Seedborne fungal pathogens associated with *Panicum miliaceum* and *Echinochloa crus-galli* in Hungary

Két egyszikű gyom magfertőző gombabetegségei Magyarországon

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Abstract: The purpose of the study was to investigate the fungal seed diseases affecting two major monocotyledonous weed species, *Panicum miliaceum* L. and *Echinochloa crus-galli* L., in Hungary. Seeds of *Panicum miliaceum* L. were collected from Keszthely in 1985 and 2007, while the seeds of *Echinochloa crus-galli* were gathered from Keszthely and Szombathely regions in 2010. The experiment was set up in two replications with 50 seeds each, resulting in a total of 100 seeds incubated and evaluated per sample. Seeds were arranged in Petri dishes on moistened filter paper, then covered, and incubated in an incubator at 24 ºC for 14 days. Visual scans for early infections were conducted on day 7 and thereafter on day 14. Infected seeds underwent both visual and microscopic examinations to identify fungal pathogens. The microscopic analysis revealed that most isolates were consistent with ascomycetous fungi. The most abundant genera identified were *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. Based on the analysis of the results, out of the 400 seeds examined, *Fusarium* was the most dominant with 44.25%, followed by *Alternaria* (21.25%) and *Aspergillus* (16.5%), respectively. *E. crus-galli* showed a higher susceptibility to *Fusarium, Alternaria,* and *Aspergillus* compared to *P. miliaceum*. Given that these monocot weeds are prevalent and serve as reservoirs for plant pathogens, causing considerable yield losses in Hungarian agricultural fields, strengthening the holistic integrated pest management (IPM) strategies is crucial. Furthermore, transitioning towards the use of non-chemical control measures for these fungal groups is recommended to reduce the damage to the environment and overreliance on fungicides.

Keywords: *seed-borne fungi; Panicum miliaceum; Echinochloa crus-galli; Fusarium; Alternaria*

Összefoglalás: A vizsgálat célja két hazai jelentős egyszikű gyomfaj, a termesztett köles gyomosító változata (*Panicum miliaceum* L.) és a kakaslábfű (*Echinochloa crus-galli* L.) magjait fertőző gombabetegségek vizsgálata volt laboratóriumi vizsgálatokban. A *Panicum miliaceum* L. magjait Keszthely térségéből 1985-ben és 2007-ben, míg az *Echinochloa crus-galli* L. magjait Keszthely és Szombathely térségéből 2010-ben gyűjtöttük. A kísérletet két ismétlésben, ismétlésenként 50 mag felhasználásával végeztük, így mintánként összesen 100 mag csíráztatására és magkórtani értékelésére került sor. A magokat nedvesített szűrőpapírt tartalmazó Petri-csészékbe helyeztük, lefedtük, majd 24 °C hőmérsékleten, termosztátban 14 napon keresztül inkubáltuk. A külső kontamináció minimalizálása érdekében az inkubálást megelőzően a magmintákat Neomagnol oldattal felületileg fertőtlenítettük. A korai fertőzések kimutatására a beállítást követő 7. napon értékeltünk, majd ezt követően a 14. napon vizuális megfigyeléseket végeztünk. A fertőzött magokat vizuális és mikroszkópos vizsgálatnak vetettük alá a gombanemzetségek azonosítása céljából. A mikroszkópos vizsgálatok eredményei alapján az azonosított gombák többsége az Ascomycetes osztályba tartozott. A leggyakrabban előforduló gombanemzetségek a *Fusarium* spp., az *Alternaria* spp. és az *Aspergillus* spp. voltak. A 400 vizsgált mag közül a *Fusarium* spp. bizonyult a legdominánsabbnak 44,25%-os előfordulási aránnyal, ezt követte az *Alternaria* spp. (21,25%) és az *Aspergillus* spp. (16,5%). Az *Echinochloa crus-galli* magjai a Fusarium, Alternaria és Aspergillus nemzetségekhez tartozó gombákkal szemben nagyobb mértékű fogékonyságot mutattak, mint a *Panicum miliaceum*. Tekintettel arra, hogy ezek az egyszikű gyomfajok széles körben elterjedtek, és kórokozók rezervoárjaként szolgálhatnak, amelyek jelentős mezőgazdasági károkat okoznak a magyarországi termőterületeken, az integrált növényvédelmi (IPM) stratégiák átfogó megerősítése kiemelt jelentőségű. Emellett a vizsgált gombacsoportok elleni nem kémiai védekezési módszerek alkalmazása is javasolt a környezeti terhelés csökkentése és a fungicidektől való túlzott függőség mérséklése érdekében.

