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Original Research

Fusarium oxysporum as a Pathogen of Pot Plants: a Case Study of the Easter Lily Cactus (*Echinopsis oxygona*) in Poland

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Abstract

Houseplants are being grown to increase the aesthetic value of indoor space but also to elevate air quality. Their infections with phytopathogens, however, not only have an impact on the plant physiology and appearance but also may lead to air contamination and, in consequence, affect human health. Present research is a case study of *Echinopsis oxygona*, known as the Easter Lily cactus, infected with a fungal pathogen. The phenotypic and molecular studies were conducted to identify the etiological agent of the lesions. Colony appearance and growth on various media, as well as the presence of fungal propagation structures were evaluated. Internal transcribed spacer (ITS) sequences from the isolated cultures were obtained, and the BLAST analysis was performed to estimate genetic similarity. The phenotypic and molecular tests allowed to identify the pathogen as *Fusarium oxysporum* and, to our knowledge, it is the first report on *E. oxygona* (as a pot plant) infected with this species in Poland. This fungus is a soil-born species and a well-known toxin producer. Therefore, it does not only reduce aesthetic value of the infected plant but also may lead to air contamination with mycotoxins and fungal structures.

Keywords: plant diseases, cactus, *Echinopsis oxygona*, *Fusarium oxysporum*

Introduction

Houseplants for centuries have served people as the adornment of house, garden or public places, and are classified into two groups: blooming and foliage plants. They provide aesthetic and soothing value, thus should be free from disease and damage caused by phytophages [1]. It has been also proved that pot plants improve physicochemical conditions of the household by increasing the humidification of the air, which lowers air temperature and cleanses it from harmful substances released from carpets or wallpapers. Such plants are also known for their ability to reduce noise [2, 3].

Some common houseplants are beneficial for health as they produce oxygen and remove toxic compounds like benzene, formaldehyde or trichloroethylene from the air [4]. Thus, indoor plant care is recommended in houses and office space due to plant air-cleaning

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properties. The plants considered as efficient air-cleaners are: Areca palm (*Chrysalidocarpus lutescens*), Lady palm (*Rhapis excelsa*), Bamboo palm (*Chamaedorea erumpens*), Rubber plant (*Ficus elastica*), Dracaena (*Dracaena decremensis* 'Janet Craig'), English ivy (*Hedera helix*), Dwarf date palm (*Phoenix roebelenii*), Ficus (*F. macleilandii* 'Alii'), Boston fern (*Nephrolepis exaltata* 'Bostoniensis'), and Peace lily (*Spathiphyllum wallisii*) [4, 5]. Moreover, some plants are especially effective in removing anhydrous ammonia, making them useful to eliminate foul odor in henhouses and pigsties. One of such plants is Yucca sp. which is often cultivated in animal husbandry farms. Succulents are another example of health-promoting plants. These popular houseplants photosynthesize through crassulacean acid metabolism (CAM) – they store CO_2 in a vacuole as the C_4 acid at night. This process contributes to the reduction of CO_2 level in the room, however the plants must be kept in water deficient conditions [2, 6].

Both outdoor and indoor plants are exposed to various biotic and abiotic conditions that may influence their development and appearance [7]. The symptoms resulting from exposure to different stress factors are quite similar, thus the determination which factor is responsible for plant damage is rather difficult. The proper diagnosis often requires time-absorbing and specialized tests [7, 8].

The example of popular houseplants in Poland are cacti, which intrinsically inhabit a broad spectrum of territories (from deserts to tropical forests) in both Americas [9]. Cacti are classified into three subfamilies (*Cactoideae*, *Opuntioideae* and *Pereskioideae*) that consist of about 100 genera and over 1500 species [10, 11]. Despite the broad ecological occurrence, these plants are sensitive to environmental changes, which resulted in placing over 1000 species of the Cactaceae family on the IUCN Red List of Threatened Species [12].

