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Aspergillus niger van Tieghem AS TOXIC AND ALLERGENIC MOULD IN DWELLING BUILDINGS AFTER FLOODS

Key words: moulds, housing buildings, flood

S u m m a r y

In last years problems of moulds in dwelling buildings becomes very important and vide broached in Europe and Poland territory. It's not only for moulds in dampness buildings, but also as effect such disasters like floods. 76 species of moulds were detected on walls in dwellings therein from buildings after floods in the Lubuskie Province of which ten are the most common ones. Among those ten moulds, there are six species that, in the order of the most common allergens, cause an inflammatory condition, namely: Penicillium chrysogenum, Aspergillus niger, Cladosporium herbarum, Aspergillus versicolor, Alternaria alternata, Aspergillus flavus. Four of them were selected for biotoxicological tests: three belonging to the class of Aspergillus and one belonging to the class of Cladosporium (Table 1). The method of the bioindicating test applying Dugesia tigrina Girard (Plathelminthes, Turbellaria, Tricladida; Paludicola) was used to determine the toxicity of the selected moulds. All bio-tests gave a positive result. It means that moulds synthesise toxins that increase the danger for the inhabitants of the infected buildings (both toxicological and allergic danger). Toxicological and allergenic danger increase in dwelling buildings after floods on account of much more species of moulds. The species that proved to be the most toxic ones were Aspergillus niger and Aspergillus versicolor, whose severe toxicological values LC 50 were very similar. On the other hand, A. niger is a species of mould that may appear in infected space along with mites (Acarina) increasing the number of allergens in the housing environment. In the performed tests, the Cladosporium herbarum proved to be the least toxic species of mould, what is good news for the inhabitants of the infected buildings, since the fungus in question is very common in housing construction, its frequency value being 5.

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Introduction

Over the last years, the number of people vulnerable to allergies has increased significantly, actually it doubled last year. The highest number of people suffering of allergies is registered in Great Britain. Allergies are most commonly associated with pollen and spores of moulds in the air, and thus special announcements are broadcast in mass media, and for specific regions of Poland there are calendars of pollen discharge and periods of the highest levels of mould's spores in the air [Piontek 2004a]. Such announcements present most commonly such species of moulds as *Alternaria* and *Cladosporium*. A separate and increasing problem is that of allergies produced by moulds and mites (*Acarina*) in housing construction. It is also important problem in dwelling housing after floods.

The source of allergens of moulds are proteins. They are one of the four different products of moulds that may present a danger for health [Nielsen, Gravesen, Nielsen, Andersen, Thrane, Frisvad 1999]. Proteins cause immediate reactions within a couple of minutes after the infection (allergies type 1) [Flannigan, Mccabe, Mcgarry 1991], [Gravesen, Frisvad, Samson 1994] and secretion of histamine without agency of IgE, non-immunological reaction – pseudo allergies [Larsen, Clementsen, Hansen, Maltbæk, Gravesen, Skov, Norn, 1996].

Inhalation of a large number of spores, fragments of mycelia and other particles floating in the air influences health and this is why we should not expect visible mildew on construction partitions not to have negative influence on the inhabitants of the infected buildings. Mildewed construction partitions give off a huge number of spores (counted in thousands). Their number may periodically increase or decrease depending on the weather, the season, heating season, sporing of mildew fungi and other factors.

Spores of moulds that are released into the air and fragments of mycelia may cause allergic diseases (allergies type 1) that include: bronchial asthma (mould asthma), allergic inflammation of alveoli (alveolitis allergica, extrinsic allergic alveolitis, hypersensivity pneumonitis), allergic rhinitis, atopic conjunctivitis, toxic syndrome caused by organic dust, described in the literature as organic dust toxic syndrome (ODTS), chronic fatigue-like syndrome [Krysińska-Traczyk 2001]. Allergens of moulds that condense in badly ventilated rooms are a real danger for atopic allergy sufferers [Bogacka 1997]. It is well-known that spores of *Aspergillus, Cladosporium* and *Penicillium* fungi, which may be encountered in damp buildings, may cause asthma and/or rhinitis among atopic allergy sufferers. Atopic diseases (hay fever, atopic asthma) are suffered by more than 10% of people. Atopic allergy is a hereditary propensity to excessively synthesise IgE antibodies as compared with antigens that are common in

the environment (e.g. moulds and mites) [Nielsen, Gravesen, Nielsen, Andersen, Thrane, Frisvad 1999].

