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TOXIN PRODUCING MICROMYCETES ON FRUIT, BERRIES, AND VEGETABLES

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Abstract: In 1999–2001 the investigations on mycological state of stored and sold fruit, berries, and vegetables grown in Lithuania and imported from other countries were performed. The samples of foodstuff were taken from storehouses, various supermarkets, and market places. Such ecological conditions lead to a rapid spreading of micromycetes and contamination of other articles of food stored and sold nearby. On fresh fruit and berries the development of microorganisms is slow. However, microorganisms penetrate into internal tissues of berries and fruit, thus becoming difficult to notice visually. Some microorganisms, especially micromycetes of some species belonging to the *Penicillium* Link, *Aspergillus* Mich. ex Fr., and other genera, are able to produce secondary metabolites (mycotoxins) of various compositions that are toxic to plants, animals, and humans. Therefore, the ability of micromycetes to synthesise and excrete toxic secondary metabolites was examined. Considering this issue, 393 micromycete strains ascribed to 54 genera and 176 species were tested. 46 strains were identified as active producers of toxic substances and were selected for further examinations. Most of them belonged to the *Penicillium*, *Aspergillus* and *Fusarium* genera. Their detection frequency on the investigated berries, fruit, and vegetables was determined, and the impact upon warm-blooded animals (BALB/c mice) was tested. Significant changes of the internal organs and blood composition were found in mice infected with toxic micromycetes. In conclusion, it was evidenced that more than 10% of micromycete strains developing on incorrectly-preserved fruit, berries and vegetables, produce toxic secondary metabolites that pose a potential health hazard for people eating or handling the foodstuff.

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Key words: berries, fruit, vegetables, micromycetes, toxic metabolites, mycotoxins, mice, organs, blood.

INTRODUCTION

One of the most essential issues at present is to improve the nutrition of people, to make it wholesome, well-balanced, and healthy. This strongly depends upon ecological environment of food processing. Therefore, the high quality of raw materials submitted for processing is of the utmost importance. They should not be contaminated by chemicals or propagules of microorganisms [9, 11, 39]. People use plenty of alimentary products of plant origin grown under local conditions and imported from other countries. These products add variety to the everyday nutrition making it wholesome and well-balanced; however, additional problems arise, which can be defined by some ecological aspects. Fruit, berries, and vegetables are grown in various types of soil, applying different agrotechnical and agrochemical means. Some conditions promote the development of various groups of microorganisms that contaminate berries, fruit, and vegetables already in places of their growth, ripening, and while harvesting. Picked fruit and berries, uprooted, dug out, or cut vegetables become a substrate easily available for microorganisms [3, 5, 6]. Microorganisms of some species intensively developing on berries, fruit and vegetables, heavily contaminate them, thus making unsuitable for consumption. This causes considerable economic loses. It has been

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estimated that because of the microorganism action about 30% of stored vegetables are deteriorate and therefore not utilised by producers [9]. Active contaminators of fruit and vegetables are micromycetes that get onto the raw material while harvesting and start their development as soon as the conditions are favourable. Another part of micromycetes gets onto food during the storage [13, 26, 27, 41]. The conditions of storage could vary significantly. It often happens that the premises intended for storage are not sufficiently attended to, even if berries, fruit, and vegetables are stored there for several subsequent years. In such cases the propagules of undesirable, often hazardous, microorganisms survive in the storehouses from the previous harvest; they contaminate and rapidly spread on the newly brought production. Micromycetes are frequently brought in together with package of berries, fruit, and vegetables. It is of particular importance when production is brought from other countries (e.g. Romania, Russia, Spain, Morocco, countries of South Africa) because new microorganisms, often toxic or even pathogenic to plants, animals, and humans, can penetrate together with the imported goods. They start to multiply and can contaminate not just the storehouse premise but a wider environment as well, and become the cause and source of infection [25, 36, 39].

On fresh fruit and berries the development of micro-organisms is slow. However, microorganisms penetrate into internal tissues of berries and fruit thus becoming difficult to notice visually. Some microorganisms, especially micromycetes of some species belonging to the *Penicillium* Link, *Aspergillus* Mich. ex Fr., and other genera, are able to produce secondary metabolites of various compositions that are toxic to plants, animals, and humans [2, 8, 10, 16, 24, 44, 51]. Recently, it has been ascertained that various aflatoxins, ochratoxins, fumitremorgins, fumigaclavins, rugulosins, patulin, emodin, verruculogen, zearalenone, trichothecenes, and other toxic compounds produced by certain micromycete species, are especially hazardous to the health of people [20, 21, 32, 42, 43].

The aim of the present study was to determine micromycete species that are most frequently detected on vegetables, fruit, and berries grown under local conditions and imported from other countries, and to evaluate their ability to produce toxic secondary metabolites (mycotoxins) hazardous to warm-blooded beings.

MATERIALS AND METHODS

Investigated premises. For mycological investigations of fruit, berries, vegetables, and other food products of plant origin 8 premises (located in Vilnius and Kaunas, Lithuania) with significantly different conditions of production preservation were chosen. The samples of foodstuff were taken in 3 storehouses: 2 of them belonged to private farms and 1 to a state owned farm. One private storehouse is established in the premises of a former livestock farm. The premises are not equipped with any modern systems of temperature and humidity regulation, or ventilation. The spoiled products are not instantly removed from the premises. Another storehouse of the private farm, where up to 500,000 tons of potatoes and onions are kept, is located in a new building; systems of temperature and humidity regulation, as well as ventilation are arranged according to modern standards. The spoiled production is instantly sorted out and taken away. The state owned storehouse consists of several premises where various vegetables and fruit intended for selling and planting are stored. These premises are only partly modernised. In the apple storing premises constant humidity and temperature regime is being maintained; movements of people are limited.

Two wholesale trade storehouses of fruit and vegetables could be attributed to another group of the checked objects. In the storehouses, production brought from other countries is usually stored, sorted, and distributed to shopping centres. They are rarely disinfected, not suitable for a long keeping of production. In one of these storehouses a separate site for selling was established, in another one fruit and vegetables were stored, sorted out, and sold in the same premises.

One of the market places was chosen as an object of a uncontrolled selling of fruit, berries, and vegetables. Articles of food brought from other countries and produced in Lithuania are being sold there. The market place is arranged in the open so that the conditions are mostly determined by environmental factors (air conditions). It is rather problematic maintaining cleanness and order there.

As an object of the controlled selling of food, the shop belonging to a large chain of supermarkets was chosen. The largest quantity of food samples was taken there. The premises are clean; decayed products are immediately removed. No disinfectants are used.

Complex investigations on the quality of food, cleanness of air, walls, and equipment were performed in the premises of food storage and processing in one of the children's medicinal institutions. The premises are damp, poorly ventilated. At the period of sampling walls, ceilings, and windowpanes were contaminated with microscopic fungi.

Isolation of micromycetes. In the above-mentioned objects, 179 samples of fruit, berries, vegetables and other foodstuff of plant origin were taken; they were analysed according to the methods described by Samson *et al.* [38], Rabie *et al.* [34] and other [1, 7, 33]. When visual observation allowed the presumption that the sample was contaminated by one infection agent, the method of plating was employed, in case of possible mixed infections the method of diluting was applied. In the first case, a piece of infected product, cut off with a sterile scalpel, was placed onto a Petri dish containing malt extract agar medium with chloramphenicol (50 mg/l). In the second case, 1 g of product was taken and placed in 10 ml of sterile water, shaken for 15 min. and a series of dilutions prepared from the obtained suspension. From each dilution series, 1 ml of suspension was drawn into 9 cm

diam. Petri dish and poured over with 15 ml (48ºC) of the same malt extract agar medium enriched with antibiotic. The dishes were kept for 4 days in a thermostat at a temperature of 28ºC, and for the next 4 days at a temperature of 20ºC; the regime of light and dark was changed every 12 hours. Pure micromycete cultures were isolated, cultivated in standard Czapek agar, standard malt, and corn extract media at a temperature of 28ºC for 5–6 days, and identified according to manuals [6, 11, 17, 30, 35]. Detection frequency (%) of each identified species was calculated.

Evaluation of the micromycete toxicity. Ability of micromycetes to synthesise and excrete secondary metabolites (mycotoxins) was tested applying the methods described by Frisvad [11]. Micromycetes were cultivated on standard Czapek, Czapek yeast extract agar (CYA), and yeast extract-sucrose agar (YES) media for 7–14 days at a temperature of 28ºC. Significant changes in the colour of fungus colony and abundant excretion of pigment into CYA and YES media, comparing with the growth on Czapek medium, in the authors' opinion allowed the supposition that the investigated strain can be a potential producer of mycotoxins. The authors indicate that the above-mentioned media particularly induce the synthesis of mycotoxins by micromycetes of the *Aspergillus*, *Penicillium*, and *Fusarium* genera.

