THESIS

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M.Sc. Plant Protection

Keszthely

2024

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M.Sc. Plant Protection

SEED-INFECTING FUNGAL DISEASES OF TWO MAJOR MONOCOT WEEDS IN HUNGARY

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Keszthely

2024

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1.0 INTRODUCTION

Weeds are an integral part of agricultural production, and they compete with cultivated crops for resources such as light and space, resulting in significant decreases in crop yields by up to 32 %. In some cases where no weed control measures are implemented, the losses can rise to 72 % (Ņečajeva et al., 2023). Weeds play a role in harboring or serving as reservoir alternate hosts for pathogens and vectors, and they can be obligate alternative hosts for some pathogens. Additionally, directly as vectors for the case of parasitic weeds, therefore spreading 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 weeds because they occupy comparable ecological niches to cereal vegetation. Cereal crops such as wheat, maize, and rice are among the 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 fungal pathogens that they host 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*, *Fusarium*, *Aspergillus*, *Alternaria*, and *Colletotrichum* that have been detected in seeds of monocot weeds in several regions of the world (Ņečajeva et al., 2023).

Proso millet (*Panicum miliaceum*) is a widely distributed weed species that causes economic damage in cereal-growing areas in the world. Barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv) is a widespread weed species causing significant losses in different crops including rice and maize. Since the European Union (EU) and Hungary are significant cereal producers on a global scale, the two weed species are among the most important monocot weeds. *Panicum* *miliaceum* and *Echinochloa crus-galli* stand out as major monocotyledonous weeds prevalent in Hungary according to the Fifth National Weed Survey on Arable Weeds of Hungary (Novák et al., 2009).

Fungal diseases that infect the seeds and spread by seeds pose a serious threat to agricultural production, especially in *Panicum miliaceum* and *Echinocloa crus-galli*, which are the most prevalent monocots weeds in Hungary producing enormous seeds as well. 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. For effective disease management and sustainable weed control strategies, addressing this gap is very important.

Fungal seed-borne diseases reduce crop yields, and quality, and increase production costs. (Nganje et al., 2004). Fungal pathogens affecting the seeds of *Panicum miliaceum* and *Echinochloa crus-galli* can potentially spread to cultivated crops. Therefore, understanding the seed-borne diseases in these two monocot weeds will help 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; Munkvold, 2003). I investigated the fungal diseases present in the seeds of *Panicum miliaceum* and *Echinochloa crus-galli*, and the findings will help 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 chemical Fungicides to an acceptable level as per the “Farm to Fork Strategy” EU agricultural policy.

The aim of my research was to investigate the various fungal genera present in the seeds of *Panicum miliaceum* and *Echinocloa crus-galli*, found in agricultural fields across Hungary.

2.0. LITERATURE OVERVIEW

Weeds are plants that grow where they are not desired and compete with cultivated plants for, space and sunlight (Zimdahl Professor Emeritus et al., 2007). Weeds play a role in harboring and spreading plant pathogens, which can impact crop disease incidence (Martínez et al., 2021). Management of monocot weeds is challenging because of their specific adaptive biological traits (Flora of North America Editorial Committee, 2007)and therefore it is essential to examine the monocot weeds in modern agriculture because they can cause large economic losses being agents of dispersal and host to pathogens.

This literature review focuses on two monocot weeds in Hungary: Proso millet (*Panicum miliaceum* L.) and barnyard grass (*Echinocloa crus galli* L. *P* Beauv). Moreover, it emphasizes the aspect of fungal infections within the seed of these weeds because this biology could help reduce the loss of yields, weed biocontrol, and ecosystem health, shedding light on sustainable agriculture.

Monocot weeds can outcompete valuable crops leading to reduced crop yields (Duke, 2012; Ziska & Dukes, 2010)and due to their advanced adaptive traits, they thrive in diverse environmental conditions, making them particularly hard to manipulate (Flora of North America Editorial Committee, 2007). Grass weeds pose a greater challenge than broad-leaved weeds because they occupy comparable ecological niches to cereal vegetation and this implies that barnyard grass, for instance, has ecological alternatives or preferences that can be like the ones of rice and their young seedlings and flowers are indistinguishable (Rono, 1996). This poses a demanding situation to farmers who ought to adopt effective strategies to manage monocot weeds and reduce yield losses through diseases spread by these weeds.

2.1. Proso millet (*Panicum miliaceum* L.)

2.1.1. Distribution

Panicum miliaceum, commonly known as the common millet or proso millet, is a type of millet with various names including wild millet, black-seeded proso millet, panic millet, broom corn, and hog millet (Sheahan, 2014). Proso millet is believed to have originated from China (Novák et al., 2009)and then spread to India, Russia, the Middle East, and Europe. Nowadays it is primarily grown and produced in India, China Russia, the Middle East, Central Asia, Africa, Eastern Europe (Russia and Ukraine), and North America (Cavers & Kane, 2016).

Proso millet is a versatile crop that is grown for various purposes such as grains, consumed as porridge, or added to meat stews, bird seed, and forage among others (Cavers & Kane, 2016). It is cultivated as a secondary crop in Hungary and intended for use as fodder (Novák et al., 2009). Proso millet is a weed that tends to spread across Hungary and the entire of Europe (Pásztor & Nádasy-Ihárosi, 2015) for instance in Hungary around 60 years ago, Proso millet was a rare arable weed but after 1947, it became a more common weed and was ranked 119^{th} during late summer of maize fields from its annual survey. Its average coverage was 0.006% and during the second weed survey, it climbed into a higher position in terms of dominance and its spread increased marginally in the last 20 years, whereby it has been ranked the tenth most important species in the maize-wheat list. Komárom-Esztergom is found to be the most to be the most county in Hungary (Novák et al., 2009).

In Hungarian maize fields, the weedy representatives of proso millet cause significant challenges in plant protection due to the heavy use of agrochemicals (Magyar et al., 2015; Novák et al., 2009).In addition to its abundance in maize fields, recently its appearance has been steadily increasing in other cultivated crops such as potatoes, and wheat among other cereals (Pásztor et al., 2018a). According to (Novák et al., 2009), *Panicum miliaceum* poses a challenge to row crops including maize in Hungary, whereby the yield loss is directly proportional to the total weed cover since it competes with maize plants for essential resources for growth like water nutrients and light. In addition, millet species of the *Panicum* genus, apart from competing with crops for nutrients and space as a weed, also facilitate the spread of cereal viruses (Pásztor et al., 2018a).

2.1.2. Biology

Proso millet is a first-growing grass that can reach a height of over 2 meters during the summer growing season (James et al., 2010), producing more seeds than any other weed found in arable crops even after the plant has matured the seed hull remains attached to the primary roots and hence seeds do not deteriorate after germination (Bough & Cavers, 2009; Strand & Behrens, 2019). Germination begins at the start of a warm season at a depth of a few centimeters (Bough & Cavers, 2009)and continues throughout the growing season followed by enormous seed production (Bough & Cavers, 2009; Eberlein et al., 1990).

The start of flowering in proso millet happens a month after the germination process, which can vary depending on the biotype, and the flowering process continues nonstop, resulting in the seed continuously ripening and falling off the seed heads or shattering mature seeds (Trivedi, 2010). Proso millet continues producing seeds until frost kills the plant in autumn and the seed remains dormant until the following spring (Strand & Behrens, 2019). According to (Strand & Behrens, 2019) proso millet produces a lot of seeds per plant, approximately 500 or more per square foot, that can be easily spread by harvesting equipment.

2.1.3. Morphology

Proso millet is an annual plant species belonging to the family Poaceae, and the plant stands erect reaching a height of over one meter in most cases in cultivated crops (Novák et al., 2009). The stems are light green and may be branched at the base having alternatively arranged leaves covered by stiff hairs, short ligules, and no auricles (Baltensperger, 1996). It has a shallow fibrous root system spreading laterally up to 120 cm and a depth of 150 cm. They produce several tillers that bear a drooping, branched, compact inflorescence that is made up of many stalked, ovoid spikelets (Cavers & Kane, 2016). The fruit of *Panicum miliaceum*, like in all grasses, is a caryopsis enclosed by a husk consisting of palea and lemma, producing seeds, which are shiny, yellow to brown, smooth, oval, and approximately 2.4 to 3mm long (Sheahan, 2014).

2.1.4. Physiology

Proso millet is a type of heat and drought-tolerant C4 plant that has a high-water use efficiency and a low transpiration ratio (Cavers & Kane, 2016)reaching a height of over 100 cm high (Baltensperger, 1996; Novák et al., 2009). This plant is sensitive to frost and may not grow well in soil with a pH level higher than 7.8 and additionally, it may not tolerate high levels of salinity (Sheahan, 2014).In terms of germination, proso millet germinates at a temperature range of 10 to 45 degrees Celsius (Sheahan, 2014), with 15 degrees Celsius being the optimum (James et al., 2010). In Hungary, proso millet needs an optimum temperature of 18 to 21 degrees Celsius to emerge at a depth of 5cm (Mándy & Szabó, 1973). It is well suitable to high altitude plateaus and can survive in colder climates (Baltensperger, 1996) but cannot survive very well under water stress since it has a shallow root system (Cavers & Kane, 2016).

