MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Hungarian University of Agriculture and Life Sciences

# *Fusarium* species isolated from sorghum (*Sorghum bicolor* L. Moench) kernels and their mycotoxigenic potential and the effect of antioxidants on the mycotoxin biosynthesis

Szabó Barbara Katalin

Gödöllő

2024

**1. melléklet. A belső címoldal hátoldalának mintája**

**PhD School**

**Name:** Növénytudományi Doktori Iskola

**Discipline**: agrártudományok

**Head:** Prof. Dr. Lajos Helyes

Professor, PhD., D.Sc., CMHA

Hungarian University of Agriculture and Life Sciences

**Témavezető:** Dr. Katalin Orsolya Körösi

associate professor, PhD.

Hungarian University of Agriculture and Life Sciences

Plant Protection Institute

Department of Integrated Plant Protection

..... .....

Approval of Head of Doctoral School

Approval of Supervisor

# 1. Introduction and objectives

Nowadays, small crops, including sorghum (*Sorghum bicolor* L. Moench), are becoming increasingly popular, thanks to their increasing role in human food and livestock feed. Due to these two uses, the presence of mycotoxin-producing fungi is unavoidable even in small crops, since mycotoxin contamination can cause problems in the grain yield of many types of cereals. However, there is a little knowledge about the mycotoxin-producing fungi that appear on the mentioned cereales.

However, so far no research has been conducted on the detection and identification of *Fusarium* species infecting the kernels of sorghum varieties grown in Hungary, while the growing areas are constantly increasing and the climatic conditions (humidity and temperature during flowering) are favorable for Fusarium Grain Mold (FGM) caused by *Fusarium* species.

Mycotoxin contamination is also unavoidable in the case of sorghum. There are no effective regulations for the extent of mycotoxin contamination in grain-sorghum-based food industry products and feed materials.

**The objectives:**

1. Examining the presence of mycotoxin-producing fungi in sorghum kernel samples, conducting macro- and micromorphological examinations, and classifying the isolated fungi:
    i. internal infection tests
    ii. macro- and micromorphological examinations
2. Molecular genetic analysis of mycotoxin-producing genera that have been isolated using specific primers for the TEF1-α and calmodulin genes (cmd5/cmd6, CL1/CL2A). Sequencing of DNAs for accurate species-level identification, as well as macro- and micromorphological examination of the identified species.

3. Examination of the mycotoxin-producing capacity of the *Fusarium* species isolated from sorghum kernels (*F. proliferatum*, *F. verticillioides*, *F. subglutinans*, *F. cf. incarnatum*, *F. graminearum*, and *F. sporotrichioides*).
4. Identification of genes involved in aflatoxin production in *Aspergillus* species (*A. falvus* and *A. oryzae*) isolated from sorghum kernels.
5. Analysis of the mycotoxin contamination of sorghum samples (FB1, FB2, BEA, ZEA, HT-2, and T-2) using the LC/MS-MS method.
6. Examination of the lipophilic antioxidant composition of sorghum samples.
7. Investigation of the effect of selected lipophilic antioxidants (α-tocopherol, γ-tocopherol, lutein, and zeaxanthin) on the fumonisin biosynthesis of selected *Fusarium proliferatum* strain.

# 2. Materials and methodes

## 2.1. Examination of internal kernel contamination of sorghum samples

We collected samples of sorghum kernels in 2021 and 2022. After surface sterilization of the collected kernels were placed on Nash & Snyder's selective culture medium (Leslie and Summerell 2006. Kernels were examined in 10 replicates and 10 biological replicates. In order to be able to identify the outgrown fungal colonies at the genus level, the outgrown fungal colonies were inoculated onto chloramphenicol-containing potato dextrose medium (Potato Dextrose Agar (PDA)). To identify them at the genus level, the colonies were examined macro- and micromorphologically.

## 2.2. Molecular identification of *Fusarium, Aspergillus* and *Penicillium* species

To identify *Fusarium* species at the species level, it is essential to prepare monosporal cultures, as conidia of several *Fusarium* species may be found in each inoculated fungus (Geiser et al. 2004). Pure cultures of members of the genera *Aspergillus* and *Penicillium* were isolated on PDA medium. We inoculated the fungi identified at the genus level onto a new PDA culture medium before performing the DNA extraction. We performed DNA extractions using the ZR Fungal/Bacterial DNA MiniPrep Kit and phenol:chloroform (v/v, 1:1, pH 8.0).

During the molecular identifications, we worked with three sequence pairs. A special primer for the translation elongation factor (TEF-1α) was used for fungi belonging to the genus *Fusarium*. Special primer sequence pairs (cmd5/cmd6 and CL1/CL2A) for the calmodulin gene (CaM) were used for fungi belonging to the genera *Aspergillus* and *Penicillium*. In the case of fungi

belonging to the genera *Fusarium*, *Aspergillus*, and *Penicillium*, standard polymerase chain reaction (PCR) was used.

### 2.3. *Fusarium* strain cultivation to determine their toxigenic potential

*Fusarium* isolates were cultivated in carboxymethyl cellulose (CMC, Sigma-Aldrich) to prepare spore suspensions. After 3-4 days of incubation in CMC at 25°C and at 180 rpm in a Multitron incubator shaker (INFORS AG, Bottmingen, Switzerland), the spores were separated using a 40 µm membrane filter (Sefar Nitex, Switzerland), counted in a Malassez chamber and diluted with sterile water to reach a final concentration of 1 x $10^4$ spores/ml.

*F. graminearum, F. sporotrichioides*, and *F. cf. incarnatum* cultures were grown in Synthetic liquid medium (MS medium) (Boutigny et al., 2009) According to *F. graminearum, F. cf. incarnatum* isolates, the extraction of type B trichothecenes (TCTB) was performed based on the description of Montibus et al. (2021). For *F. sporotrichioides*, the medium was diluted with 50% methanol, vortexed, and filtered through a 0.45 µm, 15 mm diameter filter syringe prior to HPLTC-MS/MS analysis. Then we performed the analysis.

*F. verticillioides, F. proliferatum, F. cf. incarnatum* and *F. subglutinans* isolates were prepared in GAYEP (glucose-amylopectin-yeast extract-peptone) liquid medium, and then the amount of fumonisin in the investigated isolates was determined using UPLC according to Picot et al. (2013) as described.