The main reason for allergies in people in the housing construction is supposed to be mites *(Acarina*). In mildewed flats, the risk of allergy increases, since apart from mites, bacteria and nematodes, there are also moulds. A peculiar biological film is formed with subsequent stages. Many species of moulds are the sustenance of mites, while dead mites, its excrements and exuviae provide fungi with organic substances. In the mycological tests performed by P i o n t e k [Piontek 2004a] in the Lubuskie Province such an inter-species interaction was observed in mildewed flats for a long period of time (couple of years) between *Acarina* and ten species of moulds: *Acremonium strictum, Aspergillus niger, Botryotrichum piluliferum, Chaetomium elongatum, Epicocum nigrum, Penicillium aurantiogriseum, P. chrysogenum, Rhizopus stolonifer, Trichothecium roseum* and *Ulocladium botrytis* [Piontek 2004a].

Most moulds, including allergenic ones, have the property of synthesis of secondary metabolic substances. What is important is that mycotoxins have immunosuppressive effects on the human immunological system what makes internal organs (e.g. kidneys, liver, and central nervous system) more vulnerable and negatively influences allergic reactions.

According to experimented conducted on animals, the respiratory tract leads toxins to tissues in an exceptionally efficient way. Creasia *et al.* [Creasia, Thurman, Wannemacher, Bunner 1990] demonstrated on tested animals that inhalation of toxins causes a systematic toxic effect more efficiently that their absorption through the mouth or viscerally [Flannigan 2001]. Since it is a large number of spores inhaled with the air are what causes allergies, it is worthwhile to relate the two questions – mycotoxicity and allergic reaction related to the presence of moulds in housing construction – and to continue the described investigation.

Materials and Methods

What was used in the mycotoxicological tests were four strains of moulds obtained from mass cultures in a malt extract agar (MEA), isolated from housing structures: *Aspergillus niger, Cladosporium herbarum, Aspergillus versicolor, Aspergillus flavus* (Table 1).

From the obtained biomes of moulds with substrate, water and methanol extracts were prepared GPM (fungi - laboratory substrate-methanol 80%). Toxicological tests of the mildew fungi were carried out using *Dugesia tigrina* Girard. Toxicity for tested animals was expressed as acute toxicity 240–h LC 50 (mg dm^{-3}, %), [Piontek 2004a, Piontek 2004b].

Table 1. Allergenic mildew fungi selected for biotoxicological tests

Species*	Size of spores	Selected important mycotoxins [Samson *et al*.,(12)]
Aspergillus niger	3.5 – 5 μm	malformins A,B,C, naftro-γ-pyrons, ochratoxin A
Cladosporium herbarum	5.5 – 13 x 4-6 μm	----------
Aspergillus versicolor	2 –3.5 μm	sterigmatocistine, 5-methoxy-sterigmatocistine
Aspergillus flavus	3.6 μm	aflatoxin B_1, cyclopiasonic acid, 3-nitropropionic acid, sterigmatocistine

* ordered from the most allergenic ones Schata et al.[Schata, Jorde, Elixmann, Linskens 1989]

All species of moulds mentioned in the table were isolated from the lungs during autopsy [Flannigan 2001]. The spores that are most easily inhales are those that are smaller than 7μm and have a small mass, what facilitates their floating in the air.

Results

The results of the tests concerned with toxicity of methanol extracts made of biomes of moulds with substrate (GPM) for *Dugesia tigrina* Girard were presented in the order from the most to the least toxic extract 240-h LC 50 (mg dm^{-3}, %), i.e. acute toxicity.

Table 2. Results of toxicity of mildew fungi extracts for Dugesia tigrina Girard

GPM (fungi-substrate-methanol)			
Species	240-h LC 50 (%)	240-h LC 50 (mg dm^{-3})*	Toxicity class**
A. niger	0.56	69.5	III (moderately poisonous)
A. versicolor	0.57	70.9	III (moderately poisonous)
A. flavus	0.85	106.0	IV (barely poisonous)
C. herbarum	1.24	155.2	IV (barely poisonous)

* biomass of moulds in the extract
**according to the classification by Liebmann [Liebmann 1962]

All extracts proved to be toxic for tested animals. The most toxic ones proved to be *Aspergillus niger* and *Aspergillus versicolor* (values 240-h LC 50

were very similar, being respectively 0.56 and 0.57%). The extracts proved to be two times more toxic than *Cladosporium herbarum* (240-h LC 50, 1.24%). In the biotoxicological tests in question, that species of fungi proved to be the least toxic mildew fungus, what confirms the fact that it does not synthesise any mycotoxins (Table 1). Between the most and the least toxic extract, there was a GPM extract from the mould called *Aspergillus flavus*, whose 240-h LC 50 was determined to be 0.85%.

