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EXPERIMENTAL ARTICLES

Effect of Water Activity and Relative Air Humidity on the Growth of *Penicillium chrysogenum* Thom, *Aspergillus repens* (Corda) Sacc., and *Trichoderma viride* Pers. Isolated from Living Spaces

V. B. Ponizovskaya[a], A. B. Antropova[b, 1], V. L. Mokeeva[c], E. N. Bilanenko[c], and L. N. Chekunova[c]

[a] *State Research Institute for Restoration, Russian Federation Ministry of Culture, ul. Gastello, 44, b.1, Moscow, Russia*
[b] *Mechnikov Research Institute for Vaccines and Sera, Russian Academy of Medical Sciences, M. Kazennyi per. 5A, Moscow, 105064 Russia*
[c] *Moscow State University, Moscow, 119992 Russia*

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Abstract—Original data on the growth parameters of the fungi *Penicillium chrysogenum* Thom, *Aspergillus repens* (Corda) Sacc., and *Trichoderma viride* Pers. isolated from living spaces in Moscow are presented. Spore germination, fungal growth, and the radial growth rate of the colonies were investigated upon cultivation on agarized nutrient media with different water activity (a_w) values. Spore germination and fungal growth were studied in house dust under laboratory conditions at different relative air humidity (RH). It was shown that, at decreased a_w and RH, the spore germination time increased, as did the period from germination to mycelium and conidia formation, while the radial growth rate of colonies decreased. House dust was found to be a suitable growth substrate for *A. repens* and *P. chrysogenum*, supporting their complete life cycle. It was suggested that house dust is unsuitable as a substrate for the growth of *T. viride*. The a_w and RH ranges for development of these micromycetes were determined. On this basis, the *A. repens*, *P. chrysogenum*, and *T. viride* strains isolated from living spaces were identified as xerophilic, xerotolerant, and hygrophilic ones, respectively.

Keywords: water activity, relative air humidity, micromycetes, *Aspergillus repens, Penicillium chrysogenum, Trichoderma viride*, house dust.

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The problem of influence of water activity and relative air humidity on the growth of micromycetes has many aspects. It is well known that micromycetes are cosmopolites and eurybionts. One of the most important factors limiting and strongly influencing the growth of micromycetes is the presence and availability of water, which is characterized by the relative air humidity and the water activity (a_w). A low a_w level characterized by a low water content, water crystallization, and increased concentrations of osmotically active substances is characteristic of a number of natural and artificial cenoses [1–3]. Information on the a_w and RH values at which different micromycete species are able to exist is required in many fields, e.g., storage and transportation of foodstuffs, protection of cultural heritage (monuments and items of art), prognosis for fungal growth and prevalence under natural conditions, and in the practice of biocontrol [4–7]. One important issue is studying the conditions of propagation of micromycetes in living spaces, which is urgent due to the variegated influence of the fungi on human health. The mycobiota of living spaces of the city of Moscow was shown to be a xerotolerant community including micromycetes that are capable of growth at low a_w and RH values [8]. Research on the influence of a_w and RH on micromycete growth is necessary for developing the practical recommendations on the microclimate of living spaces, as well as for the planning of preventive and avoidance measures.

The goal of the present work was to study the effect of the water activity and the relative air humidity on the growth and development of the fungi *Penicillium chrysogenum* Thom, *Aspergillus repens* (Corda) Sacc., and *Trichoderma viride* Pers. isolated from living spaces.

MATERIALS AND METHODS

Three fungal cultures isolated from living spaces in Moscow were used in the work. Since the species *Aspergillus repens* (Corda) Sacc. and *Penicillium chrysogenum* Thom predominated in the mycobiota of Moscow living spaces, they were chosen as the study subjects. *Trichoderma viride* Pers., a micromycete which is rare and not prevalent in living spaces [8], was used for comparison. Media based on wort agar (WA)

[1] Corresponding author; e-mail: antropova-a@yandex.ru

with different water activity (a_w) values (0.99, 0.95, 0.85, 0.75, and 0.65) were used as nutrient media; the a_w in WA was accepted as 0.99, and the other a_w values were obtained by the addition of glycerol [9, 10]. All the experiments were carried out at 25°C, which is the optimal or close-to-optimal temperature for the development of these fungi [11]. This temperature is typical for Moscow apartments.