### 2.4. Identification of the aflatoxin production promoting genes from *Aspergillus* species

During the PCR tests, we examined the presence of seven genes associated with mycotoxin production using the standard PCR method

described by Degola et al. (2007) and Gallo et al. (2012). During the experiment, we examined 21 isolates, of which 19 were *A. flavus* and 2 were *A. oryzae* isolates.

### 2.5. Examination of tocopherol and antioxidant composition of sorghum kernels

The studies on the tocochromanol composition were carried out by Lux et al. (2020). Tocochromanols were quantified by liquid chromatography coupled to a fluorescence detector (LC/FLD). The carotenoid composition was determined by liquid chromatography connected to a photodiode array detector (LC-DAD) (Savignac et al. 2023).

### 2.6. Impact of tocopherols and carotenoids on fungal growth of *F. proliferatum* and their mycotoxin biosynthesis in liquid media

Isolates were established and propagated in GAYEP liquid medium as described by Picot et al. (2013). The medium (GAYEP) was supplemented with 0.1 mM lutein, zeaxanthin, α-tocopherol, and γ-tocopherol. Fumonisins were extracted according to the protocol described by Picot et al. (2013).

### 2.7.Mycotoxin contamination of the sorghum samples

A total of 30 sorghum kernels were collected from 13 different places in Hungary. During the examination of mycotoxin contamination, we examined the toxins DON, T-2, HT-2, ZEA, BEA, FB1 according to Varga et al. (2021) based on the protocol.

# 3. Results

## 3.1. Results of the internal infection of sorghum kernels

Based on the internal infection values, the genus *Fusarium* was dominant in all samples, while the species of the genera *Aspergillus* and *Penicillium* appeared less frequently. Based on the tests of the two years, we found that the dominance of *Aspergillus* species increased in the year 2022.

## 3.2. Molecular identification of the detected genus

We identified a total of 9 different *Fusarium* species, of which 60 were *F. proliferatum*, 21 were *F. verticillioides*, 19 were *F. sporotrichioides*, 13 were *F. graminearum*, eight each were *F. thapsinum* and *F. cf. incarnatum*, seven *F. equiseti*, six *F. avenaceum*, and three *F. subglutinans* species were identified. The molecular genetic tests identified two *A. oryzae* and 19 *A. flavus* species. During the molecular genetic tests of *Penicillium* species, 27 *P. rubens*, 23 *P. chrysogenum*, 5 *P. allii*, 3 *P. hordei*, 1-1 *P. polonicum*, and *P. crustosum* species were identified. All the sequences have been deposited tot he NCBI GeneBank database.

## 3.3. Capacity of mycotoxin production of the identified *Fusarium* strains

Liquid culture media promoting the production of mycotoxins were used and mycotoxins were assessed in 14-day old broths. The *F. proliferatum* isolates were able to produce FB1, FB2 and FB3. The range of values of FB1, FB2 and FB3 toxins were from not detected (ND) to 14130µg/g, ND to 477µg/g, and ND to 532µg/g respectively. The average values of FB1, FB2 and FB3 toxins were 2956µg/g, 183µg/g, and 191µg/g. Regarding the *F. verticillioides* strains, only FB1 was quantified after 14 days of cultures. The range of levels was from ND to 177 µg/g, lower than that determined for *F.*

*proliferatum* isolates. Low levels of FB1 were also retrieved in *F. cf. incarnatum* and F. subglutinans broths. For *F. verticillioides*, *F. cf incarnatum* and *F. subglutinans*, traces of FB2 and FB3 with amounts under the limit of quantification were also observed.

The *F. graminearum* strains were shown to produce TCTB toxins such as deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON). The range of values of DON and 15-ADON were ND to 189µg/g. Similarly, the *F. cf. incarnatum* strains did not produce any TCTB toxin. The *F. sporotrichoides* isolates were analyzed for TCTA production; the levels of HT-2 and T-2 toxins were 0.96 µg/g and 6.79 µg/g respectively.

### 3.4. Identification of genes involved in mycotoxin production of *Aspergillus* isolates

Significant differences between the *Aspergillus* isolates were observed during the examination of the genes involved in mycotoxin production. In the case of two samples, we detected the presence of all genes, i.e. we obtained a positive result for all genes promoting mycotoxin production.

The presence of the *aflD* gene in 12 isolates of *A. flavus* and 1 isolate of *A. oryzae* were detected. The presence of the *aflM* gene was detected in the DNA of 15 *A. flavus* and 2 *A. oryzae*. We detected the *aflO* gene in 19 *A. flavus* and 1 *A. oryzae* cases. During gel electrophoresis, a 790-bp PCR product confirmed the presence of the gene. In the case of the *aflP* gene, we discovered its presence at 870 bp in 12 *A. flavus* and 1 *A. oryzae*. The presence of the *aflQ* gene associated with aflatoxin production was found in the DNA of 9 *A. flavus* and 1 *A. oryzae* of the isolated fungi. During gel electrophoresis, we detected the *aflR* gene at 1079 bp in 15 *A. flavus* and 2 *A. oryzae* samples. At 684 base pairs, the *aflS* gene was detectable in 12 A. *flavus* and 2 *A. oryzae*.

### 3.5. The main lipophilic antioxidants in sorghum kernels

The levels of carotenoids and tocochromanols were measured in grains of the two different genotypes of sorghum. The total amounts of tocochromanols in genotype1 and genotype2 were 23.87 μg/g and 23.35 μg/g which accounted for nearly 98% of the total of lipophilic antioxidants in both genotypes. The total amount of carotenoids in genotype1 and genotype2 were 0.51 μg/g and 0.43 μg/g which accounted for 2.13% and 1.84% of the total of lipophilic antioxidants, respectively. Among carotenoids, the percentages of lutein and zeaxanthin in the two different genotypes were quite similar, lutein representing about 53% and zeaxanthin 47% of the total carotenoids. Our results indicate that the main carotenoids and tocochromanols found in sorghum grain samples included lutein and zeaxanthin, γ-tocotrienol, γ-tocopherol, α-tocotrienol, α-tocopherol and δ-tocotrienol.

