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Growth of *Chaetomium globosum* fungus in laboratory conditions on agar medium and Scots pine wood

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Abstract: *Growth of Chaetomium globosum fungus in laboratory conditions on agar medium and Scots pine wood.* The *Ch. globosum* fungus (*Chg*) belongs to Chaetomiaceae molds fungi family, which very easy attacked different wood species causing their disfiguration and discoloration. It belongs to the cellulolytic fungi, which in favorable conditions and longer periods of interactions can impair the strength of wood as result of soft rot. *Ch. globosum* is a test fungus frequently used in the study of building materials and to the evaluation of their resistance to molds. The recognition the growth rate of *Chg* fungus on salt-agar medium in comparison to other filamentous fungi and assessing its ability to attack on natural and preserved with model wood preservative Scots pine wood, was the aim of this research. The *Chg* fungus was growing on the salt-agar medium quiet fast, slower only than *Trichoderma viride* fungus. The growth rate of *Chg* fungus during double culture-species tests on salt-agar medium was dependent on the second fungus species. It was slowest during culturing *Chg* with *Aspergillus niger* and very fast, faster than individual growth of *Chg*, as it was culturing together with *Penicillium funiculosum* fungus. The antagonism was not observed. The [DDA][NO_2] ionic liquid use as model wood preservative controlled the growth of *Chg* at 10g/m^2 retention to the extent that satisfies the requirements of the building procedures. The natural Scots pine wood was in the same conditions complete coverage with *Chg* on the surface.

Keywords: filamentous fungi, molds growth, wood, ionic liquid, efficacy

INTRODUCTION

Chaetomium globosum (Kunze ex Fr.) belongs to Chaetomiaceae molds fungi family. It is the most common species of Chaetomium found in buildings [Andersen and Nissen 2000]. *Ch. globosum* very easy attacked different wood species causing their disfiguration and discoloration. The fungus secrete cellulases, similar to such fungi as *Aspergillus fumigatus* and *Trichoderma viride*, so can be classified as a cellulolytic fungus. It is very important in biochemical degradation processes of organic materials under aerobic conditions, also when cellulose is encrusted with lignin, e.g. wood. The growth of *Ch. globosum* may cause the drop of the material value, financial loses as well as technical or technological problems with the processing of infested wood. *Ch. globosum* so as like and *T. viride* are commonly used to test the effectiveness of the impregnating agents used to fabrics, textile coverage and wood in order to prevent damage from cellulolytic organisms. *Ch. globosum* is also used among others in Poland for testing bio-resistance of building materials, in particularly the assessment of their resistance to molds [Instrukcja ITB…1998, Fojutowski *at al.* 2007, 2011]]. This fungus is often isolated from buildings, especially in areas damaged by water. Its growth may cause danger to human and environment because of mycotoxins, chaetoglobosin A and C, which are produced by it in the above mentioned conditions, but also on surfaces of construction materials in laboratory cultures. The mycotoxins, chaetoglobosin A were many times, repeatedly, detected in air, dust, fungal biomass and wallpaper sample, because of susceptibility of dry mold to making dispersion [Nielsen *et al.* 1999, Nielsen 2003, Fogle *et al.* 2007]. The threat from these cytotoxic compounds is associated with the inhibition of mammalian cell division and transport of glucose [Fogle *et al.* 2007, Ueno 1985].

The recognition the growth rate of *Chg* fungus on salt-agar medium in comparison to other filamentous fungi and assessing its ability to attack on natural and preserved with model wood preservative Scots pine wood, was the aim of this research.

