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Biodiversity of fungi colonizing hull-less seed squash (*Cucurbita pepo* subsp. *pepo* var. *styriaca* Greb.) cultivated in an organic farm

Bioróżnorodność grzybów występujących na dyni zwyczajnej (*Cucurbita pepo* subsp. *pepo* var. *styriaca* Greb.) o nasionach bezłupinowych, uprawianej w gospodarstwie ekologicznym

Summary. Hull-less seed squash (*Cucurbita pepo* subsp. *pepo* var. *styriaca* Greb.) has an increasing importance as a plant material to obtain valuable oil and seeds for direct consumption. Its seeds are an important source of organic and inorganic compounds. They have pro-health properties and they are used in treating a number of illnesses. The aim of studies conducted in 2014 was to estimate the species community of fungi occurring on the plants of three cultivars of hull-less seed squash grown in an ecological farm: 'Junona', 'Miranda' and 'Gleisdorfer Ölkürbis'. The mycological analysis was made of the roots, stems and leaves (leaf blades together with petioles). The studies showed that fungi *Alternaria alternata* and *Fusarium equiseti* most abundantly colonized the leaves and stems of squash in the conditions of ecological cultivation. Species *Fusarium oxysporum* was most abundantly isolated from the squash roots. In the conditions facilitating their development, these fungi can become pathogenic factors to squash plants. Among the studied cultivars, the Polish cultivar 'Miranda' was most abundantly colonized by fungi from genus *Fusarium*, while species *Alternaria alternata* was most frequently isolated from the leaves of the German cultivar 'Gleisdorfer Ölkürbis'.

Key words: oil pumpkin, pathogenic fungi, *Fusarium* spp., 'Junona', 'Miranda', 'Gleisdorfer Ölkürbis'

INTRODUCTION

Squash (*Cucurbita pepo* L.) is one of popular seasonal vegetables cultivated in the world under different climatic conditions. This vegetable is characterized by high nutritious and technological values. That has an effect on increase of acreage of this crop [Kawecki and Kryńska 1994]. Squash can be stored for several months, which enables its consumption in late autumn and in winter [Orłowski 2000]. Its flesh contains a number of valuable compounds which supplement our diet and it has also medicinal and cosmetic properties. Due to its alimentary and health benefits, it is used in fruit and vegetable processing industry [Balcerzak and Legańska 2000, Nawirska-Olszańska 2011].

In recent years squash (*Cucurbita pepo* subsp. *pepo* var. *styriaca*), also called hull-less seed or oil squash, is cultivated more and more often. Its seeds have no hull. This type of squash appeared at the beginning of the 19th century in Styria and Carinthia (Austria) as a result of a spontaneous mutation of one recessive gene. The major products obtained from this cultivation are the seeds [Korzeniewska *et al.* 2013]. The fat in them has a comparable content to that of the oil from sunflower or soybean seeds [Balcerzak and Legańska 2000, Nawirska-Olszańska 2011]. Squash seeds are characterized by a hight content of protein, assimilable lipids (phytosterols and unsaturated fatty acids), polyphenols and mineral compounds [Balcerzak and Legańska 2000, Fruhwirth and Hermetter 2008]. These compounds inhibit the accumulation of cholesterol in tissues and decrease the level of „bad" cholesterol, thus preventing artherosclerosis. Regular consumption of squash seeds helps prevent prostatic hyperplasia and cures inflammation conditions of skin and the mucous membrane. It is used for removing the parasites from the digestive canal, which is due to alkaloids (cucurbitacin) fighting different intestinal parasites [Niemirowicz-Szczytt 1993]. Cold-pressed squash seed oil has a green brownish colour and a nutty smell and taste. It contains linoleic and oleic acids as well as vitamins E, B1, B2, B6, A, C and D. It is rich in β-carotene, potassium, selenium and zinc. Lipids in squash seeds belong to full-value plant fats. Unsaturated acids are necessary for the organism and they are component necessary for the production of vitamin D, hormones and cell walls [Niemirowicz-Szczytt 1993, Danilcenko *et al.* 2004, Biesiada *et al.* 2006, Fruhwirth and Hermetter 2008].