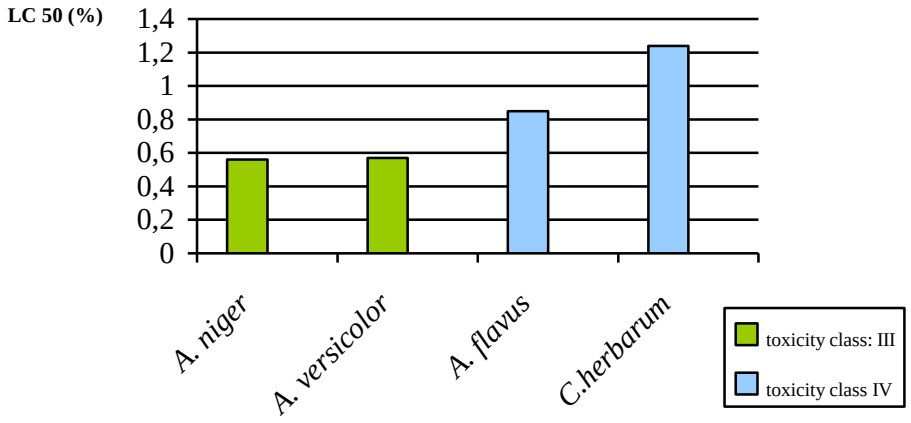

Fig. 1. LC50 values for the tested allergenic species of moulds

The level of toxicity of moulds extracts was evaluated by means of the L i e b m a n n classification [Liebmann 1962]. Moulds extracts were classified as class III of moderately poisonous compounds (*Aspergillus niger* and *Aspergillus versicolor*) and class IV of barely poisonous substances (*Aspergillus flavus* and *Cladosporium herbarum)*. None of the tested extracts was not a highly poisonous substance (toxicity class I and II).

Discussion

Four kinds of moulds present in housing structures were taken into account in the tests in question. *Cladosporium herbarum* proved to be the least toxic fungus, what is of particular importance for the inhabitants of infected buildings, since the frequency value for that fungus is high: 5 (38,4%). *Cladosporium sp.* produces a few important allergens and more than 30 others that do not have the same clinical importance [Grajewski i Twarużek 2004]. A couple of

metabolic substances (which have antimycotic properties and are inhibitors of growth of plants) were isolated from fungi belonging to the genus *Cladosporium* [Nielsen 2002]. Allergenic properties of *Cladosporium* are much lower that in the case of *Alternaria*, but in housing their condensation rises. Tests that were carried out by Piontek [Piontek 2004a] suggest that *Alternaria alternata* occurs in housing construction with the frequency of 2 (5-10%), and consequently *Cladosporium* is considered to be the main source of allergies.

Aspergillus sp. is presented as allergic moulds. The species of the genus *Aspergillus* commonly cause asthma and acute syndromes include oedema, some cases may develop into emphysema and allergic inflammation of alveoli [Twarużek 2005]. This is why three species of that genus were included in the tests. They occur with the frequency 3-rather frequently in dwelling houses (*A. flavus* – frequency 14,0%, *A. versicolor*- frequency 13,4%, *A. niger*- frequency 11,0%), [Piontek, Piontek i Bednar 2004].

All species of *Aspergillus* proved to be toxic in the tests in question, what proves that those moulds present a double risk for the inhabitants of infected houses – both in terms of toxicity as well as in those of allergies. The allergic potential increases when mites (*Acarina*) appears along with moulds. Mycological tests executed by Piontek [Piontek 2004a] suggests that such an interaction between species occurred in mildewed flats for a long period between *Acarina* and 10 species of mildew fungi. *Aspergillus niger* is mentioned among those 10 species.

The list of allergenic species of mildew fungi ordered by Schata *et al*. [Schata, Jorde, Elixmann, Linskens 1989] proves that ten among 14 species are *Penicillium* and *Aspargillus*: *Penicililum chrysogenum, P. brevicopactum, P. glabrum (P. frequentans), Aspergillus niger, Cladosporium herbarum, Aspergillus versicolor, A. fumigatus, Alternaria alternata (Alt. tenuis), Eurotium repens (*anamorphic *A. repens), E. amstelodami (*anamorphic *A. amstelodami), Aspergillus flavus, E. rubrum (A. ruber), Aureobasidium pullulans, Wallemia sebi.*

In the presented work, 4 species of mildew fungi were tested from the above mentioned list of allergenic fungi. Among them, the most allergenic one proved to be *A. niger*. At the same time, this is a species that may be found in construction partitions along with mites (*Acarina*). Biotoxicological tests that were carried out suggest that it belongs to toxicity class III (moderately poisonous). Therefore, it causes a double (toxic and allergic) risk that is increased by the correlation with mites.

