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Master of Science in Plant Protection

SEED INFECTING FUNGI OF TWO MAJOR DICOT WEEDS IN HUNGARY

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1. INTRODUCTION

Several scientific research studies and findings have shown that weeds are field pests that hinder crop growth and reduce their productive ability. Their existence in agricultural land reduces the quantity and quality of agricultural products and causes huge economic losses. In addition to competition and interference with crops for growth resources, they are important agents of plant disease spread and retention through their organs such as seeds, leaves, stems, roots, fruits, flowers etc (Ekwealor et al., 2019). Weeds have become a basic consideration in the management and control of phytopathogens of crops, as they can either be vector of a pathogen, or reservoir of a pathogen or its vector thus significantly influencing disease incidence in arable farming enterprises. They can establish ecological niches suitable for the growth and development of plant pathogens in agro-ecosystem, while some produce allelopathic toxic compounds in either their leaves, flowers, fruits, roots, rhizomes, or seeds, that directly deter growth and development of arable crops and/or are lethal. The relationship between weeds and phytopathogens influence plant disease incidence and impact in agricultural fields.

Previous scientific studies have revealed that weeds can contain / harbour various viable forms of plant pathogens in their roots, crowns, stems, leaves, flowers, fruits or seeds evidenced by lots of phytopathogenic isolations that have been obtained from numerous weed species and are capable of spreading them to infect crops.

Researchers in Plant pathology have studied and isolated several viable phytopathogens from seeds of various weed species both monocot and dicot weeds as well as from seeds of cultivated plants. Among the genera of phytopathogenic fungi that have been found in most weed seeds worldwide include *Pythium, Alternaria, Colletotrichum, Aspergillus, Penicilliun, Fusarium, Cladosporium,* *Epicoccum, Macrophomina, Diaporthe,* etc. Phytoviruses include Tobamovirus, Apple mosaic virus, Prunus necrotic ringspot virus, Tomato yellow leaf curl virus etc. Viroids include Pospiviroids, Tomato apical stunt viroid, Pepper chat fruit viroid, Citrus exocortis viroid, Tomato chlorotic dwarf viroid, Potato spindle tuber viroid etc while phytopathogenic bacteria include *Acinetobacter spp., Alcaligenes spp., Bacillus spp., Citrobacter freundii, Enterobacter spp., Erwinia amylovora, E. herbicola, Erwinia sp., Flavobacterium spp., Moraxella sp., Pseudomonas spps., Xanthomonas spp etc.* (Avinash Marwal and R.K.Gaur, 2020 ; Postic et al., 2012 ; Kremer et al., 1984; Kremer, 1986; Kremer, 1987; Malavika Dadlani & Devendra K. Yadava, 2023).

However, this study looks at *Datura stramonium and Abutilon theophrasti*, the dicot weed species that have invasively invaded grain fields of Hungary, continually introduced into Europe through increasing globalization and trade, and have become established in agricultural areas, highly competitive and difficult to control (Novak Robert et al., 2014). They have strong growth and allelopathic properties (Dafaallah, 2019; Tian et al., 2022). A 16/ m^2 density of *Datura Stramonium* can result in a reduction in the maximum 1000 seed weight of maize by 37.4% (Karimmojeni et al., 2021). Its foliage is very susceptible to viruses of Solanaceous crops and so act as a host (By & Gaire, 2008). Velvetleaf is a primary pest in spring planted row crops like corn and soybean and can cause large yield losses when the weather is dry as it is a very strong competitor than crops (McDonald et al., 2004;Tian et al., 2022).

Datura stramonium and *Abutilon theophrasti* have invaded and become established in agricultural fields of Hungary and are known to be highly competitive than crops and difficult to control (Novak Robert et al., 2014) and harbour many crop pathogens. They have strong growth and allelopathic properties that inhibit early seedling growth of crops (Dafaallah, 2019; Tian et al., 2022), but not much has been studied about potential pathogenic fungi in their seeds in Hungary, yet seeds are efficient dispersal and survival units for pathogenic fungi and other pathogens, and majority of crop diseases troubling farmers in Hungary and worldwide in fields infested by weeds are fungal (Oliveira et al., 2018). These two weed species produce thousands of viable seeds per plant capable of staying alive in the soil for too many years (Follak et al., 2017; Loddo et al., 2019; Warwick et al., 2011; Rojas-Sandoval, 2022b).

Therefore, the objective of my study is to identify potential pathogenic fungal genera in the seeds of *Datura stramonium and Abutilon theophrasti* in Hungary. The findings, therefore, can shift attention of Hungarian farmers and other stakeholders to devising strategies to curtail seed production by these two weed species thus curbing the pathogenic fungal species spread in their seeds and reduce their seedbanks in the soil. This will also reduce the usage of fungicides in crop fields in an effort to manage the fungal diseases that will be identified by this research work and as well reduce the population of these weeds.

2. LITERATURE

2.1 Datura stramonium

2.1.1 Introduction and origin

Datura stramonium is identified as a member of the Solanaceae family, found growing in cultivated fields, pastureland, around barnyards, timberyards, docks and waste places, frequenting dung-heaps, the roadsides, soil deposit sites and town & village waste disposal sites (By & Gaire, 2008).

Its nativity country is not clear. Some botanists say North America because it is a familiar plant abundantly widespread there, while others consider its origin to be South America or Asia. Early researchers say it is indigenous to the Old World, probably to the borders of the Caspian Sea (By & Gaire, 2008).

Nomenclature : (By & Gaire, 2008); Kingdom- Plantae, Sub-kingdom-Tracheobionta (Vascular plants), Super-division- Spermatophyta (Seed plants), Division -Magnoliophyta (Flowering plants), Class -Magnoliopsida (Dicotyledons), Sub-class-(Asteridae), Order -(Solanales), Family -Solanaceae, Genus -Datura L., Species -stramonium L.; Binomial name -*Datura stramonium L.*

2.1.2 Distribution and Habitat

Datura stramonium is found throughout the world except the colder Arctic regions. Gypsies are reported to have contributed to the spreading by means of its seeds from western Asia into Europe. It is only occasionally found in Great Britain and is reported that some English grew it as an ornamental plant in London (By & Gaire, 2008) . Being a warm weather plant*, Datura stramonium* is found as a weed in the warmer parts of North, Central and South America, Europe, Asia, and Africa, commonly growing in the disturbed/cultivated lands, where refuse rubble and soil is deposited, wastelands, gardens and roadsides (Mirha & Gadžo, 2017). Recently, it has been reported that *Datura stramonium* occurs in intensively farmed regions in Eastern Austria and has spread locally, but low populations in the far North-East of Austria, and absent in Western Austria (Follak et al., 2017).

