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A low nutrition medium improves the determination of fungicidal activity of AgCl on cellulose fibres

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Abstract

The fungicidal characteristics of an anti-microbial finish on cellulose fibres based on AgCl in combination with a reactive, silicon-based organic-inorganic binder was determined. The finish was applied to the cotton fabric by the exhaustion method, followed by wringing, drying and condensation of the fabric to achieve a cross linking of the reactive binder. The influence of the finish on the morphological characteristics of the fabric was studied by scanning electron microscopy. The silver concentration on the coated sample was determined by the inductively coupled plasma mass spectroscopy. The fungicide test was carried out for the fungi Aspergillus niger and Chaetomium globosum according to the DIN 53931 standard method with the use of malt extract agar (MEA) culture medium enriched by oat-meal. The results showed that MEA enriched by oat-meal is inappropriate medium for determining the toxicity of the silver coating on cotton fabric, due to its high nutritious value, which caused intensive overgrowth of the studied fungi, making the evaluation of antifungal activity impossible. Therefore, the synthetic nutrient-poor agar (SNA) culture medium was used instead. When mixed into SNA, AgCl inhibited the

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Nizko hranljivi medij izboljša določitev fungicidnega delovanja AgCl na celuloznih vlaknih

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Izvleček

Namen raziskave je bil določiti fungicidne lastnosti protimikrobne apreture, pripravljene iz AgCl v kombinaciji z reaktivnim vezivom na podlagi silicijeve spojine, na celuloznih vlaknih. Nanos apreture na bombažno tkanino je bil izveden po izčrpalnem postopku. Sledili so ožemanje, sušenje in kondenziranje tkanine, pri čemer je poteklo zamreženje reaktivnega veziva. Vpliv apreture na morfološke lastnosti tkanine je bil preučevan z vrstično elektronsko mikroskopijo, koncentracija srebra na apretiranem vzorcu pa je bila določena z masno spektrometrijo z vzbujanjem v induktivno sklopljeni plazmi. Fungicidni test je bil izveden po standardu DIN 53931 na ploščah s trdnim MEA-gojiščem, obogatenim z dodatkom ovsenih kosmičev, za glivi *A. niger* in *C. globosum*. Visoko hranilno MEA-gojišče z ovsenimi kosmiči, ki ga predpisuje standardna metoda, je manj primerno za preučevanje toksičnosti srebra v apreturi na bombažni tkanini za uporabljeni glivi. To je vodilo do modifikacije standardne metode in uporabe manj hranilnega SNA-gojišča. AgCl, dodan v SNA, učinkovito zavre rast obeh preučevanih gliv. Toksično deluje tudi v apreturi na bombažni tkanini pri uporabljeni koncentraciji 130 ppm. AgCl je učinkovitejši fungicid za glivo *C. globosum* kot za *A. niger*. Rast glive *C. globosum* v celoti zavre, medtem ko micelij glive *A. niger* prerase gojišče pod apretiranim vzorcem.

Ključne besede: celuloza, srebrov klorid, protimikrobna apretura, fungicidna aktivnost, vpliv gojišča

growth of both studied fungi efficiently. At the concentration of approximately 130 ppm, AgCl was also toxic in the coating on the cotton fabric, suppressing the growth of Chaetomium globosum more efficiently than Aspergillus niger.

Key words: cellulose, silver chloride, antimicrobial finishing, fungicidal activity, influence of culture medium

1 Introduction

Silver compounds represent an important group of anti-microbial agents [1–4]. When bound to a solid surface or in a solution, they act as an effective bactericide and fungicide even at low concentrations. Their great advantage compared to other anti-microbial agents is their biological compatibility and low toxicity to humans [5]. The anti-microbial mechanism is poorly known. However, it seems that Ag^+ ions act toxic to unicellular micro-organisms by binding easily to negatively-charged carboxyl, thiol and phosphate side groups in microbial cells [6–8] and among other, also by interacting with thiol groups of proteins. At high concentrations, silver and silver ions are also toxic to fungi [9].