Cucurbit vegetables grown under the field condition are usually infected by pathogens specific to the Cucurbitaceae family species, as well as organisms characteristic of other plants [Jamiołkowska *et al.* 2011, Rahim *et al.* 2013]. Several fungi have been identified as *Alternaria alternata* and *A. cucumerina* (Alternaria leaf spot), *Pseudoperonospora cubensis* (downy mildew), *Podosphaera xanthii*, *Erysiphe cichoracearum*, *Sphaerotheca fuliginea* (powdery mildew), *Colletotrichum orbiculare* (anthracnose), *Cladosporium cucumerinum* (scab or gummosis), *Septoria cucurbitacearum* (Septoria leaf spot), *Pythium* spp. (damping-off), *Rhizoctonia solani* (damping-off), and *Fusarium* spp. (damping-off and wilt) [Robak and Wiech 1998, Jamiołkowska *et al.* 2011, Choi *et al.* 2015]. Characterization of the population structure of fungal communities, including the pathogens, is important for the understanding of the biology of the pest and for the development of disease-control strategies.

The aim of the paper was to estimate the species composition of the fungi colonizing the plants of three varieties of hull-less seed squash (*Cucurbita pepo* subsp. *pepo* var. *styriaca)* cultivated in an ecological farm.

MATERIAL AND METHODS

Agrotechnical experiment. Studies were carried out in 2014. A field experiment was set up in an ecological farm in the village of Grądy near Ludwin, in the Lublin region (51º05′36″N 22º12′33″E). The studies included three hull-less seed oil squash cultivars (*Cucurbita pepo* subsp. *pepo* var. *styriaca*): 'Junona', 'Miranda' and 'Gleisdorfer Ölkürbis'. The squash seeds were sown in the field in the first decade of May at the spacing of 0.80 × 1.0 m. The field experiment was set up as an one-factor experiment in a random block design in 4 replications. Forty plants (4 rows with 10 plants in each) were planted for each cultivar. The plants were manually weeded in the vegetation season. No chemical preparations for plant protection were used. Spring mineral nutrition was done according to the soil analysis for the Cucurbitaceae plants family by using a nitrogen fertilizer Bioilsa 12.5 in the dose $500-700 \text{ kg} \cdot \text{ha}^{-1}$.

Mycological plants analysis. The mycological analysis of plants was made at the beginning of fruiting (first decade of August) in the laboratory of the Department of Plant Protection and Quarantine of the Life Sciences University in Lublin. Two plants from each experimental combination were randomly taken from each cultivars (experimental combination). The roots, stems and leaves (leaf blades with petioles) were analyzed. The studied material was cleaned of soil fragments and rinsed under running water for 20 minutes. The material prepared in this way was disinfected on the surface by being immersed in a 0.1% solution of sodium hypochlorite (NaClO) for one minute and next it was rinsed three times in sterile water. The disinfected material was crumbled into 3 mm fragments, 10 of which were then put on each sterile Petri dish with a potato glucose agar (PDA Difco). The medium was prepared according to the instructions and sterilized in an autoclave at the temperature of 180°C and the pressure of 1 atm for 20 minutes.

For each cultivar, 30 plates were prepared (10 for the roots, 10 for the stems and 10 for the leaves). The plates was stored in a thermostat for the period of 10 days at the temperature of 20–22°C without access of light. The fungi colonies grown from the plant fragments were placed under sterile conditions into slants with the potato glucose agar (PDA). The slants were stored under the same conditions as the Petri dishes. After the colonies grew, the segregation was performed on the basis of the colour and appearance of the colonies. The grown colonies were determined to the species by means of the available monographs and mycological keys.

RESULTS

As the result of mycological analysis 759 isolates of fungi represented by 13 species were obtained (Tab. 1–3). Totally, 245 colonies were obtained from the roots, 238 isolates from the stems and totally 276 colonies were obtained from the leaves (Tab. 1–3). Apart from fungi, numerous bacteria were also observed on the medium.