Kulcsszavak: *magfertőző gombák; Panicum miliaceum; Echinochloa crus-galli; Fusarium; Alternaria*

1 Introduction

Weeds are considered a relevant part of agricultural systems and production, competing with the cultivated crops for essential resources such as light, nutrients, water, and space, resulting in significant decreases in crop yields by up to 32%. In some cases where no weed control measures are implemented, these losses can rise to as high as 72% (Ņečajeva et al., 2023). Weeds can play a significant role as hosts and reservoirs of plant pathogens and vectors, and they can be alternative hosts for some economically important pathogens. Additionally, parasitic weeds may be direct vectors for some plant pathogens, including viruses, bacteria, and fungi, leading to disease development in cultivated crops (Suproniene et al., 2019).

Monocot weeds pose a greater challenge than broad-leaved (dicot) weeds because they occupy comparable ecological niches to cereal vegetation. Cereal crops such as wheat, maize, and rice are among the most widely produced crops worldwide. The presence of monocot weeds in agricultural fields poses a risk to cereal crops directly or indirectly. Direct risk is through their rapid growth and prolific seed production, leading to dense populations and out-competing of the cereal crops, and indirectly mounting disease pressure since the presence of fungal pathogens on weeds can potentially infect the cereal crops as well. Fungal pathogens have been observed to survive in the seeds of many monocots worldwide. Some of the most common phytopathogenic fungal genera, such as *Ustilago* spp., *Fusarium* spp., *Aspergillus* spp., *Alternaria* spp., and *Colletotrichum* spp. (seedborne pathogens) have already been detected in seeds of monocot weeds in several regions of the world (Ņečajeva et al., 2023).

Proso millet (*P. miliaceum*) is a widely distributed weed species that causes economic damage in cereal-growing regions worldwide. Similarly, barnyard grass (*E. crus-galli* L.) is a widespread weed responsible for significant losses in various crops, including rice and maize. Since the European Union (EU), with Hungary being a member, is a significant cereal producer on a global scale, the two weed species are among the most important monocot weeds. *P. miliaceum* L. and *E. crus-galli* L. stand out as major monocotyledonous weeds prevalent in Hungary according to the Fifth National Weed Survey on Arable Fields in Hungary (Novák et al., 2009).

Fungal pathogens that infect the seeds and spread by seeds pose a serious threat to agricultural production, especially in *P. miliaceum* L. and *E. crus-galli* L., which are the most prevalent monocot weeds in Hungary, producing enormous seeds as well (Novák et al., 2009). Despite their importance as weed species common in agricultural fields, as reported by previous scientists, there is a lack of comprehensive knowledge on the fungal pathogens infecting the seeds of the two monocot weeds in Hungary. Addressing this gap is crucial for developing effective disease management and sustainable weed control strategies.

Fungal seed-borne diseases reduce crop yields and quality and increase production costs (Nganje et al., 2004). Fungal pathogens affecting the seeds of *P. miliaceum* L. and *E. crus-galli* L. can potentially spread to cultivated crops. Therefore, understanding the seed-borne diseases in these two monocot weeds will help to reduce the spread of the disease and mitigate the associated economic losses. Additionally, fungal pathogens are becoming a growing concern not only for crop productivity but also for food safety. Some fungal pathogens associated with seeds, in most cases, have been recorded to produce mycotoxins, therefore becoming a major concern to food safety because they pose a risk to humans and livestock if ingested, as reported by Ferrigo *et al*. (2016) and Munkvold (2003). In this experiment, fungal pathogens present in the seeds of *P. miliaceum* L. and *E. crus-galli* L. were investigated. The findings aim to assist Hungarian farmers, government agencies, and other stakeholders in designing a holistic approach to fungal disease management to mitigate economic losses and reduce the use of fungicides to an acceptable level, as recommended in the "Farm to Fork Strategy" EU agricultural policy.

2 Materials and Methods

2.1 Experimental site

The experiment was carried out at the Hungarian University of Agriculture and Life Sciences, Georgikon Campus, at the Festetics Imre Bioinnovation Centre (Seed Pathology Laboratory) between January and March 2024.

2.2 Research materials.

Seeds of *P. miliaceum* L. and *E. crus-galli* L. were collected from agricultural fields in the Keszthely and Szombathely regions of Hungary. Seeds of *P. miliaceum* L. were collected in 1985 and 2007 from Keszthely, while *E. crus-galli* L. seeds were collected in 2010 from both Keszthely and Szombathely. Additional materials included Petri dishes of diameter 12 cm; filter paper; water; Neomagnol tablets, Numigral seed counter, and Thermostat.