### 3.6. Impact of tocopherols and carotenoids on fungal growth of F. proliferatum, and their mycotoxin biosynthesis in liquid media

The antifungal effect and mycotoxin inhibitory activity of major carotenoids and tocochromanols occurring in sorghum grains were investigated. After examining the effects of lutein (0.1 mM), zeaxanthin (0.1 mM), γ-tocopherol (0.1 mM), and α-tocopherol (0.1 mM) on dry biomass, we found that zeaxanthin did not have a statistically significant effect on the biosynthesis of fumonisins. Supplementing the strain with the above-mentioned carotenoid composition resulted in a decrease in fumonisin production, while lutein also reduced fumonisin accumulation, as the toxin tests only detected fumonisin particles. However, in terms of the effects of the examined antioxidants, we could not show a significant difference in any case. In the case of α-tocopherol (0.1 mM), the strain's fumonisin production was

74.57%, and the effect of γ-tocopherol on fumonisin biosynthesis was 3.34%. In the case of the effect on dry biomass, the amount of biomass was reduced by γ-tocopherol and α-tocopherol, as well as by lutein; however, we could not detect a significant difference in this case either.

### 3.7. Mycotoxin contamination of the sorghum kernels

The multi-mycotoxin analysis of sorghum kernels were perfomed in 3 replicates for each samples. DON and T2 toxins were not detected in the samples. Beauvericin (BEA) were found in the 40 % of the samples. The avarage vaule of BEA contamination in our sorghum kernels was 17.9 ng/g. Regarding to fumonisin B1, it was detected only in 5 samples the avarage contamination in the 5 samples was 115.0 ng/g. HT-2 and ZEA toxins contamination were detected in 2 samples of our sourghum kernels.

# 4. Conclusions and outlook

## 4.1. Examination of internal infections of sorghum kernels

Previous studies have shown that the presence of *Fusarium* species isolated from sorghum is common worldwide (Zummo 1984; Summerell et al. 2003; Little et al. 2011; Astoreca et al. 2019). As a result of our experiments with sorghum, it can be said that in most of the samples, we detected an internal infection caused by mycotoxin-producing fungi. Based on the examination of pure cultures of fungi isolated from kernels, we found that in the case sorghum, *Fusarium* species were dominant as the species causing internal kernel infection; however, *Aspergillus* and *Penicillium* species also appeared in all samples. Internal kernel infection can also be influenced by certain environmental parameters, such as cultivation technology, biotic factors, and crop rotation. The *Fusarium* species can penetrate the kernels through the so-called penetration hyphae, and in the case of the *F. graminearum* species, 24 different cell wall-degrading enzymes are distinguished, of which cutinase, lipases, and pectinases play the main role during the infection of kernels (Kikot et al. 2009). The extreme drought weather conditions of 2022 may also have contributed to the development of kernels' internal infection levels.

## 4.2. The isolated and identified mycotoxin-producing fungi

During the molecular genetic examinations 145 *Fusarium* isolates were identified. Under Hungarian cultivation conditions, the species *F. verticillioides* was identified from the stem of sorghum (Szécsi et al. 2010). In the case of sorghum kernels, based on our experiments, we came to the conclusion that *F. proliferatum* is the most frequently occurring *Fusarium* species in sorghum grains. This was followed by *F. sporotrichioides*, *F. verticillioides*, *F. graminearum*, *F. thapsinum*, *F. cf. incarnatum*, *F. equiseti*,

*F. avenaceum*, and *F. subglutinans*. Among the *Fusarium* species identified, *F. thapsinum*, *F. cf. incarnatum*, and *F. equiseti* were identified at the first time from cereales in Hungary. From the point of view of the dominance of individual *Fusarium* species, as the results of the thesis also support, the *F. proliferatum* species is dominant, *i.e.*, one of the members of the *F. fujikuroi* disease complex. In the case of domestic maize cultivation, *F. verticillioides* and *F. graminearum* species dominate, and in the case of wheat, *F. graminearum*, *F. poae*, and *F. avenaceum* species dominate; however, we do not have recent research data in this regard in the last 2-3 years, so it can be assumed that there has also been a change in dominance conditions. All identified species were identified worldwide from sorghum grain; however, in the case of sorghum grain, the species *F. andiyazi* was also identified, which we did not detect during our experiments (Bottalico and Perrone, 2002; Chala et al., 2019; Prom és Iskeit 2021; Ferrigo et al., 2022; Corallo et al., 2023). Our results are consistent with previous research related to sorghum kernels (Sharma et al., 2011; Kelly et al., 2016).

During the isolation of *Aspergillus* species, 23 pure cultures were created, from which 19 *A. flavus* and 2 *A. oryzae* were identified during molecular genetic tests. Both identified species belong to the section *Flavi*, which is extremely important from the point of view of agriculture, biotechnology, and human and animal health (Frisvad et al. 2018). *A. flavus* is a species often found on agricultural plants that is often isolated from many parts of the world, and due to their aflatoxin-producing ability, they pose a major threat during food and feed safety (Palencia et al. 2010; Riba et al. 2010). The species *A. oryzae* has also been isolated in many cases, but due to its non-aflatoxigenic properties, its presence is considered safe. Furthermore, the fermentation industry still uses the species for various fermentation processes (Chang and Ehrlich 2010). In domestic conditions, the species *A.*

*flavus* has also been identified in many cases from maize kernels (Baranyi et al. 2015; Tóth et al. 2012; Dobolyi et al. 2013; Sebők et al. 2016). A 2012 EFSA scientific announcement drew attention to the increasing number of *Aspergillus* species appearing in the kernels of some cereals as a result of extreme drought stress (Battilani et al. 2012). The scenarios presented in the report are becoming a reality today, as our research results confirm that the *Aspergillus* species are becoming more and more common, which pose various threats to the food and feed chain due to aflatoxin contamination.

Prior to molecular genetic studies of *Penicillium* species, we created 60 pure cultures, during which we identified the following species: *P. rubens*, *P. chrysogenum*, *P. polonicum*, *P. hordei*, *P. crustosum*, and *P. allii*. The species *P. rubens* and *P. chrysogenum* have been isolated from maize kernels in many cases in Hungary, and in the case of some fruits, tests of the antifungal protein of *P. chrysogenum* have also been carried out. PAF (Penicillium Antifungal Protein) can also affect filamentous fungi, yeasts and bacteria. PAF is an antifungal protein that triggers ROS-mediated apoptotic cell death in *Fusarium* and *Aspergillus* species (Kovács et al. 2014; Galgóczy et al. 2013). Knowing these facts, it is likely that the *Aspergillus* and *Fusarium* species were present in much smaller numbers from the places of origin from which the *P. chrysogenum* species were isolated (Delgado et al. 2015; Martínez-Culebras et al. 2021). The species has been isolated from sorghum grains worldwide in many cases (Vankudoth et al. 2015; Gupta 1996).