MATERIALS AND METHODS

The growth rates of individual fungi and their interactions were tested on salt-agar medium. Besides of *Chaetomium globosum* (DSM 1962), pure cultures of the following, other individual fungi, often used in testing resistance of building materials [Instrukcja ITB….1998] were used: *Aspergillus niger* van Tieghem (DSM 12634), *Ophiostoma piliferum* (Fries:Fries) H.&P. Sydow (DSM No 4920), *Penicillium funiculosum* Thom (DSM 2213), *Paecilomyces variotii* Bainier (DSM 1961) and *Trichoderma viride* Persoon:Fries (DSM 63065). A suspension of mould spores of the density of 1×10^6 conidia/cm^3 of the individual fungi was prepared. Filter paper discs of the diameter of 5 mm, immersed for 2 seconds in the suspension were placed as inoculum (infestation) on salt-agar medium in Petri dishes of the diameter of 90 mm. The inoculum was placed close to one edge of a Petri dish in the case of a one-species test or by two edges of a Petri dish, opposite to each other, in the case of double-species test (distance ~70 mm). Incubation conditions were: the temperature of 27$\pm$1ºC and relative humidity of 90%. The growth of fungi was measured in mm (also calculated in %, taking maximum of the distance as 100%) after 4, 5, 6, 7, 8, and 14 days or to the moment when one of fungus species meets the other. The interactions of fungi were observed and recorded at the moment when the fungi met, according to the following conventional scale: 0 – no further increase in fungus growth, + – further increase in fungus growth, and i – zone of inhibition (mm). A method adapted from building procedures [Instrukcja ITB....1998] was used for mycological testing of *Chaetomium globosum* (*Chg*) growth on natural and preserved wood. The materials used in the tests was Scots pine (*Pinus sylvestris* L.) sapwood of the quality meeting the requirements of [EN 113] standard and mean density of approximately 550 kg·m^{-3}. Before the exposure to fungi samples were sterilized with steam in an autoclave (20 min., 121ºC). A set of 6 wooden discs of the nominal diameter of 50mm or 25mm (tangential/radial surface) and 4 mm thick cut out from the sapwood was used for each variant of tested wood samples, i.e. natural wood (control wood) and samples treated with model wood preservative. A preservative containing [DDA][NO_2] ionic liquid was used. The samples of wood were treated with the wood preservative by brushing method to retention of 40, 25, 15, 10, 5 g·m^{-2} (on samples of 50mm diameter) and of 1 or 0,5 g·m^{-2} (on samples of 25mm diameter). A suspension of mould spores of *Chg* fungus of the density of 1×10^6 conidia·cm^{-3} was sprayed on the surface of tested wood samples placed individually on the surface of salt-agar medium in Petri dishes with 90mm diameter and outside height of 15 mm, and incubated at the temperature of 27$\pm$1ºC and relative humidity of 90%. In earlier studies we found that infection samples disposed on a substrate salt-agar medium or infection salt-agar and subsequent placement of uninfected samples on the infected surface, had no significant effect on the rate of overgrowth by fungi wood samples in the laboratory conditions [Fojutowski *at al.* 2012, 2013]. The activity of the fungi spores used in the test was evaluated by checking their growth on salt-agar medium and on control Scots pine wood samples. After 4 weeks the growth of mycelium on the surface of test samples was evaluated using the following scale:

0 – no growth of fungi on a sample visible under microscope,

1 – trace growth of fungi on a sample, hardly visible to the naked eye but well visible under microscope or growth limited to the edges of a sample, visible to the naked eye,

2 – growth of fungi on a sample, visible to the naked eye, but less than 15% of the surface is covered with fungus,

3 – over 15% of the surface is covered with fungus visible to the naked eye.

A standard evaluation was completed with the visual estimation of the percentage of the sample surface overgrown by mycelium.

As part of standard evaluation also was made visual evaluation of intensity of fungi growth on the wood surface (Fojutowski *et al.* 2014): 3 – strong, very thick mycelium, 2 – medium thick mycelium, 1 – weak, thin mycelium, 0 – lack of growth visible to the naked eye.

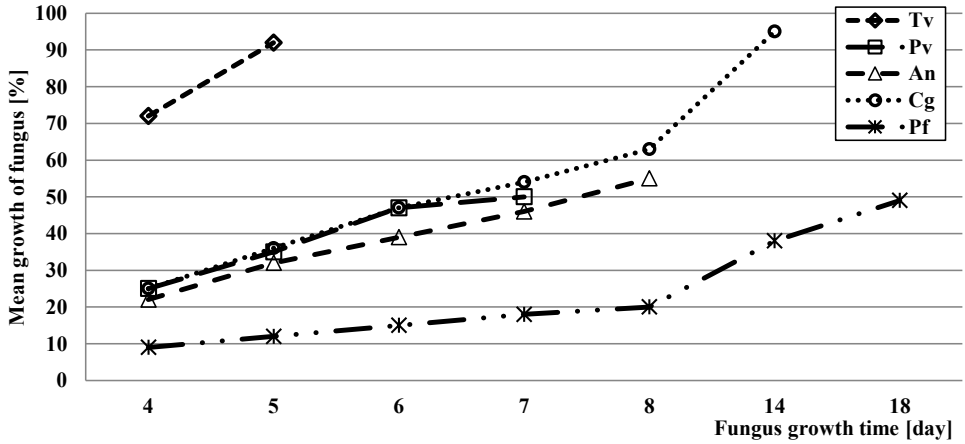
The final state of samples infestation with fungi after 4 weeks of their growth was assessed also instrumentally by measurement the surface of wood samples covered by fungi.
The determination of wood surface covered by fungi were made with computer assisted measurement of relevant part of samples. The samples with growing fungi (mycelium) were individually photographed and the boundary of fungi growth was in detail marked manually on the blow-up photograph. The part of samples covered with fungi was then very precisely automatically determined in percent of whole exposed surface of the samples with Images Plus 2.0 and OptaView 7.1.0.4 computer programs.
The final state of samples infestation with fungi was assessed also instrumentally by measurement the color of wood samples. It was made with the use of the Elrepho 2000 Data Color (on wood samples of 25mm diameter) and CR410 colorimeter (on wood samples of 50mm diameter) in CIE Lab system (Bekhta and Niemz 2003) where the coordinates of color *L, a, b* and the total color change ΔE were determined according to the formula:

$$\Delta E=[(L_1-L_o)^2+(a_1-a_o)^2+(b_1-b_o)^2]^{1/2}$$

L_1, a_1, b_1 – coordinates of color of wood before mycological test,
L_o, a_o, b_o – coordinates of color at the end of mycological test.