2.3 Experiment setup

The experiment was conducted in two repetitions with 50 seeds in each, resulting in 100 seeds germinated per sample. The seeds were thoroughly cleaned to remove foreign impurities and then counted using the Numigral seed counter. Petri dishes were prepared, each layered with a sterile filter paper moistened with approximately 15 ml of sterile distilled water to ensure germination of the weed seeds, holding of the seeds, and providing a suitable environment for fungal microorganisms.

To exclude external contamination and ensure reliable microbiological testing, the seed surfaces were disinfected using Neomagnol. For 1 liter of water, 2 tablets of Neomagnol were used, and then the samples were left in this solution for 10 minutes. After 10 minutes, the samples were rinsed twice with sterilized water and placed on the filter papers with great care, ensuring the seeds did not touch each other to avoid cross-contamination. 50 seeds of each weed species each year were arranged in a grid pattern with 5 rows having 10 seeds each within the Petri dishes, then covered as shown in Figure 1.

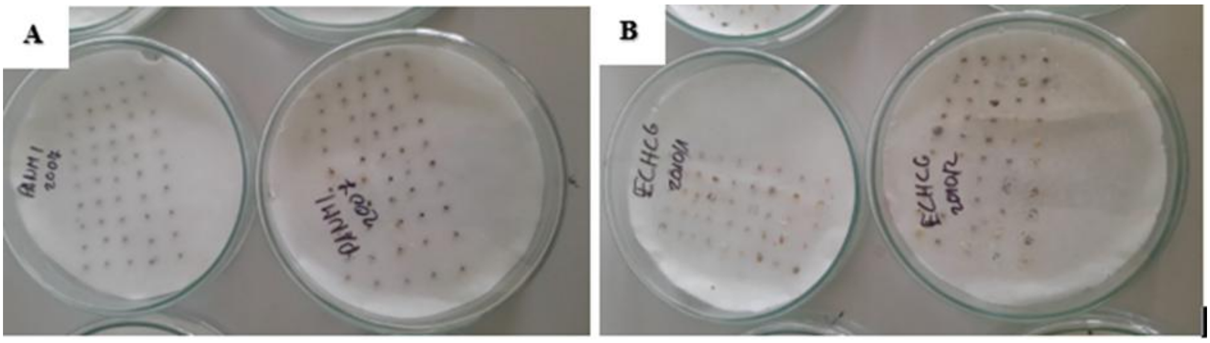

Figure 1 *(A) P. miliaceum L. seed samples on the moistened filter paper. (B) E. crus-galli L. seed samples on the moistened filter paper.*

After preparation, the samples were placed in an incubator set to 24°C (ideal for fungal germination) in sealed containers to minimise contamination. The germination lasted for 14 days. During this time, we made visual scans, and the first one was on day 7 to check for initial fungal infections. The second test was carried out on day 14, when the exact number of seeds infected with fungal microorganisms was determined and recorded. During the study, we sampled infected seed parts and prepared these samples so that we could microscopically examine them to identify the fungi as accurately as possible as shown in Figure 2. The samples in this experiment were destroyed after evaluation.

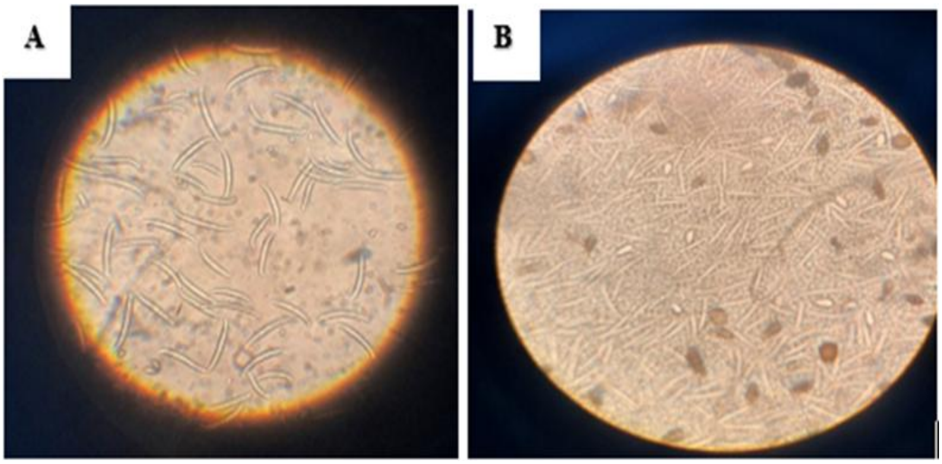

Figure 2 *(A) Sickle-shaped macroconidia of Fusarium spp. from microscopic examination. (B) Round-shaped pale brownish macroconidia of Alternaria spp. from microscopic examination (Photos by György Pásztor).*

After collecting the data from the experiment, the data were organized and analysed using Microsoft Excel 365 to obtain the average infections, trends of disease over time, percentages of infections based on the years of collection, and the locations (regions) where seeds were collected from. T-test and One-way ANOVA using the IBM SPSS Statistics software were used to analyse the comparison of fungal infections between the seeds of the two monocotyledonous weeds.