Despite the fact that silver acts as an anti-microbial agent on a wide spectrum of micro-organisms, which includes bacteria as well as fungi, it is seen from the literature [10–15] that the bactericidal action of silver has been much more widely studied than its fungicidal action. The antibacterial action of silver compounds on textile fibres is most often evaluated either by determination of reduction of colony forming units or zone of inhibition. In the case of zone of inhibition determination, a contact between the textile sample and the nutrient agar inoculated with the bacteria is established. After certain time of incubation, the assessment of degree of bacteria growth in the area where the sample was in contact with the nutrient agar and the size of the inhibition zone on the agar plate around the sample area is made. While several different established standard and modified methods are available for bacteriological tests, there are only a few methods to determine the fungicidal activity of the agents. The tests

1 Uvod

Srebrove spojine predstavljajo pomembno skupino protimikrobnih sredstev [1–4]. Vezane na trdno površino oziroma v raztopini že pri zelo nizkih koncentracijah delujejo kot učinkovit baktericid in fungicid. Njihovi veliki prednosti pred drugimi protimikrobnimi sredstvi sta biološka kompatibilnost in majhna toksičnost za človeka [5]. Kljub temu, da mehanizem protimikrobnega delovanja srebra še ni docela raziskan, je znano, da biocidno delujejo ioni Ag^+, ki so toksični za enocelične mikroorganizme. Mehanizem protibakterijskega delovanja srebrovih kationov [6–8] je povezan z njihovo afiniteto do žvepla in fosforja. Srebrovi kationi se z lahkoto vežejo na negativno nabite karboksilne, tiolne in fosfatne stranske skupine v celicah bakterij. Pri tem prizadenejo različna mesta v celici in ovirajo oziroma dezaktivirajo njene kritične fiziološke funkcije, kot so sinteza celične stene, membranski transport, sinteza nukleinskih skupin, na primer DNA in RNA, ter transport elektronov. Interakcije s tiolnimi skupinami proteinov imajo za posledico oviranje encimatskih funkcij. Vezanje srebrovih kationov v DNA bakterije povzroči izgubo njene reprodukcijske sposobnosti. Vse to je za bakterije uničujoče. Srebro in srebrovi ioni so pri dovolj visoki koncentraciji toksični tudi za glive [9].

Kljub dejstvu, da deluje srebro protimikrobno na širok spekter mikroorganizmov, ki vključuje tako bakterije kot glive, je iz literature razvidno [10–15], da je baktericidno delovanje srebra preučeno v veliko večji meri kot njegovo fungicidno delovanje. V teh raziskavah se protibakterijsko delovanje srebrovih spojin na tekstilnih vlaknih največkrat ovrednoti na dva načina. Pri prvem načinu se določi bakterijska redukcija, dobljena v suspenziji bakterij, ki so bile v stiku s tekstilnimi vlakni, oplemenitenimi s srebrom. Pri drugem načinu se vzpostavi stik med tekstilnim vzorcem in bakterijami, cepljenimi na hranilni agar. Po določenem času inkubacije se oceni stopnja rasti bakterij v območju, v katerem je bil vzorec v stiku s hranilnim agarjem, in določi velikost območja inhibicije na agar plošči ob vzorcu. V primerjavi z bakteriološkimi testi, pri katerih je na razpolago veliko različnih uveljavljenih standardnih in modificiranih metod, pa je za določitev fungicidne aktivnosti sredstev teh veliko manj. Testi se izvedejo z zakopom tekstilnega vzorca v zemljo [16], pri čemer se določi njegova odpornost proti gnitju, v primeru ploskovnih tekstilij pa lahko tudi na plošči s hranilnim agarjem, prekritim s sporami gliv. Medtem ko test z zakopom le posredno kaže odpornost tekstilije proti glivam, ki predstavljajo pomembno skupino mikroorganizmov v humusni zemlji, pa je za določitev fungicidnega delovanja sredstva za določeno glivo primeren le test na plošči, prekriti z agarjem.

