Identification and Occurrence of Potential Phytopathogenic Fungi Infecting Seeds of Invasive Allelopathic Dicot Weeds

Az invazív allelopátiás kétszikű gyomok magjait megfertőző potenciális fitopatogén gombák azonosítása és előfordulása

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Abstract: *Datura stramonium* and *Abutilon theophrasti* are among the dicot weeds that have invaded and become established in agricultural fields of Hungary, with strong competitive ability, strong growth and allelopathic properties against crops. However, the information about potential phytopathogenic fungi in their seeds is limited. Thus, the objective of this study was to identify the phytopathogenic fungi at genera level that may be inhabiting the seeds of *Datura stramonium* and *Abutilon theophrasti* in Hungary. Seed samples of *Abutilon theophrasti* and *Datura stramonium* were collected from arable fields of Keszthely in 2007 & 2010 and 2009 & 2010 respectively. Isolates obtained were *Fusarium spp.* (elongated-cylindrical whitish-yellowish macroconidia), *Alternaria spp.* (round brown mature macroconidia and circular, smooth, grayish-brownish immature conidia), and *Aspergillus spp.* (columnar pale green and bluish conidia). *Alternaria spp.* had the highest infection rate (43.25%), followed by *Aspergillus spp.* (11.75%) and least infectious was *Fusarium spp.* (4.25%). Based on the above morphological features, it was concluded that seeds of *Datura stramonium* and *Abutilon theophrasti* can harbour diseases of *Fusarium spp., Alternaria spp.* and *Aspergillus spp.*

Keywords: *Abutilon theophrasti; Datura stramonium; Seed-infecting fungi; morphological; pigmentation*

Összefoglalás: A *Datura stramonium* és az *Abutilon theophrasti* a jelentősebb kétszikű gyomnövények közé tartoznak, amelyek erős versenyképességgel, növekedési és allelopátiás tulajdonságokkal rendelkeznek a kultúrnövényekkel szemben. A magvaikkal fennmaradó és terjedő potenciális fitopatogén gombákról kevés az információ áll rendelkezésre. A vizsgálat célja volt, hogy azonosítsuk azokat a fitopatogén gombanemzetségeket, amelyek a *Datura stramonium* és az *Abutilon theophrasti* magvain fennmaradhatnak és terjedhetnek. Az *Abutilon theophrasti* és a *Datura stramonium* magmintákat Keszthely környékének szántóterületeiről gyűjtöttük 2007-ben, 2010-ben, illetve 2009-ben és 2010-ben. A magokon *Fusarium spp.*, *Alternaria spp.*, valamint az *Aspergillus spp.* gombák jelenlétét igazoltuk fénymikroszkóp segítségével. Az *Alternaria spp.* fertőzöttség 43,25%, az *Aspergillus spp.* 11,75% és a *Fusarium spp*. 4,25% volt. A *Datura stramonium* és az *Abutilon theophrasti* talajban lévő magjai hozzájárulhatnak a *Fusarium spp*, *Alternaria spp* és *Aspergillus spp* fajok fennmaradásához és terjedéséhez.

***Kulcsszavak**: Abutilon theophrasti; Datura stramonium; gyommagot fertőző gombák; morfológiai; magfertőzöttség*

1. Introduction

Weeds as field pests hinder crop growth and reduce their productive ability. The adverse effects of weeds cause huge economic losses, due to superior competition and interference properties (allelopathy) over crops for growth resources. Weeds are important agents of plant disease spread and retention (Ekwealor et al., 2019). Hence, weeds have become a basic consideration in the management and control of phytopathogens of crops due to their significant influence on disease incidence in arable fields.

Previous scientific studies have revealed that weeds can harbour various viable forms of plant pathogens in their roots, crowns, stems, leaves, flowers, fruits, or seeds. This is evidenced by phytopathogenic isolations that have been obtained from numerous weed species (monocots and dicots) worldwide and are capable of spreading them to infect crops. 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, and Diaporthe*. Phytoviruses include Tobamovirus, Apple mosaic virus, Prunus necrotic ringspot virus and Tomato yellow leaf curl virus (Avinash and Gaur, 2020; Postic et al., 2012; Kremer et al., 1984; Kremer, 1986; Kremer, 1987; Malavika & Devendra, 2023).