The study of spore germination and fungal growth on agarized nutrient medium. The media were inoculated according to the method proposed by Marin et al. [10]. For this purpose, the spores were suspended in a liquid medium of the same composition as described above, with the same a_w values. To prevent aggregation of the spores, Tween 80 was added to the media. The final concentration in the medium was 10^6 spores/ml. The fungal spore suspension obtained (0.1 ml) was applied to each plate with wort agar and spread with a glass spatula. The experiments were carried out in two replicates. The inoculated plates were sealed with a Parafilm tape to prevent the medium from drying [9] and placed in a thermostat. Every day for 35 days from the moment of inoculation, agar disks were cut out with a sterile borer, placed on a slide, and examined under a microscope. One hundred spores were studied, and the proportion of those germinated and not geminated was recorded. The spores that underwent a swelling stage and developed a germ tube were considered to be germinated. Multiple branching, when it was not possible to see the main axis, was considered an indication of mycelium formation.

The study of the radial growth rate of fungal colonies on agarized nutrient medium. The center of a plate with agarized medium with a given a_w value was stab-inoculated with the fungi. The experiments were carried out in two replicates as described above. The colony diameters in two perpendicular directions were measured daily for 35 days. The average of these measurements was taken as the colony diameter [12]. The radial growth rate was calculated as the average increment of the colony diameter (mm/day) during the exponential growth phase.

The study of spore germination and micromycete growth in house dust under the laboratory conditions at different RH was carried out in desiccators in which, using the saturated solutions of KH_2PO_4, KCl, NaCl, and $NaNO_2$, the RH 95, 85, 75, and 65%, respectively, was obtained at 25°C. The RH of 99% was created with pure water [13].

Sterile glass vials containing 2 g of sterile dust each were put in the desiccators. The house dust was collected with a household vacuum cleaner from the furniture and floors in apartments in residential buildings with central heating in different districts of Moscow. The dust sterilization regime was twice for 30 min at 0.5 atm. The desiccators were closed with ground-in covers and allowed to stand at 25°C for 3 days for the humidity balance with the desiccator environment to be established in the dust. After that, the micromycete spores (10^5 spores/g dust) collected aseptically from 14-day lawns of the fungi with a preparation needle were introduced into the vials with dust and mixed thoroughly for 5 min. The experiments were carried out in two replicates. Every day for 35 days, the dust was sampled and examined under a microscope. The share of germinating spores among 100 spores was calculated.

For graphical representation, the formation of a mycelium was conventionally designated as 100% spore germination.

The results were statistically processed using the Excel 7.0 and Statistica 6.0 software packages.

RESULTS AND DISCUSSION

Growth of micromycetes on agarized media with different a_w values. When the micromycetes grew on media with different a_w values, the duration of periods from inoculation to germ tube formation and from the beginning of germination to mycelium formation and sporulation depended both on the fungal species and a_w (Fig. 1). At $a_w = 0.65$, spore germination was not observed for any of the fungi studied (Table 1).

At a water activity of 0.99 and 0.95, all the fungi underwent a complete life cycle; the mycelium formation (conventionally designated on the graphs as 100% spore germination) was observed as early as on the first or second day of the experiment.

At a_w decreased to 0.85, complete life cycles were observed only for *A. repens* and *P. chrysogenum*. In this process, the period from the beginning of germ tube formation to the appearance of mycelium lengthened. No spore germination was observed in *T. viride* at a_w lower than 0.95. Only *A. repens* could germinate at $a_w = 0.75$. However, under these conditions, the period from inoculation to germ tube formation lengthened threefold. Mycelium formation was recorded only on the eighth day.