V raziskavi smo podrobno preučili fungicidno delovanje apreturnega sredstva, ki je vsebovalo srebrov klorid, na bombažni tkanini. Pri tem smo uporabili metodo v skladu s standardom DIN 53931, ki se izvaja na plošči, prekriti z agarjem, in je namenjena za določitev fungicidne učinkovitosti sredstev na ploskovnih tekstilijah.

include determination of resistance to biofouling after textile samples were incubated in soil [16] or placed on nutrient agar media and inoculated with fungal spores. In the case of the latter, the determination of the fungicidal activity of the finishing agent against a certain type of fungus can be made.

In the study, the fungicidal action of the finishing agent on cotton fabric containing silver chloride was studied in detail by applying the DIN 53931 standard method. This method is performed on malt extract agar plates and is used for the determination of the fungicidal efficacy of the agents on plane textiles. We aimed to determine if, at the concentration used, the finishing agent acts fungicidal and if the applied standard method is suitable for such studies.

2 Experimental

2.1 Materials

Plain-weave 100% cotton fabric with a mass of 164 g/m² was used in the experiments. In a pre-treatment process the fabric was bleached and mercerised. A pH of water extract of fabric reached a value of 6.7 when concentrated, and 7.0 when ten times diluted.

As a fungicidal agent, a commercial product iSys AG was used in combination with iSys MTX (CHT, Germany). The former is a dispersion containing AgCl (AG) and the latter is a reactive organic-inorganic binder (RV). All products can be mixed with water to any desired concentration.

2.2 Finishing of cotton fabric

The AG-RV finish was applied to cotton fabric by the exhaustion method, where 0.15% AG and 0.57% RV o. w. f. was used. The samples were immersed in AG-RV sol-gel solution with a ratio of 1 : 50 until equilibrium was achieved. Afterwards, the sample was wrung by a wet-pick-up of 80 ± 1%, dried at 120 °C and cured at 150 °C for 1 minute.

2.3 Scanning electron microscopy (SEM) and Inductively coupled plasma mass spectroscopy (ICP-MS)

The morphology of the coated cotton fabrics was studied by a JEOL JSM 5800 scanning electron

Želeli smo ugotoviti, ali apreturno sredstvo pri uporabljeni koncentraciji deluje fungicidno in ali je izbrana standardna metoda primerna za tovrstne raziskave.

2 Eksperimentalni del

2.1 Podatki o tkanini in apreturnem sredstvu

V raziskavi smo uporabili tkanino iz 100 % bombaža v vezavi platno s ploščinsko maso 164 g/m² ter gostoto osnove 30 niti/cm in gostoto votka 24 niti/cm. Tkanina je bila predhodno beljena in mercerizirana ter s tem pripravljena za plemenitenje. pH koncentriranega vodnega ekstrakta tkanine je znašal 6,7, pH desetkrat razredčenega vodnega ekstrakta pa 7,0.

Za fungicidno sredstvo smo izbrali tržni produkt iSys AG v kombinaciji z iSys MTX (CHT, Nemčija). Prvi je disperzija težko topne soli AgCl (AG), drugi pa reaktivno organsko-anorgansko vezivo na podlagi silicijeve spojine (RV). Oba produkta se za dosego ustrezne koncentracije razredčita z vodo.

2.2 Nanos apreturnega sredstva na tkanino

Apretiranje smo izvedli po izčrpalnem postopku, pri katerem smo uporabili 0,15 % AG in 0,75 % RV na maso blaga. Kopel smo pripravili v kopelnem razmerju 1 : 50 in v njej obdelovali vzorec tkanine, dokler se ni vzpostavilo ravnotežje. Nato smo vzorec oželi na dvovaljčnem fularju z 80 % ožemalnim učinkom, ga sušili pri temperaturi 120 °C in kondenzirali pri temperaturi 150 °C 1 minuto.