This study is geared towards *Datura stramonium* and *Abutilon theophrasti,* the dicot weed species that have invaded grain fields of Hungary (Novak Robert et al., 2014). They have strong growth and allelopathic properties (Dafaallah, 2019; Tian et al., 2022) over the cultivated field crops. Earlier researchers in other parts of the world have isolated several pathogens from parts of *Datura stramonium* and *Abutilon theophrasti* (Karimmojeni et al., 2021). *Datura stramonium* and *Abutilon theophrasti* produce thousands of viable seeds per plant capable of staying alive in the soil for many years (Follak et al., 2017; Loddo et al., 2019; Warwick et al., 2011; Rojas-Sandoval, 2022b). Plant seeds are efficient dispersal and survival units for pathogenic fungi and other pathogens, and majority of field diseases troubling farmers in Hungary and worldwide are fungal (Oliveira et al., 2018). However, there is limited information about potential pathogenic fungi in the seeds of *Datura stramonium* and *Abutilon theophrasti* in Hungary. On this foundation, the main objective of this study was to identify potential phytopathogenic fungal genera in the seeds of *Datura stramonium* and *Abutilon theophrasti* in Hungary, based on morphological features of fungal structures.

2. Materials and Methods

The research study investigated the potential phytopathogenic fungi in the seeds of *Abutilon theophrasti* and *Datura stramonium* in Hungary. The experiment was conducted at the Hungarian University of Agriculture and Life Sciences, Georgikon Campus, Keszthely, Hungary at the Festetics Imre BioInnovacios Kozpont (Biotechnology laboratory).

Materials used included seeds of *Abutilon theophrasti* and *Datura stramonium* collected from arable fields of Keszthely in the years 2007, 2010, and 2009, 2010 respectively; Petri dishes of diameter 12 cm; filter papers; plain water, thermostat (incubator) and a Zeiss microscope. A completely randomized design was implemented due to the homogeneity of the methodology. The experiment consisted of four experimental units with two treatments, each replicated twice. Seeds of *Abutilon theophrasti* and *Datura stramonium,* were divided into two petri dishes, with each dish containing 50 seeds. These two petri dishes served as the two replicates for each seed treatment for the years of seed collection in 2007, 2009, and 2010.

The seed samples were washed with plain water for 10 minutes and surface sterilized by immersing them in 95% alcohol for one minute (Postic et al., 2012) to remove any possible external contamination. The seed samples were counted and placed grid-wise in respective sterilized petri dishes that were first layered with two moistened filter papers to hold the seeds stably without rolling over, facilitate weed seed germination and fungal growth. Each set petri dish was covered with another cleaned and sterilized petri dish and labelled (Figure 1).

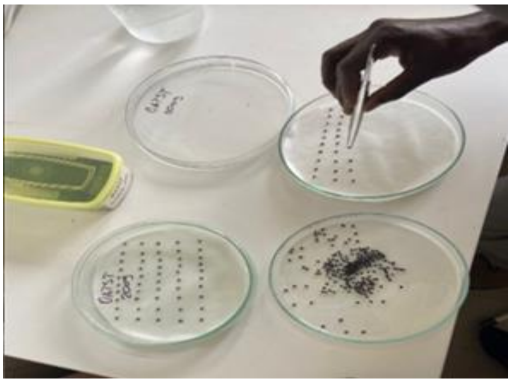

Figure 1. *Seed preparation (counting and arranging of seeds in petri dishes)*

The petri dish set ups were then randomly put in an incubator calibrated at 24^0C (suitable for fungal growth) and left to incubate for 14 days with visual inspection done after the first 7 days to check for initial fungal infections.

A sample of germinated infected seedlings were microscopically examined to determine the infecting fungi based on morphology and pigmentation of macroconidia. Macroconidia were photographed and characterized. Lastly, for each weed seed sample, the exact number of seeds infected with particular fungus was established visually by colouration and recorded. The data was processed using Microsoft excel 365, and frequency of occurrence of the fungi between the seeds of the two weed species was compared using the T test of GenStat (14th edition) software.

3. Results and Discussion

3.1. Microscopic identification of fungi in seeds of Datura stramonium and Abutilon theophrasti

Three isolates were obtained at the end of the experiment, namely Fusarium spp., Alternaria spp. and Aspergillus spp. The isolate of Fusarium spp. had elongated-cylindrical whitish-yellowish macroconidia. Isolate of Alternaria spp. exhibited round brown mature macroconidia and circular, smooth, grayish-brownish immature conidia. Isolate of Aspergillus spp. was characterised by columnar pale green and few bluish conidia. Statistically, results showed no significant differences (P>0.05) in frequencies of occurrences of Fusarium spp., Alternaria spp. and Aspergillus spp. between the seeds of Datura stramonium and Abutilon theophrasti. This could suggest that the seeds of the two weed species have no influence on their infection by the three fungi.