3 Results

3.1 Overall prevalence of *Panicum miliaceum L.* and *Echinochloa crus-galli L.*

In this study, a total of 400 seeds of *P. miliaceum* L. and *E. crus-galli* L. were examined. The results showed that a total of 177 seeds (44.25%) were found to be infected with *Fusarium* spp., 85 seeds (21.25%) were infected with *Alternaria* spp., 66 seeds (16.5%), were found to be infected with *Aspergillus* spp., and a total of 72 seeds (18%) were found to be healthy (Table 1).

Table 1 Prevalence of fungal infections on *P. miliaceum* L. and *E. crus-galli* L. collected from different locations and years. Each row represents one replicate of 50 seeds. Two replicates (n=100 seeds) were used per species, location, and year

Weed species	Collection year	Collection location	Total number of seeds	Fusarium Infection	Alternaria infection	Aspergillus infection
***P. miliaceum* L.**	1985	Keszthely	50	25	13	5
***P. miliaceum* L.**	1985	Keszthely	50	27	10	5
***P. miliaceum* L.**	2007	Keszthely	50	21	8	3
***P. miliaceum* L.**	2007	Keszthely	50	20	9	3
***E. crus-galli* L.**	2010	Szombathely	50	25	13	12
***E. crus-galli* L.**	2010	Szombathely	50	16	12	11
***E. crus-galli* L.**	2010	Keszthely	50	18	8	17
***E. crus-galli* L.**	2010	Keszthely	50	25	12	10
Total			400	177	85	66

Table 2 below shows the summarized frequency of fungal infections on the seeds of *P. miliaceum* L. and *E. crus-galli* L. per year of collection and overall percentages of infection of each fungal genus.

Table 2 Summarised table showing the total number of seeds and percentages of infection by Fusarium spp., Alternaria spp., and Aspergillus spp. collected from different locations and years

				Frequency of fungal infections			
Weed species	**Year**	**Location**	**Number of seeds**	**Fusarium**	**Alternaria**	**Aspergillus**	**Healthy seeds**
Panicum miliaceum	1985	Keszthely	100	52	23	10	15
Panicum miliaceum	2007	Keszthely	100	41	17	6	36
Echinochloa crus-galli	2010	Szombathely	100	41	25	26	11
Echinochloa crus-galli	2010	Keszthely	100	43	20	24	10
Total number of seeds			**400**	**177**	**85**	**66**	**72**
Percentage of infection				**44.25%**	**21.25%**	**16.50%**	**18%**

3.2 Panicum miliaceum L.

3.2.1 *Panicum miliaceum* L. seeds collected from Keszthely (1985)

After 14 days of incubation, the seeds collected in Keszthely in the year 1985 showed notable fungal infection. Among the observed fungal infections, *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. were the most ubiquitous pathogens identified by physical assessment based on the mycelial colour produced and the confirmation with microscopic analysis. In a total of 100 seeds, 52 seeds (52%) were found to be infected with *Fusarium* spp, 23 (23%) seeds were infected with *Alternaria* spp., and 10 seeds (10%) were infected with *Aspergillus* spp., as shown in Table 1.

3.2.2 *Panicum miliaceum* L. collected from Keszthely (2007)

In this case, *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. appeared to dominate the fungal microflora in these seeds. Out of the 100 seeds incubated for this experiment, 41 seeds (41%) were found to be infected with *Fusarium* spp., 17 seeds (17%) with *Alternaria* spp., and 6 seeds (6%) with *Aspergillus* spp. (Table 1).

3.3 *E. crus-galli* L. collected from Szombathely and Keszthely in the year 2010

After 14 days of incubation, seeds of *E. crus-galli* L. that were collected from Keszthely and Szombathely regions in Hungary in the year 2010 showed high levels of infection based on the visual identification, followed by microscopy revealing a distinct pattern of fungal infestation in seeds collected from these regions.

3.3.1 *Echinochloa crus-galli* L. seeds collected from Szombathely (2010)

According to the results obtained, a total of 41 seeds (41%) were found to be infected with *Fusarium* spp., 25 seeds (25%) were infected with *Alternaria* spp., and 26 with *Aspergillus* spp. Out of the 100 seeds for the year 2010, which were incubated in the two repetitions, 11 seeds (11%) were found to be healthy (Table 1).