2.3 Vrstična elektronska mikroskopija (SEM) in masna spektrometrija z vzbujanjem v induktivno sklopljeni plazmi (ICP-MS)

Morfološke lastnosti apretiranega vzorca tkanine smo preučili z vrstično elektronsko mikroskopijo na aparatu Jeol JSM 58000. Koncentracijo srebra na apretiranem vzorcu smo določili z masno spektrometrijo z vzbujanjem v induktivno sklopljeni plazmi (ICP-MS) na spektrofotometru Perkin Elmer SCIEX Elan DRC. Vzorce velikosti 0,5 g smo pripravili v mikrovalovnem sistemu Milestone s kislinskim razklopom z uporabo HNO_3 in H_2O_2.

2.4 Fungicidno delovanje

Fungicidno delovanje srebra na vzorcih bombažne tkanine smo določili po standardu DIN 53931 za glivi *Aspergillus niger* (*A. niger*, ATCC 6275) in *Chaetomium globosum* (*C. globosum*, ATCC 6205). V skladu s standardno metodo smo pripravili MEA-gojišče z dodatkom ovsenih kosmičev. Na plošče s hranilnim gojiščem smo ločeno nanesli suspenzije trosov obeh gliv in inkubirali 24 ur pri temperaturi 29 °C. Po inkubaciji smo na gojišče prenesli vzorce apretirane tkanine, za primerjavo pa tudi vzorce neapretirane tkanine, potem pa ponovno inkubirali 7 in 14 dni. Po inkubaciji smo določili stopnjo preraščenosti vzorca z glivo in intenziteto

microscope (SEM). The concentration of the silver on coated sample was determined by ICP-MS on a Perkin Elmer SCIED Elan DRC spectrophotometer. A sample of 0.5 g was prepared in a Milestone microwave system with acid decomposition using HNO_3 and H_2O_2.

2.4 Fungicidal activity

The fungicidal activity of the silver on cotton samples was estimated for the Aspergillus niger strain ATCC 6275 and the Chaetomium globosum strain ATCC 6205 by applying DIN 53931 Standard Method. According to this method a malt extract agar (MEA) with the addition of oat-meal is prescribed as a nutrient medium. Spore-suspensions of both fungi were spread on nutrient agar plates and incubated at 29 °C for 24 hours. Afterwards, samples of coated and untreated cotton fibres were placed on the medium and incubated at 29 °C for 7 and 14 days. After incubation, the mycelial growth on and below the surface of the cotton fibres and the intensity of sporulation was determined. The degree of fungal growth was graded from 00 to 5, where 00, indicates no growth; 0, fungal growth outside an inhibition zone surrounding the cotton specimen; [0] fungal growth up to the specimen's edge; 1, fungal growth only on and bellow the specimen's edge; 2, fungal growth on and bellow less than 25% of the specimen; 3 fungal growth on and bellow 25–75% of the specimen; 4, fungal growth on and bellow more than 75% of the specimen; and 5, 100% overgrowth of the specimen. The intensity of sporulation was assessed using the fol-

rasti glive. Stopnjo preraščenosti vzorca smo podali z ocenami od 00 do 5, pri čemer 00 pomeni, da je celotno gojišče neprerašče-no z glivami, 0 nepreraščen vzorec, ob robu katerega se je oblikovala cona inhibicije, (0) nepreraščen vzorec brez cone inhibicije, 1 preraščen rob vzorca, 2 vzorec, preraščen manj kot 25 %, 3 vzorec, preraščen od 25 do 75 %, 4 vzorec, preraščen nad 75 %, in 5 popolnoma preraščen vzorec. Intenziteto rasti gliv smo ocenili z znaki, pri čemer + pomeni zelo slabo intenziteto, samo micelij brez spor, ++ micelij, delno spore in +++ zelo močno intenziteto, močno razvite spore.