The results obtained for the a_w ranges for the growth of fungi agree with the literature data [4, 12]. *A. repens* is the most resistant to the effect of low a_w values. Its complete life cycle was observed at all a_w values, from 0.99 to 0.75. *T. viride* exhibited the least tolerance to low water activity. The range of its development was limited to $a_w = 0.99–0.95$. *P. chrysogenum* occupied an intermediate position.

The radial growth rate of the fungal colonies on agarized media also depended on the water activity. In *P. chrysogenum* and *T. viride*, the highest growth rate was observed at $a_w = 0.99$; in *A. repens*, at $a_w = 0.95$. The a_w dependence of the radial growth rate corresponded to the a_w dependence of the germination time and development for these species (Table 2, Fig. 2).

Micromycete growth in house dust at different values of relative air humidity. No data exist in the litera-

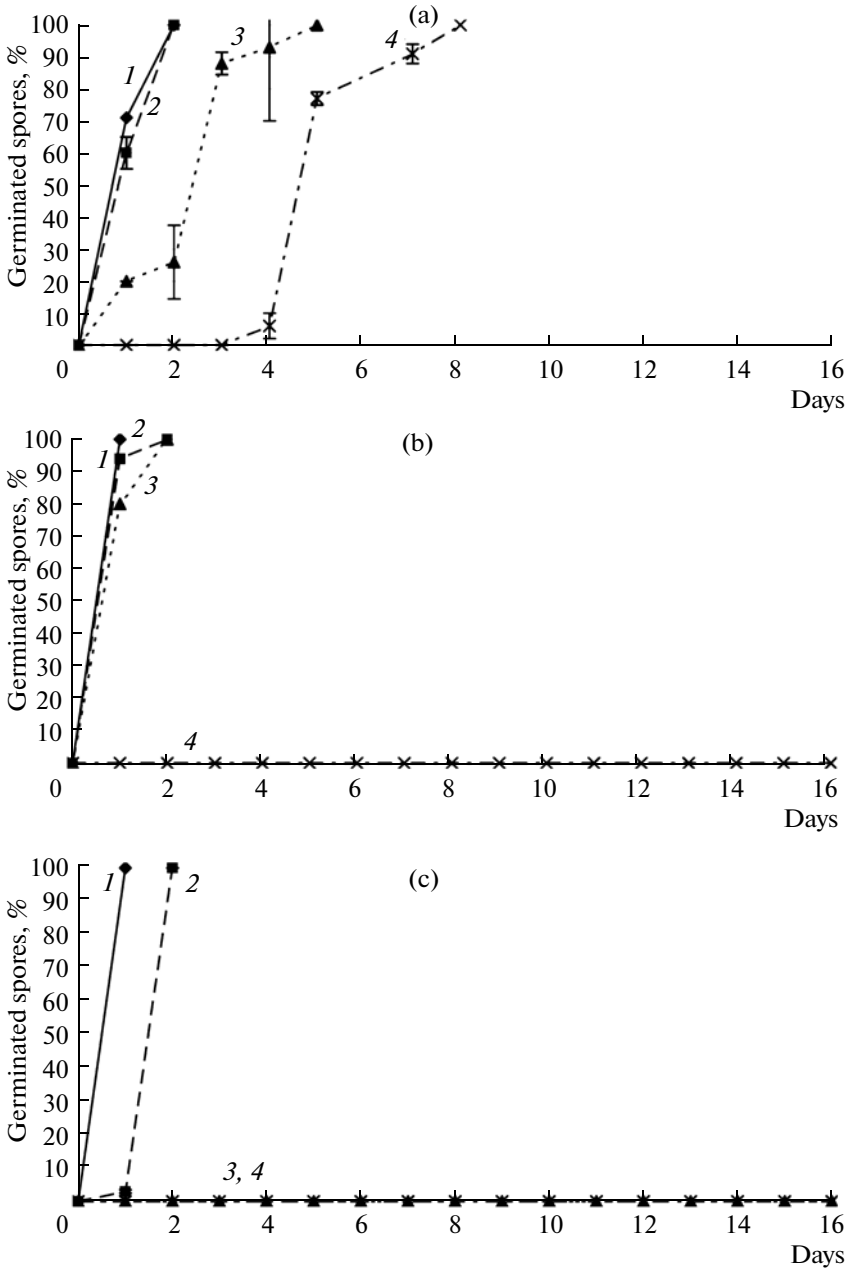

Fig. 1. Conidium germination (%) of the micromycetes *A. repens* (a), *P. chrysogenum* (b), and *T. viride* (c) on agarized media with different a_w: 0.99 (*1*), 0.95 (*2*), 0.85 (*3*), and 0.75 (*4*). Mycelium formation was conventionally designated on the graphs as 100% germination.

ture concerning spore germination and micromycete growth in house dust under the laboratory conditions. Developing in dust at different RH values, micromycetes underwent the same stages as when they grew on agarized media. The longevity of the growth phases depended on the fungal species and RH (Fig. 3). At RH = 65%, spore germination did not occur in any of the species studied (Table 1).

The minimal RH values required for spore germination in dust coincided with the relevant a_w values required for spore germination on agarized media; however, when the fungi were grown in dust, the phase duration was longer. Not all the species grew well in house dust. While *A. repens* and *P. chrysogenum* underwent a complete life cycle, at none of the RH values did *T. viride* undergo a complete life cycle. In *T. viride*, the spores germinated rather slowly even at RH = 99 and RH = 95%, and sporulation was not observed throughout two months of observation (Table 1). It must be emphasized that *A. repens* and *P. chrysogenum* formed conidial structures in house dust, which did