3.3.2 *E. crus-galli* L. seeds from Keszthely (2010)

Fungal genera *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. dominated (Table 1). Out of the 100 seeds of *E. crus-galli* L. (2010) incubated, a total of 43 seeds (43%) were found to be infected with *Fusarium* spp., 20 seeds (20%) were infected with *Alternaria* spp., and 24 seeds (24%) with *Aspergillus* spp. while 10 seeds (10%) were healthy, as shown in Table 1 above.

3.4 Comparison between the weed species and locations

Seeds of *E. crus-galli* from Szombathely showed a slightly higher incidence of *Alternaria* and *Aspergillus* than those from Keszthely, while Fusarium infection levels were similar at both locations (Table 2). The seeds of *E. crus-galli* L. showed a higher overall level of fungal infection as compared to that of *P. miliaceum*, across all sampling years, with 179 seeds of *E. crus-galli* and 149 seeds of *P. miliaceum* being infected by at least one of the three fungal species. The statistical results obtained indicated that the p-value for *P. miliaceum* L. is ($p < 0.001$), meaning there were significant differences in the infection by *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. Similarly, for *E. crus-galli* L., the p-value ($p < 0.007$) suggests a statistical difference in the infection by *Fusarium* spp., *Alternaria* spp., and *Aspergillus* species (Table 3).

Table 3 Comparison of fungal infections between the seeds of the two monocot weeds

Seed	Fungal Infections	n	Mean ± SE
Panicum miliaceum L.	*Fusarium* spp.	4	23.25 ± 1.65[a]
	Alternaria spp.	4	10.00 ± 1.08[b]
	Aspergillus spp.	4	4.00 ± 0.58[c]
Overall mean		12	12.42
P-Value			**<0.001**
Echinochloa crus-galli L.	*Fusarium* spp.	4	21.00 ± 2.35 [a]
	Alternaria spp.	4	11.25 ± 1.11[b]
	Aspergillus spp.	4	12.50 ± 1.56 [b]
Overall mean		12	14.92
P-Value			**0.007**

3.5 Distribution of fungal genera

The distribution of fungal genera (Fusarium, Alternaria, Aspergillus), the seeds of *P. miliaceum* L. and *E. crus-galli* L. in Keszthely and Szombathely regions in Hungary across the years 1985, 2007, and 2010 showed a consistent dominance of *Fusarium* spp (Figure 3).

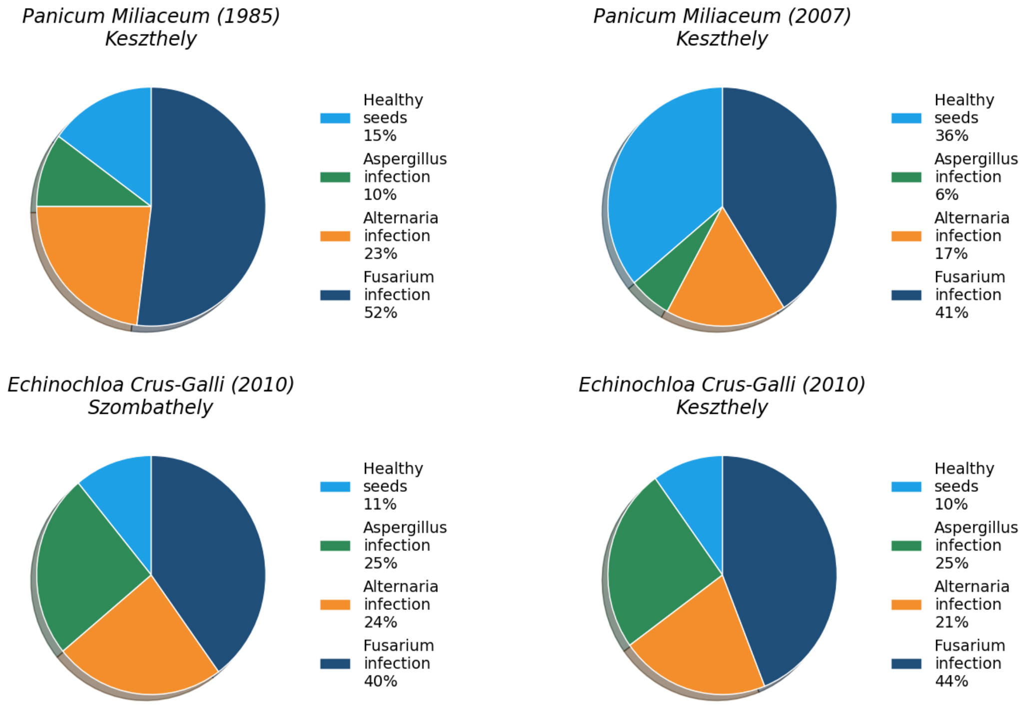

Figure 3 *Distribution of fungal pathogens in seeds of Panicum miliaceum L. and Echinochloa crus-galli L. in Keszthely and Szombathely in the years 1985, 2007, and 2010*