Tests on the acute toxicity of micromycetes that have intensively excreted the pigments into media upon the warm-blooded animals were performed with 12-weeks-old BALB/c mice of both sexes. Laboratory mice were kept according to the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes. The mice were infected with 46 strains of micromycetes according to the slightly modified methods suggested by Krikštaponis *et al.* [20], i.e. for cultivation of micromycetes YES medium was used instead of Czapek medium as it better induced the secondary metabolism. In order to obtain the inoculation mass, Petri dishes containing YES medium were inoculated with 0.5 ml of freshly prepared water suspension of fungi (concentration of 10^6 cfu/ml). The dishes were kept in a thermostat at a temperature of 28ºC for a period of 14 days. Spore suspension of each strain intended for infection was prepared in saline (up to the concentration 10^6–10^8 cfu/ml), homogenised by shaking and a dose of 3 ml was injected into the peritoneum of a mouse in the course of 12 hours in 3 equal portions of 1 ml. The mice of the control group were injected with identical doses of saline (0.85% NaCl) in the same way. In order to avoid bacterial infection, the spore suspensions and saline were supplemented with penicillin (100 units/ml). The suspensions of each micromycete strain and saline were injected each time into the group of 3 mice. The mice were observed for 14 days; alteration of the body weight was evaluated, the weight of spleen of the infected mice and mice from the control group was compared, the changes in internal organs were noted.

Blood tests of the BALB/c mice. For further investigations on the impact of toxic micromycete strains upon the mice blood, the micromycetes characterised by high level of toxicity upon the BALB/c mice were chosen. The majority of mice infected with these strains died during the period of 14 days. A half of the dose of micromycete spore suspension and saline was injected into the peritoneum of mice. Spore suspension was prepared as described above. The mice were kept in accordance with the requirements of the European Convention. Blood was taken from mice of each group after 5, 10, and 15 days. The mice once used for blood testing were no longer used in the experiment. Analysis of mice blood was performed applying the HAMAVET Multispecies Hematology Analyzer (Model 800), produced by Mascot™ for Multispecies Haematology Instruments, CDC Technologies, Inc., USA. The following parameters of blood cells were estimated: leukocyte count, erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular (erythrocyte) volume, mean corpuscular (erythrocyte) hemoglobin, mean corpuscular (erythrocyte) hemoglobin concentration, erythrocyte volume, reticulocyte count and percent, thrombocyte count, mean platelet volume, neutrophil count and percent, lymphocyte count and percent, eosinophil count and percent, monocyte count and percent, basophil count and percent. The alterations of mice body weight and spleen weight were determined [47]. Changes in the internal organs were ascertained. The abilities of fungi used for infection to remain viable in the organism of mice were tested. The YES agar medium dishes were inoculated with 0.1–0.2 ml of mice blood and kept for 5–6 days at a temperature of 28ºC; the grown micromycetes were identified.

The obtained results were processed using Microsoft Excel XP, Statistica 5.1, and Primer 5 programmes.

RESULTS

From fruit, berries, and other products of plant origin 294 micromycete species ascribed to 97 genera were isolated, their monocultures obtained and identified. Attention was chiefly focused on mycological state of vegetables grown in Lithuania, as in the centres chosen for investigations they were more abundant than the imported ones.

The stored potatoes, which are most widely used, were most frequently contaminated by *Sclerotinia fuckeliana*, *Rhizoctonia solani*, *Verticillium alboatrum*, *Fusarium equiseti*, *Geotrichum candidum*. The new-dug potatoes sold in the market place were contaminated by *Rhizoctonia solani*, *Fusarium merismoides*, *F. oxysporum*, more rarely by other fungi. In carrots infected by rots, most frequent were *Verticillium alboatrum*, *Myrothecium roridum*, *M. verrucaria*, and *Alternaria alternata*, in dill *Cladosporium cucumerinum*, *Acremonium strictum*, *Penicillium digitatum*. Leeks were mostly contaminated by *Sclerotinia sclerotiorum*, *Penicillium granulatum*, *P. expansum*, *Rhizopus stolonifer var. stolonifer*, *Fusarium moniliforme*.

The stored beetroots were heavily contaminated by fungi of the *Penicillium* Link genus. Parasitic fungi of

Verticillium alboatrum, *Sclerotinia sclerotiorum*, and *Pythium ultimum* species were also detected. Cabbages were heavily contaminated by *Aspergillus niger* fungi accompanied by *Botrytis cinerea*, *Peronospora brassicae*, *Sclerotinia sclerotiorum*, *Penicillum expansum*, and *P. granulatum*. Cucumbers sold in the market place were contaminated by *Alternaria alternata*, *Botrytis cinerea*, while paprika – by *Sclerotinia sclerotiorum*. Tomatoes sold in the market were damaged by *Fulvia fulvum*, known as a causative agent of tomato leaf spotting, often referred to as *Cladosporium fulvum*. Stored onions were contaminated by *Botrytis alli*, *B. bifurcata*, *Peronospora destructor*, *Fusarium moniliforme*, *F. oxysporum*, and *Penicillium spinulosum*. Micromycete species most often brought with the imported goods should be taken into account. Our knowledge about them is insufficient; although, as they enter the environment, get into shopping centres, food-processing premises, or living premises they can become the sources of hazardous infections or even diseases. The imported vegetables were less contaminated by micromycete propagules. Low diversity of microscopic fungi can be predetermined by the application of fungicides before harvesting or during the plant growth. Further investigations are required in order to substantiate this opinion. Only 3 species of *Penicillium* genus were isolated from onions imported from Holland: *P. fuscum*, *P. verruculosum*, and *P. italicum*. *Rhizopus oryzae*, *Geotrichum klebahnii* were isolated from tomatoes grown in Spain; *Botrytis cinerea*, *Geotrichum fermentans*, *Penicillium chrysogenum*, *Radiomycopsis embreei* were isolated from paprika. The last-named fungus, belonging to the *Mucorales* order, was detected for the first time in Lithuania. *Penicillium decumbens*, *P. expansum*, *P. oxalicum*, *P. cyaneofulvum* were isolated from aubergines. These fungi were isolated from vegetables and fruit imported also from other countries, so it could be supposed that they got onto products during transportation. High species diversity of micromycetes was detected in watermelons imported from Spain: *Alternaria cucumerina*, *Cladosporium cucumerinum*, *Geotrichum fermentans*, *Penicillium digitatum*, *Acremonium strictum*, *A. charticola*, *Exophiala mansonii*. The last-named species is known to infect respiratory and other organs [6, 12, 16, 20, 32, 42, 44].

The research showed that berries and fruit imported from various countries are contaminated by the propagules of different micromycetes; though, micromycetes of some species were isolated from berries and fruit grown in different countries. Strawberries imported from Poland were contaminated by *Mucor ramannianus* and *Penicillium granulatum*; grapes from Italy and Hungary were contaminated by *Geotrichum fermentans*. All grapes were widely contaminated by fungi of the *Penicillium* Link genus, *Eupenicillium brefeldianum* was particularly abundant. *Penicillium italicum*, *Aspergillus niger*, *Ulocladium chartarum*, *Alternaria* sp. prevailed in sweet cherries imported from Hungary, while *Absidia cylindrospora*, *Penicillium granulatum*, *Geotrichum candidum* in the ones imported from Poland. Plums brought from Hungary were contaminated by fungi of *Alternaria alternata*, *Aureobasidium prunicola*, *Penicillium expansum*, *Pleospora infectoria* species; bananas brought from Ecuador - by *Fusarium moniliforme*, *F. sporotrichioides*, *Nectria haematococca*. Oranges imported from Spain were contaminated by *Botrytis cinerea*, *Eupenicillium brefeldianum*, *Penicillium italicum*, *P. chrysogenum*, *P. janthinellum*, *P. verruculosum*. The above-mentioned fungi of the *Penicillium* Link genus, accompanied by *Leptodontium boreale*, were also isolated from mandarins brought from Morocco. Grapefruits from Africa were mostly contaminated by fungi of the *Penicillium* Link genus and *Scopulariopsis acremonium*. Lemons from Argentina were contaminated by fungi of the *Penicillium expansum*, *P. daleae*, *P. verrucosum*, *P. digitatum*, *Cladosporium macrocarpum*, *Gliocladium virens*, and a few species of the *Aspergillus* Mich. ex. Fr. genus. However, on lemons brought from Turkey *Aspergillus ochraceus*, *Eupenicillium brefeldianum*, and *Penicillium chrysogenum* dominated. Peaches imported from Greece were contaminated by a rather peculiar fungi: *Ascochyta pruni*, *Aureobasidium prunicola*, *Aspergillus candidus*, *Eurotium niveoglaucum*.