3 Rezultati z razpravo

Na sliki 1 sta prikazana SEM-mikrografa bombažne tkanine pred nanosom apreture AG-RV in po njem. Iz njiju so razvidne morfološke spremembe in porazdelitev delcev srebra po bombažnih vlaknih, ki smo jih apretirali z AG-RV (slika 1B). Srebrovi delci so bili krogelne oblike in velikosti od 100 do 500 nm. Njihova koncentracija je bila približno 130 ppm.

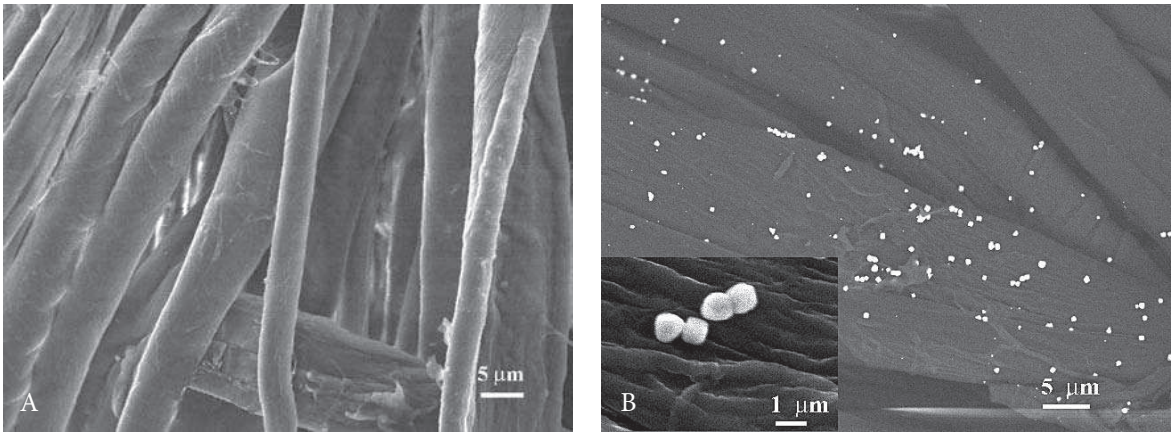

Figure 1: SEM micrograph of untreated cotton fibres (A) and fibres finished with AG-RV finish (B).

Na sliki 2 so prikazani rezultati protimikrobnega testa na gojišču MEA. Na podlagi teh rezultatov bi lahko brez dvoma hitro zaključili, da srebro ne deluje toksično za preučevani glivi, saj sta obe intenzivno prerasli površino apretiranih vzorcev. Prisotnost močno razpredenega micelija in visoka stopnja sporulacije obeh gliv na apretiranih vzorcih sta dobro razvidni s slike 3. Ob takšnih nepričakovanih rezultatih so se pojavila naslednja vprašanja: ali sredstvo AG, za katero smo že dokazali odlično protibakterijsko aktivnost [17], ne deluje fungicidno; ali je njegova koncentracija na bombažnih vlaknih prenizka za učinkovito fungicidno delovanje; ali je morda za dobljene rezultate kriva uporaba visoko hranilnega MEA-gojišča z dodanimi ovsenimi kosmiči, ki ga predpisuje uporabljena standardna metoda za teste z glivo *A. niger*? To gojišče bi zaradi svoje velike hranilne vrednosti lahko povzročilo tako agre-