Table 1. Effect of a_w and RH on the time of germination, mycelium formation, and micromycete sporulation on agarized media and in house dust

a_w/RH	Growth phases	*A. repens*	*P. chrysogenum*	*T. viride*
0.99/99	Germination	1/1	1/1	1/4
	Mycelium formation	2/3	1/3	1/5
	Beginning of sporulation	2/3	4/3	5/–
0.95/95	Germination	1/2	1/1	1/4
	Mycelium formation	2/3	2/3	2/–
	Beginning of sporulation	3/3	4/3	11/–
0.85/85	Germination	1/3	1/7	–/–
	Mycelium formation	5/4	2/12	–/–
	Beginning of sporulation	14/7	21/12	–/–
0.75/75	Germination	4/7	–/–	–/–
	Mycelium formation	8/16	–/–	–/–
	Beginning of sporulation	21/21	–/–	–/–
0.65/65	Germination	–/–	–/–	–/–
	Mycelium formation	–/–	–/–	–/–
	Beginning of sporulation	–/–	–/–	–/–

Note: "–" indicates the absence of germination, mycelium, or sporulation ".../..." indicates duration of the growth phase (days) on media/in house dust.

Table 2. Duration of the period from inoculation to the beginning of visible growth of colonies (L) (days) and the radial growth rate of the fungal colonies (V) (mm/days) on agarized media with different water activity values

Species	a_w							
	0.99		0.95		0.85		0.75	
	L	V	L	V	L	V	L	V
A. repens	<1	2.2	<1	6.5	6	1.3	6	0.1
P. chrysogenum	<1	4.9	<1	4.8	8	0.3	–	–
T. viride	<1	23.3	<1	5.6	–	–	–	–

Note: "–" indicates the absence of growth.

not differ in micromorphology from those on the standard agarized media (Figs. 4, 5).

The comparison of micromycete growth in dust and on agarized media revealed both similarities and differences.

The minimal RH and a_w values required for spore germination in dust and on agarized media were similar. In house dust, all the fungal growth phases lengthened with an RH decrease similarly to the growth of fungi on the media (Figs. 1, 3).