Fruit and berries grown in Lithuania are most frequently contaminated by *Sclerotinia sclerotiorum*, *Absidia butleri*, *Alternaria alternata*, *Drechslera biseptata*, *Spaerotheca mors-uvae*, *Aspergillus niger*, *Eurotium herbariorum*, *Geotrichum fermentans*, and various species of the *Penicillium* Link genus.

Tests of all 393 micromycete strains, isolated from various vegetables, berries, and other articles of food, showed that most active in production and excretion of secondary metabolites were micromycete strains listed in Table 1. The research results revealed that most active in production and excretion of secondary metabolites, under the research conditions, were micromycetes ascribed to various species of the *Penicillium* Link genus; their spreading on the investigated berries, fruit, and vegetables was rather wide but irregular. Most frequently determined contaminators of food were: *Penicillium biforme*, *P. chrysogenum*, *P. corylophilum*, *P. expansum*, *P. italicum*, *P. spinulosum*, *P. verrucosum*. *Aspergillus niger* fungi were widely spread on berries, fruit, vegetables, etc., *A. fumigatus*, and *A. restrictus* were also frequently found, whereas other fungi of this genus were less frequent, including some of well known producers of toxins. Some vegetables and fruits are often contaminated by fungi of the *Fusarium* Link genus: *F. moniliforme*, *F. solani*, *F. equiseti*, *F. sporotrichioides*. Micromycetes of the last-named species produce and excrete neosolonion, toxins T-2, HT-2, and other substances strongly influencing the warm-blooded animals [6, 16, 23, 48, 49]. It should be noted that during the investigation even micromycetes of such species as *Acremonium roseum*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Mucor hiemalis*, *Rhizopus stolonifer*, *Sclerotinia sclerotiorum*, which are usually not considered as active producers of mycotoxins, intensely synthesised secondary metabolites [6, 28, 39, 50].

Based on the ability of micromycetes to excrete toxic secondary metabolites and aimed at determining their toxicity to warm-blooded animals, 46 micromycete strains belonging to 10 genera and 42 species were selected (Tab. 2). Suspension (10^6–10^8 cfu/ml) of each selected micromycete was injected into peritoneum of BALB/c mice. Suspensions of various micromycetes produced different effects upon mice. Application of statistical analysis by ANOVA, where main criteria for the evaluation were survival of mice, changes in body weight, swelling or shrinkage of spleen, morphological changes of internal organs, allowed the division of investigated micromycetes into groups according to their effect upon mice. Under the influence of certain micromycetes, mice died during the period of 2 weeks. Those micromycetes were ascribed to the first group. They were: *Acremonium roseum* BL-68, *Aspergillus fumigatus* M-22, *A. niger* LR-7, *Fusarium sporotrichioides* BA-4, and M-62, *Mucor hiemalis* M-44, *Penicillium biforme* BS-1, *P. commune* LR-10, *P. expansum* O-25, *P. steckii* PS-3, *P. stoloniferum* KO-15, *P. variabile* S-11, *P. verruculosum* MA-2, *P. viridicatum* BL-77. Under the influence of the above-mentioned fungi the tested mice died at different periods of time. Mice affected by *Fusarium sporotrichioides* M-62, *Penicillium biforme* BS-1, *P. expansum* O-25 died within the first day. After mice evisceration no statistically significant changes in spleen weight were determined; however, the spleen weight of mice that also died during the first day, but were affected by *Penicillium commune* LR-10 and *P. viridicatum* BL-77, was changed.

Changes of spleen weight and alterations of other organs (swollen lymph nodes, fungal foci on liver, spleen, intestines, stomach, kidney) in mice that died after 2–3 days' post infection were evident. It is worth mentioning that under the influence of *Penicillium steckii* PS-3 mice died on the 11th day and the average of their spleen weight and body weight significantly differed compared to mice from the control group ($p < 0.05$ and $p < 0.01$, respectively). These results certify strong toxicity of these fungi to warm-blooded animals.

Under the influence of some micromycete strains during the period of 2 weeks only part of BALB/c mice died. These strains were ascribed to the second group. Evisceration of died and survived mice demonstrated evident changes in spleen weight, body weight, and the internal organs. This group contained the following micromycete strains: *Aspergillus penicilloides* GR-11, *Penicillium chrysogenum* SL-4, *P. cyclopium* PRG-1, *P. daleae* C-5, *P. expansum* KO-6, *P. implicatum* PU-3. Although these fungi were less toxic, still in the course of longer period and larger doses they could cause death of warm-blooded animals.

Micromycete strains that were not lethal to mice but caused reliable changes in body weight, spleen, and other internal organs were ascribed to the third group. The considered changes of mice body were different. Solitary instances of mice death occurred, but after evisceration it was confirmed that mice weight and other parameters had not changed. The following micromycete strains were ascribed to this group: *Aspergillus restrictus* RA-1, *Fusarium solani* JR-5, *Penicillium paxilli* JR-4, *P. aurantioviolaceum* AP-10, *P. canescens* V-6, *P. cyaneofulvum* BK-3, *P. corylophilum* SG-3, *P. notatum* BR-10, *P. stoloniferum* M-51, *Rhizopus stolonifer* SG-9, *Sclerotinia sclerotiorum* BR-19. Under the influence of these fungi the changes in the internal organs of mice were not so serious; still in some instances foci of necrosis, coalescence of the internal organs, isolated fungal foci in spleen, liver, kidney, and intestines occurred. This allows the presumption that the above-mentioned micromycetes are slightly toxic, but can be hazardous in the case of gradual and long lasting penetration into organism.

The remaining part of the tested micromycetes was ascribed to the 4th group, as they caused no particular effect on mice. The death of one mouse infected by *Penicillium lanosoviride* O-9 could be considered accidental (Tab. 2), as evisceration did not reveal any evident changes in mice organs. The mice infected with the suspension of *Alternaria alternata* MA-8 did not differ from the control group.

The impact of micromycetes upon warm-blooded animals often depends upon the amount of propagules penetrating into animals' organism. In order to determine the impact of lower concentrations of micromycete propagules upon the BALB/c mice, 24 micromycete strains characterised by high toxicity (mice died within 2 weeks), wide distribution (*Rhizopus stolonifer*), or specificity (*Sclerotinia sclerotiorum*) were selected.

The injected conidia suspensions (10^3–10^4 cfu/ml) of various micromycetes produced unequal effect on the BALB/c mice. This is confirmed by the data presented in Table 3. Under the influence of some micromycete strains the body weight of mice decreased: under the impact of *Aspergillus penicilloides* GR-11 body weight decreased by 0.4 g, *A. fumigatus* M-22 - by 1.0 g, *Penicillium variabile* S-11 - by 3.0 g, *P. stoloniferum* KO-15 - by 1.4 g, *P. lanosoviride* O-9 - by 6.5 g, *P. verruculosum* MA-2 -by 4.7 g, *Alternaria alternata* PA-3 - by 0.9 g. However, under the impact of other fungi the body weight increased: under the impact of *Penicillium chrysogenum* SL-4 the increase reached 8.1 g, *Aspergillus niger* LR-7 – 11.0 g, *Rhizopus stolonifer* SG-9 – 5.7 g, *Acremonium roseum* BL-68 – 5.4 g. Yet, mice infected by *Fusarium equiseti* BL-54 died after the first day. Under the impact of fungi the spleen weight of mice had changed. Spleen weight of mice from the control group was 0.2 g, and the spleen weight of mice affected by *Penicillium expansum* KO-6 was only 0.1 g, *P. verruculosum* MA-2 – 1.02 g, *P. viridicatum* BL-77 – 0.11 g, *P. lanosoviride* O-9 – 0.11 g, *P. chrysogenum* SL-4 – 0.13 g, *Fusarium sporotrichioides* M-62 – 0.13g. Still under the impact of other micromycetes the spleen weight of mice increased: *Penicillium expansum* O-25 – up to 0.52 g, *P. commune* LR-10 – up to 0.49 g, *P. spinulosum* PRG-1 – up to 0.43 g.

Mice infected by micromycetes were eviscerated after 15 days; evident changes in internal organs were detected: swollen lymph nodes, coalesced internal organs, the surface

Table 1. Micromycete strains producing toxic metabolites, recovered from foodstuff of plant origin (% of the total number of isolates).

