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Micromycetes on polymeric materials under natural conditions of warm humid climate and simulated tropical climate conditions

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Artificial polymer materials represent a specific ecological niche for destructive microorganisms adapted to growth and development at the expense of their components. Microorganisms quickly adapt to a wide range of materials, begin to intensively develop either due to external pollution, or due to the components of the material itself, causing microbiological damage of a different nature. Among the many types of microorganisms, micromycetes play a special role as agents of the biodegradation process. This is especially true under conditions of high humidity and temperature. Tests are carried out under various conditions in order to study the microbiological resistance of functional materials and to isolate new microorganisms-destructors. During 18 months under natural conditions of warm humid climate and conditions of tropical climate imitation there were exposed samples of polymeric materials: rubbers, sealants, and rubber-fabric materials. 16 strains of mycelial fungi belonging to 14 species of 9 genera were isolated from all studied samples of polymeric materials after exposure. For the first time micromycetes contaminating the polymeric materials were isolated under conditions of tropical climate imitation. 10 strains of micromycetes were isolated, *Penicillium lanosum* and *Cladosporium sphaerospermum* were dominant, *Penicillium* sp., *Cladosporium oxysporum* and *Aspergillus ochraceus* were less common. Under natural conditions of warm humid climate 6 strains of micromycetes were isolated, *Trichoderma viride*, *Aspergillus cervinus* and *Cladosporium oxysporum* species prevailed, *Pestalotiopsis guepinii* species was noted less often. Most of the isolated fungi are known as destructors of polymeric materials in different climatic zones and environmental conditions. Fungi cultures are of interest for testing materials for microbiological resistance, effectiveness of antiseptics and biocidal additives, studying the biological destruction of biodegradable materials, and other research.

Keywords: biodeterioration, biological destruction, microbiological resistance, microbial damage, microorganisms-destructors, ecological niche.

УДК 582.284.51+52 (571.51)

Микромицеты на полимерных материалах в естественных условиях тёплого влажного климата и имитационных условиях тропического климата

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Полимерные материалы, создаваемые человеком, представляют собой специфическую экологическую нишу для микроорганизмов-деструкторов, которые приспособились к росту и развитию за счёт их компонентов. Особенно это актуально в условиях повышенной влажности и температуры. В данном исследовании в течение 18 месяцев в естественных условиях тёплого влажного климата и условиях имитации тропического климата проходили испытания на микробиологическую стойкость образцы полимерных материалов: резины, герметики и резинотканевые материалы. Со всех исследуемых образцов полимерных материалов после экспозиции было выделено 16 штаммов мицелиальных грибов, относящихся к 14 видам 9 родов. Впервые были выделены 10 штаммов микромицетов, контаминирующих полимерные материалы в условиях имитации тропического климата, среди которых доминировали виды *Penicillium lanosum* и *Cladosporium sphaerospermum*, реже встречены *Penicillium* sp., *Cladosporium oxysporum* и *Aspergillus ochraceus*. В естественных условиях тёплого влажного климата выделено 6 штаммов микромицетов, преобладали виды *Trichoderma viride, Aspergillus cervinus* и *Cladosporium oxysporum*, реже отмечен вид *Pestalotiopsis guepinii*. Большинство выделенных грибов известны как деструкторы полимерных материалов в различных климатических зонах и экологических условиях и представляют интерес для испытаний материалов на грибостойкость, изучения биологической деструкции биоразлагаемых материалов и других исследований.

Ключевые слова: биоповреждения, биологическая деструкция, микробиологическая стойкость, микробиологические повреждения, микроорганизмы-деструкторы, экологическая ниша.

In the course of climate change and increasing anthropogenic load, artificial polymer materials have become a specific ecological niche for microorganisms-destructors, which have adapted to the growth and development due to their components. Microorganisms quickly adapt to a wide range of materials, begin to develop intensively either due to external contamination or due to the components of the material itself, causing biodeterioration. Among many types of microorganisms, micromycetes or mycelial fungi play a special role as agents of the biodegradation process on materials of different chemical nature and structure [1–3].