The differences could be seen in the following. In dust, the period from spore inoculation to the beginning of their germination at RH below 99% was longer than at the corresponding a_w values on agarized media. It is probably a result of the necessity for the micromycetes to adapt to such a specific substrate as dust. The number of germinated spores in dust increased more slowly, especially at lower RH values. The terms of mycelium formation of the fungi in dust were also longer than on solid media, with the differences being more pronounced at lower the RH values. However, in many cases sporulation occurred earlier than on the media. The earlier sporulation was probably a response to the stress conditions.

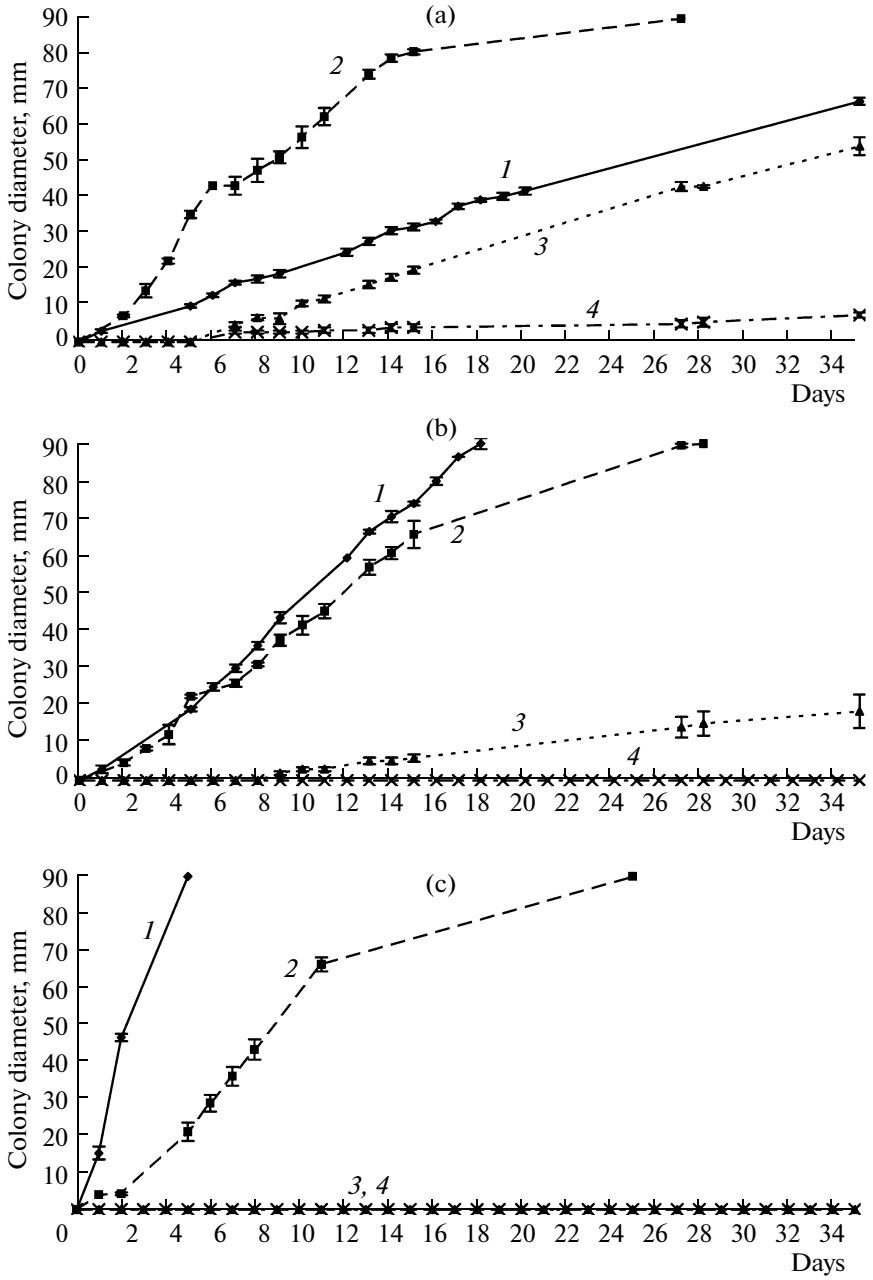

Fig. 2. Radial growth rate of the colonies of the micromycetes *A. repens* (a), *P. chrysogenum* (b), and *T. viride* (c) on agarized media with different a_w: 0.99 (*1*), 0.95 (*2*), 0.85 (*3*), and 0.75 (*4*).