2.2. Barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv)

2.2.1. Distribution

Echinochloa crus-galli, commonly known as barnyard grass, has an uncertain origin, but it is native to tropical Asia (Rojas-Sandoval J. & Acevedo-Rodríguez P., 2022). It is a weed species distributed worldwide causing significant impact in agricultural fields, and it spans from 50°N to 40°S covering the northern part of Europe through the subtropics and the tropical regions (Clayton & Renvoize, 1986). *Echinocloa crus-galli* is a troublesome weed in contemporary agriculture (Michael, 2003), having exceptional biological features for ecological adjustments to several habitats in the world, therefore listed as one of the world's worst weeds in the Global Compendium of Weeds (Randall, 2017).

Barnyard grass has been documented to be present in over 60 countries in the world and this is attributed to its features like quick germination, rapid growth, and abundant seed production (Barrett, 1983). It is a weed to over 36 crops worldwide including major crops like rice, corn, cotton sorghum, soybean, and sugarcane (Holm et al., 1977; Narayana Rao, 2021).

In North America, for instance, barnyard grass is widespread and is observed in crop fields wetlands, and disturbed habitats which is also a similar case in Ontario (Bosnic & Swanton, 1997; Flora of North America Editorial Committee, 2007). It is widely growing in proximity, to rice fields in Asia, in drylands and wetlands of Australia (Shabbir et al., 2019), and in agricultural fields and natural ecosystems in African countries (Holm et al., 1977).

In Europe, *Echinochloa crus-galli* in arable agricultural farms poses a challenge and it is the most important weed in the early summer according to the Fifth National Survey of Arable Weeds of Hungary (Novák et al., 2009). According to (Dorner et al., 2012), *Echinochloa crus-galli* is the most common weed, based on the percentage cover on both organic and conventional maize fields in Jászság region, Hungary.

2.2.2. Biology

Barnyard grass is a weed species found in various habitats in the world (Bajwa et al., 2015)exhibiting distinctive morphology, physiology ecology, and genetics play crucial roles in its adaptability and ecological success (Rojas-Sandoval J. & Acevedo-Rodríguez P., 2022). A single barnyard grass can produce up to 39000 seeds (Selig et al., 2022) with their primary dormancy decreasing with storage (Kennedy et al., 1980).

2.2.3. Classification

Echinochloa crus-galli belongs to the kingdom, subkingdom Tracheobionta, super division Spermatophyta, division Magnoliophyta, class Liliopsida, subclass Commelinidae, order Cyperales, family Poaceae/Gramineae and genus *Echinocloa* P. Beauv (Rao et al., 2019). The chromosome number reported for *Echinochloa crus-galli* varies from 2n=36,42,48,54 to 2n=72 according to FAO,2014 as cited by (Rojas-Sandoval J. & Acevedo-Rodríguez P., 2022).

2.2.4. Morphology

Barnyard grass (*Echinochloa crus-galli*) is an annual grass that can grow up to 2 meters tall, with aerial parts of the grass being spread out, decumbent or stiffly erect, with nodes that are typically smooth or slightly hairy near the base of the plant (Chin, 2001; Rojas-Sandoval J. & Acevedo-Rodríguez P., 2022). The sheaths of the plant are smooth, while the leaves grow up to 65 cm in length (Maun' & Barrett', 1986). The panicles of the plant can grow up to 25 cm in length having hairs located below the nodes of the primary axes. The spikelets are small, sterile, and separate at maturity, with upper glumes about as long as the spikelets and the anthers typically 1 mm long.

The caryopses of the plant are brownish, ovoid, or oblong having approximate lengths of 2.2 mm and 1.8 mm (Rojas-Sandoval J. & Acevedo-Rodríguez P., 2022). Discussing the seed, proso millet spikelets contain small grains surrounded by a palea, lemma, and glumes. As the grain matures, its outer layer becomes hard and forms a protective coating called caryopses coat, which is made up of the suberized nuclear membrane, aleurone, parts of the inner integument, and exo-and endocarp layers of the pericarp. Inside this protective layer lies the embryo, which is attached to the scutellum, and the endosperm (Rono, 1996).

2.2.5. Physiology

Echinochloa crus-galli is an annual tropical plant belonging to the family Poaceae, utilizing the C4 pathway of carbon fixation (Bajwa et al., 2015), exhibiting plant characteristics that require shorter periods of daylight to grow and flower under diverse environments, shifting to reproductive growth more rapidly in a 9-to 13-hour photoperiod compared to a longer 16-hour photoperiod (Maun' & Barrett', 1986). *Echinochloa crus-galli* thrives in water and requires moist soil for optimal growth and early flowering because dry soil conditions impede its growth (Bajwa et al., 2015).

Its development growth and development depend on light quality, whereby the blue light reduces the height and weight compared to white and red light. Full sunlight leads to a higher number of tillers and panicles, while heavy shading affects growth (Maun' & Barrett', 1986) Studies have shown that the thermostability and thermodenaturation of the enzyme, phosphoenolpyruvate carboxylase (PEP carboxylase), altered in response to the selective thermal pressures of the environment in the region where this species occurs hence net photosynthesis and dark respiration increase with CO_2 $\quad$ (Simon et al., 1984).

Germination in *Echinocoa crus-galli* is hypogeal (Bajwa et al., 2015), and seedlings grown in an anaerobic environment are highly active metabolically which may elaborate on barnyards' ability to germinate and emerge from flooded rice fields (Kennedy et al., 1980).

Generally, when it comes to reproduction and habitat, barnyard grass exhibits both sexual reproductions ending up in seed production and asexual reproduction through tillering and vegetative propagation. The inflorescence is a green-to-purplish apical panicle, with elliptical and pointed spikelets which can be awned awnless and it flowers all year round and propagates through seed. Barnyard thrives in wet soil with high nitrogen content and sunny places, and it is commonly found in rice fields, water edges, and marshes (Chin, 2001).

A single barnyard grass can produce up to 39000 seeds (Selig et al., 2022), with their primary dormancy decreasing with storage (Benvenuti et al., 1997a). The extent of germination is highly dependent on light conditions, and heat shock is an effective method for breaking dormancy (Benvenuti et al., 1997b). Finally, the success of barnyard grass as a weed depends on seed persistence, decay rate, and dormancy response to seasonal changes, like other annual (Rono, 1996).

2.3. Pathogens of the Proso millet (*P. miliaceum*) and Barnyard grass (*E. crus-galli*).

2.3.1. Viruses

Like any other field crops, *Panicum miliaceum* and *Echinochloa crus-galli* are infected by various viruses and these viruses have the possibility of infecting Monocotyledonous field crops that are valuable like wheat maize and rice. *Echinochloa crus-galli* and a millet species *P. capillare* are infected by wheat streak mosaic virus (WSMV)according to research done on grass species in the USA and Czech Republic (Chalupniková et al., 2017; Christian & Willis, 1993).

In Hungary, the Wheat streak mosaic virus was first identified in 1985 by Pocsai and Barabas (Pásztor et al., 2018b), thereafter specifically in Keszthely research on viruses hosted by *Panicum miliaceum* was done, and Wheat streak mosaic virus (WSMV) was detected (Pásztor et al., 2017), in addition other viruses including Barley stripe mosaic virus (BSMV), Wheat dwarf virus (WDV), Brome streak mosaic virus (BstMV) and Brome mosaic virus (BMV) were also detected. All these viruses infect cereals therefore the appearance of the weedy proso millet within the vicinity of the field crops like wheat and maize is a potential threat to the health of these crops.

2.3.2. Bacteria

Bacterial infections have been identified to infect seeds of gramineous plants including *Echinochloa crus-galli*. For instance, in Korea, *Pseudomonas avenae* which causes bacterial brown stripe disease in rice (Shakya, 1985), and *Pseudomonas syringae* infecting *E. crus-galli*, have been isolated and proved to be effective in controlling the weed in rice plantation (Sepahi & Jafaryan, 2010). Moreover, it has been documented that *Panicum miliaceum* can be infected by *Pseudomonas syringae pv. Panici* in Australia (Young & Fletcher, 1994).

2.3.3. Fungal Diseases

Seed-borne diseases pose a significant challenge to cultivated crops (Ņečajeva et al., 2023). Dormant seeds possess both physical and chemical defense mechanisms against pathogens (Dalling et al., 2011), whereby the presence of a hard seed coat serves as a mechanical defense against the decay of seeds (Pollard, 2018), and the presence of phenolic compounds serve as a chemical defense mechanism against pathogen invasion (Hendry et al., 1994).

Weed seeds vary in the extent of susceptibility to attack by microbial microorganisms and these differences vary both between different weed species and within the same species (Gardarin et al., 2010). According to molecular identification on seed-borne diseases of *Echinochloa crus-galli* in Latvia by (Ņečajeva et al., 2023), the fungal genera common in isolates were *Fusarium* and *Alternaria*, occurring on the seeds, and this was achieved by sequencing the ITS region of rDNA. Moreover, another research in Egypt, showed that in the isolates identified both morphologically and by phylogenetic analysis, *Fusarium* spp, *Alternaria* (*A. alternata*, *A. tenuissima*), *Chaetomium* (*C. globosum*), and *Nigrospora* (*N. oryzae*) were the prevalent genera (Mohammed & Badawy, 2020). According to the research work of (Manjusha et al., 2000) on the fungal microflora on seeds of proso millet (*Panicum miliaceum*) in India, *Cochliobolus nodulosus* was found to be the most dominant. In London and Ontario, six genera of fungi i.e. *Alternaria*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Epicoccum*, and *Aspergillus* were isolated from the seeds of proso millet that failed to germinate (Cavers & Kane, 2016).