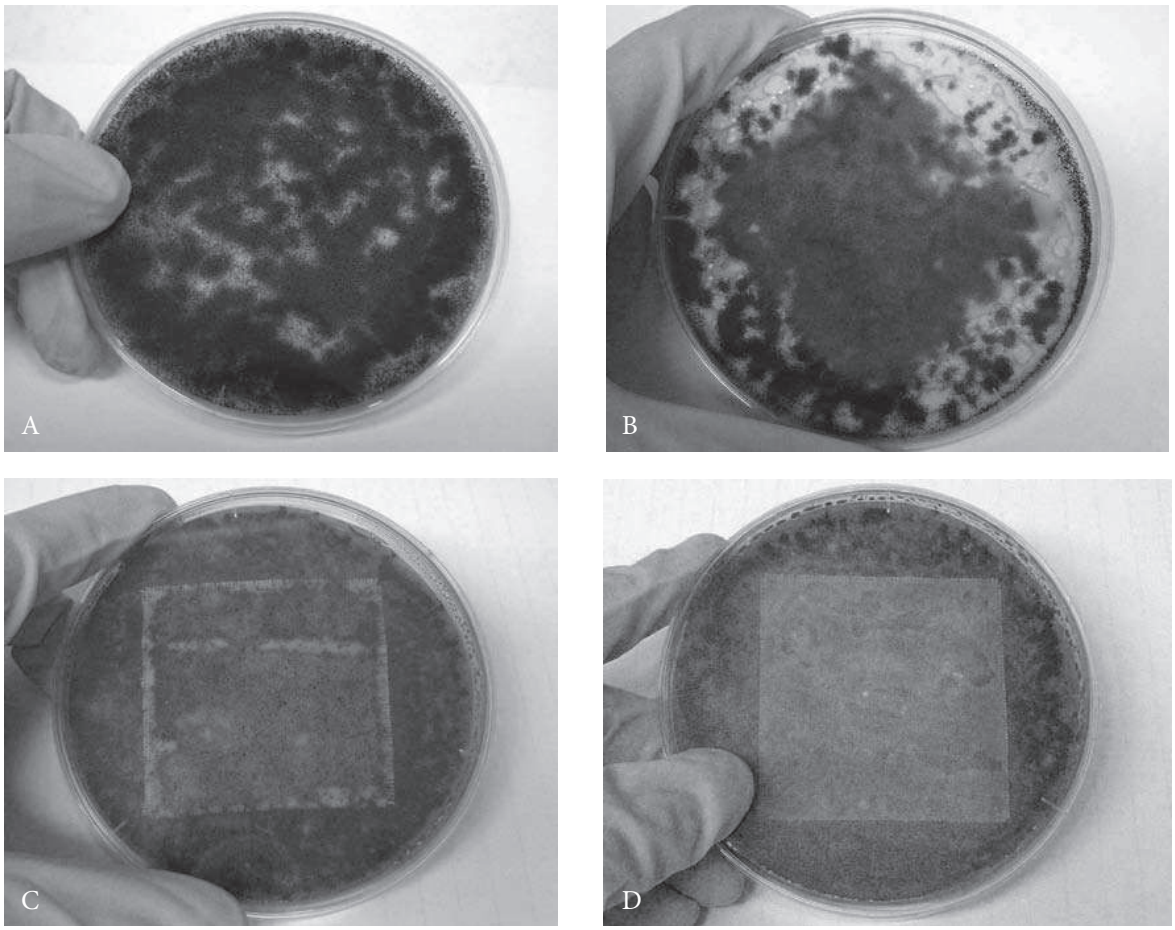

Figure 2: Growth of fungi A. niger (A and B) and C. globosum (C and D) on MEA culture medium covered with a sample of untreated cotton fabric (A and C) and an Ag-RV coated fabric (B and D) after seven days of incubation.