Fungi from *Fusarium* genus were most frequently isolated (totally, 387 isolates). The species of this genus mainly came from the roots and the stems and they amounted to respectively, 152 and 153 isolates. The greatest number of them was obtained from 'Miranda' and 'Gleisdorfer Ölkürbis' cvs (Tab. 1–3). Genus *Fusarium* was most abundantly represented by *F. oxysporum*. Numerous macro- and microconidia were visible under the microscope (Phot. 1). Totally, 184 isolates of this species were obtained (Tab. 1–3). It mainly colonized ‘Miranda’ (totally, 89 colonies) and it amounted to 37% of all isolations from this cultivar (Tab. 1–3, Fig. 1). This species was also numerously obtained from ‘Gleisdorfer Ölkürbis’, where 67 colonies (27% of all isolations) were obtained (Tab. 1–3, Fig. 1). Only 28 isolates of *F. oxysporum* were obtained from ‘Junona’, which amount to 11% of all isolations (Tab. 1–3, Fig. 1). The next species which was frequently isolated from plants was *F. equiseti* (Phot. 2). The fungus was most numerous on the plants of ‘Miranda’ (81 colonies, 33% of isolations) and it mostly was isolated from the leaves. This species was the least abundantly isolated from ‘Gleisdorfer Ölkürbis’ , where it mostly occurred on the stems (31 isolates, 13% of all isolates) (Tab. 1–3). Totally, 21 colonies of *F. avenaceum* were obtained. The fungus mainly colonized the leaves and stems of ‘Junona’ (11 colonies) (Tab. 2–3).

Table 1. Fungi occurring the roots of oil squash in 2014
Tabela 1. Grzyby występujące na korzeniach dyni oleistej w 2014 roku

Fungus species Gatunek grzyba	Number of isolates Liczba izolatów			Total Łącznie
	‘Junona’	‘Miranda’	‘Gleisdorfer Ölkürbis’	
Chaetomium funicola Cooke	–	–	2	2
Fusarium avenaceum (Fr.) Sacc.	–	–	2	2
Fusarium equiseti (Corda) Sacc.	7	–	–	7
Fusarium graminearum Schwein.	9	–	–	9
Fusarium oxysporum Schl.	17	55	62	134
Gliocladium fimbriatum Gilman Abbott	–	–	3	3
Mucor hiemalis Wehm.	65	6	9	80
Trichoderma hamatum (Bonord.) Bainier	–	8	–	8
Total/ Łącznie	98	69	78	245

Table 2. Fungi occurring the stems of oil squash in 2014
Tabela 2. Grzyby występujące na łodygach dyni oleistej w 2014 roku

Fungus species Gatunek grzyba	Number of isolates Liczba izolatów			Total Łącznie
	‘Junona’	‘Miranda’	‘Gleisdorfer Ölkürbis’	
Alternaria alternata (Fr.) Keiss.	–	31	–	31
Aureobasidium pullulans (de Bary) Arnoud	4	–	4	8
Epicoccum nigrum Link	–	–	5	5
Fusarium avenaceum (Fr.) Sacc.	9	–	7	16
Fusarium equiseti (Corda) Sacc.	50	11	31	92
Fusarium oxysporum Schl.	6	34	5	45
Mucor hiemalis Wehm.	20	–	17	37
Mucor mucedo Fresen.	–	–	4	4
Total/ Łącznie	89	76	73	238

Phot. 1. Macro- and microconidia of *F. oxysporum* (optical microscope ×40)
(photo A. Jamiołkowska)
Fot. 1. Makro- i mikrokonidia *F. oxysporum* (mikroskop optyczny ×40)
(fot. A. Jamiołkowska)

Table 3. Fungi occurring the leaves of oil squash in 2014
Tabela 3. Grzyby występujące na liściach dyni oleistej w 2014 roku

Fungus species Gatunek grzyba	Number of isolates Liczba izolatów			Total Łącznie
	'Junona'	'Miranda'	'Gleisdorfer Ölkürbis'	
Alternaria alternata (Fr.) Keiss.	26	19	91	136
Aureobasidium pullulans (de Bary) Arnoud	32	–	–	32
Chaetomium funicola Cooke	6	–	–	6
Epicoccum nigrum Link	6	5	3	14
Fusarium avenaceum (Fr.) Sacc.	2	1	–	3
Fusarium equiseti (Corda) Sacc.	3	70	1	74
Fusarium oxysporum Schl.	5	–	–	5
Humicola grisea Traaen	–	3	–	3
Mucor hiemalis Wehm.	–	–	1	1
Rhizoctonia solani Kühn.	–	–	2	2
Total/ Łącznie	80	98	98	276