Our investigations established that, for the two fungi, *A. repens* and *P. chrysogenum*, house dust was quite suitable as a substrate for growth (Figs. 4, 5). For *T. viride*, house dust did not suit as a substrate for growth. These findings agree with the assumption of Velikova and Pugacheva [14] that micromycetes probably utilize the dust components as a source of nutrients. In the cited work, it was also noted that the presence of high dust concentrations decreased the growth rate of *T. viride* on paper. The authors explained that this was because of the presence in dust of certain toxic metals, for example, copper, to which the strain used was probably less resistant.

The specific features of the life cycle of the micromycetes in house dust under the laboratory conditions agree with the data on the structure of the domination in the mycobiota of living spaces in Moscow. Thus, the occurrence and the specific abundance of *A. repens* and *P. chrysogenum* in house dust are high, whereas those of *T. viride* are insignificant [8]. Probably this is explained not only by the resistance of *A. repens* and

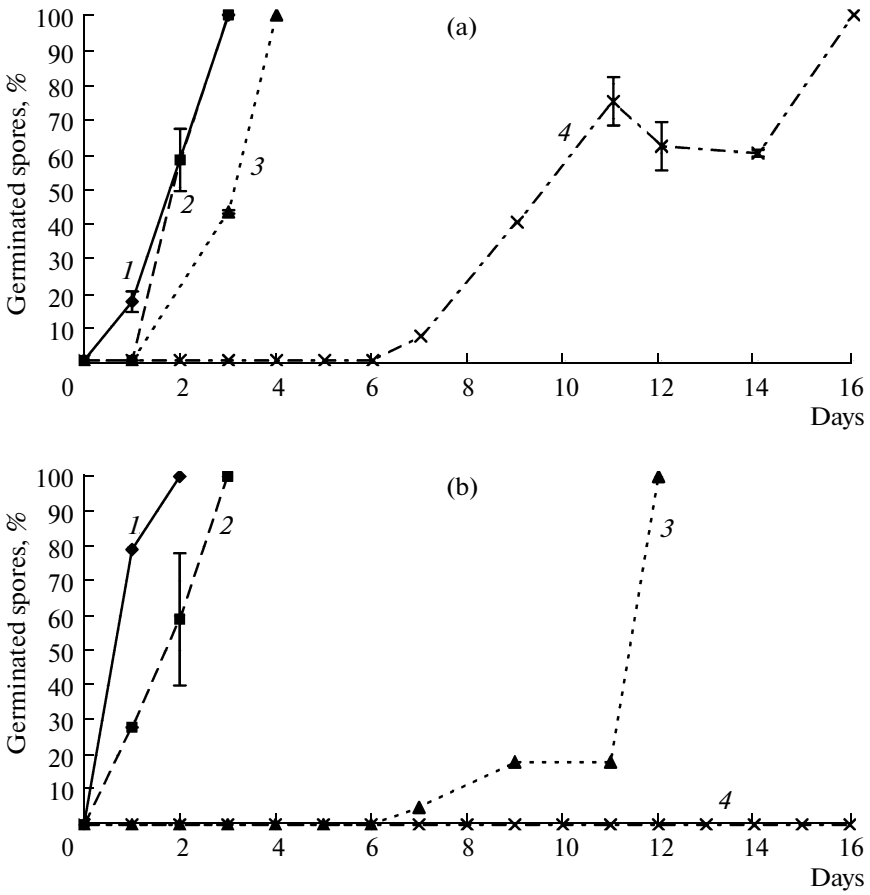

Fig. 3. Conidium germination (%) of the micromycetes *A. repens* (a) and *P. chrysogenum* (b) in house dust at different RH: 99 (*1*), 95 (*2*), 85 (*3*); and 75% (*4*). Mycelium formation was conventionally designated on the graphs as 100% germination.