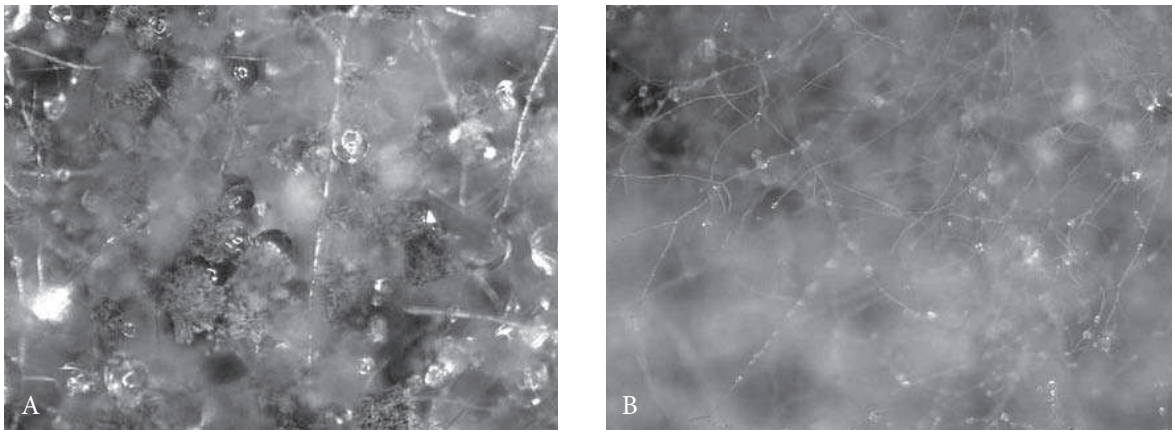

Figure 3: Growth of fungi A. niger (A) and C. globosum (B) on MEA culture medium in contact with the sample of cotton fabric coated with Ag-RV at 6.5× magnification.

lowing symbols: + weak, only mycelium; ++ noticeable growth, partly spores; and +++ strong overgrowth, extensive spore formation.

sivno rast gliv, da je tudi srebrovi ioni ne bi mogli uspešno zavreti, in v tem primeru ne bi bilo primerno za določitev fungicidnega delovanja srebra.

3 Results with discussion

From the SEM micrographs of the cotton fabric before and after the application of the AG-RV finish (Figure 1) morphological changes and the distribution of the silver particles on the AG-RV finished cotton fabric (Figure 1B) can be observed. The silver particles were spherical in shape and from 100 to 500 nm in size. Their concentration was approximately 130 ppm.

From the results of the anti-microbial test (Figure 2) one could conclude that silver does not act toxic to the studied fungi, since, despite the presence of the anti-microbial agent, they have both intensively overgrown the surface of the finished samples. The presence of a wide-spread mycelium and the high degree of sporulation of both fungi on

Da bi odgovorili na vsa zastavljena vprašanja, smo visoko hranilno MEA-gojišče z dodanimi ovsenimi kosmiči zamenjali s sintetičnim hranilnim gojiščem (SNA) [18], ki je siromašno s hranili. Pri njegovi pripravi smo med sestavine primešali sredstvo AG različnih koncentracij. Na plošče z gojiščem smo ločeno nacepili glivi *A. niger* in *C. globosum*. Po različnih časih inkubacije (3, 6 in 9 dni) pri temperaturi 29 °C smo določili prirast micelija posamezne glive (slika 4). Iz primerjave rasti micelija gliv na gojiščih brez sredstva AG in z dodanim sredstvom AG smo izračunali stopnjo redukcije rasti, *R*, iz naslednje enačbe:

$$R = (1 - \frac{M}{N}) \times 100\ (\%) \qquad (1)$$

v kateri je *M* prirast micelija v mm na SNA-gojišču, ki je vsebovalo sredstvo AG določene koncentracije, in *N* prirast micelija glive na SNA-gojišču, v katerem ni bilo sredstva AG. Vrednosti *R* za obe preučevani glivi so prikazane na sliki 5. Iz rezultatov je razvidno,