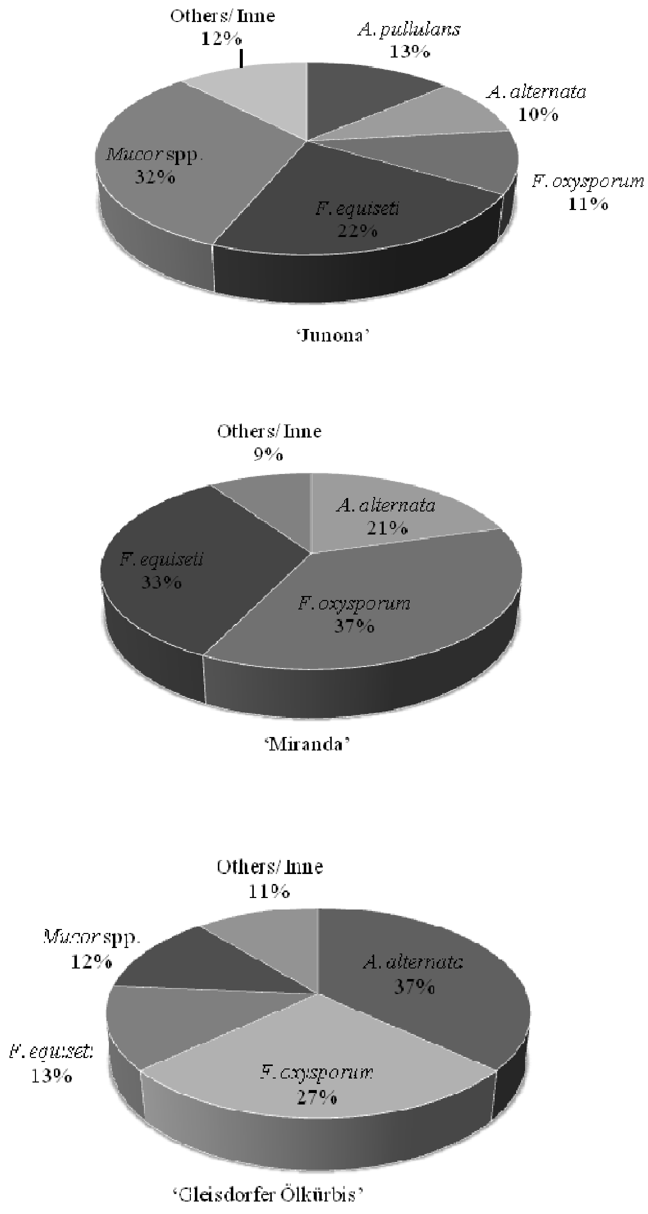
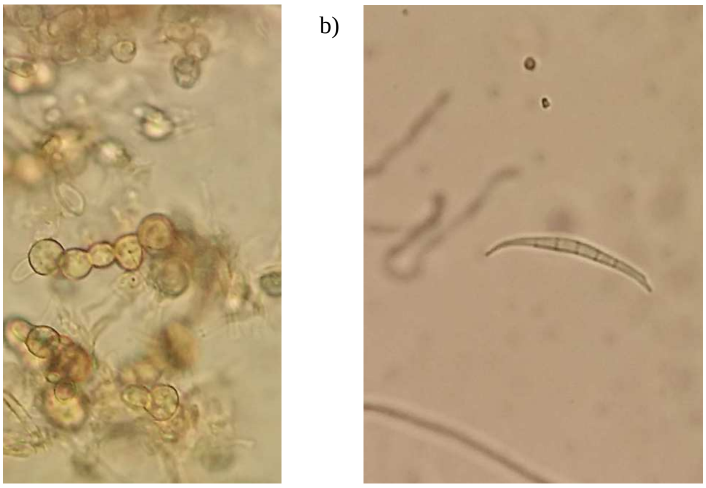