Fungal strain	Isolation source	Foodstuff where micromycetes of the same species were found	Spreading frequency of species, %
Acremonium roseum BL-68	Potatoes	Potatoes	0.31
Alternaria alternata SLV-3	Plums	Plums, raspberries, cabbages, cucumbers, avocados, vegetable marrows, paprikas	0.72
Alternaria alternata PA-3	Paprikas		
Alternaria citri MA-8	Mandarines	Mandarines	0.1
Aspergillus candidus PSK-5	Peaches	Peaches, poppy seed, coconuts	0.31
Aspergillus fumigatus M-22	Carrots	Avocados, carrots, cabbages, vegetable marrows	1.03
Aspergillus niger LR-7	Hazelnuts	Mandarines, lemons, grapefruits, apples, pears, plums, sweet cherries, grapes, gooseberries, black currants, apricots, cherries, potatoes, onions, cabbages, carrots, beetroots, cauliflowers, tomatoes, celery, avocados, courgettes, radishes, coconuts, hazelnuts, mushrooms, dried pears, dried pineapples, millet, different kinds of biscuits and cakes, carrot salad, spices	5.58
Aspergillus penicilloides GR-11	Walnuts	Walnuts	0.1
Aspergillus restrictus RA-1	Raisins	Raisins, kidney beans, hazelnuts, figs, pistachio nuts, cucurbit seed	0.72
Cladosporium cladosporioides PO-10	Tomatoes	Tomatoes, potatoes, radishes, garlics, carrot salad, plums, apricots, raspberries, gooseberries, strawberries	0.93
Eurotium niveoglaucus PSK-4	Peaches	Peaches, kidney beans	0.21
Fusarium equiseti BL-54	Potatoes	Potatoes, carrots	0.21
Fusarium moniliforme S-29	Onions	Onions, bananas, radishes, beetroots	0.41
Fusarium solani JR-5	Radishes	Radishes, carrots	0.21
Fusarium sporotrichioides BA-4	Bananas	Bananas, carrots	0.21
Fusarium sporotrichioides M-62	Carrots		
Mucor hiemalis M-44	Carrots	Carrots, cabbages, beetroots, onions	0.83
Penicillium aurantioviolaceum AP-10	Oranges	Oranges, onions	0.21
Penicillium biforme BS-1	Strawberries	Strawberries, cherries, dried apricots, walnuts, almonds, beetroots, carrots, turnip-rooted parsley	0.83
Penicillium canescens V-6	Grapes	Grapes, parsnips	0.21
Penicillium chrysogenum SL-4	Spring onions	Spring onions, tomatoes, paprikas, avocados, mushrooms, apples, mandarines, grapefruits, peaches, dried pears, walnuts, yogurt with wild berries	1.45
Penicillium claviforme S-4	Onions	Onions, leeks, carrots, parsnips, beetroots, apples, cakes with apples	1.45
Penicillium clavigerum PR-8	Leeks	Leeks, potatoes, carrots, beetroots, apples, onions	0.62
Penicillium commune LR-10	Hazelnuts	Hazelnuts, potatoes	0.31
Penicillium corylophilum SG-3	Sunflower seeds	Sunflower seeds, leeks, radishes, tomatoes, beetroots, apricots, rusks with sunflower seeds	0.72
Penicillium cyaneofulvum BK-3	Aubergines	Aubergines	0.1
Penicillium cyclopium PRG-1	Cakes	Cakes, potatoes, apples, almonds, ring-shaped roll with poppy seed	0.62
Penicillium daleae C-5	Lemons	Lemons	0.1
Penicillium expansum O-25	Apples	Apples, lemons, plums, grapes, oranges, cabbages, carrots, beetroots, celery, leeks, aubergines, onions, garlics, courgettes, turnip-rooted parsley, walnuts, bread	3.51
Penicillium expansum KO-6	Cabbages		
Penicillium frequentans V-3	Grapes	Grapes	0.1
Penicillium italicum MA-3	Mandarines	Mandarines, oranges, pears, apples, sweet cherries, peaches, apricots, onions, carrots, tomatoes, cabbages, potatoes, turnip-rooted parsley, water melons, dried apricots	2.27
Penicillium lanosoviride O-9	Apples	Apples, onions, potatoes, carrots	0.62
Penicillium notatum BR-10	Beetroots	Beetroots, tomatoes, potatoes	0.31
Penicillium spinulosum S-22	Onions	Onions, carrots, beetroots, cabbages, potatoes, turnip-rooted parsley, spring onions, sunflower seed, walnuts, dried apricots, apple jam, bread, biscuits with coconuts, rusks with raisins	1.86
Penicillium paxilli JR-4	Radishes	Radishes, turnip-rooted parsley, onions, grapes, carrot salads	0.62

Fungal strain	Isolation source	Foodstuff where micromycetes of the same species were found	Spreading frequency of species, %
Penicillium steckii PS-3	Parsnips	Parsnips, avocados, hazelnuts, noodle	0.41
Penicillium stoloniferum KO-15	Cabbages	Cabbages, carrots, oranges, walnuts	0.41
Penicillium stoloniferum M-51	Carrots		
Penicillium variabile S-11	Onions	Onions, potatoes, oranges, strawberries	0.52
Penicillium verrucosum M-1	Carrots	Carrots, potatoes, cabbages, beetroots, onions, turnip-rooted parsley, lemons	0.83
Penicillium verruculosum MA-2	Mandarines	Mandarines, hazelnuts, potatoes, beetroots, carrots, onions, radishes	0.93
Penicillium viridicatum BL-77	Potatoes	Potatoes, apples, dried apricots, dried pineapples	0.41
Rhizopus stolonifer SG-9	Sunflower seed	Sunflower seed, poppy seed, peaches, oranges, leeks, potatoes, carrots, cabbages, tomatoes, onions	1.24
Sclerotinia sclerotiorum BR-19	Beetroots	Beetroots, paprikas, leeks, cabbages, potatoes, radishes, carrots, strawberries, cherries, gooseberries, black currants, raspberries, bananas	1.34

of some organs coated, alterations in their colour, visible fungal focuses, necrotic tissues. The changes in the internal organs of mice affected by separate fungal strains are specified in the 4th section of Table 3.

One of the criteria for the evaluation of toxic impact of micromycetes upon the BALB/c mice was their blood count. Cluster Analyses Tree Diagram was used for 23 micromycetes strains and control group (unweighted pair group average, percent disagreement). While analyzing the blood of mice infected with micromycetes after 5, 10, and 15 days some regularities of the changes in blood count were noticed (Tab. 4). After 5 days in the blood of mice the number of leukocytes increased. The lowest amount of leukocytes (1.26 K/μl) ($K = 10^3$) was determined in blood of mice infected with *Fusarium sporotrichioides* M-62. The number was lower than the normal value (1.8-10.7 K/μl). The highest number of leukocytes (134.92 K/μl) was determined in blood of mouse infected with propagule suspension of *Penicillium stoloniferum* KO-15. After 5 days the number of leukocytes in the blood of mice infected by other fungi was close to their number in blood of mice from the control group, i.e. not infected by fungi. This effect was noted in mice infected with *Penicillium expansum* O-25, *P. verruculosum* MA-2, *P. lanosoviride* O-9, *P. daleae* C-5, *P. variabile* S-11, *Aspergillus fumigatus* M-22, *Acremonium roseum* BL-68 micromycetes. Although the number of leukocytes in the blood of mice infected with the above-mentioned fungi was within the normal values, in blood of some mice a higher than normal number of monocytes and eosinophils was noted, in other cases only the higher number of monocytes or monocytes and lymphocytes; more rarely higher number of monocytes and basophils was registered.

In blood of mice not infected with fungi the hemoglobin concentration was 10.0–15.1 g/dL, the number of erythrocytes - 6.36–9.42 M/μl. However, in the blood of mice infected with micromycetes these parameters significantly decreased and were below the normal values. The lowest blood hemoglobin concentration was found in mice infected with the propagule suspensions of *Fusarium sporotrichioides* M-62 and *Penicillium expansum* O-25. The lowest number of erythrocytes (0.14 M/μl) was found in the blood of mice infected with propagules of *Aspergillus penicilloides* GR-11. When mice were infected with *Acremonium roseum* BL-68 fungi no increase in the hemoglobin concentration or the number of erythrocytes was noticed; whereas in the blood of mice infected with *Penicillium viridicatum* BL-77, *P. cyaneofulvum* BK-3, *P. expansum* O-25 the hemoglobin concentration decreased and the number of erythrocytes remained close to normal. During a period of 5 days blood composition had not changed under the influence of *Penicillium steckii* PS-3, *Mucor hiemalis* M-44, *Aspergillus niger* LR-7, *P. chrysogenum* SL-4, *Rhizopus stolonifer* SG-9. The highest hemoglobin concentration (2.2 g/dL) in the blood of mice from this group was recorded after 5 days under the impact of *Penicillium chrysogenum* SL-4 propagules; the highest number of erythrocytes (8.42 M/μl and 8.49 M/μl) was registered under the impact of *Penicillium steckii* PS-3 and *Rhizopus stolonifer* SG-9, respectively.