The composition of the micromycetes cenosis on the surface of the material is determined not only by its chemical nature, but also by a set of external factors, such as operating and storage conditions of the material, as well as temperature and humidity conditions. Therefore, the effect of micromycetes on various materials in a humid tropical climate is particularly significant. Under these conditions the rapid growth and accumulation of biomass occurs, which is facilitated by high rates of temperature, humidity, and other external factors. In addition, in these climatic and geographical conditions, the highest level of biodiversity in the world, including microbiological communities, was noted, which significantly increases the likelihood of new destructors and, accordingly, the occurrence of microbiological lesions of materials that are operated in this zone. Russia does not have its own territory with a humid tropical climate, but there are areas of humid warm climate, for example, the Sochi region. Thus, there were prerequisites for comparative tests of materials' microbiological resistance and the release of microorganisms under the natural conditions of warm humid climate, as well as in the simulation of humid tropical climate. As optimal conditions for the latter were selected Tropical block in the New greenhouse of Tsitsin Main Botanical Garden of Russian Academy of Sciences. The climate of this greenhouse, equipped with modern electronic control systems, is as close as possible to the conditions of a humid tropical climate. In the Tropical block of the New greenhouse, about 550 species of woody and herbaceous plants from all regions of the globe with a tropical climate are planted in the ground [4].

The aim of this work was to identify strains of micromycetes from samples of various polymeric materials exposed under natural conditions of warm humid climate and tropical climate simulation.

Objects and Methods

Samples of the following polymeric materials were used for exposure under various conditions: rubber-fabric material used for the manufacture of a soft fuel tank, a rubber mixture based on butyl-nitrile rubber of medium viscosity, a rubber mixture based on butyl-nitrile rubber of low viscosity, thiocol sealant vulcanized with manganese salts. The samples were exposed for 18 months in mycological stands in the Yew-boxwood grove near the city of Sochi and in the New greenhouse of Tsitsin Main Botanical Garden in Moscow. During testing at both mycological sites, temperature and humidity conditions were monitored. After the end of the exposition, the samples were placed in sterile containers and transported to the laboratory, where micromycetes were isolated from their surface.

Standard methods were used for isolation of micromycetes from the samples of polymeric materials. These include prints and direct sowing on a nutrient Czapek medium and malt agar. Inspection of samples and photos were taken on the Leica M165FC stereomicroscope. The frequency of occurrence of strains is calculated as the ratio of Petri dishes number in which the strain is encountered to the total number of Petri dishes on which the isolation was carried out.

Results and Discussion

The species composition of the selected micromycetes and their occurrence on the samples of materials are presented in tables 1 and 2. On all

Table 1

Species composition of isolated micromycetes and their incidence on samples of materials after exposure to natural conditions of a warm humid climate (Sochi region)

Isolated species of micromycetes	Material				Incidence of micromycetes by materials
	rubber-fabric material	medium viscosity rubber compound	low viscosity rubber compound	thiokol sealant	
Aspergillus cervinus Massee	–	+	–	+	2
Cladosporium oxysporum Berk. & M.A. Curtis	+	–	+	–	2
Mucor plumbeus Bonord.	+	–	–	–	1
Penicillium rugulosum Thom	–	–	–	+	1
Pestalotiopsis guepinii (Desm.) Steyaert	+	–	–	–	1
Trichoderma viride Pers.	+	–	–	+	2
Species diversity of isolated micromycetes	4	1	1	3	–

Table 2

Species composition of isolated micromycetes and their incidence on samples of materials after exposure to imitation conditions of tropical climate (Greenhouse, Moscow)

