca	+	+	+	-	+
Cardinal	-	+	+	+	+

The examination of seeds harvested in the year 2022 revealed a presence of various micromycetes in the studied hybrids. These micromycetes included *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. Within this spectrum, *Penicillium* spp. and *Rhizopus* spp. were specifically identified on sunflower achenes, while the micromycete *Alternaria* spp. was observed on the caryopsis of wheat grains. Additionally, fruiting bodies associated with the genus *Fusarium* spp. were identified on the corn grains. This comprehensive analysis underscores the diversity of micromycetes present across different seed types within the 2022 harvest.

The presence of fusarium wilt in the 2022 corn crop is believed to be primarily attributed to climate changes, specifically characterized by elevated temperatures and prolonged periods of drought. This combination of factors can significantly contribute to contamination by *Fusarium* and *Penicillium*. Notably, the critical period for such contamination is during the ripening phase of corn. The summer of 2022 witnessed a particularly dry climate, marked by exceptionally high maximum temperatures, which likely exacerbated these conditions. In light of these environmental factors, it becomes increasingly essential to assess the extent of mycotoxin contamination through methods that are both rapid and precise. The phenomenon of global warming intensifies the susceptibility to fungal contamination, particularly in regions with warmer climates. This heightened temperature environment increases the risk of aflatoxin contamination in crops like corn and wheat. Aflatoxins are potent toxins produced by certain molds, such as Aspergillus, and they can proliferate more rapidly and abundantly in higher temperatures. The consequences of aflatoxin contamination are significant. It can render these crops unsuitable for use in both human food and animal feed. When crops are tainted with aflatoxins, they become a health hazard, as consuming contaminated food products can lead to severe health issues, including liver damage and an increased risk of cancer. Additionally, contaminated crops cannot be safely used as animal feed, as they can harm livestock and even enter the food chain indirectly through animal products like milk or meat. In summary, global warming exacerbates the risk of aflatoxin contamination in crops due to the elevated temperatures it brings, posing a serious threat to food safety and agricultural sustainability. This issue necessitates close monitoring and mitigation efforts to ensure the security of our food supply (as illustrated in Figure 2). This underscores the urgency of addressing the impact of climate change on crop health and safety.

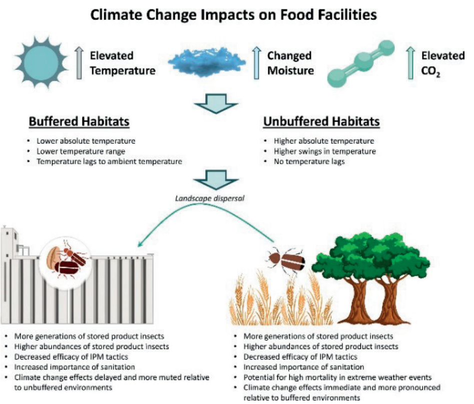

Figure 2. Climate change impact on food facilities (source: Gerken & Morrison, 2022)

The emergence of mycotoxins produced by *Fusarium* head blight (FHB) in wheat grains can be attributed to a multifaceted interplay of various factors. Among these factors, the climatic context plays a pivotal role, encompassing elements such as temperature, humidity, and precipitation patterns. Additionally, the choice of wheat variety, the specific cropping techniques employed, and the precision and reliability of mycotoxin measurement methodologies all contribute significantly to the complex matrix influencing mycotoxin occurrence in wheat grains. In summary, the occurrence of mycotoxins in wheat grains is influenced by a complex interplay of factors, including the choice of wheat variety, agricultural practices, and the accuracy of mycotoxin measurement methods. Furthermore, *Aspergillus* and *Fusarium* species, known pathogens in wheat, corn, and barley, can produce mycotoxins that pose serious health and safety risks, emphasizing the importance of monitoring and management in agriculture.

The most common isolated pathogenic species belong to the genus *Aspergillius* spp and *Fusarium* spp and have colonized the wheat seeds, corn and barley (Figure 3). The *Alternaria* spp fungus was identified on the wheat seeds and sun flowers. The *Peniciullium* spp fungus was identified on the Carioca and Cardinal strains, and the *Fusarium* spp fungus was identified on the Glosa variety (Figure 4). The *Rhizopus* spp fungus were identified on the Glossa, Experto and Cardinal.

Figure 3. *Aspergillus* spp. isolates from P9889

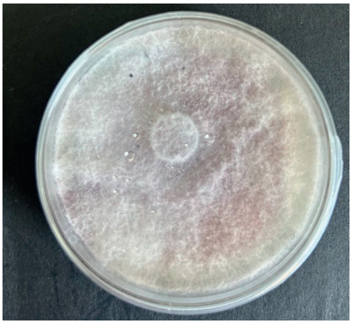

Figure 4. *Fusarium* spp. isolates from Glossa

CONCLUSIONS

In conclusion, our research underscores the dynamics of mycotoxin contamination in agricultural products, which can originate both in the field during growth and during storage. The year 2022 posed significant challenges for corn cultivation due to the adverse effects of high temperatures and prolonged dry periods, particularly during critical phases like pollination and ripening, which had an impact also on contaminatin with mycotoxigenic fungi. Prevalent species responsible for grain spoilage were *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp., and *Rhizopus stolonifera*. Warmer climates pose increased risks of aflatoxin contamination in crops like corn and wheat, potentially rendering them unfit for consumption or use as animal feed. Thus, addressing the impact of climate change on crop health and safety becomes an imperative.

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