P. chrysogenum to the effect of low a_w and RH values, but also by the fact that house dust, in contrast to *T. viride*, is a suitable substrate for them.

Our data on the conidium germination time and the minimal and optimal RH values required for growth of *A. repens, P. chrysogenum*, and *T. viride* agree with the literature data [4, 12]. However, in the work of Zlochevskaya et al. [12], spore germination of the *A. repens* isolates obtained from parchments and leather 110 days after the beginning of the experiment was noted even at $a_w = 0.65$. We did not note this, possibly due to the shorter observation time or due to strain differences. The spore germination time of *A. repens* and *P. chrysogenum* in dust was much shorter than on parchment at the same RH values, which may give evidence of a greater suitability of house dust as a food substrate for these fungi.

The data of our own investigations and the literature analysis suggest significant influence of the water activity and the relative air humidity on the growth and development of micromycetes. At decreased a_w and RH, the periods from inoculation to spore germination and from the beginning of germination to mycelium formation and sporulation increased, while the colony radial growth rate decreased. However, the sensitivity to decreased a_w and RH values varies in different fungi [5, 10, 12, 15–17]. The accumulation of osmotically active substances for cell protection from dehydration is known to be one of the strategies of xerophilic and xerotolerant organisms. Yeasts and mycelial fungi predominantly utilize glycerol as an osmolyte [18, 19]. However, it is not known to what degree the fungi are able to utilize the glycerol contained as a substrate constituent and to what degree they carry out the de novo synthesis.

Based on the work done, we conclude that the micromycete strains isolated from living spaces may be assigned to the following ecological groups: *A. repens*, to xerophiles, because it is able to undergo a complete life cycle at a_w below 0.80 with optimum growth $a_w = 0.95$; *P. chrysogenum*, to xerotolerant organisms due to its ability to undergo a complete life cycle at a_w below 0.90; and *T. viride*, to hygrophiles incapable of growth at a_w below 0.95. It was established that the micromycetes *A. repens* and *P. chrysogenum* are able to undergo a complete life cycle in house dust forming conidial structures that do not differ in micromorphology from those on the standard agarized media.