2.1.3 Plant description.

Datura stramonium is a broad leaved annual erect herb with a bushy growth in rich soil due to its branchy morphology. Its body is smooth, except the younger parts, which are covered with short hairs. It emits strong odour from the leaves when bruised, but the flowers are sweet-scented (Soni et al., 2012 ; By & Gaire, 2008). The root system is large and whitish with many long and thick fibers. The stout, smooth stem, and branches can be green or purple. In the forks of the branches emerge a leaf and a single, erect flower. Leaves are simple, alternate, wide and ovate with pinnately shallow tip pointed lobes. The margin may have secondary lobes/coarse dentate teeth. Young leaves may be hairy but become hairless with age. The flowers are large, white or faint-violet and eye catching when fully open, growing singly on short stalks that spring out from the axils of the leaves or at the forking of the branches. Sepals are 5 fused into tubular shape while petals are also 5 fused at the base and folded into funnel shape. The flowers open in the evening for the attraction of night-flying moths and emit a powerful fragrance. The fruit is a large, oval, green seed capsule covered with numerous sharp spines. The fruits become brown upon ripening then open outwardly from the tops to scatter the dark-brown seeds (Kuete, 2014)**.**

2.1.4 Biology of *Datura stramonium.*

Datura stramonium propagates by seed and grows well in wide climates and soil conditions, but clayey, acidic, water-logged or moisture deficient soils are unfavourable (By & Gaire, 2008). It prefers full or partial sun, moist conditions, and rich fertile soil. Its seeds stay viable in the ground for many years and aerial parts of perennial species die back during the winter (if frosted) and will grow back from the roots in spring (By & Gaire, 2008). The optimum temperature for its seed germination is reported to be 30 °C and with scarification of testa, germination efficiency is very high. The hard testa slows down germination because it is nearly impervious to water and the diffusion of oxygen, and embryo may be maintained in the dormant condition till the testa ruptures (Marwat et al., 2005; Benvenuti & Macchia, 1997; Nikolić et al., 2022). The flowers are insect pollinated by Sphinx moths, flea beetles and bees (By & Gaire, 2008).

2.2 *Abutilon theophrasti*

2.2.1 Introduction, origin and distribution

Abutilon theophrasti is a tall erect hairy summer annual herb, belonging to the mallow family (Malvaceae), and reproduces by seed. Its habitats can be orchards, vineyards, crop fields, nursery fields, gardens, roadsides and other disturbed areas. It is said to be native to southern Asia, where it is cultivated in northern China for its strong, jute-like fiber in the erect stem for craft work. It is said to be widely naturalized in warmer regions of North America, where it is said to be a pronounced farm weed (Britannica, 2023 and Susan Sahr, 2023). It is reported to be the only species in temperate climates, while others are in tropical and subtropical climates (Rojas-Sandoval, 2022b). It is distributed throughout Asia, Europe and North America (Tian et al., 2022), and reported to be also frequent in crop fields of Hungary in Sugar beets and Maize (Novak et al., 2009**,** Novak Robert, 2012). Today, larger populations of *Abutilon theophrasti* can be found in agricultural areas in the lowlands in eastern Austria (Pannonian Basin), but also commonly along the Danube valley (Upper Austria) and locally in the Alpine foreland, while in the south, it occurs infrequently (Follak et al., 2017). In Austria, the Czech Republic, and Slovakia, continuous increase has been recorded since its introduction in the 19th century where it has invaded farm fields much more frequently, mainly sugar beet and maize fields (Follak et al., 2014).

Nomenclature: (Britannica, 2023); Domain -Eukaryota, Kingdom -Plantae, Phylum -Spermatophyta, Subphylum -Angiospermae, Class -Dicotyledonae, Order -Malvales, Family -Malvaceae, Genus -*Abutilon*, Species -*theophrasti*; Binomial name -*Abutilon theophrasti Medik.*

2.2.2 Morphological description:

Seedlings have one round and one heart-shaped cotyledons and hairy on all surfaces including the stem. The root system is a shallow, branching, white taproot and fibrous roots. The first true leaves are heart-shaped, covered with soft hairs on both surfaces, and toothed margins. Leaves are hairy, large, heart-shaped with pointed tips, bluntly toothed edges, alternate arrangement, with long, stout leaf stalks. Clear leaf veins emerge from the point of petiole attachment. Stems branch towards the top of the plant and are covered with soft, velvety hairs. The flowers are yellow or yellow-orange and grow from the upper leaf axils on long stalks, singly or in clusters. The flower is wide and has five green sepals and five shallowly notched petals that are fused at the base. Fruits (pod-like capsules) consist of a cup-like ring formed by woody segments. Young capsules are light green and turn brown to black as they mature. A capsule contains grey-brown, kidney shaped seeds.

2.2.3 **Biology of *Abutilon theophrasti***

It is reported that soil disturbance triggers seed germination and the plant quickly grows, developing larger alternate, palmate, heart-shaped leaves that taper to a point with prominent veins. In mid-summer, flowers are produced, then the fruits (the capsules) grow and turn brown or black on maturity. Seed pod contains 5 to 15 seeds and one plant can produce 700 to 17,000 viable seeds that can stay viable in soil for over 50 years (Britannica, 2023; Susan Sahr, 2023). Upon release from parent plant, over 57% seeds are dormant due to a hard seed coat that impedes water entry (Loddo et al., 2019) and seeds produced in a shady environment have thinner seed coats and a substantially lower percentage dormancy (Bello et al., 1995; Nurse & DiTommaso, 2005). It is said that as dormant seeds age, the micropyle cracks open and allows water to enter and germination is triggered thus sporadic germination over many years. The seed coat contains germination inhibitors, but this appears to be unimportant in maintaining dormancy in the field (LaCroix & Staniforth, 1964). It is also reported that a few seeds can germinate at 8 °C, but germination is best at 24-30 °C and declines above 35 °C (Šoštarčić, 2019; Leon et al., 2004). Beyond 50 °C, seed coat permeability increases and so the mortality of seeds (Nishida et al., 1999). Velvetleaf emerges primarily from mid-spring to early summer, but a few seedlings emerge sporadically later in the growing season (Loddo et al., 2019; Werle R. & Sandell D. Lowell, 2014). Flowers can pollinate the day they open and a few seeds become viable 12 days after flowering and essentially all are viable within 15 days, but capsules won't disperse seeds until 18-23 days after flowering, and pollination is primarily self, but some cross pollination by insects may be there too (Warwick et al., 2011). It is reported that dispersal modes include feed corn which tends to be usually contaminated with seeds of *Abutilon theophrasti* which pass through the digestive tract of livestock and get spread with manure, soil clinging to tyres and tillage implements (Warwick et al., 2011), farm machinery and accidentally via contamination of grains and crop seeds (Rojas-Sandoval, 2022a).

2.3 Weed Seed-Infecting Fungi

One of the setbacks of weeds - crops co-existence in an agroecosystem is disease risk that may increase when weed plant species are hosting pathogens (Dentika et al., 2021). Pathogenic fungi are the main cause of diseases in plants and have been recorded and reported to use seeds as an efficient means of survival and dispersal (Oliveira et al., 2018). Seedborne fungi can either directly or indirectly harm seeds by causing seed rot or discoloration, as well as causing seedling diseases that compromise plant growth. Fungal pathogens are made of hyphae, filamentous strands that make up the body and their infection is identified by mycelia (masses of hyphae) and various fungal fruiting bodies or overwintering structures that produce and give off spores (Dell'Olmo et al., 2023; Oliveira et al., 2018). (Dentika et al., 2021) reported ten fungal genera as the most associated with weed seeds that is, *Alternaria, Cladosporium, Curvularia* and *Fusarium* as the highest in that order, while *Aspergillus, Bipolaris, Nigrospora, Papularia and Rhizopus, Pythium* as least from seeds of weed members of Asteraceae, Convolvulaceae, Malvaceae, Poaceae and Rubiaceae. *Colletotrichum lindemuthianum,* and other *Colletotrichum* spp. cause Anthracnose disease in legumes and are Ascomycetes that can overwinter in the seeds particularly in the seed coat and more rarely, in the embryo. *Macrophomina Phaseolina* as microsclerotia*, Rhizoctonia solani as mycelium* inside the seed coat and rarely in the embryo are other reportedly isolated seed borne pathogenic fungi in legume weed seeds capable of infecting legume crops (Dell'Olmo et al., 2023; Lockhart & Wiseman, 2023).