Cacti, both as pot and naturally growing plants, are vulnerable to infections, including those caused by microscopic fungi [13]. The most frequent diseases are: stem rot caused by *Fusarium oxysporum* and *Bipolaris cactivora*, soft rot (*Helminthosporium* sp.), internal soft rot (*Pythium* sp.), phyllosticta pad spot (*Phyllosticta* sp.), anthracnose (*Colletotrichum* sp.), gold spot (*Alternaria* and *Ascochyta* sp.), prickly-pear black spot (*Colletotrichum gloeosporioides* or *Psedocerospora opuntiae*), and cactus late blight disease (*Phytophthora cactorum* and *P. nicotianae*) [14-16]. It should also be noted that some European countries, as well as Israel and Southern Asia, classified some cacti pathogens as quarantine organisms that comprise *F. oxysporum*, *B. cactivora*, *P. cactorum*, and *P. nicotianae* [13].

The main goal of this research was to identify an etiological agent responsible for the lesions of the Easter Lily Cactus (*Echinopsis oxygona*). Moreover, the phenotype and genotype of the phytophage isolated from the diseased tissue was characterized.

Experimental

The studied biological material was isolated from the lesions on *E. oxygona* growing in the Department of Mycology and Genetics, University of Wrocław.

Isolation of an Etiological Agent Responsible for the Infection

The isolation of an etiological factor responsible for the lesions on the Easter Lily Cactus (Fig. 1) was performed on PDA medium (*Potato Dextrose Agar*, Biocorp), in Petri dishes. The infected fragments were detached from the plant with a scalpel and pincer, treated with 0.5% NaOCl for 1 min (or untreated), and placed on PDA. The plates were incubated for 14 days at 24±0.5ºC. The NaOCl-untreated fragments were additionally incubated in a damp chamber made of sterile glass, in Petri dishes for 28 days at 24±0.5ºC.

Identification of an Etiological Agent

To identify the isolated fungus, macroscopic and microscopic observations on PDA, Sabouraud, Czapek-Dox (1.2% agar, Biocorp), MEA (*malt extract agar*, Biocorp), and CYA (*Czapek yeast autolysate agar*) were made [17]. The observed features included colony growth, as well as the color and occurrence of specific morphological structures like spores. The observations were analyzed according to monographs [18-20]. The fungus specimens placed on microscope slides were dyed with LPCB (lactophenol cotton blue, Sigma-Aldrich). For microscopic imaging, Axio Image.M1 (Zeiss) was used, and macroscopic observations were documented using Nikon cooplix S3700.

To confirm species affiliation, ITS (internal transcribed spacer) was sequenced. DNA was isolated from fungal colonies cultured on PDA according to the original, hexadecyltrimethylammonium bromide (CTAB)-based method, with minor modifications [21]. In brief, the volume of the buffer was elevated from

Fig. 1. Lesions on *Echinopsis oxygona.*

400 to 700 µl, and the extraction with chloroform : isoamyl alcohol (24:1; 500 µl) was performed twice instead of once. Fungal rDNA was amplified using the primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR was performed in T100 Thermal Cycler (Bio-Rad), according to Dyląg et al. [22]. The PCR products were verified by electrophoretic separation on a 1.2% agarose gel and, subsequently, purified using Clean-UP (A&A Biotechnology) and sequenced by Macrogen Europe (Netherlands).

Bioinformatics Data Analysis

Sequenced fragments were analyzed using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/). The obtained rDNA sequence was deposited in the National Center for Biotechnology Information database. MEGA program was used to analyze genetic similarity to other fungal ITS in the NCBI database. The genetic distance between analyzed sequences was evaluated using likelihood method. Phylogenetic tree was constructed using neighbor-joining (NJ) technique and confirmed by bootstrap with 1000 repetitions [23].