Epidemiological research and obtaining appropriate extracts from allergenic mildew fungi is particularly difficult. They are concerned with:

– detection of fungus spores and their identification;

– obtaining properly standardised allergenic extract for the purposes of diagnosis and medical treatment, repeatable concentration of main and secondary allergens;
– determination of clinical criteria that will predictably be correlated with the degree of exposal to the allergen of the fungus [Bogacka 1998].

Isolation, cleaning and standardisation of allergens that are synthesized by moulds are the principal problems that make us ignore the value of erroneous measurements in the tests [Jacob, Ritz, Gehring, Koch, Bischof, Wichmann, Heinrich 2002].

Conclusions

Aspergillus niger is a species that may be found in construction partitions along with mites (*Acarina*). Biotoxicological tests that were carried out suggest that it belongs to toxicity class III (moderately poisonous). This mould produced a highly nephratoxic component ochratoxin A, which may pose a significant indoor problem. Therefore, causes a double (toxic and allergenic) risk that is increased by the correlation with mites.

Moulds, and therefore also mycotoxins, are present in the environment. If the concentration of spores and fragments of mycelium in the air is preserved at a lower level, it does not cause any reactions in a healthy organism. However, if the concentration rises, then the human and animal organism may be exposed to many diseases: allergies, mycosis, mycotoxicosis. The minimum concentration of mycotoxins of different mildew fungi species in closed rooms and the time of exposal to their effects that may cause disadvantageous results for health. Without a doubt, elimination of moulds, and therefore of mycotoxins and also allergenic proteins from dwelling houses, especially from buildings after floods may reduce the pathogenic symptoms and sometimes eradicate them [Johanning 2002].

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ASPERGILLUS NIGER VAN TIEGHEM JAKO TOKSYCZNY I ALERGOGENNY GRZYB PLEŚNIOWY W OBIEKTACH BUDOWLANYCH PO POWODZIACH

Słowa kluczowe: grzyby pleśniowe, budownictwo mieszkaniowe, powódź

S t r e s z c z e n i e

W ostatnich latach na obszarze Europy i Polski problem z występowaniem grzybów pleśniowych w budynkach mieszkalnych stał się bardzo ważny i szeroko omawiany. Nie tylko ze względu na występowanie grzybów pleśniowych w zawilgoconych budynkach, ale także w budynkach uszkodzonych na skutek takich katastrof jak powódź. Na terenie województwa Lubuskiego oznaczono 76 gatunków grzybów pleśniowych pochodzących z obiektów budowlanych, w tym także z budynków popowodziowych. Spośród oznaczonych gatunków grzybów pleśniowych, 10 z nich występuje w budownictwie z największą frekwencją. Wśród tych 10 gatunków sześć z nich powoduje alergie. W kolejności od najbardziej alergogennych gatunków są to: Penicillium chrysogenum, Aspergillus

niger, Cladosporium herbarum, Aspergillus versicolor, Alternaria alternata, Aspergillus flavus. Do przeprowadzenia testów biotoksykologicznych zostały wybrane cztery gatunki grzybów pleśniowych: trzy należące do rodzaju Aspergillus i jeden należący do rodzaju Cladosporium. Testy bioindykacyjne przeprowadzono przy użyciu wypławków Dugesia tigrina Girard. Wszystkie biotesty dały pozytywne rezultaty. Stwarza to podwójne (toksykologiczne i alergogenne) niebezpieczeństwo dla mieszkańców zainfekowanych pomieszczeń. W budynkach popowodziowych zagrożenie toksykologiczne i alergogenne wzrasta ze względu na występowanie większej ilości gatunków grzybów pleśniowych. W przeprowadzonych testach najbardziej toksyczny okazał się Aspergillus niger i Aspergillus versicolor. Najmniejszą wartość LC 50 wykazał Cladosporium herbarum, co jest dobrą wiadomością dla mieszkańców zainfekowanych pomieszczeń, ponieważ grzyb ten występuje w obiektach budowlanych z wysoką frekwencją 5.