### 4.3. Macro- and micromorphological studies of the identified Fusarium species

In the case of the nine identified *Fusarium* species, we found during the macro- and micromorphological studies that the species we detected and

identified have characteristic properties for the given species (Leslie and Summerell 2006).

### 4.4. Capacity of mycotoxin production of the isolated Fusarium species

*Fusarium* species are known to produce various mycotoxins. However, the extent of mycotoxin production can vary between and within species (Crous et al. 2021). Fumonisins (FBs) are the main mycotoxins produced by *F. verticillioides*. The identified *F. verticillioides* species, with the exception of the INVT_035 isolate, are capable of producing FB1 toxin. It was previously shown that *F. proliferatum* species can produce different amounts of FB1, FB2, and FB3 toxins (Corallo et al. 2023), which were isolated from sorghum of different genotypes. In addition, a significant number of *F. proliferatum* isolates are capable of producing highly toxic fumonisins (Sharma et al. 2011; Vismer et al. 2019; Corallo et al. 2023). The *F. proliferatum* INVT_018 isolate we identified proved to be the highest producer of FB1, FB2, and FB3. Research results on the production of fumonisin by *F. subglutinans* are different; in some cases, fumonisin production has also been observed in the case of the species *F. subglutinans* (Stępień et al. 2013; Wang et al. 2014; Fumero et al. 2015). The *F. subglutinans* strain lacks the *Fum* genes required for fumonisin biosynthesis, according to the literature (Fumero et al. 2020). The *F. subglutinans* species identified in our study was able to synthesize a small amount of FB1 toxin. During our experiments, it is likely that the tested isolate possessed the *Fum* gene and therefore could produce a small amount of mycotoxin.

*F. cf. incarnatum* is a member of the *Fusarium incarnatum-equiseti* species complex (FIESC), which is able to produce both fumonisins and type B trichothecenes (Villani et al. 2016; Villani et al. 2019). During our research,

the identified and selected *F. cf. incarnatum* isolates were investigated for their mycotoxinogenic potential for fumonisins and TCTB toxins. In our study, the isolated *F. cf. incarnatum* isolate INVT_007 is capable of producing small amounts of FB1 and FB3 toxins; isolate INVT_009 produced only FB1; and strain INVT_008 did not produce any of the toxins named above or tested at all. The *F. cf. incarnatum* has different regulatory genes responsible for the production of type B trichothecenes, such as the *tri5, tri4, tri13*, and *tri7* genes, and also has genes responsible for fumonisin production, such as *tri3* and *tri11*. It is important to highlight that the *tri4* and *tri5* genes are also involved in fumonisin biosynthesis (Desjardins 2006).

Corallo et al. (2023) isolates of *F. graminearum* identified from sorghum kernels produced mostly zearalenone (ZEN) and deoxynivalenol (DON) toxins, and in some cases nivalenol (NIV). Contrary to the literature, our selected F. graminearum isolates, especially the INVT_010, INVT_011, and INVT_012 isolates, produced large amounts of 15ADON and DON toxins; however, during our experiments, we did not experience the synthesis of ZEN in the given isolates, which may presumably indicate the absence of the *zeb1* gene (Alexander et al. 2003), and the production of mycotoxins by Fusarium species can be affected by many other factors, such as environmental factors such as moisture and high temperature. The year 2021 was a wetter year, which may have affected the mycotoxin production ability of the isolated pathogens.

According to Edwards et al. (2012), *F. sporotrichioides* typically produces type A trichothecenes (TCTA), such as HT-2 and T-2 toxins, and at the same time, the *F. sporotrichioides* isolate we identified (INVT_026) is also capable of producing HT-2 and T-2 toxins; however, strain INVT_024 did not produce TCTA toxins.

Potential mycotoxin production can cause problems in both the food and feed chains. Due to the reasons mentioned, it is necessary to pay special attention to the *Fusarium* species that cause grain mold (FGM).

### 4.5. Examination of genes associated with aflatoxin production in isolated Aspergillus species

According to Gallo et al. (2012) the *aflQ* gene could be detected in large numbers in non-aflatoxigenic isolates. The *aflQ* gene was amplified 10 times in the samples we examined, which suggests that the 10 *Aspergillus* species probably belong to the group considered non-aflatoxigenic in Gallo et al.'s 2012 research. However, since the presence or absence of a single gene does not clearly determine the mycotoxin production capacity, it is also important to evaluate the results of the other tested genes.

In the case of the *aflR* and *aflS* genes, the close correlation between the presence of the genes and the ability to produce aflatoxin was proven by most of them (Degola et al. 2007; Gallo et al. 2012). Thus, in the case of our own samples, the presence of these genes those isolates can be considered as aflatoxigenic isolates.

### 4.6.Impact of tocopherols and carotenoids on fungal growth of F. proliferatum, and their mycotoxin biosynthesis in liquid media

The main carotenoid and tocopherol compounds found in our sorghum grain samples are lutein and zeaxanthin, γ-tocotrienol, γ-tocopherol, α-tocotrienol, α-tocopherol, and δ-tocotrienol. The main composition of tocopherols in sorghum grains is γ-tocopherol and α-tocopherol, which contain lutein and zeaxanthin from carotenoids. Several studies demonstrate that sorghum contains carotenoids and tocochromanols (tocopherols and tocotrienols) (Shahidi and Costa de Camargo 2016; Elvira-Torales et al.