Under the impact of different micromycete strains the decrease in the number of thrombocytes in the blood of infected mice was determined. In the blood of healthy BALB/c mice the number of thrombocytes varies from 592 to 2972 K/μl. These parameters remained within such limits for 5 days under the impact of the following fungi: *Penicillium expansum* O-25, *P. steckii* PS-3, *P. chrysogenum* SL-4, *P. lanosoviride* O-9, *Mucor hiemalis* M-44, *Aspergillus niger* LR-7, *A. penicilloides* GR-11, *Acremonium roseum* BL-68, *Fusarium sporotrichioides* M-62. After 5 days the highest number of thrombocytes (915 K/μl) was registered under the influence of *Aspergillus niger* LR-7, while the lowest number (132 K/μl) under the influence of *Aspergillus fumigatus* M-22.

After 10 days the increase in the number of erythrocytes in the blood of mice was observed under the influence of *Penicillium expansum* O-25 (18.5 M/μl) and *P. variabile* S-11 (65.58 M/μl). After 5 days this parameter was within the normal values. Hemoglobin concentration and the number of erythrocytes in the blood of these mice fell below the normal values. Most probably the response of mice organism towards the impact of these fungi was somewhat delayed.

Table 2. Survival of BALB/c mice infected by microscopic fungi and their body and spleen weight changes analysed by ANOVA.

No	Injected micromycete strain	Change of the mice body weight	Weight of mice spleen after the experiment	Survival
1	*Acremonium roseum* BL-68	*	**	+
2	*Alternaria alternata* SLV-3	NS	**	-
3	*Alternaria alternata* PA-3	NS	NS	-/+
4	*Alternaria citri* MA-8	NS	NS	-
5	*Aspergillus candidus* PSK-5	NS	**	-
6	*Aspergillus fumigatus* M-22	**	**	+
7	*Aspergillus niger* LR-7	**	*	+
8	*Aspergillus penicilloides* GR-11	*	*	-/+
9	*Aspergillus restrictus* RA-1	*	**	-
10	*Cladosporium cladosporioides* PO-10	**	NS	-
11	*Eurotium niveoglaucus* PSK-4	*	NS	-
12	*Fusarium equiseti* BL-54	*	*	+
13	*Fusarium moniliforme* S-29	NS	*	-
14	*Fusarium solani* JR-5	*	**	-
15	*Fusarium sporotrichioides* BA-4	**	**	+
16	*Fusarium sporotrichioides* M-62	*	NS	+
17	*Mucor hiemalis* M-44	**	**	+
18	*Penicillium paxilli* JR-4	*	**	-
19	*Penicillium aurantioviolaceum* AP-10	*	**	-
20	*Penicillium biforme* BS-1	*	NS	+
21	*Penicillium canescens* V-6	*	**	-
22	*Penicillium chrysogenum* SL-4	**	**	-/+
23	*Penicillium cyaneofulvum* BK-3	NS	*	-/+
24	*Penicillium clavigerum* PR-8	NS	**	-
25	*Penicillium claviforme* S-4	NS	**	-
26	*Penicillium commune* LR-10	**	*	+
27	*Penicillium corylophilum* SG-3	**	*	-
28	*Penicillium cyclopium* PRG-1	**	**	-/+
29	*Penicillium daleae* C-5	*	**	-/+
30	*Penicillium expansum* KO-6	*	*	-/+
31	*Penicillium expansum* O-25	*	NS	+
32	*Penicillium frequentans* V-3	NS	**	-
33	*Penicillium implicatum* PU-3	**	**	-/+
34	*Penicillium italicum* MA-3	NS	**	-
35	*Penicillium lanosoviride* O-9	NS	NS	-/+
36	*Penicillium notatum* BR-10	**	**	-
37	*Penicillium spinulosum* S-22	NS	**	-
38	*Penicillium steckii* PS-3	**	*	+
39	*Penicillium stoloniferum* KO-15	*	NS	+
40	*Penicillium stoloniferum* M-51	**	NS	-/+
41	*Penicillium variabile* S-11	**	**	+
42	*Penicillium verrucosum* M-1	NS	**	-
43	*Penicillium verruculosum* MA-2	*	*	+
44	*Penicillium viridicatum* BL-77	*	**	+
45	*Rhizopus stolonifer* SG-9	**	**	-
46	*Sclerotinia sclerotiorum* BR-19	*	**	-

NS: difference compared with a control group was not statistically significant; *: difference compared with a control group was statistically significant ($p < 0.05$); **: difference compared with a control group was statistically significant ($p < 0.01$); +: all mice in the groupe died; -/+: some mice in the group died; -: all mice in the group survived.

Table 3. Reaction of BALB/c mice to the suspensions of micromycetes strains, injected in amounts equal to half of the lethal dose (see Table 2).

Injected micromycete strain	Changes of the internal organs of mice	Change of the mice body weight	Weight of mice spleen after the experiment
Acremonium roseum BL-68	Swollen lymph nodes, fungal foci on intestines, liver whitish, all internal organs coalesced	NS	NS
Alternaria alternata PA-3**	Swollen lymph nodes, fungal foci on spleen and liver, necrotic foci, spleen, intestines, peritoneum and liver coalesced	*	NS
Aspergillus fumigatus M-22	Swollen lymph nodes, fungal foci on spleen, liver, intestine and kidney, spleen coated coalesced with intestines and stomach	*	*
Aspergillus niger LR-7	Fungal foci visible on liver, peritoneum and stomach coalesced	**	NS
Aspergillus penicilloides GR-11	Distinctly swollen lymph nodes, mouse enlarged, liver coated with fungus, abundance of fluid in abdomen	NS	*
Fusarium equiseti BL-54	No change	Mice died after 24 hours	
Fusarium sporotrichioides M-62	Swollen lymph nodes, fungal foci on intestines and liver, liver coalesced with intestines and stomach	NS	NS
Mucor hiemalis M-44	Swollen lymph nodes, fungal foci on liver, spleen coated and coalesced with stomach, one kidney whitish	**	NS
Penicillium biforme BS-1	Swollen lymph nodes, fungal foci on spleen and liver, spleen and intestines coalesced	NS	NS
Penicillium chrysogenum SL-4	Swollen lymph nodes, spleen and stomach coalesced, fungal foci visible on intestines	**	NS
Penicillium commune LR-10	Swollen lymph nodes, spleen coated coalesced with intestines and peritoneum, kidney and liver coalesced	*	*
Penicillium cyaneofulvum BK-3**	Swollen lymph nodes, fungal foci on spleen, liver and intestines, spleen, liver and stomach coalesced, necrotic foci visible on spleen	**	*
Penicillium cyclopium PRG -1	Swollen lymph nodes, fungal foci on intestines and liver, spleen coated, liver, spleen and stomach coalesced	NS	*
Penicillium daleae C-5	Swollen lymph nodes, fungal foci on liver and peritoneum, spleen and peritoneum coalesced	NS	NS
Penicillium expansum O-25	Swollen lymph nodes, coating visible on spleen and liver	NS	*
Penicillium expansum KO-6**	Fungal foci on spleen and liver, intestines, liver and spleen coalesced	NS	NS
Penicillium lanosoviride O-9**	Fungal foci on spleen, liver, intestines, kidney, intestines coalesced with liver and peritoneum	NS	NS
Penicillium steckii PS-3	Swollen lymph nodes, fungal foci on liver, spleen coated and coalesced with stomach, one kidney whitish	NS	*
Penicillium stoloniferum KO-15*	Fungal foci on liver and intestines, place of injection covered with fungal foci	**	NS
Penicillium variabile S-11	Swollen lymph nodes, fungal foci on spleen and liver, spleen, intestines, stomach and liver coalesced	*	NS
Penicillium verruculosum MA-2	Fungal foci on spleen, liver and kidney, spleen, liver, intestines and stomach coalesced, spleen and peritoneum coalesced	NS	**
Penicillium viridicatum BL-77*	Swollen lymph nodes, place of injection covered with fungal foci	*	NS
Rhizopus stolonifer SG-9	Swollen lymph nodes, fungal foci on liver	NS	NS
Sclerotinia sclerotiorum BR-19**	Liver distinctly necrotic with abundant fungal foci, coalesced with thorax, diaphragm, stomach, peritoneum, spleen and peritoneum coalesced	*	NS

*: after 5 days; **: after 10 days (mice from these groups did not survive until 15 days).