***Fusarium* head blight**

The genus *Fusarium* is an ascomycetous fungus widely distributed inhabiting a wide range of ecological niches. It comprises a variety of species that serve as pathogens to plants and animals, including human beings by producing mycotoxins. The mycotoxins contaminate food making it unfit for human consumption and more importantly causing economic losses in cereals by reducing the yield, according to (Agrios, 2005; Costa et al., 2021; Kvas et al., 2009; P. E. Nelson et al., 1994).

Fusarium species cause a variety of plant diseases, sometimes in combination with other pathogens. These diseases can affect seedlings, roots, crowns, stems, stalks, fruits, vascular systems, and most importantly cereal head blights. Therefore, the manifestation of these effects in plants that are both economically and ecologically important poses a great risk (Leslie & Summerell, 20006; Zemankoa & Lebeda, 2001).

The *Fusarium* species have been found to act as endophytes, saprophytes, or pathogens in most crops, and grasses (Costa et al., 2021; Szécsi et al., 2013).In fact, various *Fusarium* spp have been identified and documented on various types of millets, for instance, proso millet (*Panicum miliaceum*), pearl millet (*Pennisetum glaucum*), foxtail (*Setaria italica*), and finger millet (*Eleusine coracana*). As reported by (Akanmu et al., 2013),14 species of *Fusarium* species have been documented to infect millets. An example is *Fusarium anthophilum* which was isolated from barnyard grass and studied in Iran in 2006 according to (Mojaradi et al., 2006).

Fusarium species such as *Fusarium equiseti* (Corda.) Sacc. and *Fusarium poae* (Peck.) Wollenw. has been found in the seeds of *Panicum miliaceum* in Ontario (Cavers & Kane, 2016).Similarly in the United States species such as *Fusarium acuminatum* Ellis & Everh. *Fusarium bulbigenum* Cooke & Massee; *Fusarium equiseti* (Corda) Sacc. *Fusarium Oxysporum* Schltdl.; *Fusarium moniliforme* J. Sheld., and *Fusarium poae* (Peck.) Wollenw. have been identified, according to (Farr et al., 2013).

Research conducted by (Szécsi et al., 2013), revealed that the most common species of *Fusarium* that were isolated from leaves and stems of grass species in Hungary were *Fusarium compactum*, *Fusarium equiseti*, *Fusarium graminiarum*. Among these species, *Fusarium graminiarum* and *Fusarium compactum* were isolated from *Panicum miliaceum* whereas *Fusarium compactum* was isolated from *Echinochloa crus-galli*. Additionally, *Panicum maximum*, a close relative of *Panicum miliaceum* has also been found to be infected by *Fusarium* according to (Marchi et al., 2010).

In terms of Morphology of the genera *Fusarium,* Mycologists use the fruiting bodies and spores to identify and categorize fungal microorganisms at the genera level (Ajmal et al., 2022). *Fusarium* is a genus of fast-growing fungi with very flourishing, bright colonies, and a uniform layer on top. It also develops spindle-shaped, curved, and coiled macroconidia whose tip is pointed while microconidia are mostly 0 to 1 septum in length. The chlamydospores however vary from species to species being tick-walled structures may either be present or absent. Most of the fungal species belonging to the genera *Fusarium* have a structure called sporodochia that produces macroconidia -asexual spores on their surface which are important for the propagation and survival of fungi serving as an asexual means of reproduction (Zemankoa & Lebeda, 2001).

With reference to Physiology of *Fusarium* species, to understand the physiology of *Fusarium* species, exploring their life cycle, growth conditions, reproduction and their pathogenic mechanism is paramount. *Fusarium* species follow diverse reproductive strategies for survival and spread which is a common trait among the filamentous ascomycetous fungi where reproduction can be sexual or asexual (Hornok, 2007).

Some *Fusarium* species are known to have a documented sexual cycle and manifest in either homothallic or heterothallic mating, which subsequently progresses into meiosis followed by ascospore production. However, many pathogenic species such as *Fusarium equiseti*, *Fusarium poae, Fusarium avenaceum, Fusarium cerealis,* and *Fusarium culmorom* lack recognized sexual stage (Kerényi et al., 2004). Sexual reproduction occurs in less than 20 percent of the species in *Fusarium*, Asexual spores are produced continuously throughout their lifecycle provided favorable environmental conditions and food are available. Thereby this technique of balance between sexual and asexual reproduction reflects the diverse methods used by fungal microorganisms to adapt to be changing conditions of the environment (Ajmal et al., 2022).

Fusarium species have several reproductive strategies including sexual spore generation which varies across several species. The ascospores of *Fusarium graminiarum* serve as primary inocula initiating the infection process and ending up causing *Fusarium* head blight in cereals such as wheat and barley (Agrios, 2005; Ajmal et al., 2022).In sexual reproduction, the ascospores are generally produced in perithecia and released into the environment, and on encountering susceptible plants they initiate the infection (Ajmal et al., 2022). *Fusarium* species can produce fruiting structures in the laboratory but the occurrence of sexual fruiting structures in the field is limited, for instance, with the *Gibberella* teleomorphs, only *G. zeae* and *G. fujikuroi* commonly produce fruiting bodies in the fields while other species do not (Ajmal et al., 2022).In asexual reproduction, *Fusarium* species employs three different types of mitotic spores -Macroconidia, microconidia, and chlamydospores, and this serves as an efficient method of reproduction and aid in survival during adverse weather conditions (Ajmal et al., 2022).

The life cycle of most of the *Fusarium* species including *Gibberella zeae* and *G. fujikuroi* - are known to cause blight of corn seedlings and small grains-including the production of ascospores or conidia depending on the environmental conditions. The life cycle starts with survival structures like chlamydospores or hyphae, residing in soil, perithecia ascospore), and plant debris which germinate under favorable environmental conditions giving rise to vegetative hyphae (Agrios, 2005).

The vegetative hyphae grow producing various asexual spores i.e. microconidia, macroconidia, and chlamydospores which will be dispersed in the spring by wind or water, upon landing on a plant surface they germinate and penetrate the plant through the wounds therefore causing infection. The conidia produced by infected plants serve as the secondary inoculum and the cycle starts again (Agrios, 2005).

Alternaria blight

Alternaria is one of the widespread fungal genera in cereal found in all parts of the world and is known to produce mycotoxins which are harmful to humans (Tralamazza et al., 2018; Woudenberg et al., 2014). *Alternaria* exists as a saprophyte, endophyte, and a pathogen in diverse variety of plants, where over 400 plant species have been reported to be susceptible (Tralamazza et al., 2018). Nearly 300 species of *Alternaria* have been documented as pathogens to several plant species whereby *Alternaria alternata*, *Alternaria tenuissima*, *Alternaria porri*, *Alternaria radicina*, *Alternaria solani Alternaria arborescens*, *Alternaria brassicae,* and *Alternaria infectoria* are the most ubiquitous species of *Alternaria* (Lee et al., 2015). According to (Jasim & Juber, 2010), *Alternaria alternata* was found to be the most frequent fungal pathogen on the seeds of barnyard grass among the 26 species of fungi tested. *Alternaria* contaminate cereals including wheat, sorghum, and proso millet among other crops, taking possession of the ripening ears and the product is a black pointed grain (Agrios, 2005; Lee et al., 2015).

Taxonomically, *Alternaria* is a, melanized fungus, characterized by the presence of dark grey colonies and in some cases brown and has been commonly classified based on morphological characteristics of the genus level (Tralamazza et al., 2018). Large conidia develop from inconspicuous conidiophores having distinct and longitudinal septa and elongated apical cells and conidia can occur individually or in chain patterns (Kustrzeba-Wójcicka et al., 2014).

Traditionally the genus is divided into two main categories based on conidial spore size, where small conidia are less than 60 micrometres and large conidial spores are less than 100 micrometres (Tralamazza et al., 2018; Woudenberg et al., 2014). Due to minute differences in the species of *Alternaria*, classification based on morphological characteristics is becoming challenging (Woudenberg et al., 2014).

Analysis of the secondary metabolite profile, produced by various species of *Alternaria* can be used in the identification and classification of the species of *Alternaria* having small spores like *Alternaria alternata*, *Alternaria infectoria*, and *Alternaria tenuissima* (Andersen & Thrane, 1996; Frisvad et al., 2008), but it is not a reliable method since some of the closely related species shown diversity in their metabolite profile. With the arrival of molecular analysis, phylogenetic analysis, and reassessment of classification was done and the results showed no correlation between the phylogenies and *Alternaria* preciously identified morphologically (Wang et al., 2011), more so in the small-conidial spore (Andrew et al., 2009).

The polyphasic approach lately has been used in the identification of various groups of fungi, and this method utilizes scientific techniques coupled with knowledge and approaches such as micro-and macro-morphology, biochemical analysis, and molecular biology. The use of the polyphasic approach in taxonomy emerges to be a promising tool in the identification of species of the genus *Alternaria* since it utilizes various techniques in identification rather than solely relying on one technique (da Cruz Cabral et al., 2017).

The smuts

Smut fungi, Basidiomycete parasitic biotrophs belonging to order Ustilaginales, that live inside and between the plant cells producing thick, ashy clumps of teliospores. Smuts consist of several economically important genera namely, *Ustilago*, *Tilletia*, *Entyloma,* and *Urocystis*. On artificial media, some forms of smut fungi can produce a slimy yeast-like growth. Grasses and cereals within the Poaceae family are generally considered susceptible to smut, with approximately 53 % of over 1450 described affected, leading to significant losses. Furthermore, many fungus species are capable of infecting sedges, according to (McKenzie & Vánky, 2001).