The principal phytopathogens are fungi, bacteria, and viruses that can be preserved by weedy plant species that are inside the crop field or in nearby unmanaged land, in their seeds, leaves, stems etc (Marshall et al., 2003; Ekwealor et al., 2019; Lockhart & Wiseman, 2023). This has led to various researchers doing studies on various weed species and weed parts of interest to investigate possible pathogens that can be in a given chosen weed species as and this study literature reviews previous works focused on *Abutilon theophrasti* and *Datura stramonium* seed infecting fungi. For instance;-

In central Missouri, fungi of the genera *Fusarium* and *Alternaria* were identified in scentless bug-attacked seeds of *Abutilon theophrasti* and this insect feeding and fungal infection had greatly decreased seed germination. *Fusarium oxysporum* infection of seeds of *Abutilon theophrasti* increased (88%) following infestation of seeds by scentless bug and reduced seed viability and production (Kremer & Spencer, 1989).

In 2008 and 2009, weed seeds, roots and lower parts of stalk and crop plant debris were collected from 12 locations in eastern Slavonia, Croatia and included *Abutilon theophrasti* and *Datura stramonium* to determine the diversity of *Fusarium* species in them. They were cleaned by washing with tap water for 10 minutes and surface sterilized by immersing them in 95% alcohol for one minute and then placed on sterile filter paper in 9-cm Petri dishes which were then placed in incubators at 22°C and a 12/12 day/night schedule for 7days. These were examined daily and moistened with distilled water as need be and by the end of 7 days, mycelia with and without conidia that appeared to belong to a *Fusarium spp.* were transferred to potato dextrose agar (PDA) plates and incubated at 22°C for another 7 days under fluorescent lights on a 12/12 day/night schedule. A total of three hundred isolates obtained were assigned to 14 *Fusarium* species based on morphological and molecular features with *F. graminearum* being the most frequent (20.3%), *F.* verticillioides(18.4%), *F. oxysporum(*5.7%), *F. subglutinans,*12.7%), *F. proliferatum* (11%) and *F. avenaceum* (7.7%),*F.acuminatum*(4%), *F. solani* (2.6%), *F. semitectum* (2.3%), *F. equiseti* (1.7%) in that order. *F. venenatum (1.3%), F. sporotrichioides* (1.3%), *F. concolor* (0.7%) and *F. crookwellense* (0.3%) were found less common. They found that 7% of the isolates came from *Abutilon theophrasti* and 0.4% came from *Datura stramonium* and it was reported that *Abutilon theophrasti* and *Chenopodium album* yielded the highest number of Fusarium isolates being quite the commonest weeds in agricultural fields in Croatia and were common in all locations considered. They further explained that the relatively large number of isolates got from these two weeds may indicate that they are more readily colonized by Fusarium spp. than are the other weeds sampled (Postic et al., 2012).

Meanwhile a research study at Orbost, Victoria in south-eastern Australia, also reported the isolation of *Alternaria crassa* (VPRI20404) from *Datura stramonium* using standard procedures. It was grown on V-8juice agar and incubated at 25°C in a monolayer under Osram Wattsaver 36 Warm White fluorescent tubes (12 h photoperiod). Spores rather than mycelial fragments were tested on seedlings of *Datura stramonium* which succumbed to it as well as tomato, eggplant, soybean and ornamental species got infected. Microscopic examination was done after inoculation and indicated that spores germinated, formed appressoria and penetrated equally on the species (Stewart-Wade et al., 1998). Mina Shulz, 1968 in Israel also found the same fungus as seed-borne in *Datura metel* var. where the infected seeds were gray, and the fungus caused pre-emergence death of seedlings. *Datura* seeds were disinfected with Sodium hypochlorite and sown on potato dextrose agar (PDA) and incubated for 6 days at 20^0C under 8hour daylight regime for sporulation. She reported that the fungal mycelium penetrated the seed coat causing chlorotic cotyledons and is responsible for leaf and pod blight of *Datura spp*. and other solanaceous plants (Halfon-Meiri, 1973).

In the municipality of Maglić, Vojvodina Province, five fungal isolates were obtained from *Datura stramonium* where identity was based on morphological characteristics which included circular, smooth, gray to brownish black with concentric zones of very intensive sporulation; branched, solitary brown conidiophores of 125-230 µm in size, and mature conidia were olivaceous brown, long ovoid or long ellipsoid, 102 to 113 µm long and 16 to 29 µm wide, with five to 12 transverse septa and zero to three longisepta; all isolates were identified as *Alternaria protenta* Sorauer. These were isolated by placing surface-sterilized infected seed tissues (immersed in 1% sodium hypochlorite for 1 min and rinsed with sterilized water) on V8 medium for 7 days at 23°C with a 12-h photoperiod (Blagojević et al., 2019).

In the study of the relationship between microorganisms and seeds of *Abutilon theophrasti* in contact with soil in Columbia, it was found that *Alternaria alternata* (Fr.) Keissl., *Cladosporium cladosporioides* (Fres.) de Vries., *Epicoccum purpurascens* Ehrenb. Ex Schlecht, *Aspergillus flavus* and *Fusarium spp.* were associated with over 50% of the seeds during 32 days of incubation. It was further reported that soilborne microorganisms did not establish on these infected seeds due to the effective barrier accorded by seedborne microorganisms, but the viability of these velvetleaf seeds exceeded 90%. It was then suggested that *Abutilon theophrasti's* seedborne microorganisms may enhance seed longevity on and in soils by providing a barrier to potential seed decomposers found in the soil (Kremer et al., 1984; Kremer, 1986).

In eastern Croatia, Vrandecic et al., (2004) acknowledged *Phomopsis longicolla* as a very harmful seed pathogenic fungus of *Abutilon theophrasti* and other plants though they also isolated it from diseased stem tissue of *Abutilon theophrasti* that was collected from Sugar beet and soybean fields. They used a standard Phyto pathological method, that is culturing in a moist chamber and isolation on acidified potato dextrose agar at 25 ° C, with a 12/12-hour light/dark regime. They based on the cultural characteristics, pattern of stromata, the formation of only alpha conidia, biometrical comparisons of pycnidia and conidia and prominent pycnidial beaks, to identify *Phomopsis longicolla* from *Abutilon theophrasti.*

At the University of Illinois Ecological Research Area, 6 km northeast of Urbana, seedborne mycoflora were examined from seeds of *Abutilon theophrasti* Medic, *Datura stramonium* L., *Ipomoea hederacea* Jacq., and *Polygonum pensylvanicum* L. collected from adjacent maize field. They disinfected one hundred seeds from each species in a 1.25% solution of sodium hypochlorite for 4 minutes, then in 70% ethyl alcohol for 2 minutes and rinsed twice in autoclaved distilled water. Seeds were placed on potato-dextrose agar supplemented (PDAS) with 100 mg of potassium penicillin and 25 mg streptomycin sulfate per litre of agar and then incubated at 20, 25 and 30 ^{0}C for 5 days. They made hyphal tip isolations from emerging fungi which were identified to genus level. They reported isolates of *Altanaria sp* in *Datura stramonium* (43.3%) *and Abutilon theophrasti* (2.7%), *Chaetophoma sp* in *Datura stramonium* (0.3%),*Cladosporium sp* in *Datura stramonium* (5.3%), *Dendrophom sp* in *Datira stramonium (0.7%), Fusarium sp. A, Fusarium sp. B, Fusarium sp. C* in *Datura stramonium* (2.3%, 3.0%, 3.0% respectively), *Nigrospora sp in Datura stramonium* (0.7%), *Abutilon theophrasti* (0.3%) (Kirkpatrick & Bazzaz, 1979 ; Kremer, 1987).