4 Discussion

In this study, a microscopic assessment of fungal pathogens in the seeds of two noxious monocotyledonous weeds, – *P. miliaceum* L. and *E. crus-galli* L. – the results showed that the seeds of these weeds may be heavily infected with seedborne fungal pathogens. Varying degrees of incidence and prevalence of the three phytopathogenic fungal genera were observed. Out of the 400 seeds examined from both weed species, 177 were found to be infected with *Fusarium* spp., 85 with *Alternaria* spp., and 66 with *Aspergillus* spp. (Table 2) and these results confirmed that the weed seeds can act as important pathogen reservoirs.

In this study, we discovered that the seeds of two weed species from the Poaceae family, i.e., *P. miliaceum* L. and *E. crus-galli* L., were infected with *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. These results align with previous studies conducted by Nelson (2018), who found that most of the isolated fungal seed endophytes from Poaceae members were typically filamentous ascomycetes, including *Alternaria, Fusarium, Cladosporium, Phoma, Aerobasidium*, and *Stemphylium.* Similar findings were reported in Latvia by Ņečajeva et al. (2023), using molecular techniques to sequence the ITS region of rDNA, which showed that *Alternaria,* Fusarium*, Sarocladium,* and *Cladosporium* were the most abundant genera colonizing the seeds of *E. crus-galli* L. Additionally, this study supports research conducted by Cavers and Kane (2016) in London and Ontario, who found six genera of fungi – *Alternaria, Aspergillus, Cladosporium, Fusarium, Helminthosporium,* and *Epicoccum* from the seeds of *P. miliaceum* L. that failed to germinate. Although our study was focused on pathogen detection rather than seed viability, fungal infection does not necessarily inhibit germination, and infected seeds may still emerge and contribute to pathogen carryover in the field.

Dominance of *Fusarium* spp. in monocot weed seeds

Fusarium spp. and *Alternaria* spp. showed a higher prevalence than *Aspergillus* spp. In both weed species, with *Fusarium* spp. as the most dominant with 52% and 41% in the *P. miliaceum* L. seeds collected from Keszthely in the years 1985 and 2007, respectively, and in *E. crus-galli* L., the percentage of infection was 43% in Keszthely and 41% in Szombathely (Figure 3). Additionally, in terms of the overall average mean of infection, *Fusarium* spp. showed a significant difference in the degree of infection compared to *Alternaria* spp. and *Aspergillus* spp. In the seeds of both weed species (Table 3). The results from this research align with findings from Simonin et al. (2022), indicating that Fusarium is one of the most widespread fungal genera associated with the infection of seeds, not only weed seeds but also cereal crops. These findings also align with research by Ņečajeva et al (2023), indicating that *Fusarium* and *Alternaria* were the most prevalent genera in the seeds of *E. crus-galli* L. and *Avena fatua* collected in the same location in 2020 and 2021 in Latvia.

Occurrence of *Alternaria* spp. and *Aspergillus* spp.

High frequency of occurrence of *Alternaria* spp. in the seeds of both species, with infection of 23% and 17% from the *P. miliaceum* L. seeds collected in Keszthely in the years 1985 and 2007, respectively. Seeds of *E. crus-galli* L. with 21% from Keszthely and 24% from Szombathely, collected in 2010, showed a relatively higher infection in Szombathely than in Keszthely (Figure 3). Comparing the degree of infection among the fungal infections identified, *Alternaria* spp. showed a higher degree of infection compared to *Aspergillus* spp. in the seeds of both weed species (Table 3) above. The results align with the previous studies of Jasim and Juber (2010), who described *Alternaria* as the most frequent fungal pathogen on the seeds of *E. crus-galli* L.

among the 26 species of fungi tested. *Alternaria* frequently colonizes seeds, exists as a saprophyte, endophyte, and a pathogen in a variety of plants, and is reported in over 400 plant species (Simonin et al., 2022). The most ubiquitous were *Alternaria alternata*, *Alternaria tenuissima,* and *Alternaria porri*, contaminating cereals and other crops, according to Lee *et al*. (2015) and Tralamazza et al. (2018). Additionally, *Aspergillus* spp. showed a lower infection rate in the previous years, that was, 10% and 6% in *P. miliaceum* L. in 1985 and 2007, respectively, and 25% from Szombathely and Keszthely in the year 2010. The identification of *Aspergillus* in research studies in India, where seeds of 5 minor millet species (*Paspalum scrobiculatum*, *Panicum miliaceum*, *Panicum trypheron, Setaria italica*, and *Echinochloa crus-galli* L.) were tested for susceptibility to *Aspergillus flavus,* and the results showed that indeed all these minor millets are susceptible to *Aspergillus flavus,* leading to the production of toxins (Ansari and Shrivastava, 1991).