Results

Lesions on the Easter Lily Cactus were visible as white-yellow discoloration and progressed from the root to the top of the plant. As a consequence, the plant rotted from the inside, roots were completely brown and rotten (Fig. 1). Finally, the growth was inhibited and the plant fell down in about 2-3 months. Two fungal cultures with slightly different phenotypes were isolated on PDA from the surface and the inside of the diseased tissue (Fig. 2). The first culture produced numerous cauliflower-shaped dark sclerotia (Fig. 2a), whereas the second one exhibited less numerous sclerotia and, additionally, orange sporodochia (Fig. 2b). Further research was conducted on two isolates (UWR_151 and UWR_152) which represented both types of cultures. First, the appearance of the colony on various media (CYA, Czapek-Dox, MEA, PDA, Sabouraud, and YPG) was evaluated, confirming distinctive phenotypes of both cultures – isolates differed slightly in colony morphology and reverse color, aerial mycelium structure, and the growth rate (Fig. 3).

Microscopic observations of 4-week cultures on PDA showed that both isolates probably belong to one species (Fig. 3). The presence of characteristic structures like conidiophores, chlamydospores, and macro- and microconidia was noted on the microscope slides. Conidiophores were present in the substrate and aerial mycelium, usually in small groups, and occasionally conglomerated into sporodochia (Fig. 4A1 and Fig. 4B1). Conglomerated microconidia formed heads exhibiting

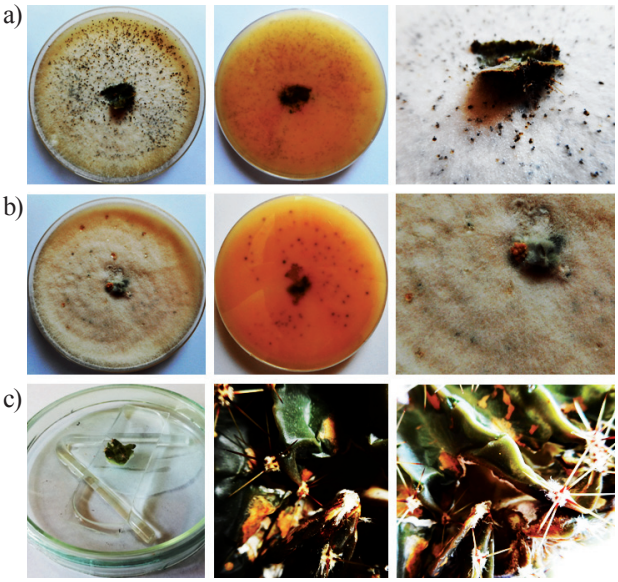

Fig. 2. The etiological agent of lesions on *Echinopsis oxygona* on PDA (a – UWR_151 culture, b – UWR_152 culture) and grown as light fungal mycelium in a damp chamber c). 6-week culture (a, b) and 3-week culture c) at 24±0.5ºC. Numerous dark sclerotia of cauliflower-like shape a), less numerous sclerotia and orange sporodochia b).