2019). However, knowledge about the composition of carotenoids and tocochromanols in grain sorghum samples is still limited, and the results are mostly related to sorghum varieties grown in semi-arid areas (Kean et al. 2007; de Morais Cardoso et al. 2015; Mawouma et al. 2022). The aforementioned studies showed that xanthophylls (lutein and zeaxanthin) and tocopherols were the dominant compounds within the carotenoid and tocochromanol families. At a concentration of 0.1 mM, γ-tocopherol, α-tocopherol, lutein, and zeaxanthin were tested for their effect on fumonisin production and dry biomass of F. proliferatum strain I58. The effect of lipophilic antioxidants depended on the type of antioxidant. In this study, 0.1 mM lutein reduced fumonisin production by strain I58. Other research looked at how α-tocopherol, lutein, zeaxanthin, β-carotene, and ferulic acid affected the production of fumonisin and the amount of dry biomass in *F. verticillioides* (Picot et al. 2013). In this study, lutein significantly reduced fumonisin accumulation. During our tests, we could not detect any significant differences in the effect of antioxidants on fumonisin biosynthesis. However, as detailed by Savignac et al. (2022, 2023), there are strong arguments for these compounds to potentially play a key role in plant defense, considering their ability to alleviate ROS-induced oxidative stress, their ability to interfere with plant hormone signaling, and their ability to reduce fungal growth and mycotoxin contamination. During the evaluation of the bioactivity of α- and γ-tocopherol, as well as lutein and zeaxanthin, against *F. proliferatum*, no fungal growth inhibition was observed, regardless of *Fusarium* species and lipophilic antioxidants. Furthermore, only lutein showed a decrease in FB yield. The inhibitory effect of lutein on FB biosynthesis has already been reported (Picot et al. 2013). The oxidative stress-relieving ability of lutein and zeaxanthin, demonstrated to stimulate the biosynthesis of various mycotoxins (Montibus et al. 2015), could potentially explain this inhibitory effect.

However, Elvira-Torales et al. 2019 recognize tocochromanols as strong antioxidant compounds, and their lack of effect clearly indicates the existence of additional mechanisms underlying the bioactivity of carotenoids.

### 4.7.Mycotoxin contamination of sorghum kernels

During mycotoxin contamination tests, 40% of grain sorghum samples were contaminated with beauvericin (BEA). Fusarium species producing BEA are: *F. sambucinum*, *F. acuminatum*, *F. oxysporum*, *F. poae*, *F. equiseti*, *F. avenaceum* (Munkvold et al. 1998). However, previous studies reported that both F. proliferatum and F. subglutinans can produce BEA (Logrieco et al., 1993; Plattner et al., 1994; Moretti et al., 1996). BEA belongs to the "new" group of mycotoxins, the so-called „emerging" mycotoxins. Fumonisin B1, HT-2 and zearalenone contamination was detected in some samples. The presence of *Fusarium* species in sorghum grains poses a huge risk to food and feed safety due to the mycotoxinogenic potential of these species. Regarding the contamination of HT-2 and T-2 toxins, the European Commission has defined an indicative limit value (EU 2013/165).

# 5. Új tudományos eredmények

1. We proved the presence of mycotoxin-producing fungi capable of mycotoxin production on sorghum kernels from domestic cultivation areas using a selective culture medium in laboratory conditions, during which we identified them using molecular genetic methods and deposited them in the NCBI GeneBank:
    i. nine *Fusarium* species: *F. proliferatum, F. verticillioides, F. subglutinans, F. cf. incarnatum, F. equiseti, F. graminearum, F. thapsinum, F. sporotrichioides, F. avenaceum,*
    ii. two *Aspergillus* species: *A. flavus, A. oryzae,*
    iii. six *Penicillium* species: *P. chrysogenum, P. rubens, P. allii, P. hordei P. polonicum és P. crustosum*.
2. We were the first to isolate *F. cf. incarnatum, F. equiseti, F. thapsinum* pathogens from sorghum kernels in Hungary, which was also supported by molecular genetic methods. The mentioned *Fusarium* species have not yet been identified from other cereals in Hungary.
3. We found that in our country *F. proliferatum* is the dominant *Fusarium* species in sorghum kernels.
4. We found that the *Fusarium* species isolated from the Hungarian production site are also capable of producing A and B type trichothecenes and fumonisins (B1, B2, B3) in sorghum kernels.
5. We found that the mycotoxins beauvericin, fumonisin B1, HT-2 toxin and zearalenone are present in Hungarian sorghum kernels. It should be highlighted that this is the first study that confirmed the presence of beauvericin as an emerging mycotoxin in the case of cereal from domestic production sites.
6. We found that grain sorghum is capable of producing lipophilic antioxidants to a large extent, these lipophilic antioxidants are lutein and

zeaxanthin, γ-tocotrienol, γ-tocopherol, α-tocotrienol, α-tocopherol and δ-tocotrienol. A research result similar to this has not yet been produced in the case of grain sorghum in Europe.

7. We found that lutein, as a lipophilic antioxidant, has a negative effect on the fumonisin biosynthesis of the *F. proliferatum* strain.

# 6. References


1. Alexander, N.J., Proctor, R.H., McCormick, S.P. (2009): Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in *Fusarium*. *Toxin Reviews*, 28 (3) 198-215.p.

2. Astoreca, A.L., Emateguy, L.G., and Alconada, T.M. (2019): Fungal Contamination and Mycotoxins Associated with Sorghum Crop: Relevance Today. *European Journal of Plant Pathology*. 155 381-392. p.

3. Baranyi, N., Despot, D. J., Palágyi, A., Kiss, N., Kocsubé, S., Szekeres, A., Kecskeméti, A., Bencsik, O., Vagvölgyi, C., Segvic Klaric, M., Varga, J. (2015): Identification of *Aspergillus* species in Central Europe able to produce G-type aflatoxins. *Acta Biologica Hungarica*, 66 339-347. p.

4. Battilani, P., Rossi, V., Giorni, P., Pietri, A., Gualla, A., Van der Fels-Klerx, H. J., Booji, A., Moretti, A., Logrieco, A., Miglietta, F., Toscano, P., Miraglia, M., De Santis, B., Brera, C. (2012): Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate change. *EFSA Supporting Publications*, 9 (1) 223E.

5. Bottalico, A., Perrone, G. (2002): Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology*, 108 611-624.p.

6. Boutigny, A-L., Barreau, C., Atanasova-Penichon, V., Verdal-Bonnin, M-N., Pinson-Gadais, L., Richard-Forget, F. (2009): Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and Tri gene expression in *Fusarium* liquid cultures. *Mycological Research*, 113 (6-7) 746–753. p.