Typically, smuts produce two main types of spores, namely Basidiospores and Teliospores (McKenzie & Vánky, 2001). Spores gather in masses called sori which look like black blisters and can be found in many parts of the plant such as seeds, leaves, stems, flower parts, and bulbs. When sori break up they release black powder that can be carried away by wind, some smut fungi infect embryos and seedlings and develop throughout the plant only becoming visible near maturity (Britannica, 2015).

Smutted kernels of *Echinochloa crus-galli* were observed in Egypt and upon isolation, it was identified as *Ustilago trichophoron* (Link). The fungus creates clusters of flowers and bulging structures on stems that are covered by a rough membrane made up of both the fungus and the host materials. These bulging structures can range in size from a few millimeters to approximately 10 cm (Osman et al., 1999). Grain smuts caused by *Ustilago panici-frumentacei* have been isolated and identified as a disease that infects the grain seed of *Echinochloa crus-galli* with the sori developing the grain. Transformation of the ovaries into velvety, gall-like sori which are larger compared to normal grain follows, and the color of the sorus on the ear changes to black on drying (Tonapi, 2016). Moreover, according to (Cavers & Kane, 2016), *Ustilago destruens* Schltdl., *Ustilago panici-miliacei* (Pers.), *Ustilago syntherismae,* and *Ustilago crameri* Korn. causing smut on *Panicum miliaceum* in British Columbia and Canadian provinces were isolated in the years,1986 and 1992.

Pale greyish sori on flowers which later turns black has been seen in *Panicum miliaceum* and was later identified to be caused by a basidiomycete *Ustilago crameri*. This fungus affects most of the grains in the ears and sori are produced at the basal parts of the palea in flower then later raptures and black mass can be seen on the ears generally in the millet (Nagaraja, 2007).

Head smuts in proso millet and barnyard is also a common disease affecting the seeds, caused by *Sorosporium paspali-thunbergii*, characterized by a transformation of the whole panicle into long sorus. The panicles that have been infected are commonly covered by flag leaf and thereafter the envelope membrane bursts to release spores (Tonapi, 2016). The susceptibility of cultivated *Panicum miliaceum* to head smut (*Sporisporium destruens*) in Hungary was found to vary from 3 % to 87% according to (Kovacs et al., 1997)

Seed rots (*Rhizoctonia* spp, *Pythium* spp)

Rhizoctonia solani

Rhizoctonia, a genus of necrotic fungal microorganisms belonging to the division Basidiomycota; order Cantharellales, causes several illnesses in several cereal crops including rice and proso millet (Patro et al., 2018; Senapati et al., 2022). *Rhizoctonia solani* (Kuhn) is a species that causes banded blight in proso millet, having *Thanatephorus cucumeris* (Frank)Donk, as the teleomorph stage (Patro et al., 2018). *Rhizoctonia solani* produces sclerotia of different sizes which remain inactive for an extended period (Senapati et al., 2022).In addition to taking advantage of the high host specificity, the pathogen often survives in harsh environments by living on different hosts and may persist not only in soil but also over dead plant debris through the formation of durable structures such as sclerotia which serves as primary inoculum (Senapati et al., 2022).

The fungus causes a range of symptoms in different crops upon infection, such as sheath blight, foliar blight, head rot, bottom rot, and brown patch (Patro et al., 2018).In proso millet, the disease is identified by uneven lesions on the lower leaf sheath that expand rapidly and emerge to cover large areas of both the sheath and leaf lamina, followed by a series of brown bands across the leaves (Patro et al., 2018). After infecting the leaf sheath, the disease extends to infect the panicles and seeds (Sivalingam et al., 2006).

Discussing the disease cycle,*Rhizoctonia solani*, a pathogen transmitted through seeds and seeds and soil, with survival structures such as sclerotia mycelia occurring in infected seeds or the soil in tropical regions while in the temperate regions sclerotia in the soil and crop residue serves as a source of inoculum (Kozaka, 1970). Sclerotia germinates into mycelia when the conditions are conducive and upon establishing contact with the plant, these mycelia grow and generate infection structures like infection cushions and lobate appressoria which facilitate penetration into plant tissues moreover, penetration can also take place through the stomata. After that the mycelia grows and the spread from plant to plant is by mycelia which is carried by water. Infected seeds act as agents of dispersal of the fungus, where the seed can also transfer to the seedling, and this can be seen as lesions (Sivalingam et al., 2006). Wind disperses the basidiospores which act as secondary inoculum (Senapati et al., 2022).

***Pythium* spp**

Pythium is another genus of fungal microorganisms that infect the seeds, and the aftermath is seed rot. *Pythium* is an oomycete belonging to the kingdom Chromista and through its several species causes seed rots, seedling dumping off, and root rot of all types of plants (Agrios, 2005). All spring and winter small grains including Wheat (*T. aestivum*), millet, ryegrasses, and forage grasses are susceptible to *Pythium* spp.

In wheat and rye grasses *Pythium irregulare*, *Pythium vanterpoolii*, *Pythium* v*exans*, *Pythium acanthophoron*, *Pythium echinulatum*, and *Pythium violae* have been successfully isolated (Dewan & Sivasithamparam, 1988).*Pythium debaryanum* R. Hesse., *Pythium graminicola, Pythium monospermum* Pringsh. *Pythium ultimum* Trow. has been documented to cause browning root rot in some parts of Manitoba, Canada, and in the United States. (Cavers & Kane, 2016).

Discussing disease development,Pythium species produces a rapidly growing white mycelium, developing a sporangium, and germinates directly producing a soft hypha, finally producing a balloon-like structure (vesicle), which is a secondary sporangium. Inside the vesicle, several zoospores are produced and released which later germinate producing a germ tube thereafter penetrating the host and starting a new infection. On encountering the seeds and seedlings spore germ tubes or saprophytic mycelium, enter by direct penetration with the help of pectinolytic enzymes which dissolve the pectin that holds the cells together leading to maceration of cells. The protoplasts are broken down by the proteolytic enzymes of the infected cells hence causing complete collapse and disintegration of cell walls therefore the seeds or seedling dies resulting in a rotten mass made up of the oomycete, suberin, and lignin which cannot be broken down by the pathogen (Agrios, 2005).

Anthracnose (*Colletotrichum* spp)

Anthracnose disease, caused by an Ascomycetous fungi of the genus *Colletotrichum*, poses a challenge to the health of cereals and grasses worldwide, generally infecting over 42 genera of the family Poaceae. Knowledge of evolution, interaction with the host plant, and discovery of new *Colletotrichum* species have been increasing since the onset of 20^{th} C (Crouch & Beirn, 2009). Several species of *Colletotrichum* have been isolated in grasses including barnyard and Proso millet. For example, in China, a strain isolated from *Echinochloa crus-galli* was identified as *Colletotrichum echinochloae* in the year 2018 through single-spore isolation followed by the application of Koch postulates to determine the pathogenicity on *Echinochloa crus-galli*. This strain produces hyaline colonies with pink mycelium, black acervuli, and black sclerotia on PDA media (Gu et al., 2023). Additionally, *Colletotrichum jacksonii* and *Colletotrichum graminicola*, have been isolated from *Echinocloa crass-galli* (Crouch & Beirn, 2009). Furthermore, *Colletotrichum* species have been isolated from *Panicum miliaceum* whereby *Colletotrichum navitas* with characteristic hockey-stick-shaped conidia has been isolated (Crouch & Beirn, 2009).*Colletotrichum graminicola* (Ces.) G.W. Wilson was documented in 2013 to have been isolated in the United States of America, according to (Cavers & Kane, 2016)

Aspergillus

Aspergillus flavus, being the most predominant species, is a soil fungus that contaminates seed crops with aflatoxin, posing a threat to human safety and causing aspergillosis (Amaike & Keller, 2011). In a research work about the susceptibility of minor millets to Aspergillus, *Panicum miliaceum* and *Echinocloa crus- galli* was found to be infected with *Aspergillus flavus* in India (Ansari & Shrivastava, 1991). Moreover, *Aspergillus flavus* was isolated from the seeds of *Echinochloa crus-galli* in the year 2004 in Rajasthan, India where they were found to be producing aflatoxins (Rekha Purohit & Purohit, 2003). Several fungal pathogens were detected in the seeds of barnyard grass, which included *Aspergillus*, *Cladosporium*, and *Penicillium* genera, according to (Marchi et al., 2010).

3.0. MATERIAL AND METHODS

3.1. Experimental site

The experiment was carried out at the Hungarian University of Agriculture and Life Science, Georgikon Campus, at the Festetics Imre BioInnovacios Kozpont (Biotechnology laboratory) between January 2024 and March 2024.

3.2. Research materials.

The following materials were used for this study; -Seeds of *Panicum miliaceum* and *Echinocloa crus galli* collected from agricultural fields in the Keszthely and Szombathely regions of Hungary. Seeds of each plant were collected at different times with *Panicum miliaceum* seeds from 1985 and 2007 in Keszthely and *Echinochloa crus-galli* in the year 2010 from Keszthely and Szombathely; Petri dishes of diameter 12 cm; filter paper; water; Neomagnol tablets, Numigral seed counter; Thermostat.