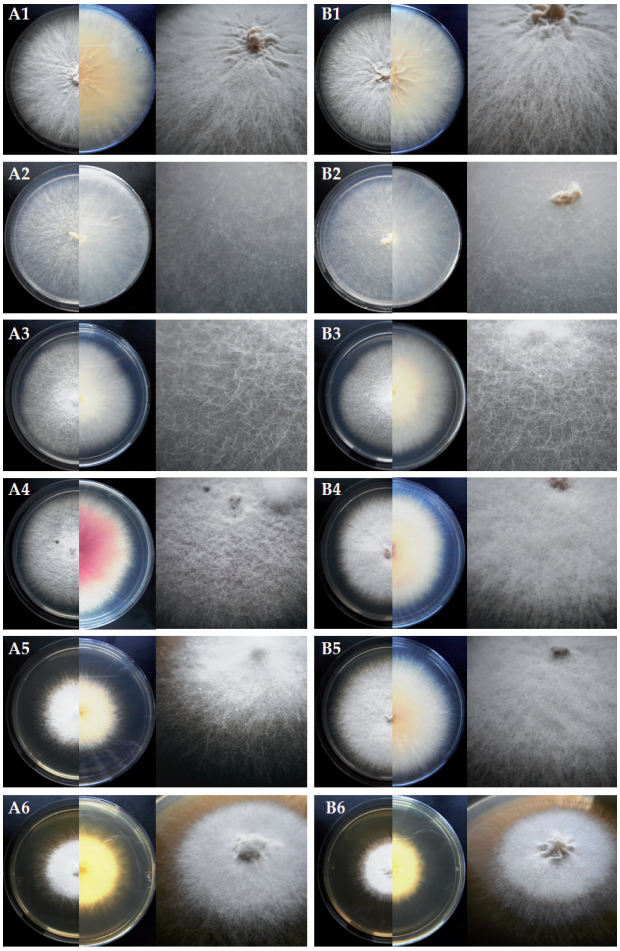

Fig. 3. A one-week culture of UWR_151 (A) and UWR_152 (B) on CYA (1), Czapek-Dox (2), MEA (3), PDA (4), Sabouraud (5) and YPG (6) at 24±0.5ºC.

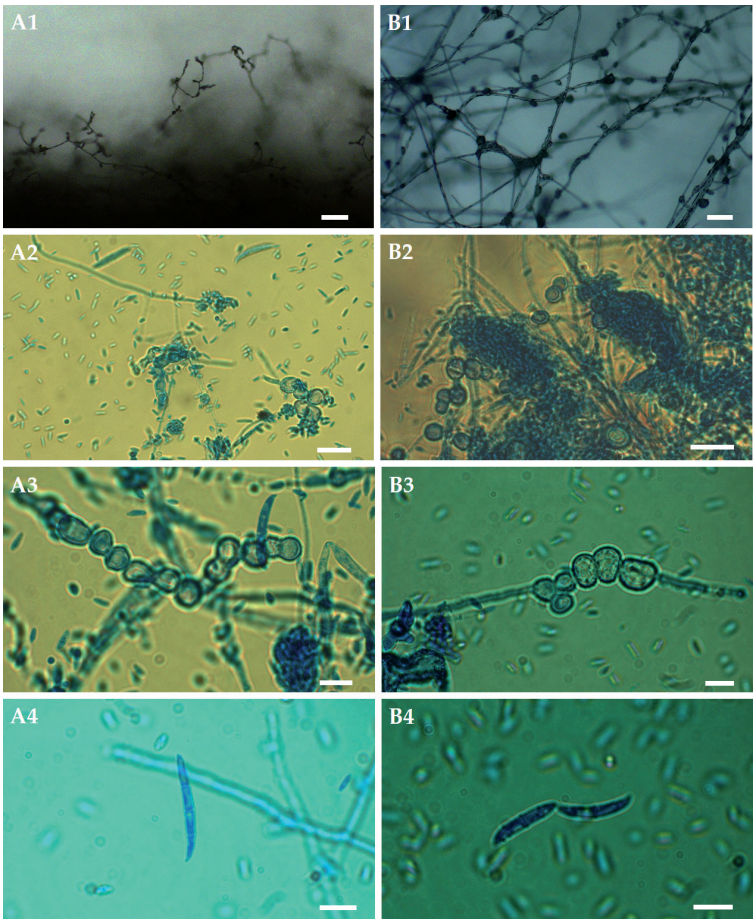

Fig. 4. Characteristic structures of 4-week cultures of UWR_151 (A) and UWR_152 (B) on PDA at 24±0.5 °C observed on the Petri dish (1) and on the slides dyed with LPCB (2, 3, 4). Scale bars: 50 µm (A1, B2), 20 µm (A2, B2), 10 µm (A3, A4, B3, B4).

shape from elliptic to cylindrical, occasionally with the curve (so called allantoidal). They were transparent, thick-walled, and mostly unicellular with the size of 5.2-8.9/2.5-3.4 µm (Fig 4A2-A4 and 4B2-B4). Macroconidia were present both in sporodochia and aerial mycelium. They were transparent, slightly bent in the middle, and mainly three-septate. The shape of their basal and top cells was pedicel- and hook-like, respectively. The size of macroconidia was of 25-40/3.2-4.7 µm (Fig. 4A4 and Fig. 4B4). Numerous circular (or close to circular), smooth-walled chlamydospores with the size of 7.5-10 µm were observed (Fig. 4A3 and Fig. 4B3).