Isolated species of micromycetes	Material				Incidence of micromycetes by materials
	rubber-fabric material	medium viscosity rubber compound	low viscosity rubber compound	thiokol sealant	
Acremonium sp.	–	–	+	–	1
Aspergillus ochraceus G. Wilh.	+	+	–	—	2
Aspergillus terreus Thom	–	+	–	+	2
Cladosporium oxysporum Berk. & M.A. Curtis	+	–	–	–	1
Cladosporium sphaerospermum Penz.	–	+	+	+	3
Penicillium lanosum Westling	+	+	+	–	3
Penicillium sp.	+	–	–	–	1
R*hizopus oryzae* Went & Prins. Geerl	+	+	–	–	2
Stachybotrys chartarum (Ehrenb.) S. Hughes	+	–	–	–	1
Trichoderma viride Pers.	+	–	–	–	1
Species diversity of isolated micromycetes	7	5	3	2	–

studied samples of materials after the exposure the fungi growth of varying degrees was observed. The most susceptible to microbiological damage were samples of rubber-fabric material, on the surface of the samples the formation of fungal mycelium was observed with the naked eye, and when viewed under a microscope, a well-developed sporulation was found (Fig. 1). In addition, the samples of rubber-fabric material showed the greatest species diversity of micromycetes. Samples of rubber compounds and thiocol sealant were also affected by micromycetes (Fig. 1, 2, see color tab).

For all selected species, the total frequency of occurrence (percentage) was calculated as presented in Figures 3 and 4.

As can be seen from the diagrams, the most common species *Trichoderma viride*, *Aspergillus cervinus* and *Cladosporium oxysporum* are found on material samples after exposure under natural conditions. *Trichoderma viride* is often isolated from samples of non-metallic materi-

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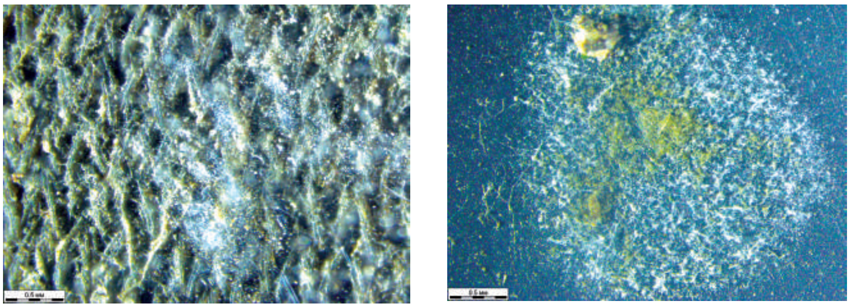
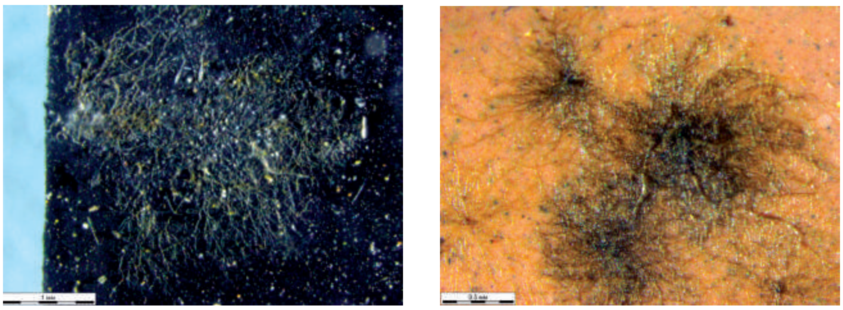


A B

Fig. 1. Growth of micromycetes on the surface of samples of rubber-fabric material (A) and medium viscosity rubber (B)



A B

Fig. 2. Growth of micromycetes on the surface of samples of low viscosity rubber compound (A) and thiokol sealant (B)