3.3. Experiment setup.

The experiment was set in two repetitions with 50 seeds in each; therefore about 100 seeds were germinated per sample. The seeds were cleaned as much as possible to remove foreign impurities. The seeds were then counted using the Numigral seed counter. To exclude external infestation of the seeds and for microbiological tests, it was necessary to disinfect the surface of the seed areas, this was achieved by Neomagnol disinfection. 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 between the filter papers with great care to prevent the eyes from touching each other, thus avoiding cross-infections. Petri dishes with a diameter of 12 cm were prepared, each layered with a filter paper moistened with approximately 15 ml of water to ensure germination of the weed seeds, hold the seeds, and allow germination of fungal microorganisms. Fifty seeds of each weed species from each year were arranged grid pattern with 5 rows having 10 seeds each within the Petri dishes then covered as shown in (Figure 1 and Figure 2).

Then after preparation, the samples were placed in a cooling-heating thermostat-calibrated at 24 degrees (suitable for fungal germination) Celsius in an airtight package to eliminate over-contamination. The germination lasted for 14 days. During this time, we made visual scans, and the first one was on day 7. This study was used 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 determine the infectious species as accurately as possible (Figure 3 and Figure 4). The samples in this experiment were destroyed after evaluation.

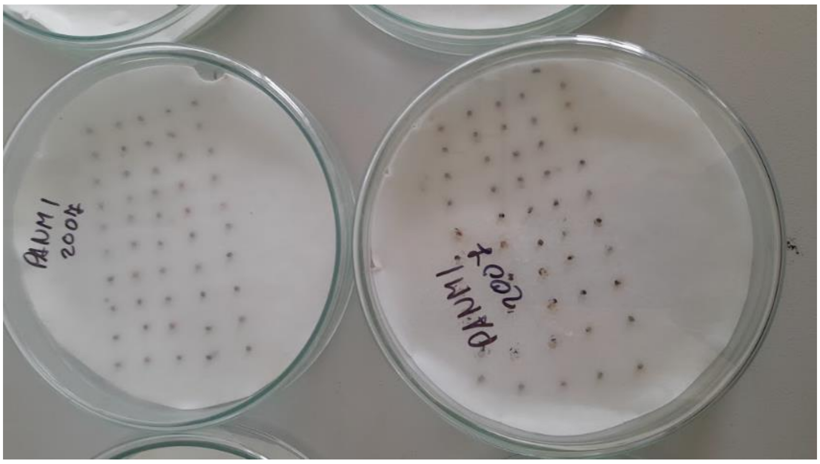

Figure 1 Panicum miliaceum seed samples on the moistened filter paper.Source: own

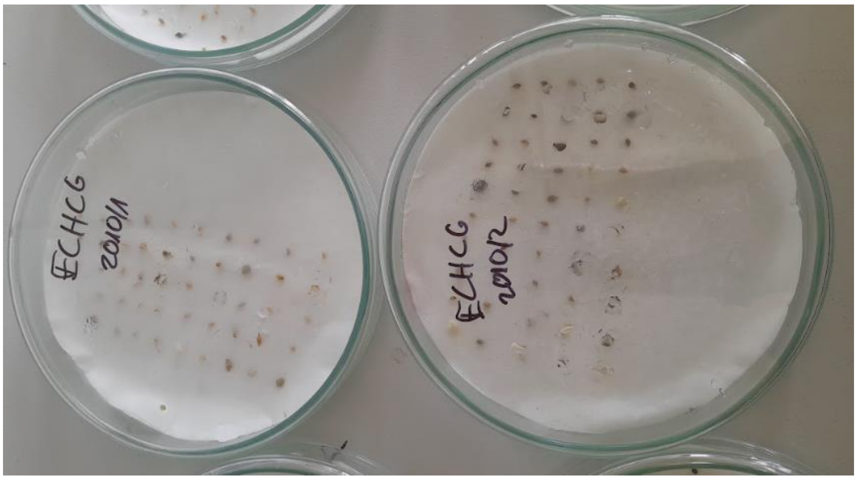

Figure 2 Echinocloa crus galli seed samples on the moistened filter paper. Source: own

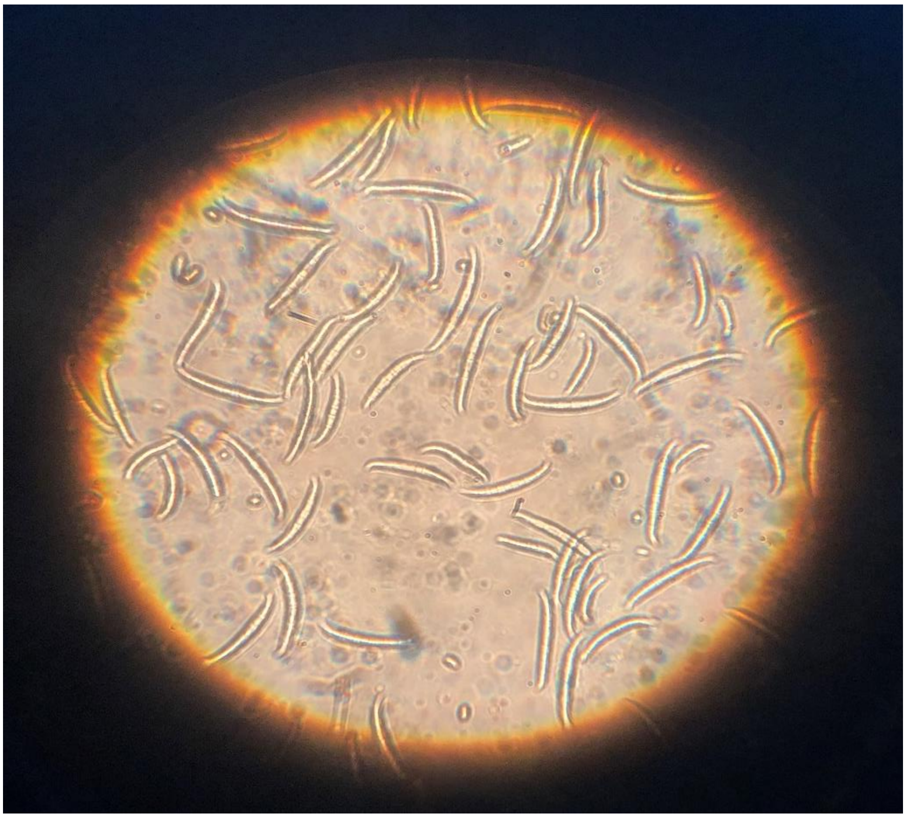

Figure 3 Sickle-shaped macroconidia of Fusarium spp from microscopic examination, Source: Own

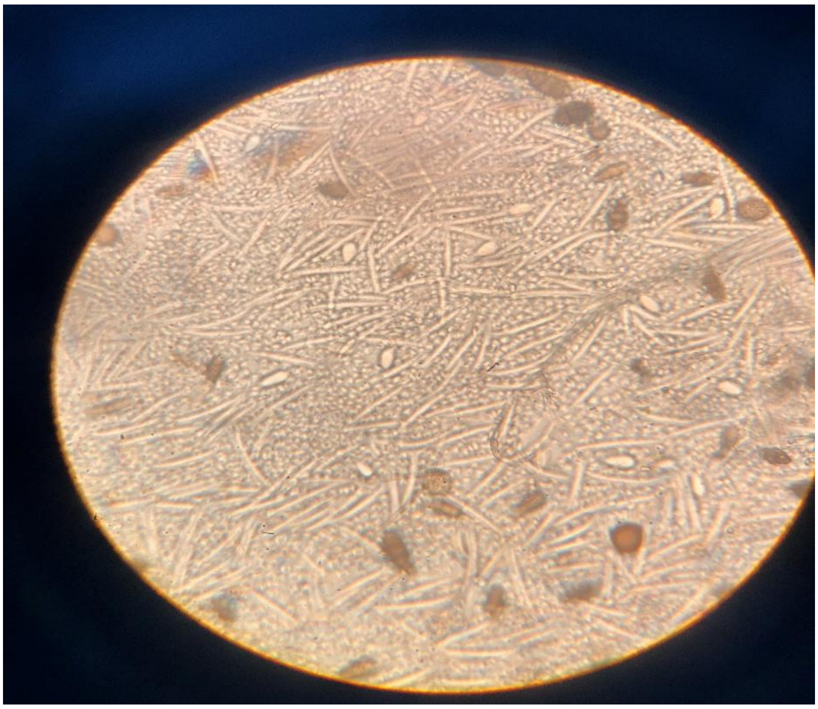

Figure 4 Round-shaped pale brownish macroconidia of Alternaria spp from microscopic examination, Source:Own

3.4 Statistical Analysis

The data collected from the experiment were organized and analyzed using Microsoft Excel 365 to obtain the average infections ,trends of infection over time ,percentages of infection and Visualization of the data based on the years of collection, and the locations (regions) were seeds where collected.

4.0. RESEARCH RESULTS AND THEIR ASSESSMENT.

The table below displays the number of seeds of *Panicum miliaceum* and *Echinochloa crus-galli* infected by fungal pathogens examined in this experiment. The three most frequently identified genera are *Fusarium*, *Alternaria*, and *Aspergillus* with different infection rates in different years and locations where the seeds were collected.