The phenotypic analysis allowed for the identification of an etiological factor causing the lesions on *E. oxygona* as the filamentous fungus of the *Fusarium* genus, most likely belonging to *F. oxysporum* Schltdl. To confirm preliminary classification, molecular studies were conducted. The obtained rDNA ITS sequences were compared with those deposited in the National Center for Biotechnology Information database using the BLAST algorithm (Table 1). Sequences obtained from both isolates were highly similar to the deposited sequence of *F. oxysporum*; the UWR_151 isolate was 99.08% identical and overlaid with the isolate from Philippines at the level of 97% (the highest known percentage of identity), whereas the UWR_152 isolate was 99.27% identical with 93% similarity with to the isolates from USA, India and Korea.

Phylogenetic trees were obtained using neighbor-joining, based on the isolate sequences, as well as those deposited in the NCBI database (10 sequences for each isolate). Studied isolates were divided into two groups: UWR_151 was most closely related to MH879861.1 isolated in Pakistan, and UWR_152 – to MK508868.1 isolated in India (Fig. 5).

Discussion

Echinopsis oxygona (Link) Zucc. ex Pfeiff. & Otto (Easter Lily Cactus) is a very popular large caespitose cactus, widely grown for its huge nocturnal flowers. It is the best described and most commonly grown globular cactus [24]. It occurs naturally in Southern Brazil, Uruguay, and province of Entre Rios, Argentina. The Easter Lily Cactus occurs in grassy plains or in low hills in lowland up to 1000 m a.s.l. with other cacti of the *Notocactus*, *Gymnocalycium*, *Frailea*, *Cleistocactus* and *Cereus* genera [24, 25]. The present study shows

Table 1. BLAST analysis of ITS sequences obtained from studied isolates.

Species from NCBI database			Studied culture			
Name	Accession No.	Country of origin	Query Cover [%]	Identity [%]	Accession No.	Isolate number
Fusarium oxysporum	KP714275.1	Philipines	97	99.08	MK733287.1	UWR_151*
	MK685126.1	Malesia	96	98.37		
	MK416124.1	China	96	98.37		
	MH311044.1	China	96	98.37		
	MK268137.1	USA	96	98.37		
	MK268134.1	USA	96	98.37		
	MK267446.1	USA	96	98.37		
	MK267445.1	USA	96	98.37		
	MK268138.1	USA	96	98.37		
	MK226163.1	RPA	96	98.37		
	MK508868.1	India	93	99.27	MK733288.1	UWR_152**
	KT828535.1	USA	93	99.27		
	JN624887.1	Korea	93	99.27		
	MH055398.1	United Arab Emirates	93	99.03		
	MF460362.1	Turckey	93	99.03		
	MH879861.1	Pakistan	93	99.03		
	MH879586.1	Pakistan	93	99.03		
	MG272267.1	China	93	99.03		
	KF730784.1	China	93	99.03		
	KU939031.1	China	93	99.03		

* 10 first sequences of at least. 96% overlay level and 98.37% identity were used for the analysis
** 10 first sequences of at least. 93% overlay level and 99.03% identity were used for the analysis

that this species, when grown as a decorative indoor plant, is exposed to fungal infections.