RESULTS

The *Ch. globosum* fungus was growing on the salt-agar medium in one-species test quiet fast, slower only than *Trichoderma viride* (*Tv*) fungus, which was showing the fastest growth rate among tested fungi (Fig. 1). *Penicillium funiculosum (Pf)* fungus characterized by the slowest growth. The growth rate of *Chaetomium globosum* (*Cg*) fungus was similar to that of *Paecilomyces variotii*, however the range growth of *Ch. globosum* was almost two-fold great than that of *Pv*.



[1)] *Tv – Trichoderma viride, Pv – Paecilomyces variotii, An – Asperillus niger,Chg – Chaetomium globosum, Pf – Penicillium funiculosum,*
[2)] y-axis - the percent of maximum distance possible for the fungus growth

Figure 1. The individual growth of tested filamentous fungi from one inoculum on salt-agar medium in Petri dishes

The growth rate of *Chg* fungus during double culture-species tests on salt-agar medium was dependent on the properties of second fungus species growing in the same culture vessel (Table 1). The fungus *Ch. globosum* grew faster than the three of the fungus tested together with it (*An, Pv, Pf*) and slowly only in comparison with the growth of the fungus *Trichoderma viride*. It was

slowest during culturing *Chg* with *Aspergillus niger* and very fast, faster than individual growth of *Chg*, as it was culturing together with *Penicillium funiculosum* fungus. The *Chg* growth rate of 83% and 87% of that stated in condition of *Chg* individual growth was stated in case of growth with *Tv* and *Pv* fungi respectively. The antagonism was not observed. *An, Pv* and *Tv* fungi limited to some extent the growth rate of *Chg* fungus in comparison to it individual growth. The main effect of the interaction between fungi is no further increase of growth for each side after contact mycelium. Only in the case of the *Trichoderma viride* it was stated a slight continuation of growth by *Chaetomium globosum* despite contact mycelium of different species.

Ch. globosum fungal showed good activity for developing on wood. It was growing fast and completely cover the surface of nutrient medium in Petri dishes within 3-5 days after infection, as it is required in the test procedure. The fungus in test with wood was also covered completely the surface of control wood, however according to the test procedure a 75% coverage of control wood surface by fungus/fungi is sufficient for test validity. It was stated not only at the end of the test period (28 days), but even earlier (Table 2). The fungus covered the surface of control wood in the highest grade of 3.0, meaning more than 15% of the wood surface, but in fact it was 100%, with grade 3 of the growth intensity. The results of susceptibility tests of Scots pine sapwood treated against mould fungi with different retentions of model wood preservative (Table 2) showed that *Chg* fungus was able to attack the surface of all tested wood samples with retention of [DDA][NO_2] not greater than 10g·m^{-2}; however to different extents depending on the preservative retention and with weak or mean intensity – grade 1 to almost 2.

Table 1. The growth of tested filamentous fungi in double-species test in relation to *Chaetomium globosum* in Petri dishes (two different inoculum on salt-agar medium opposite each other)

Main fungus: Chaetomium globosum/Test fungus[1)]	Fungi growth time [day]						State after both fungi met[2)]	Growth rate [mm/day]
	4	5	6	7	8	14		
		Distance overgrown by fungi [mm]						
Chg	20	27	33	36	36	37	0	2.64
An	18	25	31	32	32	33	0	2.36
***Chg** (individual)*	**20**	**29**	**37**	**43**	**50**	**76**	not applicable	**5.43**
Chg g	20	26	34	37	38		0	4.75
Pv	15	24	31	31	31		0	3.88
Chg	20	28	35	42	50		0	6.25
Pf	8	11	12	16	20		0	2.50
Chg	18						-	4.50
Tv	50						0	12.50

[1)] legend as to Table 1; [2)] 0 – lack of fungi growth after they met, + further growth of test fungus after meeting with main fungus i.e. *Chg*; - further growth of main fungus i.e. *Chg* after meeting with test fungus

The 10g·m^{-2} retention of [DDA][NO_2] was however sufficient to control the growth of *Chg* to the extent that satisfies the requirements of the building procedures (standard grade ≤1). The greater retentions of model wood preservative completely controlled growth of Chg fungus (a lack of growth). The computer assisted measurement of surface covered with the Chg fungus indentified it very distinctly and clearly, similar to visual assessment. The measurement of total color change of wood as result of fungus growth were also very clear, but much less diverse than the results of the remaining marks growth of the fungus. Total change of color samples overgrown by fungus *Ch. globosum* was 30.3 to 36.3 for samples not preserved or unsatisfactorily preserved, for the effective preserved wood 12.3 to 15.0. The boundary between

the growth of the fungus meeting the requirements of building procedures and does not meet these requirements, was however in total color wood change, also very clear. Generally, the results confirm different susceptibility of wood to *Chg* attack depending on wood preservative retention.