After 10 days, in the blood of mice infected with *Fusarium sporotrichioides* M-62, the number of leukocytes increased and became close to normal value; meanwhile, the hemoglobin concentration and the number of erythrocytes remained lower than normal. The number of thrombocytes in the blood of these mice slightly decreased (about 200 K/μl) but remained within normal values. The results of further tests showed that after 15 days, in the blood of mice infected with this fungus, the number of leukocytes again fell below the normal (1.44 K/μl); other blood parameters also decreased: the number of erythrocytes to 3.4 M/μl, hemoglobin concentration - 4.4 g/dL, thrombocytes - 428 K/μl.

Table 4. Haemoglobin concentration and numbers of leukocytes, erythrocytes, and thrombocytes in the blood of BALB/c mice infected with the tested micromycete strains, after 5, 10, and 15 days.

Tested strains	Days post infection	Average concentration numbers			
		Hemoglobin, g/dL (normal range 11.00–15.10)	Erythrocytes, M/μl (normal range 6.36–9.42)	Leukocytes, K/μl (normal range 1.80–10.70)	Thrombocytes, K/μl (normal range 592–2972)
Acremonium roseum BL-68	5	8.30	6.40	4.68	605
	10	5.60	4.38	2.14	663
	15	11.00	5.74	16.60	949
Alternaria alternata PA-3	5	6.00	4.48	16.84	469
	10	10.80	6.61	48.00	638
	15	+	+	+	+
Aspergillus fumigatus M-22	5	3.90	3.60	2.14	132
	10	5.70	3.55	3.74	351
	15	12.00	9.00	9.80	758
Aspergillus niger LR-7	5	11.10	6.48	119.16	915
	10	11.60	8.23	13.36	500
	15	5.50	4.29	2.42	463
Aspergillus penicilloides GR-11	5	10.40	0.14	19.44	728
	10	7.00	6.19	9.82	726
	15	6.30	3.82	13.66	466
Fusarium sporotrichioides M-62	5	3.90	4.15	1.26	807
	10	6.80	5.33	3.82	613
	15	4.40	3.40	1.44	428
Mucor hiemalis M-44	5	12.10	7.5	125.66	799
	10	8.50	15.55	51.36	333
	15	5.80	4.80	5.20	471
Penicillium biforme BS-1	5	10.60	5.74	101.34	528
	10	6.40	3.56	53.32	438
	15	10.10	7.92	10.42	885
Penicillium chrysogenum SL-4	5	12.20	6.97	118.86	621
	10	9.20	6.42	7.90	385
	15	12.90	2.40	4.04	306
Penicillium commune LR-10	5	9.80	1.97	90.86	439
	10	9.30	5.82	30.74	526
	15	6.60	5.65	7.40	354
Penicillium cyaneofulvum BK-3	5	10.90	7.69	15.56	345
	10	8.40	6.73	10.76	888
	15	+	+	+	+
Penicillium cyclopium PRG-1	5	5.90	4.55	6.64	404
	10	6.00	5.33	10.74	445
	15	11.40	8.22	23.70	566
Penicillium daleae C-5	5	5.60	4.90	2.98	498
	10	5.30	3.74	6.10	361
	15	9.40	5.60	1.12	450
Penicillium expansum O-25	5	9.90	7.37	2.56	820
	10	4.80	3.02	18.50	713
	15	10.90	4.98	132.82	444
Penicillium expansum KO-6	5	6.80	5.50	11.02	383
	10	11.6	6.57	5.18	675
	15	+	+	+	+
Penicillium lanosoviride O-9	5	6.60	5.78	3.44	665
	10	5.80	5.05	4.18	613
	15	-	-	-	-
Penicillium steckii PS-3	5	11.50	8.42	7.12	606
	10	8.70	5.87	8.22	409
	15	12.60	7.85	9.48	337
Penicillium stoloniferum KO-15	5	10.90	6.31	134.92	347
	10	-	-	-	-
	15	-	-	-	-
Penicillium variabile S-11	5	5.40	4.45	5.50	564
	10	8.80	5.81	65.58	564
	15	8.00	1.81	2.18	266

Tested strains	Days post infection	Average concentration numbers			
		Hemoglobin, g/dL (normal range 11.00–15.10)	Erythrocytes, M/μl (normal range 6.36–9.42)	Leukocytes, K/μl (normal range 1.80–10.70)	Thrombocytes, K/μl (normal range 592–2972)
Penicillium verruculosum MA-2	5	6.00	4.90	2.66	588
	10	5.40	4.98	6.10	681
	15	3.80	3.72	6.34	500
Penicillium viridicatum BL-77	5	10.30	6.70	33.88	548
	10	+	+	+	+
	15	+	+	+	+
Rhizopus stoloniferum SG-9	5	11.80	8.49	13.56	393
	10	12.30	8.99	5.70	1028
	15	10.30	0.76	12.26	398
Sclerotinia sclerotiorum BR-19	5	9.50	5.49	87.66	326
	10	7.50	5.62	3.90	788
	15	+	+	+	+
Control group	5	11.10	7.10	3.26	677
	10	7.50	8.31	4.69	629
	15	11.93	7.76	4.24	776

+: all mice died.

After 10 days, in the blood of mice infected with certain micromycete strains (*Penicillium chrysogenum* SL-4, *P. expansum* KO-6, *Aspergillus penicilloides* GR-11, *Sclerotinia sclerotiorum* BR-19, *Rhizopus stolonifer* SG-9), the number of leukocytes decreased to normal values. Some fungi did not change that parameter, even after 15 days. However, under the influence of *Aspergillus penicilloides* GR-11 the number of leukocytes increased again (13.66 K/μl). Evisceration of mice (after 15 days) infected with *Aspergillus penicilloides* GR-11 strain demonstrated evident swelling of lymph nodes, developing ascites; abundant fungal coating was visible on the liver. These symptoms proved the existence of inflammation processes in the abdominal cavities.

In the blood of mice infected with *Penicillium biforme* BS-1, *P. variabile* S-11, *Mucor hiemalis* M-44, *Aspergillus niger* LR-7 micromycetes, after 15 days the number of leukocytes decreased to the limits of normal values. Meanwhile, in the blood of mice infected with *Penicillium expansum* O-25 and *P. spinulosum* PRG-1, after 15 days further development of leukocytosis, neutrophilia, lymphocytosis, monocytosis, eosinophilia and basophilia was determined. After 15 days the increase in the number of leucocytes was noted in the blood of mice infected with *Rhizopus stolonifer* SG-9, and *Acremonium roseum* BL-68 micromycetes. After 10 days the highest number of leukocytes (65.58 K/μl) was determined under the impact of *Penicillium variabile* S-11, the lowest number (3.74 K/μl) was determined under the impact of *Aspergillus fumigatus* M-22. After 15 days the highest number of leukocytes (132.82 K/μl) was revealed in the blood of mice infected with *Penicillium expansum* O-25 strain, the lowest number (1.12 K/μl) - under the impact of *P. daleae* C-5. In the blood of these mice the number of other blood cells also decreased.

According to the influence of micromycetes upon the number of erythrocytes in the blood of the tested mice (after 10 days) all investigated fungal strains could be divided into 4 groups: 1) under the influence of *Alternaria alternata* PA-3, and *Penicillium expansum* KO-6 mice did not survive 15 days although the number of erythrocytes during this period reached the normal values; 2) under the impact of *Penicillium expansum* O-25, *P. steckii* PS-3, *Mucor hiemalis* M-44, *Acremonium roseum* BL-68 the number of erythrocytes in the blood of mice fell below the normal value, but mice did not die; 3) in the blood of mice infected with *Aspergillus niger* LR-7, *Penicillium chrysogenum* SL-4, *P. cyaneofulvum* BK-3, *Rhizopus stolonifer* SG-9 the number of erythrocytes remained within normal limits, all mice survived; 4) under the impact of other investigated micromycete strains the number of erythrocytes in the blood of mice was lower than normal during the whole period of investigation. The highest number of erythrocytes (8.99 M/μl) was determined in the blood of mice infected with *Rhizopus stolonifer* SG-9 strain, the lowest number (3.02 M/μl) - under the impact of *Penicillium expansum* O-25.

The tests of mice blood performed after 15 days revealed the ternary changes in the number of erythrocytes: 1) under the impact of *Penicillium steckii* PS-3, *P. spinulosum* PRG-1, *Aspergillus fumigatus* M-22 the number of erythrocytes in mice blood approached the normal value; 2) in the blood of mice infected with *Penicillium expansum* O-25, *P. commune* LR-10, *P. verruculosum* MA-2, *P. daleae* C-5, *P. variabile* S-11, *Mucor hiemalis* M-44, *Fusarium sporotrichioides* M-62, *Acremonium roseum* BL-68 the number of erythrocytes remained below normal; 3) under the impact of other micromycete strains (Tab. 3) the number of erythrocytes in mice blood decreased and was below normal. After 15 days the highest number of erythrocytes (9.0 M/μl) was registered in the blood of mice infected with *Aspergillus fumigatus* M-22 propagules, the lowest number (1.81 M/μl) - in blood affected by *Penicillium variabile* S-11.