Table 1 Prevalence of fungal infections on Panicum miliaceum and Echinochloa crus-galli collected from different locations and years.Source: own

Seed type	Collection year	Collection location	Total number of seeds	*Fusarium* infection	*Alternaria* infection	*Aspergillus* infection
Panicum miliaceum	1985	Keszthely	50	25	13	5
Panicum miliaceum	1985	Keszthely	50	27	10	5
Panicum miliaceum	2007	Keszthely	50	21	8	3
Panicum miliaceum	2007	Keszthely	50	20	9	3
Echinochloa crus-galli	2010	Szombathely	50	25	13	12
Echinochloa crus-galli	2010	Szombathely	50	16	12	11
Echinochloa crus-galli	2010	Keszthely	50	18	8	17
Echinochloa crus-galli	2010	Keszthely	50	25	12	10
Total			**400**	**177**	**85**	**66**

The table below shows the summarised frequency of fungal infections on the seeds of *Panicum miliaceum* and *Echinochloa crus-galli* per year of collection and overall percentages of infection of each fungal genera.

Table 2 Table showing the total number of seeds and percentages of infected by Fusarium, Alternaria, and Aspergillus.Source: own

			Number	Frequency of fungal infections			
Seed type	**Year**	**Location**	**of seeds**	***Fusarium***	***Alternaria***	***Aspergillus***	**Healthy seeds**
Panicum miliaceum	1985	Kesthely	100	52	23	10	15
Panicum miliaceum	2007	Kesthely	100	41	17	6	36
Echinocloa crus-galli	2010	Szombathely	100	41	25	26	11
Echinocloa crus-galli	2010	Kesthely	100	43	20	24	10
Total number of seeds			**400**	**177**	**85**	**66**	**72**
Percentage of Infection				**44.25**	**21.25**	**16.5**	**18**

4.1 *Panicum miliaceum* seeds

After incubation for 14 days, out of the 200 Seeds of *Panicum miliaceum* that were collected from two different periods, i.e. 1985 and 2007 in Keszthely were examined for fungal infestation. The results revealed varying degrees of ascomycete fungal infection in the seeds with *Fusarium*, *Alternaria*, and *Aspergillus* being the most common genera.

4.1.1 *Panicum miliaceum* seeds 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*, *Alternaria*, and *Aspergillus* were the most ubiquitous pathogens identified by physical assessment based on the mycelial color produced and with the confirmation with microscopic analysis. In a total of 100 seeds 52 seeds were found to be infected with *Fusarium* species,23 seeds infected with *Alternaria*, and 10 seeds infected with *Aspergillus* ,as seen in (Table 1) above.

4.1.2 *Panicum miliaceum* collected from Keszthely (2007)

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

4.2 *Echinochloa crus-galli* seeds

Seeds of *Echinochloa crus-galli* that were collected from Keszthely and Szombathely regions in Hungary in the year 2010 were incubated for 14 days and visual identification followed by microscopy was done and this revealed distinct patterns of fungal infestation in seeds collected from these regions.

4.2.1 *Echinochloa crus-galli* seeds collected from Szombathely (2010)

A significant number of fungal infections from the seeds collected from Szombathely in the year 2010 was recorded, with the genera *Fusarium*, *Alternaria*, and *Aspergillus* being the most identified. According to the results obtained, a total of 41 seeds were found to be infected with *Fusarium* species,25 seeds infected with *Alternaria*, and 26 with *Aspergillus* species out of the 100 seeds for the year 2010, that were incubated in the two repetitions.11 seeds were found to be healthy (Table 2).

4.2.2 *Echinocloa crus galli* seeds from Keszthely (2010)

Fungal infections were detected from seeds collected from Keszthely, even though to a lesser extent compared to those seeds collected from Szombathely. Fungal genera *Fusarium*, *Alternaria*, and *Aspergillus* dominated as well with White/black, pink, and green mycelial colours respectively. Out of the 100 seeds of *Echinocloa crus galli* (2010) incubated, a total of 43 seeds were found to be infected with *Fusarium*,20 infected with *Alternaria*, and 24 with *Aspergillus*.10 seeds were healthy as seen in (Table 2) above.

In general, out of a total of 400 seeds of both *Panicum miliaceum* and *Echinocloa crus galli* examined in this study,177 seeds were found to be infected with genera *Fusarium*,85 seeds infected with *Alternaria*, and finally, 66 seeds were found to be infected with *Aspergillus*. A total of 72 seeds were found to be healthy as seen in (Table 2) above.

The charts below show the distribution of Fungal genera (*Fusarium*, *Alternaria*, *Aspergillus*) in the seeds of *Panicum miliaceum* and *Echinochloa crus-galli* in Keszthely and Szombathely regions in Hungary in the years 1985,2007 and 2010.

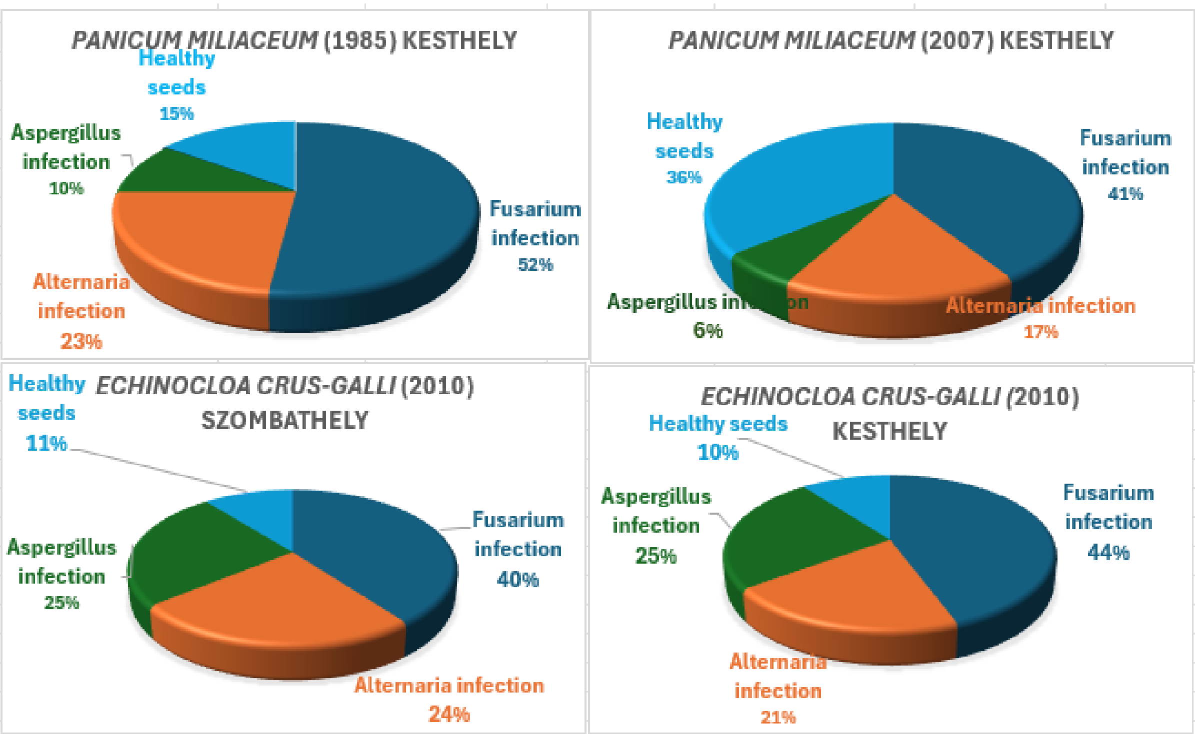

Figure 5 Distribution of Fungal diseases in seeds of Panicum miliaceum and Echinochloa crus-galli in Keszthely and Szombathely in the years 1985,2007 and 2010. Source: Own

The figures 6 ,7,and 8 below shows a graph of *Fusarium* ,*Alternalia* and *Aspergillus* infections over time from the seeds of *Panicum miliaceum* and *Echinochloa crus-galli* collected in 1995 ,2007 and 2010 in Keszthely and Szombathely.