Phenotypic research (micro- and macroscopic observations) allowed for the classification of an infectious agent isolated from the lesions on *E. oxygona* as *Fusarium*. This genus was first described by Link in 1809 as *Fusisporium*, based on the characteristic sickle shape of conidial spores, and is presently known as *Fusarium*, referred to as *Fusarium* sensu Wollenweber [18, 26]. This microscopic filamentous fungus belongs to the *Ascomycota* phylum, *Ascomycetes* class, *Hypocreales* order. *Fusarium* is the name of the anamorph, and its teleomorphs are mostly classified in the genus *Gibberella* and, less often, in the *Hemanectria* and *Albonectria* genera [18, 27]. *Fusarium* are cosmopolitan fungi, inhabiting all ecological niches, with the main reservoir being soil. The genus contains typical saprotrophs, endosymbionts and polyphages of crop and decorative plants and trees, as well as increasingly occurring human and animal pathogens [18, 28, 29]. The biologically active substances produced by *Fusarium* are often toxic and, thus, undesirable in agriculture and industry [29, 30]. The simultaneous plant colonization by various *Fusarium* species is a common phenomenon, an example being Fusarium head blight (FHB). The production of various secondary metabolites, as well as the ability to co-exist with other species enable *Fusarium* to infect various plants [31]. The infection might occur at any stage of plant development, from germinating seeds to mature vegetative tissue, and physical damage enhances the probability of infection [32].

The combination of phenotypic and molecular studies enabled the identification of the isolated fungal cultures as *F. oxysporum* Schltdl. According to the literature, this species forms white colonies on PDA, sometimes with purple shade and dark sclerotia or/and orange sporodochia, while the reverse is bright to dark purple [20]. The macroscopic observations in present work are consistent with the available data. Similarly, the morphological structures were identical with those described in the literature. Typical *F. oxysporum* microconidia are unicellular, transparent and thick-walled with the size range of 5-9(-13)/2.4-3.5 µm.

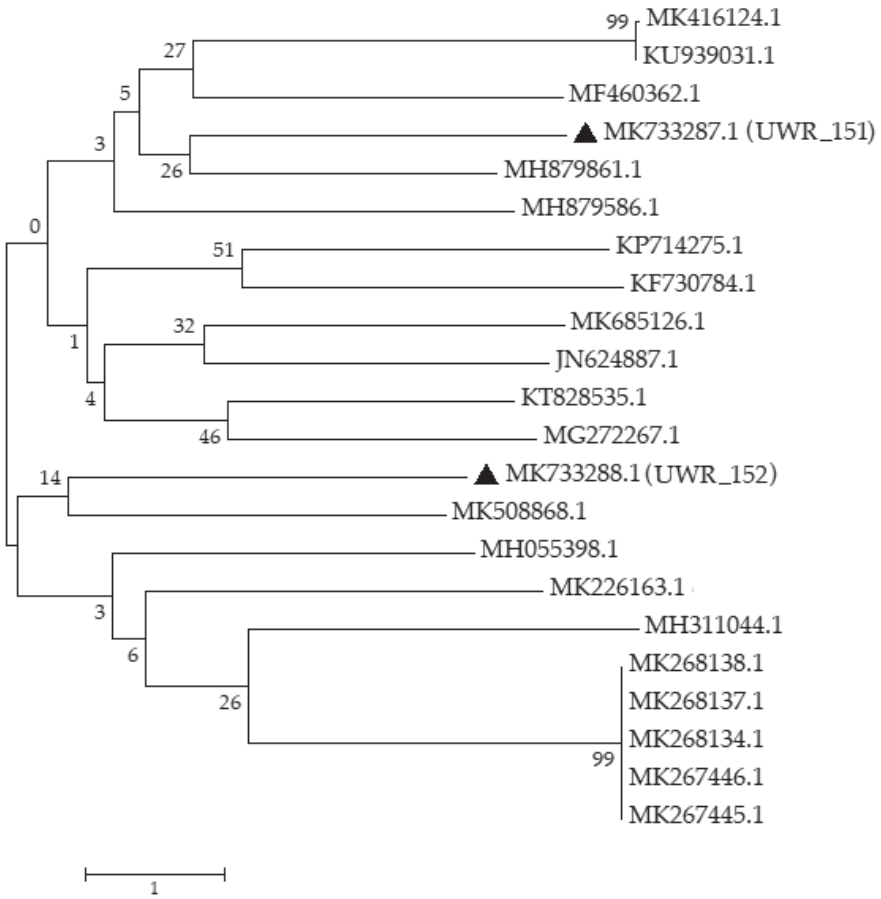

Fig. 5. Genetic similarity tree of both tested isolates (*Fusarium oxysporum* MK733287.1 – UWR_151 and *F. oxysporum* MK733288.1 – UWR_152) in relation to those deposited in NCBI database. For UWR_151 10 first sequences of at least 96% overlay level and 98.37% identity were used for the analysis, whereas for UWR_152 10 first sequences of at least 93% overlay level and 99.03% identity were used for the analysis.