Changes in the blood hemoglobin concentration of mice infected with micromycetes were also noted.

Immediately after the injection of micromycete propagule suspension the hemoglobin concentration in mice blood increased, but gradually this parameter approached normal. Under the impact of certain micromycete strains (*Aspergillus niger* LR-7, *Penicillium expansum* KO-6, *Rhizopus stolonifer* SG-9) at the beginning of the investigation, hemoglobin concentration in the blood of infected mice was within the normal values, but after 15 days in the blood of mice infected with *Aspergillus niger* LR-7 and *Rhizopus stolonifer* SG-9 an evident decrease in the hemoglobin concentration was noted. After 15 days normal hemoglobin concentration was determined in the blood of mice infected with *Penicillium steckii* PS-3, *P. chrysogenum* SL-4, *P. spinulosum* PRG-1, *Aspergillus fumigatus* M-22, *Acremonium roseum* BL-68. The highest hemoglobin concentration (12.3 g/dL) was determined after 10 days in the blood of mice infected with *Rhizopus stolonifer* SG-9 and the lowest (4.8 g/dL) - in the blood affected by *Penicillium expansum* O-25. After 15 days the highest hemoglobin concentration (12.9 g/dL) was detected in the blood of mice infected with *P. chrysogenum* SL-4, and the lowest (3.8 g/dL) - under the impact of *P. verruculosum* MA-2.

Analysis of the number of thrombocytes in the blood of mice infected with micromycetes revealed that on the 5th day of the experiment the number of thrombocytes in the blood of the majority of mice was normal, but on the 10th day their number considerably decreased and remained lower than normal value until the end of the experiment (15th day). Under the impact of certain micromycete strains (*Aspergillus penicilloides* GR-11, *Penicillium lanosoviride* O-9, *Fusarium sporotrichioides* M-62) the decrease in the number of thrombocytes was noticed not on 5th or 10th day, but only on the 15th day. During the experiment, a lower value of thrombocytes was determined in the blood of mice infected with *Penicillium commune* LR-10, *P. spinulosum* PRG-1, *P. daleae* C-5, *P. variabile* S-11. Under the influence of *Penicillium biforme* BS-1 and *Aspergillus fumigatus* M-22 after 5 and 10 days a decrease in the number of thrombocytes was registered although after 15 days the number reached the normal value. On the 10th day the highest number of thrombocytes (1028 K/μl) was registered in the blood of mice infected with *Rhizopus stolonifer* SG-9, the lowest number (361 K/μl) - under the impact of *Penicillium daleae* C-5. The highest number of thrombocytes (949 K/μl) after 15 days was determined in the blood of mice affected by *Acremonium roseum* BL-68, the lowest number (266 K/μl) - under the impact of *P. variabile* S-11.

Analysis of the research results showed that the most evident effect of micromycetes upon the composition of mice blood was registered on the 10th day: the number of leukocytes increased, the number of erythrocytes and hemoglobin concentration decreased, the tendency towards decrease in the number of thrombocytes was revealed. After 15 days gradual normalization of blood composition was noted. However, the impact of certain micromycete strains and the reaction of mice organism

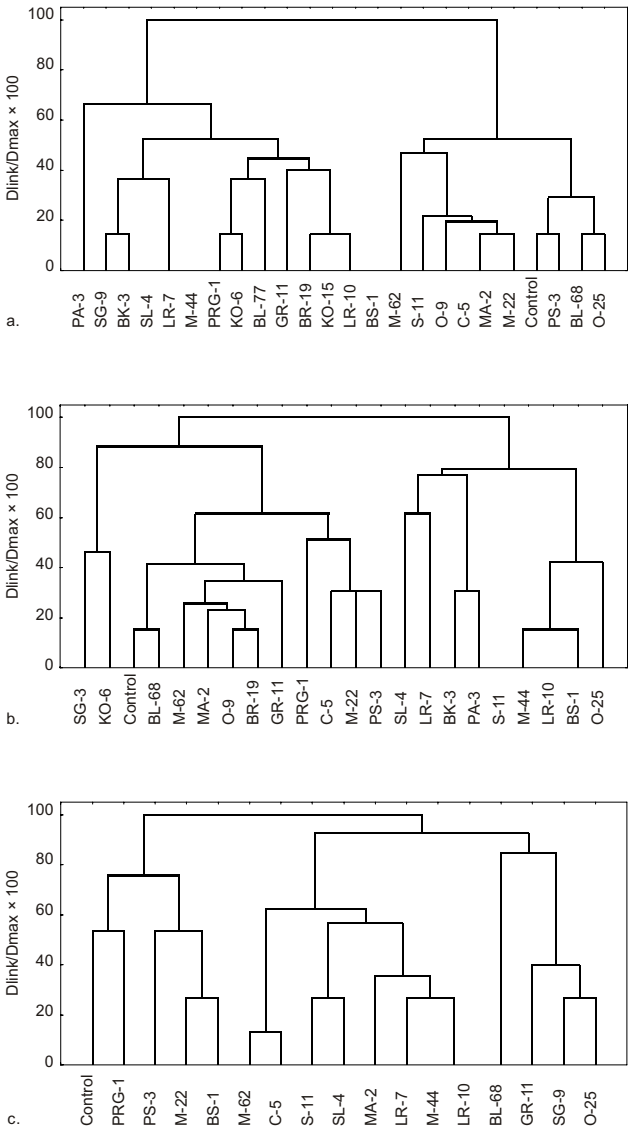

Figure 1. Differences between blood composition of mice infected by various micromycetes after: a - 5 days, b - 10 days, c - 15 days (strain codes in tables). Vertical axis denotes the linkage distance between objects. Scale tree to Dlink/Dmax × 100 - tree plot is to a standardize scale.

towards the impact of micromycete propagules is a peculiar and specific process determined by biological peculiarities of micromycete strain. For example, under the impact of *Penicillium expansum* O-25 during the whole period of the experiment in the blood of mice, hemoglobin concentration and the number of erythrocytes were the lowest, and the number of leukocytes was the highest. Meanwhile, in blood of mice infected with *Rhizopus stolonifer* SG-9 the highest numbers of erythrocytes, thrombocytes, as well as the highest hemoglobin concentration were determined.

Aimed at clarifying which micromycete species cause most evident changes in blood and whether these changes are similar under the influence of separate fungi species, the cluster analysis of the obtained results was performed. This allowed grouping the investigated fungi according to

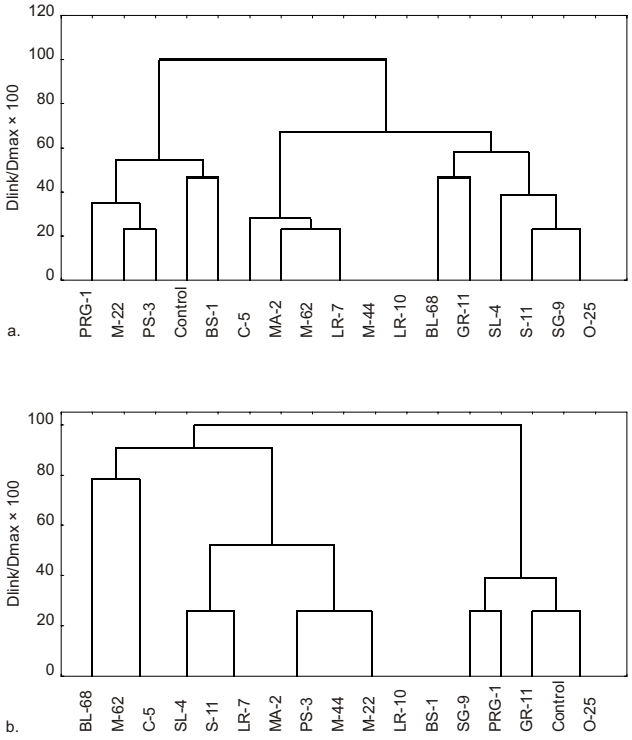

Figure 2. Differences between influence of various micromycetes after 15 days on: a - erythrocytes parameters, b - leukocytes parameters (strain codes in tables). Vertical axis denotes the linkage distance between objects. Scale tree to Dlink/Dmax × 100 - tree plot is to a standardized scale.

the character of their impact (Fig. 1a, 1b, 1c). Thus, based on the character of impact after 5 days, the investigated micromycetes fell into 2 groups (Fig. 1a). Similar effect upon the mice was revealed between systematically allied micromycete species *Penicillium expansum* KO-6 and *P. cyclopium* PRG-1. Similar character of impact was revealed also between systematically different micromycete species, e.g. *Rhizopus stolonifer* SG-9 and *Penicillium cyaneofulvum* BK-3; between *Sclerotinia sclerotiorum* BR-19 and *Penicillium stoloniferum* KO-15, *P. commune* LR-10; between *Penicillium verruculosum* MA-2 and *Aspergillus fumigatus* M-22, as well as between *Penicillium expansum* O-25 and *Acremonium roseum* BL-68. *Penicillium biforme* BS-1 strain was distinguished by a completely different character of impact. After 5 days the smallest changes were determined in the blood of mice infected with *Penicillium steckii* PS-3. Blood count of these mice was close to that of mice from the control group.