Fig. 1. Percentage (%) of chosen fungi groups obtained from squash plants (leaves, stems, roots) of 'Junona', 'Miranda', 'Gleisdorfer Ölkürbis' cultivars in 2014

Rys. 1. Udział procentowy (%) wybranych grup grzybów uzyskanych z roślin dyni (łącznie liście, łodygi, korzenie) odmian 'Junona', 'Miranda', 'Gleisdorfer Ölkürbis' w 2014 roku

a) b)



Phot. 2. Macroconidia (a) and chlamydospores (b) of *F. equiseti* (optical microscope × 40)
(photo A. Hamood Thanoon)
Fot. 2. Makrokonidia (a) i chlamydospory (b) *F. equiseti* (mikroskop optyczny × 40)
(fot. A. Hamood Thanoon)

Alternaria alternata species was abundantly isolated from analyzed parts of plants (Phot. 3). Totally, 167 colonies of the fungus were obtained. It mainly colonized the leaves of ‘Gleisdorfer Ölkürbis’ (Tab. 1–3) and amounted to 37% of all isolations obtained from this cultivar (Fig. 1). Totally, 50 isolates of this fungus came from the leaves and stems of ‘Miranda’ cv., which amounted to 21% of all isolations of this cultivar (Fig. 1). The smallest number of *A. alternata* colonies came from the leaves of ‘Junona’ (26 isolates) (Tab. 3, Fig. 1). This species did not occur on the roots of the studied cultivars.

Phot. 3. Mycelium and conidia of *A. alternata* (optical microscope × 40)
(photo A. Jamiołkowska)
Fot. 3. Grzybnia i zarodnik konidialny *A. alternata* (mikroskop optyczny × 40)
(fot. A. Jamiołkowska)

Fungi from *Mucor* genus (totally, 122 isolates) were also isolated from the plants (Tab. 1–3). This genus was represented by *M. hiemalis* and *M. mucedo*. *M. hiemalis* most frequently occurring on the roots of 'Junona' (65 isolates) and *M. mucedo* was isolated only from stems of 'Gleisdorfer Ölkürbis' (4 isolates) (Tab. 1–2).

The saprotrophic fungi with antagonistic properties also included *Aureobasidium pullulans, Epicocum nigrum*, *Gliocladium fimbriatum* and *Trichoderma hamatum.* A number of 57 colonies of *A. pullulans* were isolated (Tab. 1–3), mainly from the leaves of 'Junona' cv., where they amounted to 13% of all isolations from this cultivar (Fig. 1). The species was also present abundantly on the stems of 'Gleisdorfer Ölkürbis' (5 isolates) (Tab. 2). *Gliocladium fimbriatum* colonized only the roots of 'Gleisdorfer Ölkürbis' cv., while *Humicola grisea* was isolated from the leaves of 'Miranda' and *Trichoderma hamatum* was present only on the roots of 'Miranda' (Tab. 1, 2).

'Miranda' was the most frequently colonized by *Fusarium* spp. (70% of all isolations). *F. oxysporum* and *F. equiseti* were mainly isolated from 'Junona' and 'Gleisdorfer Ölkürbis' cultivars. Totally, they amounted to 33–40% of all fungi from the studied cultivars (Fig. 1). The predominant species on the leaves of the studied cultivars was *Alternaria alternata*, which was most frequently isolated from 'Gleisdorfer Ölkürbis' (37% of all isolations) (Fig. 1). The species was less abundant on 'Miranda' and 'Junona' cultivars. *Aureobasidium pullulans*, which amounted to 13% of all isolations, was abundantly isolated from the leaves of 'Junona' (Fig. 1).

DISCUSSION

Hull-less seed squash has an increasing importance as a vegetable due to the seeds which have no hull and to the ease of obtaining them. Ecological seeds produced in Poland are the main material exported to the EU countries.

The studies presents the species and numbers of fungi colonizing hull-less seed squash plants cultivated in an ecological farm. The analyses showed that the plants were mostly colonized by *Fusarium* spp. and *Alternaria alternata*.