Macroconidia, on the other hand, are transparent and slightly curved in the center, three-septate (rarely with 4-5(-7) septa) with the size of (18-)27-42(-54)/3-5 µm. Chlamydospores, another characteristic structures of *F. oxysporum*, measure 7-11 µm [19, 20].

Fusarium oxysporum belongs to the *Elegans* section and is an ubiquitous species – it inhabits various ecological niches with distinct environmental conditions and nutrient availability [18]. The species consists of many strains, including saprotrophs used in plant protection, but also pathogens of monocotyledonous and dicotyledonous plants, as well as emerging animal and human pathogens (mainly of immunocompromised patients). Due to this species diversity, it is also referred to as the *F. oxysporum* complex. The main symptoms of the *F. oxysporum*-driven plant infection are: root, stem, and fruit rots and vascular wilt, as well as the storage rots [18, 33, 34, 35]. The literature data report that *F. oxysporum* is an incurable fungus that enters through the roots, then slowly spreads into the cactus and may cause stem rot [36], what was also confirmed in the present study. On the other hand, there is no information on *F. oxysporum* as an etiological agent of the lesions on indoor *E. oxygona*. Thus, the present research is the first report on *F. oxysporum* as the pathogen of the Easter Lily cactus grown as an indoor pot plant.

Fungi belonging to *Fusarium* genus are well known mycotoxin producers. *Fusarium oxysporum* alone may excrete various toxic metabolites like fumonisin B1, moniliformin, diaxetoxyscirpenol, T-2 toxin, zearalenone and their derivatives [37, 38]. Both mycotoxins and spores (numerously produced by *Fusarium*) may be released into the air and enter the alveoli (along with mycelium fragments of suitable diameter), however, this phenomenon is rather rare due to their low agility [18, 39]. The inhalation of mycotoxins has many times greater effect than ingestion or skin penetration [40, 41]. It is attributed to the greater contact surface and easier diffusion through capillary walls in the alveoli [42, 43].

People tend to spend 80-90% of their time indoors, thus it should be kept in mind that the risk of intoxication is higher after the exposition to indoor air contaminants rather than polluted outdoor environment [44]. Therefore, one can postulate that diseased plants not only lower the aesthetic value of the area, but

also might be the source of dangerous air pollutants, especially in case of the fungi-infected houseplants grown in small, humid rooms with poor ventilation. Long-lasting exposition to fungal structures and their secondary metabolites might lead to allergies, infections, sick building syndrome and even cancer [44-47]. The contact with those agents is especially dangerous to children, young adults, elderly people, as well as patients with respiratory and circulatory deficiencies [48].

Conclusions

A microscopic filamentous fungus was shown to be an etiological agent of the lesions on the Easter Lily Cactus (*Echinopsis oxygona*), represented by white-yellow discoloration and internal rot. Phenotypic and molecular studies have led to the identification of the fungus as *Fusarium oxysporum*. It is probably the first report of *F. oxysporum* as a pathogen of this popular house plant. This fungus is a soil-born species with toxin-producing properties, and may infect plants, animals and humans. Houseplants generally enhance the indoor air quality, however, it should be noted that plant infection with fungal phytopathogens may not only reduce their aesthetic value, but also lead to air contamination with mycotoxins and fungal structures. Since it has been proved that the exposition to such contaminants may lead to the sick building syndrome, our future studies will include the correlation between houseplants, their infections with pathogenic fungi, and indoor air quality.

Conflict of Interest

The authors declare no conflict of interest.

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