7. Chala, A., Degefu, T., Brurberg, M. B. (2019): Phylogenetically diverse *Fusarium* species associated with sorghum (*Sorghum bicolor* L. Moench) and finger millet (*Eleusine coracana* L. Garten) grains from Ethiopia. *Diversity*, 11 (6) 93.p.

8. Corallo, A.B., Del Palacio, A., Oliver, M., Tiscornia, S., Simoens, M., Cea, J., de Aurrecoechea, I., Martínez, I., Sanchez, A., Stewart, S., Pan, D. (2023): *Fusarium* Species and Mycotoxins Associated with Sorghum Grains in Uruguay. *Toxins,* 15 (8) 484. p.

9. Crous, P.W., Lombard, L., Sandoval-Denis, M., Seifert, K.A., Schroers, H-J., Chaverri, P., Gené, J., Guarro, J., Hirooka, Y., Bensch, K., Kema, G.H.J., Lamprecht, S.C., Cai, L., Rossman, A.Y., Stadler, M., Summerrbell, R.C., Taylor, J.W., Ploch, S., Visagie, C.M., Yilmaz, N., Thines, M. (2021): *Fusarium*: more than a node or a foot-shaped basal cell. *Studies in Mycology*, 98 1-184. p.

10. de Morais Cardoso, L., Pinheiro, S.S., da Silva, L.L., de Menenzes, C.B., de Carvalho, C.W.P., Tardin, F.D., Vieira Queiroz, V.A., Duarte Martino, H.S., Pinheiro-Sant'Ana, H.M. (2015): Tocochromanols and carotenoids in sorghum (*Sorghum bicolor* L.): Diversity and stability to the heat treatment. *Food Chemistry,* 172 900-908. p.

11. Degola, F., Berni, E., Dall'Asta, C., Spotti, E., Marchelli, R., Ferrero, I., Restivo, F.M. (2007): A multiplex RT-PCR approach to detect aflatoxigenic strains of Aspergillus flavus. *Journal of Applied Microbiology*, 103 (2) 409–417. p.

12. Delgado, J., Owens, R. A., Doyle, S., Asensio, M. A., & Núñez, F. (2015): Impact of the antifungal protein PgAFP from *Penicillium chrysogenum* on the protein profile in *Aspergillus flavus*. *Applied Microbiology and biotechnology*, 99 (20) 8701-8715. p.

13. Desjardins, A.E. (2006): Fusarium mycotoxins, chemistry, genetics, and biology. *American Phytopathological Society APS Press*, St. Paul Minnesota, USA. 260. p.

14. Dobolyi, C. S., Sebők, F., Varga, J., Kocsubé, S., Szigeti, G., Baranyi, N., Szécsi, Á., Tóth, B., Varga, M., Kriszt, B., Szoboszlay, S., Krifaton, C.,

Kukolya, J. (2013): Occurrence of aflatoxin producing *Aspergillus flavus* isolates in maize kernel in Hungary. *Acta Alimentaria*, 42 (3) 451-459. p.

15. EC 1881/2006 – Commission Regulation – setting maximum levels for certain contaminants in foodstuffs.

16. Edwards, S.G., Imathiu, S.M., Ray, R.V., Back, M., Hare, M.C. (2012): Molecular studies to identify the *Fusarium* species responsible for HT-2 and T-2 mycotoxins in UK oats. *International Journal of Food Microbiology*, 156 (2) 168-175.p.

17. Elvira-Torales, L.I., García-Alonso, J., and Periago-Castón, M.J. (2019): Nutritional Importance of Carotenoids and Their Effect on Liver Health: A Review. *Antioxidants,* 8 (229) 1-23. p.

18. FAOSTAT (2023): Food and Agriculture Organization of the United Nations – Food and agriculture data

19. Ferrigo, D., Mondin, M., & Raiola, A. (2022): Pathogenic and Genetic Characterization of *Fusarium verticillioides* Strains Collected from Maize and Sorghum Kernels. *Agriculture,* 13 (1) 105. p.

20. Frisvad, J., Hubka, V., Ezekiel, C., Hong, S.-B., Nováková, A., Chen, A., Arzanlou, M., Larsen, T., Sklenář, F., Mahakarnchanakul, W. (2018): Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. *Studies in Mycology,* 93 (1) 1–63. p.

21. Fumero, M. V., Villani, A., Susca, A., Haidukowski, M., Cimmarusti, M. T., Toomajian, C., Leslie, J.F., Chulze, S.N., Moretti, A. (2020): Fumonisin and beauvericin chemotypes and genotypes of the sister species *Fusarium subglutinans* and *Fusarium temperatum*. *Applied and Environmental Microbiology,* 86 (13) e00133-20.

22. Fumero, M.V., Reynoso, M.M., Chulze, S. (2015): *Fusarium temperatum* and *Fusarium subglutinans* isolated from maize in Argentina. *International Journal of Food Microbiology*, 199 86-92.p.

23. Galgóczy, L., Virágh, M., Kovács, L., Tóth, B., Papp, T., & Vágvölgyi, C. (2013): Antifungal peptides homologous to the *Penicillium chrysogenum* antifungal protein (PAF) are widespread among Fusaria. *Peptides*, 39, 131-137. p.

24. Gallo, A., Stea, G., Battilani, P.F., Logrieco, A., Perrone, G. (2012): Molecular characterization of an *Aspergillus flavus* population isolated from maize during the first outbreak of aflatoxin contamination in Italy. *Phytopathololgia Mediterranea*, 51 (1) 198−206. p.

25. Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N., Kuldau, G.A., O'Donnell, K. (2004): FUSARIUM-ID v 1.0.: A DNA sequence database for identifying Fusarium. *European Journal of Plant Pathology*, 110: 473-479.

26. Kean, E.G., Ejeta, G., Hamaker, B.R. and Ferruzzi, M. (2007): Characterization of Carotenoid Pigments in Mature and Developing Kernels of Selected Yellow-Endosperm Sorghum Varieties. *Journal of Agricultural and Food Chemistry,* 55 (7) 2619-2626. p.

27. Kelly, A., Proctor, R.H., Belzile, F., Chulze, S.N., Clear, R.M., Cowger, C., Elmer, W., Lee, T., Obanor, F., Waalwijk, C. (2016): The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from Fusarium graminearum. *Fungal Genetics and Biology*, 95 39–48. p.

28. Kikot, G.E., Hours, R.A., Alconada, T.M. (2009): Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: a review. *Journal of basic microbiology*, 49 (3) 231-241.p.