Differences between weed species

Based on the findings of the experiment, it was discovered that fungal infections occurred differently in *P. miliaceum* L. and *E. crus-galli* L. seeds. While both species were susceptible to *Fusarium* and *Alternaria*, seeds of *E. crus-galli* L. showed a higher likelihood of infection, accompanied by rapid degradation of the seed. A total of 179 seeds of *E. crus-galli* L. were found to be infected by the three fungal genera, compared to a total of 149 seeds of *P. miliaceum* L. infected by the three fungal genera out of the total 400 seeds examined. Therefore, in terms of percentage, *E. crus-galli* L. seeds showed an infection rate of 44.75% and *P. miliaceum* L. with 37.25%. On further analysis based on the overall mean infection, *E. crus-galli* L. recorded 14.92 against 12.42 in *P. miliaceum* L., meaning *E. crus-galli* L. was more susceptible to the three fungal genera compared to *P. miliaceum* L. (Table 3). These observations are similar to what Ņečajeva et al (2023), documented about the high degrading capacity of Alternaria species on the seeds of *E. crus-galli* L., where specifically *Alternaria alternata* and *Alternaria tenuissima* were proven to have a high degrading capacity on the seeds of *E. crus-galli* L. and therefore it has been proposed that this two species can be used as promising biocontrol agents against barnyard grass which is a noxious weed in agricultural fields worldwide. Additionally, the varying susceptibility levels of *E. crus galli* and *P. miliaceum* to *Alternaria* could be attributed to differences in the characteristics of the seed, composition, or ecological preferences of the weed species contributing to overall physical and chemical defense against fungal pathogens (Pollard, 2018).

Co-infection and seed degradation

Some seeds of *P. miliaceum* L. and *E. crus-galli* L. were identified to be co-infected by both Fusarium and Alternaria. In this case, the seeds appeared to be more degraded than those inhabited by one of the genera of phytopathogenic fungi. This correlates with previous studies observed in the research work of Ņečajeva et al (2023) where seeds of *E. crus-galli* L. and *Avena fatua* (both belonging to family Poaceae) were found to be co-infected by the species of the two common fungal genera *Fusarium* and *Alternaria*, and these seeds showed a higher rate of degradation compared to the seeds inhabited by one of the fungal genera. The rapid degradation of the infected seed when co-infected by both *Alternaria* and *Fusarium* is that the species of the two fungal genera may engage in competitive interactions due to their antagonistic mycotoxin production, which can destroy the seed rapidly (Müller et al., 2015). These observations show how fungal pathogen interaction is complex in the natural setting, and this is important for us to understand because it is also the case with cultivated crops and might cause high yield loss and high contamination of the cereal crops.

5 Conclusions

This study identified three fungal genera, demonstrating that the seeds of *Panicum miliaceum* L. and *Echinochloa crus-galli* L. could harbour fungal phytopathogens. The three fungal phytopathogen genera identified in the seeds of both weed species were *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. Among the three identified common genera in the seeds of both weed species, *Fusarium* was the most prevalent, with 44.25% of the total seeds examined in the experiment compared to *Alternaria* and *Aspergillus*. This was seen to be consistent in the two locations, Szombathely and Keszthely, for the years 1985,2007 and 2010, where the seeds were collected. *Alternaria* was the second most identified genus in proso millet and barnyard grass, accounting for 21.25% of the total seed examined in this study. Notably, some seeds were co-infected by the two fungal genera *Fusarium* and *Alternaria,* and these seeds appeared to be highly degraded. While the seeds of both *P. miliaceum* L. and *E. crus-galli* L. were susceptible to *Fusarium* spp. and *Alternaria* spp., seeds of *E. crus-galli* L. showed a higher likelihood of infection, accompanied by rapid degradation of seeds, where both *Fusarium* spp. and *Alternaria* spp. co-infected the seeds.