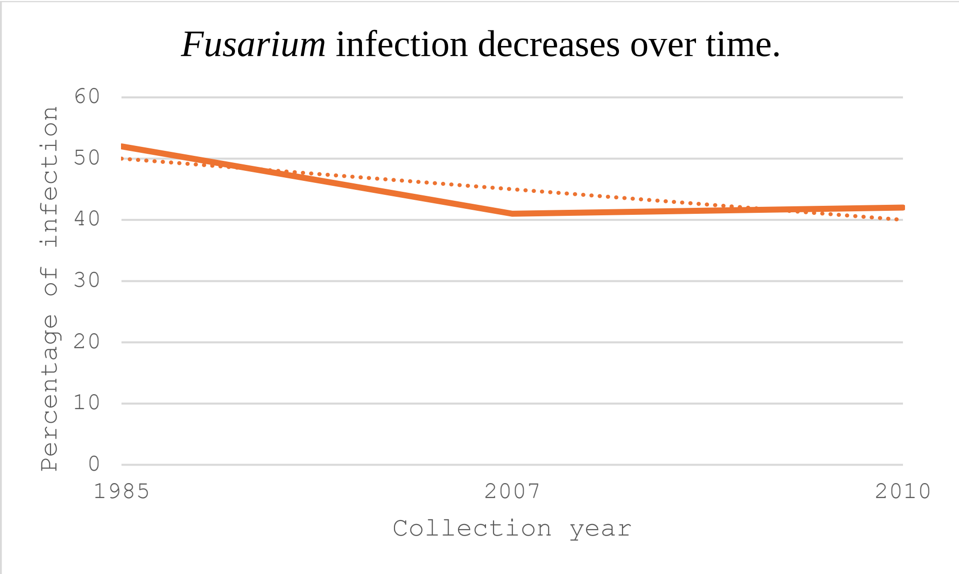

Figure 6 Trend of Fusarium infection over time.Source: own

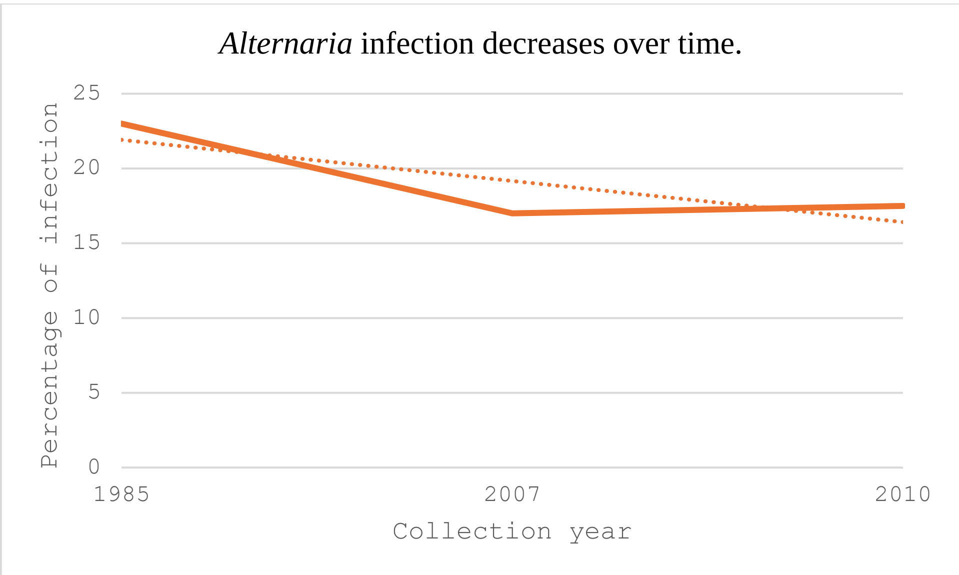

Figure 7 trend of Alternaria infection over time.Source:own

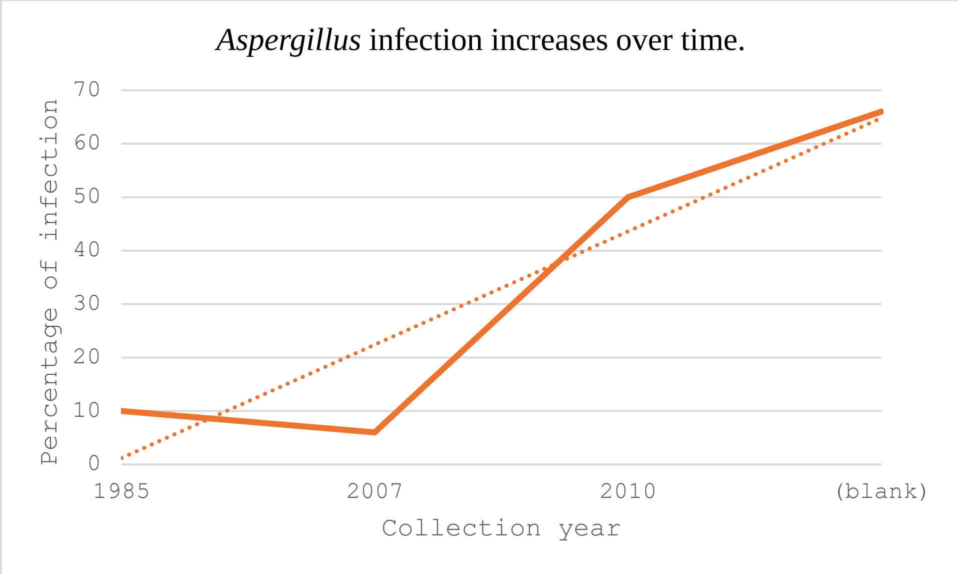

Figure 8 Trend of Aspergillus infection over time.Source: own

4.3. Discussion of results

In this study, a microscopic assessment of Fungal pathogens found in the seeds of two noxious monocotyledonous weeds, *Panicum miliaceum,* and *Echinochloa crus-galli* was carried out. The results showed that the seeds of proso millet (*Panicum miliaceum*) and barnyard grass (*Echinochloa crus-galli*) collected in Hungary are infected with fungal pathogens. Varying degrees of incidence and prevalence of the three phytopathogenic fungal genera were observed from the results of this study. The fungal genera found on the seeds of these weeds were compared about the weed species, year of collection of seeds, and the locations where the seeds were collected for the case of *Echinochloa crus-galli* which ws collected in Keszthely and Szombathely. Out of the total 400 seeds from *Panicum miliaceum* and *Echinochloa crus-galli*, a total of 177 seeds were found to be infected with *Fusarium*,75 seeds were infected with *Alternaria* 66 seeds were infected with *Aspergillus*.

In this study, it was discovered that the seeds of two weed species *Panicum miliaceum* and *Echinochloa crus-galli* from the Poaceae family; *Panicum miliaceum* and *Echinochloa crus-galli* were infected with three types of fungi: *Fusarium*, *Alternaria*, and *Aspergillus*. These results are consistent with previous studies conducted by (E.B. Nelson, 2018), which 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 also 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 barnyard grass (*Echinochloa crus-galli*). Moreover, this study supports research conducted in London and Ontario by (Cavers & Kane, 2016), which found six genera of fungi - *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Helminthosporium*, and *Epicoccum* - were isolated from the seeds of *Panicum miliaceum* that failed to germinate.

Fusarium and *Alternaria* showed a higher prevalence rate than *Aspergillus* in both weed species, with *Fusarium* as the most dominant with 52 % and 41 % in the *Panicum miliaceum* seeds collected from Keszthely in the years 1985 and 2007 respectively, and in *Echinochloa crus-galli*, the percentage of infection was 43 % in Keszthely and 41 % in Szombathely (Figure 5). 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 the weed seeds but also cereals. 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 *Echinochloa crus-galli* and *Avena fatua* collected in the same location in 2020 and 2021 in Latvia. Similarly, research by (Szécsi et al., 2013) showed that *Fusarium* species were found in 62.3 % of the 43 different stems and leaves of grass species including *Panicum miliaceum* and *Echinochloa crus-galli* collected from different regions of Hungary therefore showing a close relationship with the results obtained in this experiment.

High frequency of occurrence of *Alternaria* in the seeds of both species; with infection of 23 %, and 17 % from the *Panicum miliaceum* seeds collected in Keszthely in the years 1985 and

2007 respectively. Seeds of *Echinochloa crus-galli* with a 21 % from Keszthely and 24% from Szombathely, collected in 2010, showed a relatively higher infection in Szombathely than in Keszthely (Figure 5). The results align with the previous studies of (Jasim & Juber, 2010), who established that *Alternaria* was the most frequent fungal pathogen on the seeds of Barnyard grass(*Echinochloa crus-galli*) among the 26 species of fungi tested. *Alternaria* frequently colonizes seeds (Simonin et al., 2022), exists as a saprophyte, endophyte, and a pathogen in variety of plants, and is reported in over 400 plant species, the most ubiquitous were *Alternaria alternata*, *Alternaria tenuissima*, and *Alternaria pori*, contaminating cereals and other crops, according to (Lee et al., 2015; Tralamazza et al., 2018).

Based on the findings of the experiment, it was discovered that fungal infections occurred differently in proso millet (*Panicum miliaceum)* and barnyard grass (*Echinochloa crus-galli)* seeds. While both species were susceptible to *Fusarium* and *Alternaria*, seeds of barnyard grass (*Echinochloa crus-galli*) showed a higher likelihood of infection accompanied by rapid degradation of the seed. A total of 179 seeds of *Echinochloa crus-galli* were found to be infected by the three fungal genera compare to a total of 149 seeds of Panicum miliaceum infected by the three fungal genera out of the total 400 seeds examined.Therefore meaning in terms of percentage, *Echinochloa crus-galli* seeds showed an infection rate of 44.75% and *Panicum miliaceum* with 37.25% .These observations is similar to what (Ņečajeva et al., 2023), documented about the high degrading capacity of *Alternaria species* on the seeds of barnyard grass (*Echinochloa crus-galli*), where specifically *Alternaria alternata* and *Alternaria tenuissima* were proved to have a high degrading capacity on the seeds of barnyard grass (*Echinochloa crus-galli*) and therefore it has been proposed that this two species can be used as a biocontrol against barnyard grass which is a noxious weed in agricultural fields. Additionally,the varying susceptibility levels of barnyard barnyard grass and proso millet 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 (Ņečajeva et al., 2023; Pollard, 2018).

Some seeds of *Panicum miliaceum* and *Echinochloa crus-galli* were identified to be co-inhabited by both *Fusarium* and *Alternaria*. For this case, the seeds appeared to be more degraded than the ones inhabited by one of the genera of phytopathogenic fungi. This correlate with previous studies observed in the research work of (Ņečajeva et al., 2023), where seeds of *Echinochloa crus-galli* and *Avena fatua* (both belonging to family Poaceae) were found to be co-inhabited 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-inhabited 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 this happens also in the case of cultivated crops and might cause high yield loss and high contamination of the cereal crops.