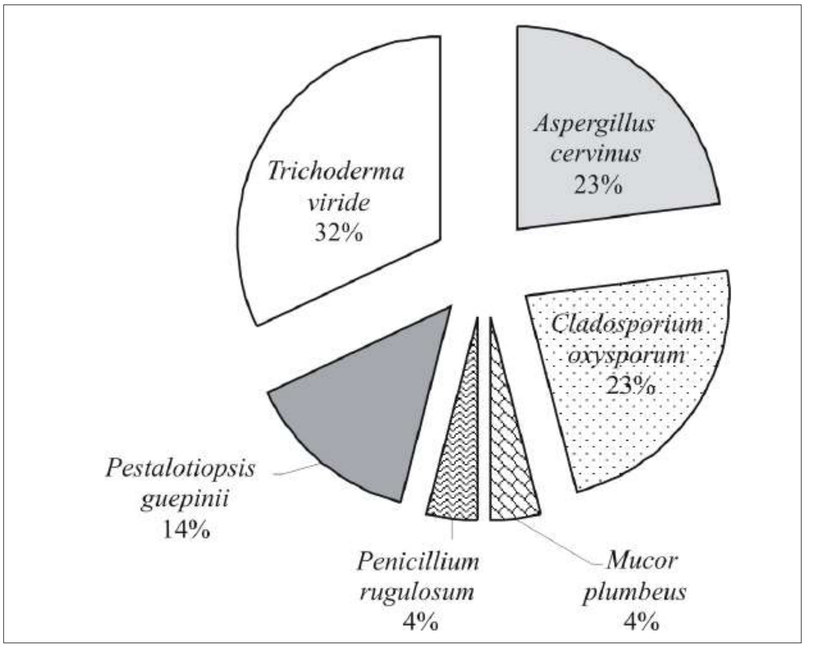


Fig. 3. The frequency of occurrence of micromycete species isolated from samples of materials after exposure to natural conditions

als in different zones from works of art and books in storage. It is noted its ability to grow due to the components of polymeric materials [5–8]. In addition, the species of *Trichoderma viride* is a part of the test cultures used by many standards for testing materials and products for fungal resistance, including GOST 9.048, GOST 9.049, etc. Micromycetes of the genera *Penicillium, Cladosporium* and *Aspergillus* are the most common fungi among micromycetes destructors. They are often isolated from various materials and structures that have undergone biodeterioration: from the surface of buildings, building materials, works of art, books and documents in book depositories, etc. [5–12]. Micromycetes of these genera are also isolated from metal materials, in particular, from the structural surfaces of spacecraft, their activity as biocorrosion agents is shown [13]. Species of these genera are part of the test cultures according to Russian and foreign standards for testing the fungal resistance of materials and products.

Pestalotiopsis guepinii is found a little less often. Species of the genus *Pestalotiopsis* is often isolated from various substrates in areas with warm humid climate, among them there are also destructors. So, for instance, the species *Pestalotiopsis microspora* has a set of enzymes that can destroy plastic. *Pestalotiopsis* sp. strains decompose hydrocarbons of oil [14, 15]. The lowest frequency of occurrence was noted for two species of fungi: *Mucor plumbeus* and *Penicillium rugulosum*. According to the literature, these species are often found on samples of polymeric materials under various environmental conditions [5, 8, 11, 12].

Among the fungi isolated from the materials after exposure in a greenhouse, the frequency of occurrence was dominated by species *Penicillium lanosum* and *Cladosporium sphaerospermum*. Species *P. lanosum* is isolated from samples of rubber of both brands and rubber-fabric material and noted in the literature as a destructor of polymeric materials of different chemical composition. *C. sphaerospermum* is isolated from both rubber and sealant grades and is also known for its destructive activity. Less often, but nevertheless repeatedly, three species were encountered: *Penicillium* sp., *Cladosporium oxysporum,* and *Aspergillus ochraceus*. Species of fungi *Rhizopus oryzae, Aspergillus terreus, Acremonium* sp., *Stachybotrys chartarum* are met almost sporadic. It is known that the species *Aspergillus terreus* often settles on the surface of polymeric materials in warehouses. Isolated strains of this species can actively destroy cellulose. Species of the genus *Rhizopus* and *Acremonium* often contaminate the surface of polymeric materials, but their role in the destruction of materials has not been studied enough. Fungi of species *Stachybotrys chartarum* are also often isolated from samples of non-metallic materials in different zones, its ability to grow due to the components of polymeric materials is noted [5, 16].