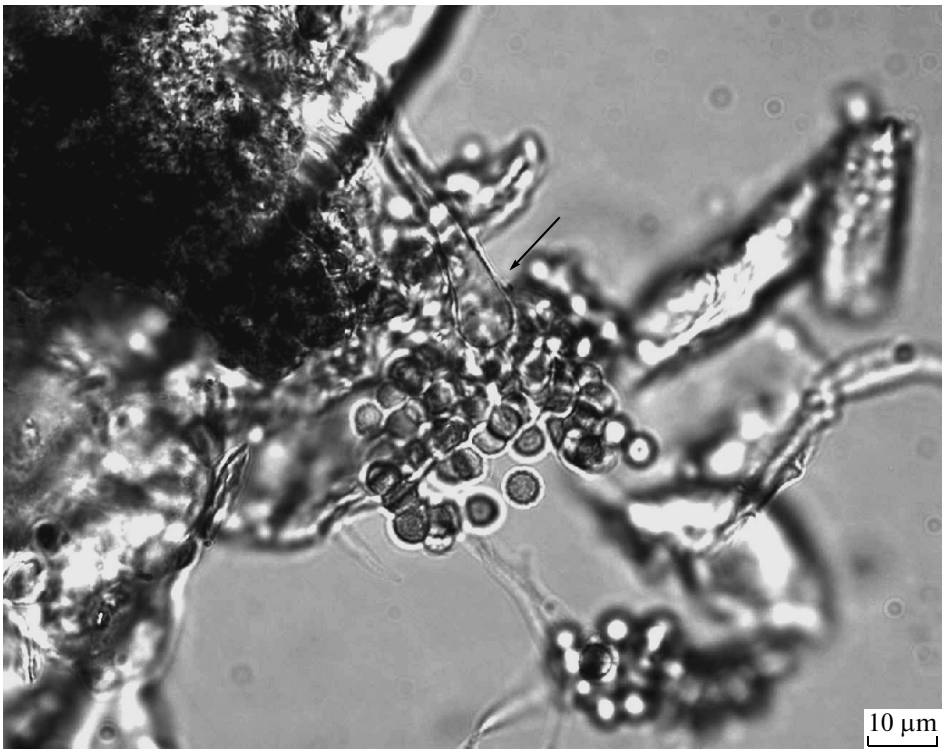

Fig. 4. *A. repens* in house dust at RH 99%, day 3. The arrow shows the conidial structures.

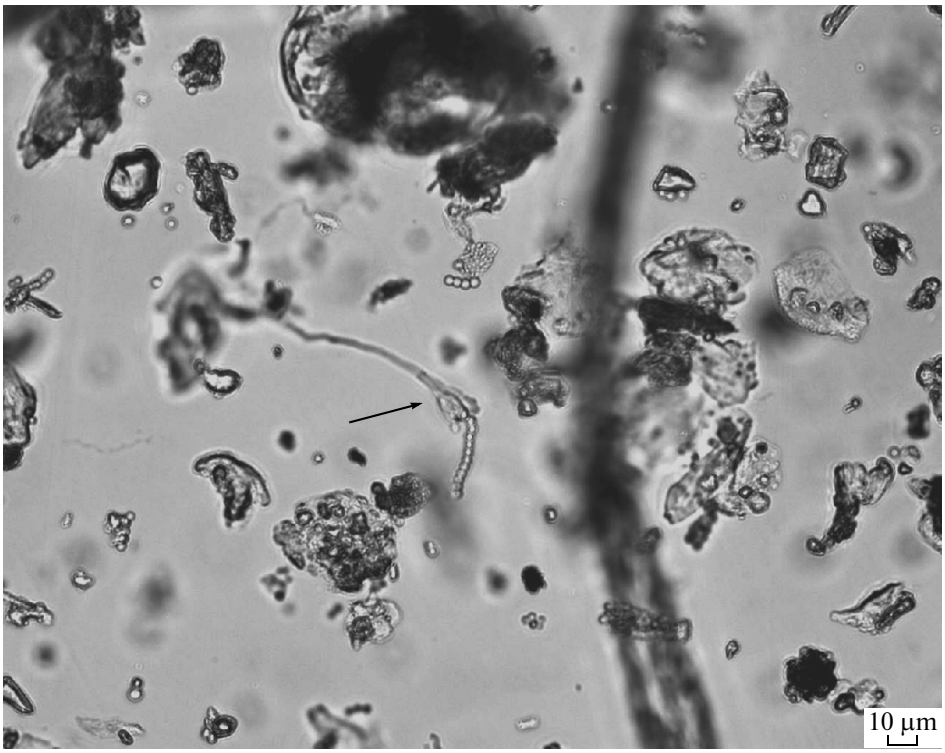

Fig. 5. *P. chrysogenum* in house dust at RH 99%, day 3. The arrow shows the conidial structures.

According to our data, the RH level in the living spaces of megalopolises varies significantly, approximately between 30 and 100%. Humidity depends on a number of factors, such as seasons, the presence or absence of central heating, emergency situations (for example, leakage of water and building errors), etc. The level of this parameter also varies inside the same apartment, for example, in living rooms and bathrooms. Thus, knowledge of the physiological characteristics of the predominant species of micromycetes will make it possible to predict the periods of their development and to plan the implementation of preventive and avoidance measures.

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