After 5 and 10 days the blood composition of mice infected with *Acremonium roseum* BL-68 only slightly differed from mice of the control group. At this period, based on the character of influence, the investigated fungi fell into different groups: similar changes in mice blood were determined under the impact of *Sclerotinia sclerotiorum* BR-19 and *Penicillium lanosoviride* O-9 as well as between *Fusarium sporotrichioides* M-62 and *Penicillium verruculosum* MA-2. Another group contained mice infected with *Mucor hiemalis* M-44, *Penicillium variabile* S-11, *P. commune* LR-10, *P. biforme* BS-1. Similar changes in mice blood were noted under the impact of *Penicillium daleae* C-5, *P. steckii* PS-3, *Aspergillus fumigatus* M-22. According to the impact upon mice blood *Alternaria alternata* PA-3 and *Penicillium cyaneofulvum* BK-3 formed a separate group.

At the end of the experiment (after 15 days), the investigated micromycetes, according to the impact upon mice blood, fell into 3 groups. The first group comprised *Aaspergillus fumigatus* M-22, *Penicillium biforme* BS-1, *P. steckii* PS-3, *P. cyclopium* PRG-1. The above- mentioned fungi species produce roquefortines, epipolythiopiperazine-3,6-diones, kojic acid, tremorgen group toxins, secondary metabolites derived from amino-acids, and other toxic metabolites. The second group comprised *Fusarium sporotrichioides* M-62, *Penicillium daleae* C-5, *P. variabile* S-11, *P. chrysogenum* SL-4, *P. verruculosum* MA-2, *P. commune* LR-10, *Aspergillus niger* LR-7, and *Mucor hiemalis* M-44. These fungi produce various toxic metabolites, often of different chemical composition: trichothecenes, tremorgenes, secondary metabolites derived from amino-acids, various organic acids (kojic, aspergillic, penicillic), etc. The research showed that their impact upon the blood of BALB/c mice was similar. *Acremonium roseum* BL-68, *Aspergillus penicilloides* GR-11, *Rhizopus stolonifer* SG-9, *Penicillium expansum* O-25 were ascribed to the 3rd group. Micromycetes of these species produce toxic metabolites of different origin: oosporein from the group of Penicillium toxins (*Acremonium roseum*), compounds of the aflatoxins group (*Rhizopus stolonifer*), citrinin, patulin (*Penicillium expansum*) [4, 18, 19, 22, 37, 39, 51].

The data related to changes in the number of separate blood cells in the blood of mice infected with micromycetes and the cluster analysis demonstrated that, according to the impact upon the number of erythrocytes, all tested micromycetes could be grouped similarly to the tests concerning the impact upon the blood count (Fig. 2a). Meanwhile, the distribution of investigated species according to their effect upon the number of leukocytes considerably differed. Regarding this characteristic, after 15 days micromycetes fell into 3 groups. The first group comprised *Acremonium roseum* BL-68, *Fusarium sporotrichioides* M-62, *Penicillium daleae* C-5, the second - *Penicillium chrysogenum* SL-4, *P. variabile* S-11, *P. verruculosum* MA-2, *P. steckii* PS-3, *P. commune* LR-10, *P. biforme* BS-1, *Aspergillus fumigatus* M-22, *A. niger* LR-7, *Mucor hiemalis* M-44, the third - *Rhizopus stolonifer* SG-9, *Aspergillus penicilloides* GR-11, *Penicillium cyclopium* PRG-1, *P. expansum* O-25 (Fig. 2b). The obtained data of the tests proved evident impact of the investigated micromycetes upon warm-blooded animals, their functions, and ratio of essential blood constituents.

DISCUSSION

One of the most essential tasks at present is to improve the nutrition of people, making it salubrious, beneficial, and healthy. People consume many products of plant origin grown under various ecological conditions. This

promotes the development of various microorganism groups in their growing environment, and contamination of fruit and vegetables already in the period of their growth, ripening, and harvesting. Picked fruits, berries, and harvested vegetables could become a substrate more or less easily available for micromycetes. Microorganisms of some species, intensively developing on berries and fruit, heavily contaminate them, thus making them unsuitable for consumption, thereby causing considerable economic losses. Micromycetes that get into storehouses together with fruit, berries, vegetables, grain and flour, destroy a considerable part of foodstuffs of plant origin. Fruit, berries, and vegetables are often kept in unsuitable conditions with high humidity, temperature favourable for microorganism spread, and an abundance of contamination sources: infected fruit, berries, garbage of different kinds. It often happens that the premises intended for storage do not get enough attention, even if berries, fruit, and vegetables are stored there for a few subsequent years. In such cases, the propagules of undesirable, often hazardous, microorganisms survive in the storehouses from the previous harvest; they contaminate and rapidly spread on the newly brought fruit, berries, vegetables, and other products. The above research results and suppositions are confirmed by numerous literature sources [9, 41, 45]. It was noticed that the majority of microorganisms develop rather slowly on fresh fruit, berries, and vegetables. However, some microorganisms penetrate into inner tissues of products, thus making them difficult to notice visually. In the case of their intense development on fruit, berries, and vegetable, micromycetes of certain species of the *Penicillium*, *Aspergillus*, *Fusarium* and other genera can produce mycotoxins of different chemical composition that are toxic to plants, animals, and humans. Results of this research correspond to the results presented by other authors [10, 14, 29, 32, 40, 46]. The results of our research correspond with those of Jesenska and Pieckova [15] who reported the development of *Eupenicillium brefeldianum* and *Eurotium herbariorum* on fresh and conserved fruit. The Armenian scientists Osipjan and Batikjan [31] indicate intensive development of micromycetes producing mycotoxins on apples and their conserves. Micromycetes of these species were recorded also on apples grown in Lithuania and imported from Poland. It often happens that after heat-treatment of fruit, berries and vegetables, some heat-resistant micromycete propagules remain viable and present a real hazard to people who consume the foodstuffs.

CONCLUSIONS

1. From fruit, berries, vegetables, and food articles of plant origin grown under various ecological conditions, 294 species of micromycetes were isolated and identified. Micromycete species ascribed to the *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus* genera dominated. Micromycetes of the *Alternaria*, *Fusarium*, *Verticillium*, *Cladosporium*, *Botrytis*, *Sclerotinia*, *Acremonium*, *Geotrichum*, *Paecilomyces* genera were also frequent; *Chrysosporium*, *Myrothecium*, *Trichoderma*, *Pythium*, *Absidia*, *Sporotrichum*, *Gliocladium*, *Mortierella*, *Phoma* were found rarely.

2. The ability of 394 strains, belonging to various genera, to synthesise and excrete into surrounding toxic secondary metabolites (mycotoxins) was tested. Most micromycete strains actively producing mycotoxins were of the *Penicillium*, *Aspergillus*, *Fusarium* genera, although rather active producers of toxins were also determined in the *Alternaria*, *Acremonium*, *Mucor*, *Rhizopus*, *Sclerotinia* genera.

3. Toxicity of 46 most active micromycete strains upon warm-blooded animals was tested. The strongest impact upon the BALB/c mice was produced by *Penicillium biforme* BS-1, *P. commune* LR-10, *P. cyclopium* PRG-1, *P. expansum* O-25, *P. stoloniferum* KO-15, *P. verruculosum* MA-2, *P. viridicatum* BL-77, *Fusarium sporotrichioides* M-62 and BA-4, *F. equiseti* BL-54, *Acremonium roseum* BL-68 micromycete strains; under the influence of these strains the mice died within 2 days. Death of mice within the period of 15 days was also caused by micromycete strains of other species, of which 7 strains belonged to *Penicillium*, 3 - to *Aspergillus*, 1 - to *Alternaria*, and 1 - to *Mucor* genera.

4. Micromycetes, characterised by acute toxicity, injected in mice in half reduced doses caused mice weight decrease, alterations of internal organs, changes in the blood count, and shrinkage of the spleen. In some cases, the micromycetes previously injected into mice organism were isolated from their internal organs. This confirms conditional pathogenicity of these fungi.

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