Genus *Fusarium* includes the species pathogenic towards many cultivated plants. *F. equiseti* was manly isolated from the stems and leaves of the studied cultivars. That fungus commonly occurs in temperate climate and it causes the root rot, stem, fruits and bulbs necrosis of many plants [Sadowska 2012]. From the studied squash cultivars this species was isolated both from the stems, leaves and roots. Jamiołkowska *et al.* [2011] showed that *F. equiseti* was the reason for the rot roots and the wilting of zucchini seedlings and the isolates of that species were heavily pathogenic. High abundance of *F. equiseti* may be a considerable threat to plants. It is an important fungus from the economic point of view and its importance is increasing. *F. equiseti* produces equisitine, which is a substance of antibiotic effect towards some Gram-positive bacteria and which has a phytotoxic effect on the seeds and seedlings of certain plants. Like other species from genus *Fusarium*, the pathogen produces a number of toxins dangerous to people and animals. Especially dangerous are trichothecenes (deoxynivalenol), which act as the inhibitors of protein synthesis, as well as fusarochromanon and zearalenone [Sadowska 2012]. *F. oxysporum* was frequently isolated among *Fusarium* spp. It mainly occurred on the roots and stems of 'Miranda'. As reported by Dobrzańska and Dobrzański [1991], the pathogenic fungus *F. oxysporum* causes fusarium wilting of the underground part of plants. The symptoms of the disease are the growth inhibition, wilting, yellowing and drying out of the lower leaves as well as reversible wilting of plants. Permanent wilting and fast decay of plants occur at the later phase of the disease. White-pink sporulation of the fungus is sometimes visible on the decaying stem tissues. Jamiołkowska and Sawicki [2011] showed that *F. oxysporum* occur frequently on zucchini roots and its isolates are strongly pathogenic towards zucchini seedlings similarly to *F. equiseti.* Of considerable importance in the development of the disease are the toxins produced by *F. oxysporum*, namely fusarium acid, lycomarasmine and diacetoxyscirpenol. They affect the permeability of cell membranes as well as other metabolic processes. Together with gum substances, tyloses and the mycelium, they block the vessels, thus leading to the wilting and necrosis of the vascular bundles. *F. oxysporum* at present contain over 150 special forms and races [Rataj-Guranowska 2012]. Both pathogenic and non-pathogenic strains found within the species. *F. avenaceum* and *F. graminearum* were also isolated from the squash plants but not frequently. As reported by Pieczul and Pukacka [2003], these are the fungi mainly occurring on different species of cereals and grasses, which is probably why they could be less important as the fungi pathogenic towards squash.

Alternaria alternata occurred frequently on the aboveground part of the studied squash cultivars. It colonized the leaves of all cultivars, mainly of ‘Gleisdorfer Ölkürbis’.*A. alternata* is a polyphagus and a weakness pathogen. As reported by Robak and Szwejda [2008], it is considered the cause of the necrosis of the Cucurbitaceae seedlings, commonly called blackleg. *A. alternata* also occurs on the surface of the leaves, stems and fruits, causing alternariosis [Dobrzańska and Dobrzański 1991]. *A. alternata* colonizes plants asymptomatically. Weakened or damaged plants become susceptible to this fungus toxins [Jamiołkowska and Sawicki 2011]. *A. alternata* is the parasite of damaged or decaying organs. It colonizes necrotic and dead tissues which were earlier infected by the main pathogens. In the infection process, the fungus produces toxins which have a destructive effect on the host plant [Rotem 1994]. Toxins are released from the germinating spores and they cause changes in the plasmatic membrane of the plant, which facilitates infection. They cause also the physiological and histological changes in the cells of the host plant. We distinguish host-specific toxins – HST (AM-toxin, AC-toxin, AK-toxin), and non-host specific toxins – NHST, which do not depend on the kind of the infected host plant (alternarior, alternarior acid, methyl alternarior ethyl) [Rotem 1994].

Rhizoctonia solani occurred only on the leaves of ‘Gleisdorfer Ölkürbis’. It is one of the major pathogens to Cucurbitaceae plants [Jamiołkowska and Sawicki 2011]. It infects plants under conditions of high humidity and big density, especially in cultivations under covers. *R. solani* causes the dumping-off of seedlings and the rot of the stems and leaf petioles of zucchini [Jamiołkowska and Sawicki 2011, Stępniewska-Jarosz 2012].