On analysis of the overall infection rate of *Fusarium* on the two weeds over time, the trend of *Fusarium* infection on Proso millet (*Panicum miliaceum*)and barnyard grass(*Echinochloa crus-galli*) over time decreased and with the trend line, a drop in the percentage of infection from 1985 to 2007 to 2010 is observed (Figure 6). Similarly, Overall, the trend of infection of seeds by *Alternaria* in the years 1985, to 2010 (Figure 7) tended to decrease as also witnessed in *Fusarium*. The decrease in the rate of infection of seeds by *Fusarium* and *Alternaria* may be attributed to several factors such as changes in climate and weather patterns, and changes in agricultural practices for instance farmers adopting new practices that reduce the build-up of fungal spores on plant surfaces and soil, improvement in disease management for instance early detection, and advancement in research.

When it comes to *Aspergillus*, the study showed a lower infection rate in the previous years, that is, 10 % and 6% in *Panicum miliaceum* in 1985 and 2007 respectively, and 25% from Szombathely and Keszthely in the year 2010 (Figure 8). Results on the identification of *Aspergillus* in this experiment were also obtained in two research studies in India, where in one case seeds of 5 minor millet species (*Paspalum scrobiculatum*, *Panicum miliaceum*, *Panicum trypheron*, *Setaria italica,* and *Echinochloa crus-galli*) 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 & Shrivastava, 1991).

5.0. CONCLUSIONS AND RECOMMENDATIONS

There were three fungal genera identified in this study, indicating that *Panicum miliaceum* and *Echinochloa crus-galli* in Hungary are infested with fungal phytopathogens. From this research mainly class Ascomycetes, a group of fungi was found to be more prevalent, which was also like what previous scientists (E.B. Nelson, 2018- Ņečajeva et al., 2023) found to be a common group of fungi isolated in the seeds of members family Poaceae. The three fungal phytopathogen genera that were identified both in the seeds of Proso millet (*Panicum miliaceum*) and barnyard grass (*Echinochloa crus-galli*) are *Fusarium*, *Alternaria* and *Aspergillus*.

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 in which the seeds were collected. *Alternaria* was the second most identified genera in both proso millet and barnyard grass, accounting for 21.25% of the total seed examined in this study (Table 2). In some cases, seeds were seen to be co-inhabited by the two fungal genera *Fusarium* and *Alternaria* and these seeds appeared to be highly degraded ,these results were similar to the research on (Ņečajeva et al., 2023) where the seeds of *Echinochloa crus-galli* that were examined for endophytic and epiphytic fungal infections showed a higher degradation in the case where the species of both *Fusarium* and *Alternaria* were present in a seed. While the seeds of both *Panicum miliaceum* and *Echinochloa crus-galli* were susceptible to *Fusarium* and *Alternaria*, seeds of barnyard grass showed a higher likelihood of infection accompanied by rapid degradation of seeds moreover whenever both *Fusarium* and *Alternaria* co-inhabit the seed .Generally,this rapid degradation of seeds is because two fungal genera may engage in competitive interactions due to their antagonistic mycotoxin production, which can destroy the seed rapidly (Müller et al., 2015).

Finally, Aspergillus, commonly found in seeds and grains in most parts of the world was also identified genera infecting the seeds of *Panicum miliaceum* and *Echinochloa crus-galli* from Keszthely and Szombathely regions with a 16.50 % of the seeds infected. Seeds of *Echinochloa crus-galli* were more susceptible to this filamentous fungus, and this was observed in the seeds collected from Keszthely and Szombathely showing a similar percentage of infection (Figure 5).

Based on findings from this study, seeds of *Panicum miliaceum* collected in 1985 and 2007 in Keszthely and *Echinochloa crus-galli* collected in 2010 in Keszthely and Szombathely, are infected with the most important fungal genera *Fusarium*, *Alternaria*, and *Aspergillus*. Since the fungal genera identified in this study cause significant economic losses on crops, therefore Integrated Pest Management (IPM) practices such as crop rotation, field sanitation, and seed treatment should be implemented because they focus on the holistic approach to disease control. This reduces reliance on chemical fungicides, which is a requirement in the new EU agricultural policy.

In this study I identified the fungal pathogens at the genera level and therefore further research can be done on molecular identification of the fungal pathogens in the seeds of *Panicum miliaceum* and *Echinochloa crus-galli* at the species level, using advanced molecular techniques such as PCR, DNA sequencing, and metagenomics to identify and characterize this 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.

6.0. SUMMARY

The purpose of this experiment was to investigate the fungal seed diseases present in the seeds of two monocotyledonous weed species- *Panicum miliaceum* and *Echinochloa crus-galli*-in Hungary. The seed samples of *Panicum miliaceum* used were collected in the years 1985 and 2007 in Keszthely and the seed samples of *Echinochloa crus-galli* were collected in the year 2010 in Keszthely and Szombathely regions. A total of 100 seeds per year of the collection 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 between the filter papers with great care to prevent the eyes from touching each other, thus avoiding cross-infections. Petri dishes with a diameter of 12 cm were prepared, each layered with a filter paper moistened with approximately 15 ml of water to ensure germination of the weed seeds, hold the seeds, and allow germination of fungal microorganisms. The seeds were germinated on Petri dishes in the laboratory at a temperature of 24 °C for 14 days. During this time, we made visual scans, and the first one was on day 7. This study was used to check for initial fungal infections. The second visual test was carried out on day 14 where the exact number of germinated seeds infected with fungal microorganisms was determined and recorded. Thereafter the seeds showing symptoms of infection were prepared for microscopic examination. A fraction of the seeds of both weed species were infected with fungal species of three genera belonging to Ascomycete group of pathogenic fungi. The most prevalent genera identified to be present in the germinated seeds were *Fusarium*, *Alternaria*, and *Aspergillus*. 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 in which the seeds were collected. *Alternaria* was the second most identified genera,

accounting for 21.25% of the total seed examined, and finally, *Aspergillus* was the third with 16.50%. While the seeds of both proso millet (*Echinochloa crus-galli*)and barnyard grass (*Echinochloa crus-galli*) were susceptible to *Fusarium* and *Alternaria*, seeds of barnyard grass (*Echinochloa crus-galli*) showed a higher likelihood of infection accompanied by rapid degradation of seeds moreover whenever both *Fusarium* and *Alternaria* co-inhabit the seed. A total of 179 seeds of *Echinochloa crus-galli* were found to be infected by the three fungal genera compared to a total of 149 seeds of *Panicum miliaceum* infected by the three fungal genera out of the total 400 seeds examined. Therefore in terms of percentage, *Echinochloa crus-galli* seeds showed an infection rate of 44.75% and *Panicum miliaceum* with 37.25%. Based on the location of collection (region), seeds of *Echinochloa crus-galli* collected from Keszthely showed a relatively higher percentage of infection with *Fusarium* compared to the seeds from Szombathely while the percentage of *Alternaria* infection in Szombathely was relatively higher than Keszthely and the percentage of *Aspergillus* infection was found to be similar in the seeds collected the same year (2010)from Szombathely and Keszthely. These fungal phytopathogens cause serious economic losses to farmers, and therefore better holistic approaches such as Integrated Pest Management (IPM) to control the fungal disease are needed to mitigate economic losses and reduce the use of chemical fungicides hence promoting the EU "Farm to Fork Strategy" which at the heart of the European Green Deal.

Acknowledgment

I wish to express my deepest gratitude to the **Almighty God** for the gift of life and for keeping me healthy during my studies.

I would like to express my sincere gratitude to my mentor and thesis supervisor, **Dr. Habil Takacs András Péter**, (Associate Professor, Keszthely). His expertise in the field of Plant protection and guidance during my thesis writing and studies have greatly improved my knowledge in the field. I would like to acknowledge the invaluable support provided by **Dr. Pásztor György**, (Assistant Professor, Keszthely), throughout my thesis work. Your resourcefulness, time, and constructive criticisms during my thesis preparation were instrumental in helping me complete this thesis work. Thanks to my lecturers, Dr. Zsolt Marczali and Dr. Pacseszákné Kazinczi Gabriella Márta for their advice and motivation during my coursework.

I am very grateful to the **Stipendium Hungaricum Scholarship** and **Tempus Public Foundation** for awarding me the scholarship grant that made my studies possible in Hungary, not forgetting the **Hungarian University of Agriculture and Life Sciences (MATE)-Georgikon campus** for admitting me into the Master of Science in Plant Protection Course.

Furthermore, my heartfelt gratitude goes to my Parents, **Christopher Koech**, and **Ann Chebet**, they have been amazing parents to me for their love, protection, and support in any way they could to see that I accomplish many important steps in my life. I would also like to thank my siblings **Mercy**, **Nancy**, **Faith**, **Franklin**, and **Hyline**, they have been a big motivation to me and because of them, the home has been the best and warmest place to be, I would also like to thank my friends and course mates, **Simon**, **Gideon,** and **Quyen** for their unwavering support throughout this academic endeavours, their friendship has made this journey more meaningful and enjoyable. May God bless you all!

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STUDENT DECLARATION

Signed below, **Kiprop kelvin,** a student of the Georgikon Campus of the Hungarian University of Agriculture and Life Sciences, at the MSc. Course of **Plant Protection** declare that the present Thesis is my own work and I have used the cited and quoted literature in accordance with the relevant legal and ethical rules. I understand that the one-page-summary of my thesis will be uploaded on the website of the Campus/Institute/Course and my Thesis will be available at the Host Department/Institute and in the repository of the University in accordance with the relevant legal and ethical rules.

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As primary supervisor of the author of this thesis, I hereby declare that review of the thesis was done thoroughly; student was informed and guided on the method of citing literature sources in the dissertation, attention was drawn on the importance of using literature data in accordance with the relevant legal and ethical rules.

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