29. Kovács, B., Hegedűs, N., Bálint, M., Szabó, Z., Emri, T., Kiss, G., Antal, M., Pócsi, I., Leiter, É. (2014): *Penicillium* antifungal protein (PAF) is involved in the apoptotic and autophagic processes of the producer

*Penicillium chrysogenum*. *Acta Microbiologica et Immunologica Hungarica*, 61 (3) 379-388. p.

30. Leslie, J.F., Summerell, B.A. (2006): The Fusarium Laboratory Manual. Blackwell Publishing, Oxford, UK. 388. p.

31. Little, C. R., Perumal, R., Tesso, T. T., Prom, L. K., Odvody, G. N., Magill, C. W. (2011): Sorghum pathology and biotechnology-A fungal disease perspective: part I. Grain mold, head smut, and ergot. *European Journal of Plant Science and Biotechnology*, 6 (Special Issue 1) 10-30. p.

32. Logrieco, A., Moretti, A., Ritieni, A., Chelkowski, J., Altomare, C., Bottalico, A., Randazzo, G. (1993): Natural occurrence of beauvericin in preharvest Fusarium subglutinans infected corn ears in Poland. *Journal of Agricultural and Food Chemistry*, 41 (11) 2149-2152.p.

33. Lux, P.E., Freiling, M., Stuetz, W., von Tucher, S., Carle, R., Steingass, C.B., Frank, J. (2020): (Poly) phenols, carotenoids, and tocochromanols in corn (*Zea mays* L.) kernels as affected by phosphate fertilization and sowing time. *Journal of Agricultural and Food Chemistry*, 68 (2) 612-622.p.

34. Martínez-Culebras, P.V., Gandía, M., Boronat, A., Marcos, J.F., Manzanares, P. (2021): Differential susceptibility of mycotoxin-producing fungi to distinct antifungal proteins (AFPs). *Food Microbiology*, 97 103760.p.

35. Mawouma, S., Condurache, N.N., Turturică, M., Constantin, O.E., Croitoru, C., Rapeanu, G. (2022): Chemical composition and antioxidant profile of sorghum (*sorghum bicolor* (L.) moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) grains cultivated in the far-North region of Cameroon. *Foods*, *11* (14) 2026. p.

36. Montibus, M., Pinson-Gadais, L., Richard-Forget, F., Barreau, C., Ponts, N. (2015): Coupling of transcriptional response to oxidative stress and

secondary metabolism regulation in filamentous fungi. *Critical reviews in microbiology*, 41 (3) 295-308. p.

37. Montibus, M., Vitrac, X., Coma, V., Loron, A., Pinson-Gadais, L., Ferrer, N., Verdal-Bonnin, M.N., Gabaston, J., Waffo-Téguo, P., Richard-Forget, F., Atanasova, V. (2021): Screening of wood/forest and vine by-products as sources of new drugs for sustainable strategies to control Fusarium graminearum and the production of mycotoxins. *Molecules*, 26(2), 405.p.

38. Moretti, A., Logrieco, A., Bottalico, A., Ritieni, A., Fogliano, V., Randazzo, G. (1996): Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (Fusarium section Liseola). *Sydowia*, 48 (1) 44–56.p.

39. Munkvold, G., Stahr, H.M., Logrieco, A., Moretti, A., Ritieni, A. (1998): Occurrence of fusaproliferin and beauvericin in *Fusarium* contaminated livestock feed in Iowa. *Applied Environmental Microbiology*, 64 (10) 3923–3926. p.

40. Palencia, E.R., Hinto, D.M., Bacon, C.W. (2010): The black *Aspergillus* species of maize and peanuts and their potential for mycotoxin production, *Toxins*, 2 (4) 399-416. p.

41. Picot, A., Atanasova-Pénichon, V., Pons, S., Marchegay, G., Barreau, C., Pinson-Gadais, L., Roucolle, J., Daveau, F., Caron, D., Richard-Forget, F. (2013): Maize Kernel Antioxidants and Their Potential Involvement in Fusarium Ear Rot Resistance. *Journal of Agricultural and Food Chemistry*, 61 (14) 3389-3395. p.

42. Plattner, R.D., Nelson, P.E. (1994): Production of beauvericin by a strain of Fusarium proliferatum isolated from corn fodder for swine. *Applied and Environmental Microbiology*, 60 (10) 3894-3896.p.

43. Prom, L.K., Isakeit, T. (2021): Diversity and frequency of fungal genera from sorghum lines inoculated with Alternaria alternata alone and in

combination with Curvularia lunata and Fusarium thapsinum in a field infected with anthracnose. *Journal of Agricultural and Crop Research,* 9 (11) 264-267.p.

44. Riba, A., Bouras, N., Mokrane, S., Mathieu, F., Lebrihi, A., Sabaou, N. (2010): *Aspergillus* section *Flavi* and aflatoxins in Algerian wheat and derived products. *Food and Chemical Toxicology*, 48 (10) 2772-2777.p.

45. Savignac, J. M., Atanasova, V., Chereau, S., Ducos, C., Gallegos, N., Ortega, V., Ponts, N., Richard-Forget, F. (2023): Carotenoids Occurring in Maize Affect the Redox Homeostasis of Fusarium graminearum and Its Production of Type B Trichothecene Mycotoxins: New Insights Supporting Their Role in Maize Resistance to Giberella Ear Rot. *Journal of Agricultural and Food Chemistry,* 71 (7) 3285-3296. p.

46. Savignac, J.M., Atanasova, V., Chéreau, S., Ortéga, V., Richard-Forget, F. (2022): Role of tocochromanols in tolerance of cereals to biotic stresses: Specific focus on pathogenic and toxigenic fungal species. *International Journal of Molecular Sciences,* 23 (16) 9303. p.

47. Sebők, F., Dobolyi, C., Zágoni, D., Risa, A., Krifaton, C., Hartman, M., Cserháti, M., Szoboszlay, S., Kriszt, B. (2016): Aflatoxigenic *Aspergillus flavus* and *Aspergillus parasiticus* strains in Hungarian maize fields. *Acta Microbiologica et Immunologica Hungarica,* 63 (4) 491-502. p.