3.2. Segregated incidence by year of Fusarium spp., Alternaria spp. and Aspergillus spp. in seeds of Datura stramonium and Abutilon theophrasti

There are differences in the incidence of *Alternaria spp., Aspergillus spp.* and *Fusarium spp.* in the different years as shown in Figure 2. The average infections by *Alternaria spp., Aspergillus spp.* and *Fusarium spp.* in the three years is 40%, 11.5% and 04% respectively. This could suggest that years have influence on infection rate by the three fungi on the seeds of the two weed species

A) B)

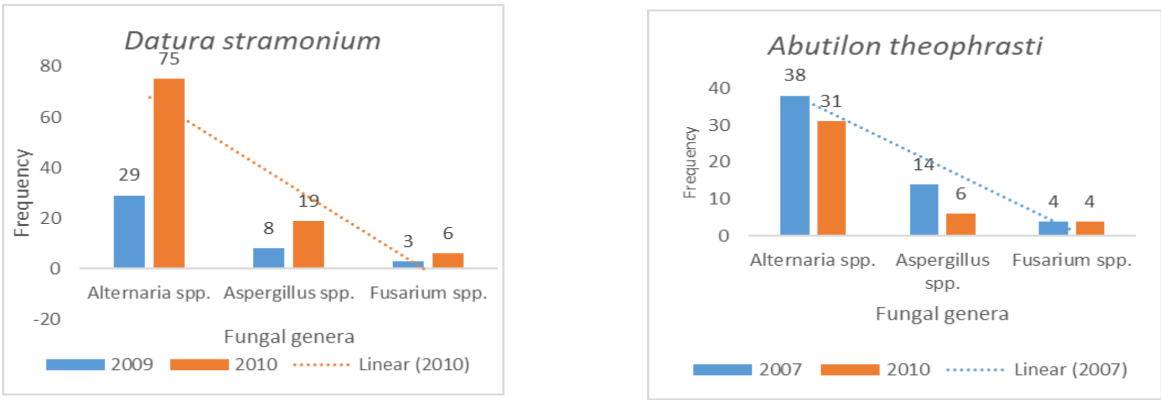

Figure 2. *Incidence by year of Fusarium spp., Alternaria spp. and Aspergillus spp. in seeds of A) Datura stramonium) and B) Abutilon theophrasti*

The results from this experiment confirmed the presence of *Alternaria spp.*, *Aspergillus spp.*, and *Fusarium spp*. in the seed samples of *Abutilon theophrasti* and *Datura stramonium*. This finding is similar to findings from previous studies like a study on the relationship between microorganisms and *Abutilon theophrasti* seeds in contact with the soil. In this study, *Alternaria alternata, Cladosporium cladosporioides, Epicoccum purpurascens, Aspergillus flavus* and *Fusarium spp.* were associated with over 50% of the seeds during 32 days of incubation suggesting that these weed species serve as vectors for the survival and spread of these fungal genera (Kremer et., 1984 and Kremer, 1986).

The infection rates of weed seeds by *Alternaria spp.*, *Fusarium spp.*, and *Aspergillus spp.* varied. Out of 400 sampled weed seeds, 43.25% were infected with *Alternaria spp.*, 11.75% with *Aspergillus spp.*, and 4.25% with *Fusarium spp*. *Alternaria spp.* had the highest seed infection rate in each of the years for both weed species, followed by *Aspergillus spp.* and least *Fusarium spp*. This finding is also reported by Postic et al. in 2012, and Kremer, 1987 whose research also showed a similar trend. According to Kirkpatrick and Bazzaz, 1979 and Kremer, 1987, isolates of *Alternaria spp.* in *Datura stramonium* were 43.3% and *Abutilon theophrasti* 2.7%, *Fusarium spp*. A, *Fusarium spp*. B, *Fusarium spp*. C in *Datura stramonium* were 2.3%, 3.0%, and 3.0% respectively in their examination of the seed-borne microflora from seeds of *Abutilon theophrasti*, *Datura stramonium* L., *Ipomoea hederacea* and *Polygonum pensylvanicum* L. The differences in infection rates could be due to factors such as the seed species' physical nature, the weed species' population and climatic conditions (Nishikawa et al., 2006).The rate of the weed seed infection therefore, depends on the prevalence of the fungi and the weed species in the fields, which is influenced by other factors like weather, environmental conditions and agronomy practices as also mentioned by Nishikawa et al., (2006).

4. Conclusions

The identification of *Alternaria spp.*, *Aspergillus spp.* and *Fusarium spp*. isolates from the seeds of *Abutilon theophrasti* and *Datura stramonium* in Hungary confirmed that both weed species are capable of harbouring and transmitting diseases of these fungal genera to crops. It is therefore worthy devising strategies to prevent seed production by *Datura stramonium* and Abutilon theophrasti to reduce incidence of these pathogenic fungal species, reduce their soil seedbank, hence also reducing the usage of fungicides in crop fields. The fact that morphological characteristics tend to be plastic, it is recommended that molecular identification of these fungal species be done.

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