2.3.1 Overview of some frequently isolated weed seed infecting Fungal Genera

2.3.2 *Alternaria Spp.*

This genus was identified in 1817 with *Alternaria alternata* (first called *A. tenuis*) being the only isolate, but now it is a genus of the phyllum Ascomycota, known with major plant pathogens, saprobes and are common allergens to humans, causing plant damage in the field and postharvest. It has over 360 accepted species which are divided into 29 sections. This genus was first put in the division of mitosporic fungi (the phylum Fungi Imperfecti) due to unidentified sexual stage for majority of *Alternaria* species (Logrieco et al., 2009; Li et al., 2022; Thomma, 2003). It is characterized by production of large, multicellular, dark-coloured (melanized) conidia with longitudinal and transverse septa (phaeodictyospores) (Hong et al., 2006; Thomma, 2003). It produces conidia in single or branched chains on short, erect conidiophores, coming out as protrusions of the protoplast through pores in the conidiophore cell wall, and tend to be broadest close to the base and gradually taper to an elongated beak, forming a club-like shape (Thomma, 2003; Mamgain et al., 2013;Woudenberg et al., 2015;Bessadat et al., 2020). Alternaria species are mostly foliar pathogens causing damage of host tissues by reducing photosynthetic ability with an infection causing necrotic lesions that may or may not have target-like appearance. The fungus stays in the centre of the lesion having an invaded chlorotic halo. Alternaria species often cause quiescent infections, that is, stays dormant in the host tissue till favourable conditions for infection. Alternaria species generally do not specifically target roots or vascular vessels, and the sexual stage and ability to overwinter as spores are not known yet, instead it survives as mycelium or spores on decaying plant debris for a long time, or as a latent infection in seeds. When seed-borne, the fungus attacks the seedling after germination, and when spores have been produced, they are mainly spread by wind onto plant surfaces where infection can occur. The major sources of spread of Alternaria species are infected seeds with spores on the seed coat or the presence of mycelium under the seed coat. The dissemination of spores occurs by wind, water, tools and animals. The fungus can survive in susceptible weeds or perennial crops (Mamgain et al., 2013;Bessadat et al., 2020) and weakened host tissues by stress factors are more susceptible to Alternaria infection than healthy host tissues (Thomma, 2003). Though there are taxonomic and pathogenic differences among Alternaria species, they cause similar infection patterns. Walls of dormant spores are highly melanized, and under favourable conditions, they can produce one or more germ tubes which penetrate stomata, cuticle or wounds with or without the formation of small appressoria. In less virulent species, wounds and stomata are targeted, while more virulent species can also penetrate directly. Enzymatic Alternaria infections target the cuticle which is the first defense line of the host to overcome by the directly penetrating fungal pathogens. It also produces toxins which in the end trigger host cell death (Thomma, 2003; Hong et al., 2006; Logrieco et al., 2009;Woudenberg et al., 2015;Pinto & Patriarca, 2017). Among the diseases caused by Alternaria species are early blight of potato, leaf spot disease by *Alternaria alternata*, *Alternaria crassa sacc.*on tomato, parsley, jimson weed and many other plants. It also causes upper respiratory infections in AIDS patients, asthma in sensitive humans and has been implicated in chronic rhinosinusitis (Mamgain et al., 2013). *Alternaria arborescens* causes stem canker of tomato, *Alternaria arbusti* causes leaf lesions on Asian pear, *Alternaria brassicae* infects many vegetables and roses, *Alternaria brassicicola* grows on cole crops, *Alternaria brunsii* causes cumin bloosem blight, *Alternaria carotiincultae* causes leaf blight on carrot, *Alternaria gaisen* causes ringspot disease of pears, *Alternaria molesta* causes skin lesions on porpoises, *Alternaria petroselini* causes parsley leaf blight, *Alternaria radicina* causes carrot decay), *Alternaria senecionis, Alternaria solani* cause early blight in potatoes and tomatoes, etc. Among the different diseases, blight disease is one of the most dominant that causes average yield loss of over 47% (Mamgain et al., 2013; Bessadat et al., 2020).

2.3.3 Fusarium spp.

Fusarium species are known as soil-borne vascular infecting pathogens, but are also seedborne (Ekwomadu & Mwanza, 2023) and are among the most important phytopathogenic and toxigenic fungi that cause great crop yield losses through loss of biomass and buildup of mycotoxins in affected parts. Mycotoxins contaminate seed grains and cause health risks to humans and animals. Structurally, they are filamentous and belong to the Class Ascomycetes and Family Hypocreaceae. They have three asexual reproductive spores, that is, macroconidia, microconidia and chlamydospores, and mycelia (that are cottony with shades of pink, yellow, and purple) all as propagules capable of infecting host plants. Their life cycle has dormant, parasitic and saprophytic stages, so the genus has harmless saprobes (species) and parasitic (pathogenic) species and some that produce mycotoxins on plants. Its pathogenic species are known to be highly host specific, hence the emergence of the "formae specialis" concept. The colour of the colony, length and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the differentiation of Fusarium species. The major toxin groups produced by most Fusarium phytopathogens like *F. oxysporum* complex are trichothecenes, zearalenone and fumonisins (Dawidziuk et al., 2014;Bahadur, 2022; Okungbowa & Shittu, 2012).

Examples of Fusarium plant diseases include Fusarium wilts caused by several species that include *F. eumartii, F. oxysporum, F. avenaceum, F. solani, F. sulphureum and F. tabacinum;* crown rot, head blight and scab on cereal grains caused by *Fusarium graminearum* ; vascular wilts on a wide range of horticultural crops; root rots; cankers; pokkah-boeng on sugarcane and bakanae disease of rice.

Also potato dry rot (one of the important fungal storage rots) caused by various *Fusarium* spp. like Fusarium coeruleum (syn. *F.solani* var. *coeruleum*), *F. sambucinum* (formerly *F. sulphureum*), *F. avenaceum*, *F. culmorum* (Okungbowa & Shittu, 2012; Peters et al., 2008; Ekwomadu & Mwanza, 2023). *Fusarium* spp. cause seedling rot, root rot, crown rot and stem and ear rot at any stage of plant development (Ekwomadu & Mwanza, 2023). Fusarium wilt disease is of great concern as it causes great economic losses in a wide variety of crops with visible symptoms of stunted growth, yellowing and wilting of the leaves, reddish discolouration of the xylem vessels and in wet conditions, white, pink or orange fungal growth on the outside of affected stems, root decay and stem decay. This disease is difficult to control because physical, chemical and cultural methods are ineffective and expensive leaving breeding for resistant cultivars as the effective option (Okungbowa & Shittu, 2012).