48. Shahidi, F., Costa de Camargo, A. (2016): Tocopherols and Tocotrienols in Common and Emerging Dietary Sources: Occurance, Applications, and Health Benefits. *International Journal of Molecular Sciences,* 17 (1745) 1-29. p.

49. Sharma, R., Thakur, R.P., Senthilvel, S., Nayak, S., Reddy, S.V., Rao, V.P., Varshney, R.K. (2011): Identification and characterization of toxigenic Fusaria associated with sorghum grain mold complex in India. *Mycopathologia*, 171 223-230.p.

50. SORGHUM ID (2022): európai, szakmaközi cirokszervezet. https://www.sorghum-id.com/hu/fooldal/ (Megtekintés időpontja: 2022. február 22.)

51. Stępień, Ł. (2014): The use of Fusarium secondary metabolite biosynthetic genes in chemotypic and phylogenetic studies. *Critical Reviews in Microbiology*, 40 (2) 176–185. p.

52. Summerell, B. A., Salleh, B., Leslie, J. F. (2003): A utilitarian approach to Fusarium identification. *Plant disease,* 87 (2) 117-128. p.

53. Szécsi, Á., Szekeres, A., Bartók, T., Oros, G., Bartók, M. Mesterházy, Á. (2010): Fumonisin B1-4 producing capacity of Hungarian Fusarium verticillioides isolates. *World Mycotoxin Journal,* 3 (1) 67-76. p.

54. Tóth, B., Török, O., Kótai, É., Varga, M., Toldiné Tóth, É., Pálfi, X., Háfra, E., Varga, J., Mesterházy, Á. (2012): Role of *Aspergilli* and *Penicillia* in mycotoxin contamination of maize in Hungary. *Acta Agronomica Hungarica*, 60 (2) 143-149. p.

55. Vankudoth, K. R., Sivadeveuni, G., & Reddy, S. M. (2015): Influence of different species of *Penicillium* and their culture filtrates on seed germination and seedling growth of sorghum. *Journal of Biochemical Technology,* 5 (4) 832-837. p.

56. Varga, E., Fodor, P., Sörös, Cs. (2021): Multi-mycotoxin LC-MS/MS method validation and its application to fifty-four wheat flours in Hungary. *Food Additives and Contaminants: Part A,* 38 (4) 670-680. p.

57. Villani, A., Moretti, A., De Saeger, S., Han, Z., Di Mavungu, J.D., Soares, C.M., Proctor, R.H., Venancio, A., Lima, N., Stea, G., Pciolla, C., Logrieco, A.F., Susca, A. (2016): A polyphasic approach for characterization of a collection of cereal isolates of the *Fusarium incarnatum-equiseti* species complex. *International Journal of Food Microbiology*, 234 24-35.p.

58. Villani, A., Proctor, R.H., Kim, H.S., Brown, D.W., Logrieco, A.F., Amatulli, M.T., Moretti, A., Susca, A. (2019): Variation in secondary metabolite production potential in the *Fusarium incarnatum-equiseti* species complex revealed by comparative analysis of 13 genomes. *BMC Genomics*, 20 1-22.p.

59. Wang Y., Zheng W., Bian X., Yuan Y., Gu J., Liu X., Liu Z., Bian J. (2014): Zearalenone induces apoptosis and cytoprotective autophagy in primary Leydig cells, *Toxicology Letters*, 226 (2) 182-191. p.

60. Yu J., Chang, P.K., Ehrlich, K.C., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Payne, G.A., Linz, J.E., Woloshuk C.P., Bennett, J.W. (2004): Clustered pathway genes in aflatoxin biosynthesis. *Applied and Environmental Microbiology*, 70 1253-1262.p.

61. Zummo, N. (1984): Sorghum diseases in western Africa. Washington DC 20250. USA, USDA, USAID 32.p.

# 7. Publications and scientific activities

**Peer-reviewed papers:**

**Szabó B.**, Fejős A., Pálinkás Z., Turóczi Gy., Körösi K. (2020): Cirok szemtermésen megjelenő mikotoxin termelő gombák. Növényvédelem (81):9. 398-404. p.

**Szabó, B.K.**, Molnár, K.D., Vlaskality, S.D., Körösi, K. (2023): Occuring Fusarium spp. in sorghum (Sorghum bicolor L. Moench) grains. Acta Phytopathologica et Entomologica Hungarica, Volume: 58, Issue: 2, doi: https://doi.org/10.1556/038.2023.00168

**Szabó, B.K.**, Körösi, K. (2024): Storage mycotoxin producing fungi in Hungarian sorghum (Sorghum bicolor L. Moench) samples – molecular approach of Fusarium spp. Journal of Plant Pathology – (IF: 2,64) doi: https://doi.org/10.1007/s42161-024-01624-0

**Peer-reviewed papers – under decision:**

**Szabó, B.K.,** Atanasova, V., Ducos, C., Kismányoky, A., Pinson-Gadais, L., Ponts, N., Savignac, J-M., Körösi, K., Richard-Forget, F. (2024): Potential health risk related to the occurrence of toxigenic Fusarium species in sorghum (Sorghum bicolor L. Moench) cultivated in Hungary – submitted: 31. January 2024; Under revision

**Conference proceedings – presentations, posters and abstracts:**

Bozóki, B., **Szabó, B.** Körösi, K. (2022): Szemes- és silócirkon megjelenő mikotoxin-termelő gombák, valamint egyes Fusarium fajok patogenitásának vizsgálata. EFOP-3.6.3.-VEKOP-16-2017-00008 Szerk: Pepó Péter, Debrecen 2022

**Szabó, B.K.**, Kiss, J., Molnár, K., Körösi, K. (2022): The case of sorghum and its phytosanitary aspects in Hungary. European Scientific Conference: Towards Pesticide Free Agriculture. 2-3. June 2022. France, Dijon (poster)

**Szabó B.**, Bozóki B., Körösi K. (2021): Potenciális mikotoxin termelő gombanemzetségek gyakorisága cirok szemtermésen. 67. Növényvédelmi Tudományos Napok, Budapest February 2021, Növénykórtan szekció, abstract

**Szabó, B.K.**, Körösi, K. (2024): Naturally occurring Aspergillus species and their mycotoxigenic potential from Hungarian sorghum (Sorghum bicolor L. Moench) kernels. Georgikon for Agriculture – poster – XXXIII. Keszthelyi Növényvédelmi Fórum