Table 2. The state of attack by *Chaetomium globosum* fungus on Scots pine sapwood samples treated with [DDA][NO_2] after 4 weeks from infestation, determined by visual grading and computer assisted measurement of mould fungi growth on wood surface and by instrumental measurement of the color change on wood

Fungus	Model wood preservative retention [g·m^{-2}]	Visual evaluation of fungal growth on the samples surface			Computer assisted measurement of fungus growth	Total color change ΔE
		Grade	Intensity	[%]	[%]	
Chaetomium globosum	40*	0.0	0.0	0.0	0.0	15.0
	25*	0.0	0.0	0.0	0.0	12.6
	15*	0.0	0.0	0.0	0.0	12.3
	10*	0.7	0.7	<1	1.0	14.2
	5*	0.8	0.7	2.0	2.0	13.9
	0*	3.0	3.0	100	100	30.3
	1**	3.0	1.3	100	100	32.7
	0,5**	3.0	1.9	100	100	33.6
	0**	3.0	3.0	90-100	100	36.6

* samples of 50 mm diameter
**samples of 25 mm diameter

CONCLUSIONS

1. The growth of *Chaetomium globosum-Chg* fungus on salt-agar medium is very easy and fast, both in individual (slower only than *Trichoderma viride-Tv* but faster than *Aspergillus niger-An*, *Paecilomyces variotii-Pv* and *Penicillium funiculosum-Pf*) and double-species cultivation (slowest with *An*, fastest with *Pf*).
2. It was not antagonism between *Chaetomium globosum* and *Aspergillus niger* or *Paecilomyces variotii* or *Penicillium funiculosum* or *Trichoderma viride* in double-species tests on salt-agar medium; however *An, Pv* and *Tv* limited, to some extent, the growth of *Chg*.
3. The Scots pine sapwood treated with [DDA][NO_2]-model wood preservative at 10g·m-^{2} retention was however sufficient to control the growth of *Chg* to the extent required by the building procedures.
4. The assessment of fungal growth on the surface of samples Scots pine wood by computer assisted measurement and total wood colour change measurement is more distinct but general similar to visual evaluation, so instrumental method may supplement a descriptive assessment.

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Streszczenie: *Wzrost grzyba Chaetomium globosum w warunkach laboratoryjnych na pożywce agarowej i drewnie sosny.* Grzyb *Ch. globosum* (*Chg*) należy do rodziny grzybów pleśniowych Chaetomiaceae, które bardzo łatwo atakują drewno różnych gatunków drewna powodując ich zniekształcenie i przebarwienie. Zaliczany jest do grzybów celulolitycznych, które w sprzyjających warunkach i długotrwałym działaniu mogą pogarszać wytrzymałość drewna w wyniku rozkładu szarego. *Ch. globosum* jest grzybem testowym często stosowanym w badaniach materiałów budowlanych i ocenie ich odporności na pleśnienie. Celem badań było rozpoznanie wzrostu grzyba *Chg* na pożywce solno-agarowej w porównaniu z innymi grzybami strzępkowymi i ocena jego zdolności do atakowania naturalnego drewna bielu sosny zwyczajnej i zabezpieczonego modelowym środkiem ochrony drewna. Grzyb *Chg* rósł na pożywce solno-agarowej całkiem szybko, wolniej jedynie niż grzyb *Trichoderma viride*. Szybkość wzrostu *Chg* w hodowli dwukulturowej na pożywce solno-agarowej była zależna od drugiego gatunku grzyba. Była wolniejsza w hodowli *Chg* z *Aspergillus niger* i bardzo duża, szybsza, niż wzrost *Chg* w hodowli indywidualnej, podczas hodowli z *Penicillium funiculosum.* Nie stwierdzono antagonizmu. Ciecz jonowa [DDA][NO_2] zastosowana, jako modelowy środek ochrony drewna, przy retencji $10 g \cdot m^{-2}$ ograniczyła wzrost *Chg* do poziomu spełniającego wymagania procedur budowlanych pod względem odporności na pleśnienie. Naturalne drewno bielu sosny zwyczajnej w tych samych warunkach zostało całkowicie pokryte na powierzchni przez *Chg*.

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