Micromycetes, which contaminate the surface of samples and can be potential destructors,

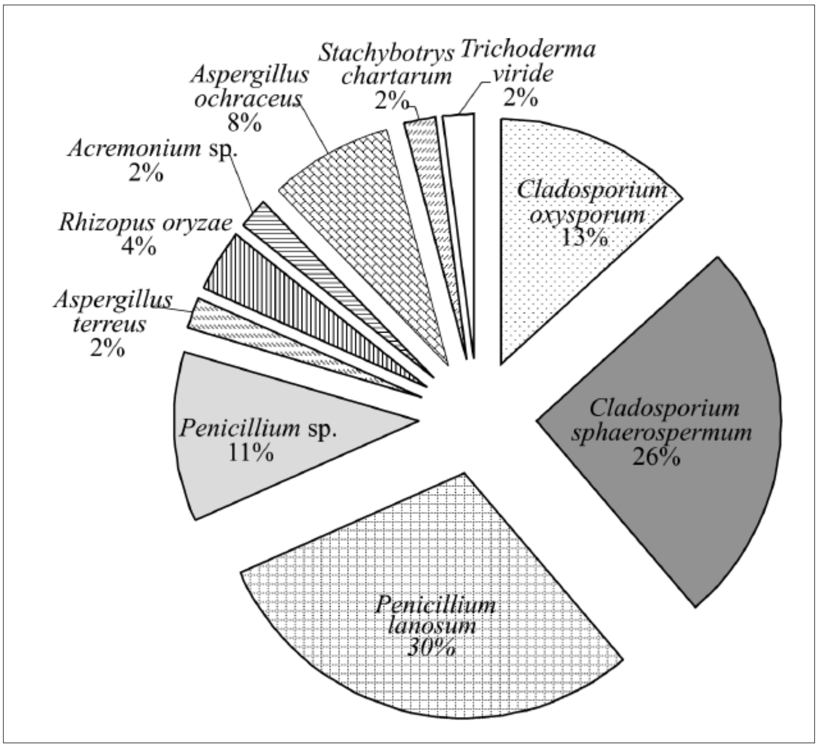


Fig. 4. The frequency of occurrence of micromycetes species isolated from samples of materials after exposure to greenhouse conditions

in nature are inhabitants of soil, plants, litter and other natural substrates. In the greenhouse, the species composition of microorganisms will be influenced by a number of factors, such as the type of soil used, plant species, the composition of mineral fertilizers, biocides used, irrigation regime, and many other factors. According to the results of this study, the species list of micromycetes isolated from the materials in the greenhouse is not inferior to the number of species isolated in the natural conditions of warm humid climate, so further study of the biodiversity of microorganisms that contaminate materials under simulation conditions is of great interest. From a practical point of view, the results of our study allow us to speak about the effectiveness of specific tests of materials for microbiological resistance, since most of the samples of non-metallic materials were subjected to biodeterioration.

Conclusion

Thus, after 18 months of exposure of polymeric materials under natural conditions of warm humid climate and conditions of tropical climate imitation, a total of 16 mycelial fungi strains belonging to 14 species of 9 genera were isolated. There were first isolated micromycetes, contaminated polymeric materials under conditions simulating a tropical climate. These include 10 strains of micromycetes, among which *Penicillium lanosum* and *Cladosporium sphaerospermum* species were dominant, less common were *Penicillium* sp., *Cladosporium oxysporum*, and *Aspergillus ochraceus*. Under natural conditions of warm humid climate 6 strains of micromycetes were isolated; *Trichoderma viride*, *Aspergillus cervinus*, and *Cladosporium oxysporum* species prevailed, *Pestalotiopsis guepinii* species was less abundant. Most of the isolated fungi are known as destructors of materials and products in various climatic zones and environmental conditions.

All the obtained cultures of micromycetes are deposited in the collection of microorganisms of All-Russian Scientific Research Institute of Aviation Materials and will be used for testing the fungal resistance of materials, testing the effect of protective agents and other studies. In addition, these cultures are of interest not only from the point of view of studying the biodiversity of micromycetes inhabiting various polymeric materials, but also from the point of view of material protection and for their use as agents of biological destruction of the materials used.

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