Since the fungal genera identified in this study cause significant economic losses to crops, Integrated Pest Management (IPM) practices are strongly recommended. Strategies such as crop rotation, field sanitation, and seed treatment should be implemented because they focus on a holistic approach to disease control. This reduces reliance on chemical fungicides, which is consistent with the efforts to reduce fungicide dependence in sustainable European agriculture. Further research could involve molecular identification of the fungal pathogens in the seeds of *Panicum miliaceum* L. and *Echinochloa crus-galli* L. at the species level, using advanced molecular techniques such as PCR, DNA sequencing, and metagenomics to identify and characterize these fungal species. Additionally, research on exploring molecular and epigenetic mechanisms underlying fungal infections in the seeds of the two weed species can be done. By investigating the genetic and epigenetic factors influencing susceptibility to fungal diseases, host-pathogen interaction can be understood, leading to specific target strategies for the management of these pathogens and weeds.

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References

Ansari, A. A. and Shrivastava, A. K. 1991. Susceptibility of minor millets to *Aspergillus flavus* for aflatoxin production. *Indian Phytopathology*. **44** (3) 350–353.

Cavers, P. B. and Kane, M. 2016. The biology of Canadian weeds: 155. *Panicum miliaceum* L. *Canadian Journal of Plant Science*. **96** (6) 939–988. https://doi.org/10.1139/cjps-2015-0152

Ferrigo, D., Raiola, A. and Causin, R. 2016. *Fusarium* toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules*. **21** (5) 1–22. https://doi.org/10.3390/molecules21050627

Jasim, K. A. and Juber, K. S. 2010. Detection of fungi associated with the seeds and plants of barnyard grass and evaluating their pathogenic efficiency. *The Iraqi Journal of Agricultural Sciences*. **41** (2) 34–42.

Lee, H. B., Patriarca, A., and Magan, N. 2015. Alternaria in food: Ecophysiology, mycotoxin production and toxicology. *Mycobiology*. **43** (2) 93–106. https://doi.org/10.5941/MYCO.2015.43.2.93

Müller, M. E. H., Urban, K., Köppen, R., Siegel, D., Korn, U. and Koch, M. 2015. Mycotoxins as antagonistic or supporting agents in the interaction between phytopathogenic *Fusarium* and *Alternaria* fungi. *World Mycotoxin Journal*. **8** (3) 311–321. https://doi.org/10.3920/WMJ2014.1747

Munkvold, G. P. 2003. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. European Journal of Plant Pathology. **109** 705–713. https://doi.org/10.1023/A:1026078324268

Nelson, E. B. 2018. The seed microbiome: Origins, interactions, and impacts. *Plant and Soil.* **422** (1–2) 7–34. https://doi.org/10.1007/s11104-017-3289-7

Nganje, W. E., Bangsund, D. A., Leistritz, F. L., Wilson, W. W. and Tiapo, N. M. 2004. Regional economic impacts of *Fusarium* head blight in wheat and barley. *Review of Agricultural Economics*. **26** (3) 332–347. https://doi.org/10.1111/j.1467-9353.2004.00183.x

Novák, R., Dancza, I., Szentey, L., Karamán, J., Béres, I., Kazinczi, G. and Gólya, G. 2009. *Arable weeds of Hungary. Fifth national weed survey (2007–2008)*. Ministry of Agriculture and Rural Development, Budapest, Hungary.

Pollard, A. T. 2018. Seeds vs fungi: An enzymatic battle in the soil seedbank. *Seed Science Research.* **28** (3) 197–214. https://doi.org/10.1017/S0960258518000181

Simonin, M., Briand, M., Chesneau, G., Rochefort, A., Marais, C., Sarniguet, A. and Barret, M. 2022. Seed microbiota revealed by a large-scale meta-analysis including 50 plant species. *New Phytologist*. **234** (4) 1448–1463. https://doi.org/10.1111/nph.18037

Suproniene, S., Kadziene, G., Irzykowski, W., Sneideris, D., Ivanauskas, A., Sakalauskas, S., Serbiak, P., Svegzda, P., Kelpsiene, J., Pranaitiene, S. and Jedryczka, M. 2019. Asymptomatic weeds are frequently colonized by pathogenic species of Fusarium in cereal-based crop rotations. *Weed Research*. **59** (4) 312–323. https://doi.org/10.1111/wre.12367

Ņečajeva, J., Borodušķe, A., Nikolajeva, V., Seņkovs, M., Kalniņa, I., Roga, A., Skinderskis, E. and Fridmanis, D. 2023. Epiphytic and endophytic fungi colonizing seeds of two Poaceae weed species and *Fusarium* spp. Seed degradation potential in vitro. *Microorganisms*. **11** (1) 184. https://doi.org/10.3390/microorganisms11010184

Tralamazza, S. M., Piacentini, K. C., Iwase, C. H. T. and Rocha, L. de O. 2018. Toxigenic Alternaria species: Impact in cereals worldwide. *Current Opinion in Food Science*. **23** 57–63. https://doi.org/10.1016/j.cofs.2018.05.002