2.3.4 *Aspergillus Spp*

This is another genus that has been found in seeds of some weed plants and is one among the many with a really diverse group of fungi in the world. Some species produce teleomorphs (sexual states) which are cleistothecial ascomycetes with inordinately arranged ascospores in dehiscent asci (Klich, 2007). This genus is divided into sections; - *Aspergillus flavus* belongs to section *Flavi* which has major has members of great importance in production of aflatoxins (*A. flavus and A.parasiticus*) while less aflatoxin-producing species in flavi section are *A. nomius, A. pseudotamarii, A. bombysis and A. parvisclerotigenus. A. ochraceoroseus*, A. *rambellii*, *Emericella venezuelensis* and *E. astellata* also produce aflatoxins though not in flavi section, but the latter two have *Aspergillus* anamorphs (asexual states) (Klich, 2007; Krijgsheld et al., 2013; Mehl et al., 2012). Aspergillus spp starts growth from spore germination which ends into a vegetative mycelium that colonizes a substrate. Its hyphae found within the mycelium are very heterogeneous in regard to gene expression, growth, and secretion. Aspergilli are capable of reproducing asexually and sexually with conidiophores and ascocarps that yield conidia and ascospores, respectively (Krijgsheld et al., 2013; Klich, 2007;Gourama & Bullerman, 1995;Mehl et al., 2012). *Aspergillus flavus* is a soil saprophyte that infects and contaminates crop seeds and wide variety of agricultural products in the field, storage areas, and processing plants and during distribution. *A. flavus, A.flavus subsp. parasiticus, A. nomius, Aspergillus niger* are known moulds producing af1atoxins (Al-Abdalall, 2005; Mehl et al., 2012;Klich, 2007). *Aspergillus oryzae* and *Aspergillus tamarii* are nontoxic (Gourama & Bullerman, 1995). These aflatoxins are carcinogenic aflatoxin (secondary metabolite) while some pathogenic Aspergillus species cause aspergillosis diseases, such as avian aspergillosis and bovine mycotic abortion while the mold's (Aspergillus) spores cause hypersensitivity reactions in sensitive patients, fibrosis, and hypersensitivity pneumonitis, allergies, mycotoxicoses in human and livestock. *A. flavus* var. *flavus*, *A. flavus* subsp. *parasiticus*, *A. fumigatus* and *A. nomius* are all able to produce aflatoxins (Krijgsheld et al., 2013; Gourama & Bullerman, 1995; Klich, 2007;Al-Abdalall, 2005;Mehl et al., 2012) Though regarded as pathogens and spoilage organisms, many Aspergillus species are beneficial for example some species are used to prepare fermented foods (Gourama & Bullerman, 1995;Al-Abdalall, 2005). Section *Flavi* also includes the food fermentation/industrial species - *A. oryzae* and *A. sojae*, which are closely related to *A. flavus* and *A. parasiticus* at molecular level, but they are morphologically distinct and do not produce aflatoxin (Mehl et al., 2012; Klich, 2007).

Aspergillus niger (black mould) was found in higher amounts on onion seeds produced from hot climates, which transmitted to the cotyledons and first true leaves of seedling onions grown under controlled conditions, and the seedlings grown from contaminated seeds had longer roots but shorter shoots compared to those grown from healthy seeds (Hayden N.J., & Maude R.B., 1992).

The production of phialides and foot cells show the presence of Aspergillus species, but the production of phialides alone is not enough to characterize aspergilli because they are also formed by Penicillium species (Gourama & Bullerman, 1995). The conidial head features like color, shape, and size are key for diagnosis of Aspergillus group. Its conidial heads vary from columnar to radiate and globose. The color of the conidia in a chain determines the color of the conidial head; thus, the color of *A. flavus* is light to deep yellow green, or olive green. Its conidiophore is thick-walled arising perpendicularly from the foot cell. The outer surface of the conidiophore can be smooth or rough for example that of *A.flavus* group are rough and hyaline (nonpigmented) (Gourama & Bullerman, 1995;Mehl et al., 2012)

Examples of diseases caused by *Aspergillus flavus* in crops; - *Aspergillus flavus* is a pathogen of plants, livestock and insects, and causes storage rots in numerous crops. In corn, it causes an ear rot; in peanuts, it causes a seedling disease called yellow mould of seedlings or aflaroot with symptoms of necrotic lesions, chlorosis of above-ground parts and lack of development of secondary roots, ‘aflaroot’- probably due to aflatoxin toxicity which has been found to inhibit root hair development in tobacco. It may also cause the rot of mature peanuts in the soil. In cotton, *A. flavus* affects cotton quality by causing boll rot and fibre infection is described as yellow spot disease due to the bright greenish yellow (BGY) fluorescence seen on the cotton fibres under long-wave ultraviolet light. *A. flavus* infection of cottonseed lowers seed viability by about 60% and its aflatoxin lowers cotton seed viability by 71%. Its aflatoxin B_1 inhibits seed germination of wheat, corn, mustard, mung and gram.(Mehl et al., 2012)

A research study by Robert J. Kremer in Central Missouri isolated various bacterial genera from seeds of *A. theophrasti, Datura stramonium, lpomoea hederacea*, *Polygonum pensylvanicum, Xanthium strumarium, and G. max* harvested in the field during 1982, 1983, and 1984 at Boone and Osage counties and were tested for the presence of microorganisms within 10 days post-harvesting. He surface-sterilized seeds by immersing in 1.25% (w/v) sodium hypochlorite, rinsed in sterile distilled water, immersed in 70% (v/v) ethanol, rinsed 5 times in a total of 1-liter sterile distilled water, and finally blotted on autoclaved paper towels. He then placed sterilized seeds on the surface of nutrient agar at five seeds/plate and incubated them in the dark at 27^0C and then examined them every 24 hours for 5 days. He randomly chose bacterial isolates from outgrowths on and around seeds, streaked them on nutrient agar and glucose peptone agar to get pure cultures for characterization and identification using Bergey's Manual of Determinative Bacteriology. The study recorded 26.4% seeds with Bacterial cultures from all the five weed species and soybean with seeds of *Abutilon theophrasti and Datura. stramonium* having the highest incidence of bacteria. Only two out of 262 seeds of *P. pensylvanicum* had bacteria and over 95% of the bacteria were isolated as single species compared to as mixtures, from seeds. It is also reported that bacteria in infected seeds of *Abutilon theophrasti* lived within the sub-palisade cell layer of the seedcoat while majority of seedborne bacteria were from germinating and imbibed seeds. The study also found that over 50% of the seedborne bacteria from *Abutilon theophrasti* was isolated from the hard-seeded component. The isolated bacterial genera and species from seeds of *Abutilon theophrasti* and *Datura stramonium* included – *Acinetobacter spp., Alcaligenes spp., Bacillus megatarium, B.pumilus, B. subtilis, Citrobacter freundii, Enterobacter spp., Erwinia amylovora, E. herbicola, Erwinia sp., Flavobacterium spp., Moraxella sp., Pseudomonas acidovarans, P. alcaligenes, P. cepacian, P. fluorescens, P. putida, P. stutzeri, P. syringae, Pseudomonas spp. and Xanthomonas spp.* (Kremer, 1987).

3. MATERIALS AND METHODS

3.1 Scope of study and Place of conduction.

The research study was purely experimental, to investigate the potential phytopathogenic fungi in the seeds of *A. theophrasti and D. stramonium* in Hungary. The experiment was conducted at the Hungarian University of Agriculture and Life Sciences, Georgikon Campus, Keszthely city, Hungary at the Festetics Imre BioInnovacios Kozpont (Biotechnology laboratory) from January 2024 to March 2024.