Apart from the potentially pathogenic species, the saprotrophic fungi occurred in the community of fungi colonizing squash plants. One of the more important was *Aureobasidium pullulans.* It was most frequently represented on ‘Junona’ and from other cultivars was isolated only sporadically. *A. pullulans* is a desirable species in the community, because as reported Rotem [1994], it is a strong antagonist and primary colonizer of many plants. It is also used as a biological control agent. It is a widely spread epiphyte occurring in the phyllosphere of many plant species. It is commonly known as „black yeast”. The fungus is connected with different environments, both air and water ones. Certain isolates of *A. pullulans* show antagonistic activity towards phytopathogenic fungi such as *Botrytis cinerea*, *Penicillium expansum* and *Pezicula malicorticis* and it is used in the protection against apple diseases [Wagner *et al.* 2013]. Basing on the strain *A. pullulans* L47 obtained in the southern part of Italy from the surface of grapevine fruits, a biopreparation with the commercial name of Boni Protrect Forte GmbH. It contains *A. pullulans* blastospores stored in the natural medium [Jamiołkowska 2013].

Considerable importance should be paid to fungi from genera *Trichoderma* and *Gliocladium*. They were isolated from 'Miranda' and 'Gleisdorfer Ölkürbis'. They are antagonists to many of pathogens, including also *F. oxysporum.* Those fungi release antibiotics and they parasitize on the hyphae of pathogenic species (mycoparasitism). They also affect positively the growth of roots and the development of plants [Mishra *et al.* 2013].

CONCLUSIONS

1. The roots of hull-less seed squash were most frequently colonized by *Fusarium oxysporum*.

2. *Fusarium equiseti* and *Alternaria alternata* were most frequently isolated from the stems and leaves of squash.

3. *Fusarium oxysporum*, *F. equiseti* and *Alternaraia alternata* can be the main causes of the diseases of hull-less seed squash cultivated in an ecological farm.

4. 'Miranda' was the hull-less seed squash most frequently colonized by fungi *Fusarium* spp. 'Junona' and 'Gleisdorfer Ölkürbis' were colonized by these fungi to a lower extent.

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Streszczenie. Dynia zwyczajna (*Cucurbita pepo* subsp. *pepo* var. *styriaca* Greb.) o nasionach bezłupinowych ma coraz większe znaczenie jako surowiec do pozyskiwania cennego oleju i nasion do bezpośredniego spożycia. Jej nasiona są źródłem ważnych związków organicznych i nieorganicznych. Mają właściwości prozdrowotne i stosowane są w leczeniu wielu schorzeń. W 2014 roku przeprowadzono badania mające na celu ocenę składu gatunkowego grzybów występujących na roślinach trzech odmian dyni zwyczajnej bezłupinowej uprawianej w gospodarstwie ekologicznym: ‘Junona’, ‘Miranda’ i ‘Gleisdorfer Ölkürbis’. Analizie mykologicznej poddano korzenie, łodygi oraz liście (blaszki liściowe wraz z ogonkami). Przeprowadzone badania wykazały, że grzyby *Alternaria alternata* i *Fusarium equiseti* najliczniej zasiedlały liście i łodygi dyni w warunkach uprawy ekologicznej. Z korzeni najliczniej izolowano zaś gatunek *Fusarium oxysporum*. Wymienione grzyby w warunkach sprzyjających ich rozwojowi mogą stać się czynnikami chorobotwórczymi dla dyni. Wśród badanych odmian najliczniej zasiedlaną przez grzyby rodzaju *Fusarium* była polska odmiana ‘Miranda’, zaś gatunek *Alternaria alternata* najczęściej izolowano z liści odmiany niemieckiej ‘Gleisdorfer Ölkürbis’.

Słowa kluczowe: dynia oleista, grzyby chorobotwórcze, *Fusarium* spp., ‘Junona’, ‘Miranda’, ‘Gleisdorfer Ölkürbis’