3.2 Research materials

The following materials were used for this study; - Seeds of *Abutilon Theophrasti* that were collected from arable fields of Keszthely in the years 2007 and 2010, seeds of *Datura stramonium* that were also collected from arable fields of Keszthely, but in the years 2009 and 2010; Petri dishes of diameter 12 cm; plain tap water and thermostat, Microscope.

3.3 Procedure of data collection (Experimental setup)

The seed samples were first cleaned as much as possible to remove any external contaminations. This was done by washing with tap water for 10 minutes and surface sterilized by immersing them in 95% alcohol for one minute. This cleaning procedure was derived from Postic et al., (2012).

For each year's weed seed sample, two replicates were used each with 50 (fifty) seeds making a total of 100 (one hundred) weed seeds of a given year for the weed species in question.

Each sterilized petri dish meant to hold the seeds was first layered with two filter papers followed by moistening the filter papers with 15ml of plain tap water to hold the seeds stably without rolling over, for weed seed germination and facilitate fungal growth. For each of the weed seed samples, fifty cleaned seeds were counted and placed grid-wise in the respective prepared and ready petri dishes. The weed seed arrangement on the filter papers in petri dish was five rows and ten columns of seeds, for easy and clear observation. Each of these set petri dishes was covered with another cleaned petri dish and labelled.

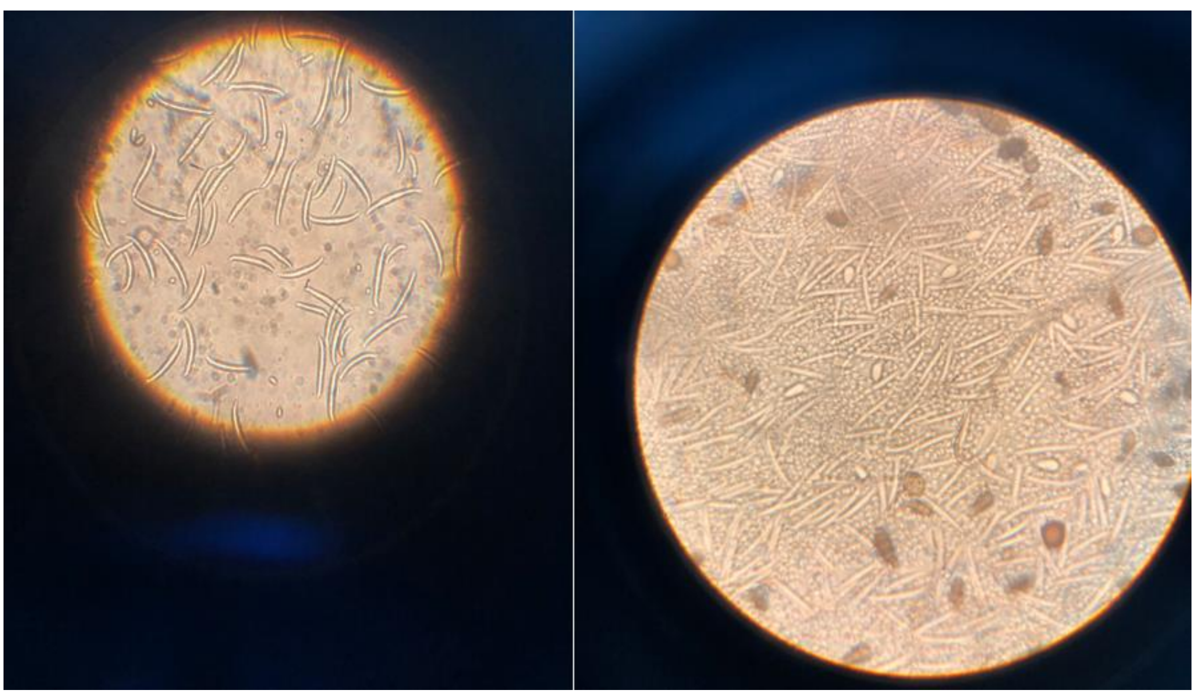
The figure 1 below shows the counting and arrangement of seeds in petri dishes.

Figure 1: counting and arranging of cleaned seeds on moistened filter papers laid in petri dishes. (Source: own, 20th February, 2024).

The ready prepared petri dish set ups were then put in a cooling-heating thermostat, calibrated at 24^0C (suitable for fungal growth) for incubation in an airtight package to avoid any possible contaminations. The set weed seed samples were left to incubate for 14 (fourteen) days, but with a visual inspection done after the first 7 days just to check for initial fungal infections. The second, but final inspection was done on the fourteenth (14th) day, the very day data was taken. The seeds that germinated were visually observed for fungal infection. A sample of infected seeds were prepared and microscopically examined to determine the real infecting fungi. Then finally, for each weed seed sample, the exact number of seeds infected with particular fungal microbes was determined and recorded. The weed seed samples used in the experiment were all carefully destroyed after result evaluation. The figure 2 below shows the microscopic images of *Fusarium sp.* and *Alternaria sp.*

Figure 2: elongated macro conidia of Fusarium sp. and roundish brown macro conidia of Alternaria sp. from microscopic examination.

(Source: Own 5th March, 2024).



3.4 Result analysis

The analysis of the data was done with Microsoft excel 365 software to come up with the percentages and graphical trends of infection.

4. RESEARCH RESULTS AND THEIR ASSESSMENT

4.1 Results

Table 1: Frequency of fungal infections on seeds of Datura stramonium and Abutilon theophrasti collected in different years

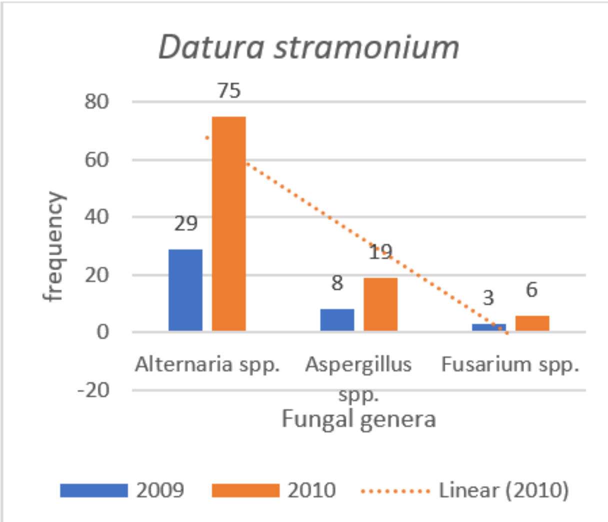
Weed seed species	**Year of weed seed collection**	**Place of weed seed collection**	**Number of seeds tested**	**Infecting Fungal spp. observed**					
				Alternaria spp.		***Aspergillus spp.***		***Fusarium spp.***	
				Freq.		**Freq.**		**Freq.**	
Datura stramonium	2009	Keszthely	100	29		08		03	
	2010	Keszthely	100	75		19		06	
Abutilon theophrasti	2007	Keszthely	100	38		14		04	
	2010	Keszthely	100	31		06		04	
Total	-	-	**400**	**173**		**47**		**17**	

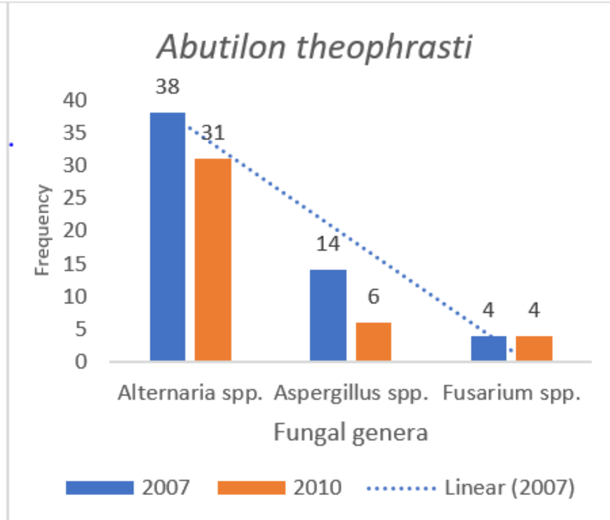
The results showed that some seed samples for the weed species under study actually contained *Alternaria spp, Aspergillus spp and Fusarium spp.* (Table 1). These were also isolated by several scholars such as Kremer & Spencer, (1989), Blagojević et al., (2019), Kremer et al., (1984) and Kremer, (1986). It was also observed that some seeds were infected by more than one of these fungi. The results also showed that seed infection by these fungi was not at same rate or degree (Table 1) which is similar to the findings of other scholars such as Postic et al., (2012), Kirkpatrick & Bazzaz, (1979) and Kremer, (1987). *Alternaria spp* had the highest seed infection rate in all the years for both weed species, followed by *Aspergillus spp.* and least *Fusarium spp*. (Table 1). Postic et al., (2012), and Kremer, (1987) also recorded *Alternaria sp. as* the highest infecting fungi for seeds of *Datura stramonium* and *Abutilon theophrasti.* For *Datura stramonium*, seeds collected in 2010 were more infected by each of the fungi than those collected in 2009 while for *Abutilon theophrasti,* seeds collected in 2007 were more infected by the each of the fungi than those collected in 2010 (Table 1, page 25; Figure 3, page 27). Generally, seeds of *Datura stramonium* were more infected by these fungi than seeds of *Abutilon theophrasti* (Figure 3, page 27*).* These findings are similar to those of Kirkpatrick & Bazzaz, (1979 and Kremer, (1987). In general, out of 400 sampled weed seeds tested, 43.25% were infected with *Alternaria spp,* 11.75% were infected with *Aspergillus spp.* and 4.25%) were infected with *Fusarium spp.* These therefore suggests that, for these two dicotyledonous weed species, *Alternaria spp* is more seed infectious than *Aspergillus spp.* while *Fusarium spp.*is more less infectious in Hungary, similar to the findings of Kirkpatrick & Bazzaz, (1979 and Kremer, (1987) and other scholars. Out of 200 seeds of *Datura stramonium* used in the study, 52% were infected with *Alternaria spp.* compared to 43.3% findings of Kirkpatrick & Bazzaz, (1979), 13.5% were infected with *Aspergillus spp.* and 4.5% were infected with *Fusarium spp.* compared to 3.0% findings of Kirkpatrick & Bazzaz, (1979). For *Abutilon theophrasti,* out of 200 seeds used in the study, 34.5% were infected with *Alternaria spp,* 10% were infected with *Aspergillus spp.* and 04% were infected with *Fusarium spp.* The results also showed that at least all the seeds of *Datura stramonium* collected in 2010 had a fungal infection (Table 1, page 25).

The figure 3 below shows the infection trends of seed samples by the respective fungal genera.

Figure 3: the trend of fungal genera infection

(Source: own)





4.2 Results discussion & Assessment

The results showed that some seed samples for *Datura stramonium* and *Abutilon theophrasti* contained *Alternaria spp, Aspergillus spp and Fusarium spp.* (Table 1, page 25), suggesting that the seeds of these weed species are vectors for survival and spread of plant diseases of these fungal genera. These fungal genera were also found by Kremer et al., (1984) & Kremer, (1986) in their study of the relationship between microorganisms and velvetleaf seeds in contact with soil in Columbia, where *Alternaria alternata* (Fr.) Keissl., *Cladosporium cladosporioides* (Fres.) de Vries., *Epicoccum purpurascens* Ehrenb. Ex Schlecht, *Aspergillus flavus* and *Fusarium spp.* were associated with over 50% of the seeds during 32 days of incubation. They further reported that soilborne microorganisms did not establish on these infected seeds due to the effective barrier accorded by seedborne microorganisms, and the viability of these *Abutilon theophrasti* seeds exceeded 90%. They then suggested that *Abutilon theophrasti's* seedborne microorganisms may enhance seed longevity on and in soils by providing a barrier to potential seed decomposers found in the soil. Again Kremer & Spencer, (1989) in central Missouri isolated the genera of *Fusarium* and *Alternaria* from scentless bug-attacked seeds of velvetleaf and reported that this insect feeding, and fungal infection had greatly decreased seed germination, and *Fusarium oxysporum* infection of seeds of *Abutilon theophrasti* increased (88%) following infestation of seeds by scentless bug and reduced seed viability and production. Kirkpatrick & Bazzaz, (1979) & Kremer, (1987) also reported isolates of *Altanaria sp* in *Datura stramonium* (43.3%) *and Abutilon theophrasti* (2.7%) and *Fusarium sp. A, Fusarium sp. B, Fusarium sp. C* in *Datura stramonium* (2.3%, 3.0%, 3.0% respectively), at the University of Illinois Ecological Research Area, Urbana in their examination of the seedborne mycoflora from seeds of *Abutilon theophrasti* Medic, *Datura stramonium* L., *Ipomoea hederacea* Jacq., and *Polygonum pensylvanicum* L. collected from adjacent maize field.

It was also observed that some seeds were infected by more than one of these fungi and the seedlings from this case of seeds were so damaged and weak suggesting that species of these three fungal genera are compatible with each other and so can bring massive destruction to susceptible host crops.

The results also showed that seed infection by these fungi was not at same rate or degree possibly due to the differences in physical nature of the seed species or some other factors. *Alternaria spp* had the highest seed infection rate in all the years for both weed species (Figure 3, page 27). Similarly, Kirkpatrick & Bazzaz, (1979), Mina Shulz, (1968), Mina Shulz and Halfon Meiri, (1973), Kremer, (1987) and Blagojević et al., (2019) also had this result's observation. This finding could be attributed to *Alternaria spp.* like "*Alternaria alternata* being well adapted, rigorous, and dominant in agricultural fields" (Nishikawa et al., 2006) and could suggest that *Alternaria* species are the most important *Abutilon theophrasti* and *Datura stramonium* fungal seedborne pathogens in Hungary.

The observed differences in the rate of weed seed infection, and the fungal flora in these seeds collected in different years for both *Datura stramonium* and *Abutilon theophrasti* could suggest that there were differences in the prevalence and incidence of these fungal genera in different years possibly due to changes in climatic conditions over years. This is related to Nishikawa et al., (2006) idea that the fungal flora in seeds is highly influenced by locality and year of harvest, which tend to have different climatic and environmental conditions which influence prevalence and incidence of pathogens and infection too.

Generally, seeds of *Datura stramonium* were more infected by each of the fungi identified than seeds of *Abutilon theophrasti,* (Table 1, page 25, Figure 3, page 27), a similar finding by Kirkpatrick & Bazzaz, (1979), Blagojević et al., (2019) and Kremer, (1987). Additionally, at least all the seeds of *Datura stramonium* collected in 2010 had a fungal infection (Table 1, page 25). This then suggests that these fungi (*Alternaria spp, Aspergilus spp and Fusarium spp.),* find *Datura stramonium* as a better alternative host than *Abutilon theophrasti* and so a better alternative host for survival and its seeds could spread more of these fungal diseases than seeds of *Abutilon theophrasti.* Therefore, a need for a study to find out why these fungi infect *Datura stramonium seeds more than Abutilon theophrasti* seeds.

In general, out of 400 sampled weed seeds tested, 43.25% were infected with *Alternaria spp,* 11.75% were infected with *Aspergillus spp* and 4.25% were infected with *Fusarium spp.* This therefore suggests that, for these two dicotyledonous weed species, *Alternaria spp* is more seed infectious than *Aspergillus spp.* while *Fusarium spp.*is more less infectious, in Hungary.

Fusarium spp. was the least observed in all the seed samples (Figure 3 , page 27) and was also observed by Nishikawa et al., (2006) who isolated only a few species of fusarium and suggested that longevity of *Fusarium spp*. on seeds is less than 9 years and that this is influenced by their inability to form rough-walled chlamydospores. Also, *Fusarium spp*. was the least found during the seedborne mycoflora examination from seeds of *Abutilon theophrasti* Medic, *Datura stramonium* L., *Ipomoea hederacea* Jacq., and *Polygonum pensylvanicum* L. by Kirkpatrick & Bazzaz, (1979); Kremer, (1987). They made hyphal tip isolations from emerging fungi which were identified as *Altanaria spp* in *D.stramonium* (43.3%) *and A. theophrasti* (2.7%), *Fusarium spp. A, Fusarium spp. B, Fusarium spp. C* in *D.stramonium* (2.3%, 3.0%, 3.0% respectively). So, these findings could support my earlier suggestion that these fungal species prefer seeds of *Datura stramonium* to those of *Abutilon theophrasti.* Meanwhile Postic et al., (2012) isolated more species of fusarium from seeds, roots and lower parts of stalk of weeds that included *Abutilon theophrasti* and *Datura stramonium* collected in 2008 and 2009 from 12 locations in eastern Slavonia, Croatia. A total of three hundred isolates obtained were assigned to 14 *Fusarium* species based on morphological and molecular features. They found that 7% of the isolates came from *Abutilon theophrasti* and 0.4% came from *Datura stramonium* and they explained that it was because of the commonness and abundance of *Abutilon theophrasti* in agricultural fields of Croatia and all locations considered. These findings could probably prove Nishikawa et al., (2006) suggestion that longevity of *Fusarium spp*. on seeds is less than 9 years as they were least found in theirs and my studies with these dicot weed seeds that were collected and stored for more than 9 years.

All in all, the research results show that seeds of both weed species are capable of holding and transmitting diseases of these fungal genera particularly *Alternaria spp* and *Fusarium spp* in crop fields while *Aspergillus spp* seem to be so much of soil saprophytes that infect and contaminate crop seeds and wide variety of agricultural products in the field, storage areas, processing plants and during distribution (Mehl et al., 2012).

5. CONCLUSION AND RECOMMENDATIONS

The research study was conducted to investigate the possible phytopathogenic fungal genera in seeds of *Abutilon theophrasti* and *Datura stramonium* in Hungary. The analysis of results showed presence of *Alternaria spp, Aspergillus spp and Fusarium spp.* These fungi infect weed seeds at different rates (Table 1, page 25). For these two dicotyledonous weed species, *Alternaria spp* is more seed infectious than *Aspergillus spp.* while *Fusarium spp.*is more less infectious (Figure 3, page 27). In fields where there were high populations of *Datura stramonium* and *Abutilon theophrasti,* there may be possibility of *Alternaria* diseases being more prevalent than *Fusarium* and *Aspergillus. Datura stramonium* seeds are more susceptible to fungal infection than *Abutilon theophrasti* seeds in Hungary. Different years or age, localities and abundance of these weeds may influence rate of infection of their seeds by these identified fungal genera. Seeds of *Datura stramonium* and *Abutilon theophrasti,* can harbour *Alternaria spp, Aspergillus spp. and Fusarium spp*. in crop fields and so are vectors of diseases of these fungal genera. The seed samples for *Abutilon theophrasti* and *Datura stramonium,* used in this research study were collected from arable fields of Keszthely in 2007, 2009 & 2010. The researcher recommended that a similar study be done with seeds collected in other agrarian parts of Hungary to ascertain nationwide, the prevalence of these three fungal genera or even more and their species. Another study should be conducted using recently harvested seeds of these weed species to ascertain the effect of their seed age and storage time on survival and persistence of these fungi in seeds. A similar study to mine can also be done using seeds from the soil seedbank. There is need to investigate why seeds of *Datura stramonium* were more infected than seeds of *Abutilon theophrasti*. There is also need to investigate why *Alternaria spp* is more seed infectious than *Aspergillus spp.* and *Fusarium spp.* for these weed species. Finally, the researcher recommended that the efforts of stakeholders be towards devising strategies to curtail seed production by these two weed species to curb the pathogenic fungal species spread in their seeds and reduce their seedbanks in the soil. This will also reduce the usage of fungicides in crop fields in an attempt to manage diseases caused by *Alternaria spp.*, *Fusarium spp.* and *Aspergillus spp.*.

6. SUMMARY

This research was conducted to identify the phytopathogenic fungal genera in seeds of *Datura stramonium* and *Abutilon theophrasti* in Hungary. Their seed samples were collected from arable fields of Keszthely in 2009 & 2010, and 2007 & 2010 respectively. The seed samples were first cleaned, and for each year's weed seed sample, two replicates were used each with 50 (fifty) seeds. The seeds were placed grid-wise in the respective sterilized petri dishes that were layered with two filter papers followed by moistening the filter papers with 15ml of plain tap water and covered with another sterilized petri dish and labelled. The set ups were put in a cooling-heating thermostat, calibrated at 24^0C (suitable for fungal growth) for incubation for 14 (fourteen) days. A first visual inspection was done on day 7 (seven) and the second was done on the fourteenth (14th) day and data taken. A sample of infected seeds was prepared and microscopically examined to determine the real infecting fungal genera and the exact number of seeds infected with particular fungal microbes was determined and recorded. The results presented in table 1 page 22, showed presence of *Alternaria spp, Aspergillus spp and Fusarium spp.* at different rates. *Alternaria spp* had the highest seed infection rate (43.25%), followed by *Aspergillus spp.* (11.75%) and least *Fusarium spp*. (4.25%). For *Datura stramonium*, seeds collected in 2010 were more infected by each of the fungi than those collected in 2009 while for *Abutilon theophrasti,* seeds collected in 2007 were more infected by each of the fungi than those collected in 2010. Generally, seeds of *Datura stramonium* were more infected by these fungi than seeds of *Abutilon theophrasti.* Also observed were some seeds being infected by more than one of these fungi. Seeds of *Datura* species are more susceptible to these fungal infections than seeds of *Abutilon theophrasti.* Finally, seeds of *Datura stramonium* and *Abutilon theophrasti,* can harbour and are vectors of diseases of *Alternaria spp, Aspergillus spp.* and *Fusarium spp.* in crop fields of Hungary.

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STUDENT DECLARATION

Signed below, **Okwangan Simon,** a student of the Georgikon Campus of the Hungarian University of Agriculture and Life Sciences, at the MSc. Course of **Plant Protection** declares 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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