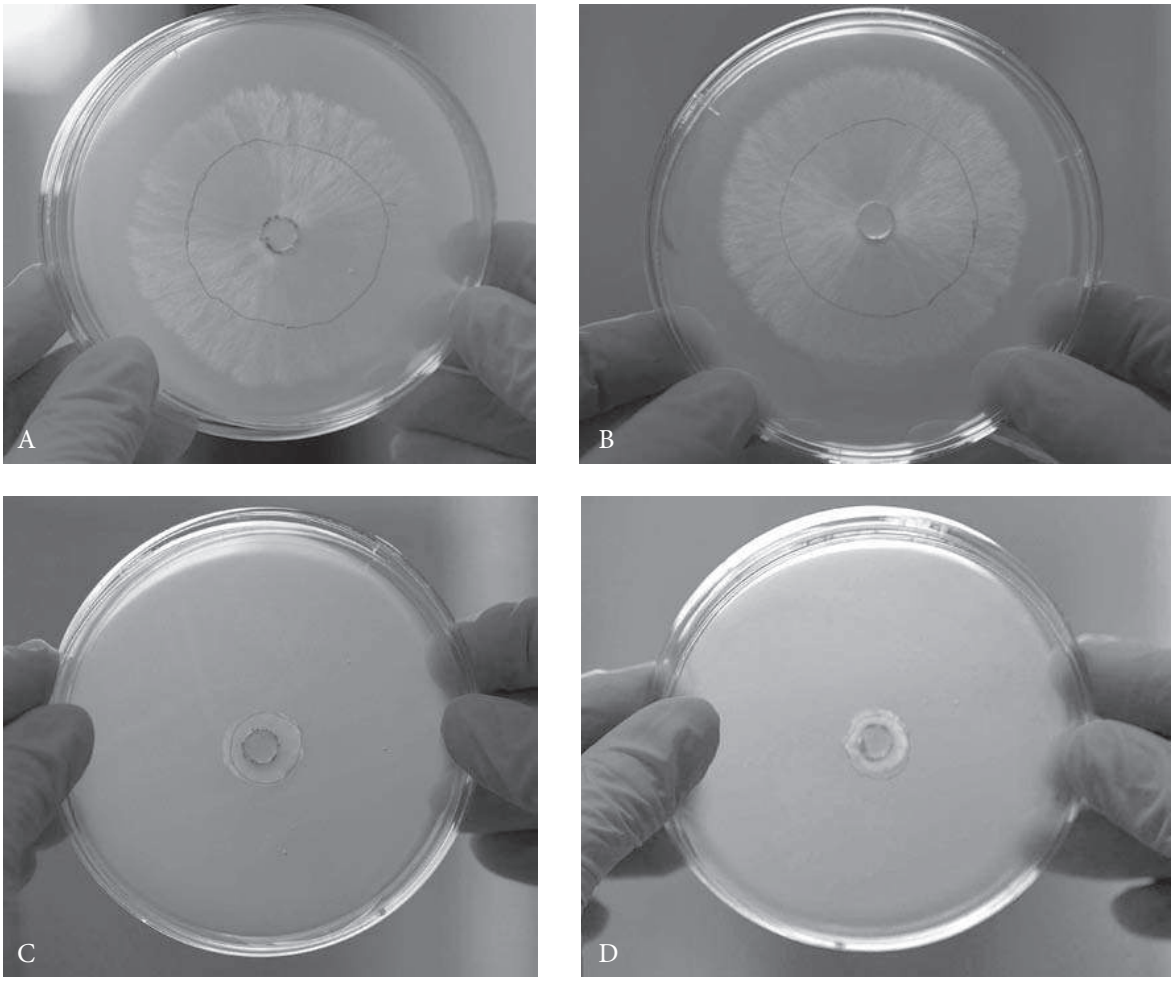

Figure 4: Mycelium growth of fungus C. globosum on SNA culture medium containing different concentrations of AG agent: (A) 0 g/l, (B) 0,5 g/l, (C) 2,0 g/l, (D) 3,0 g/l. Time of incubation was 6 days, red pen denotes growth of mycelium after 3 days of incubation.

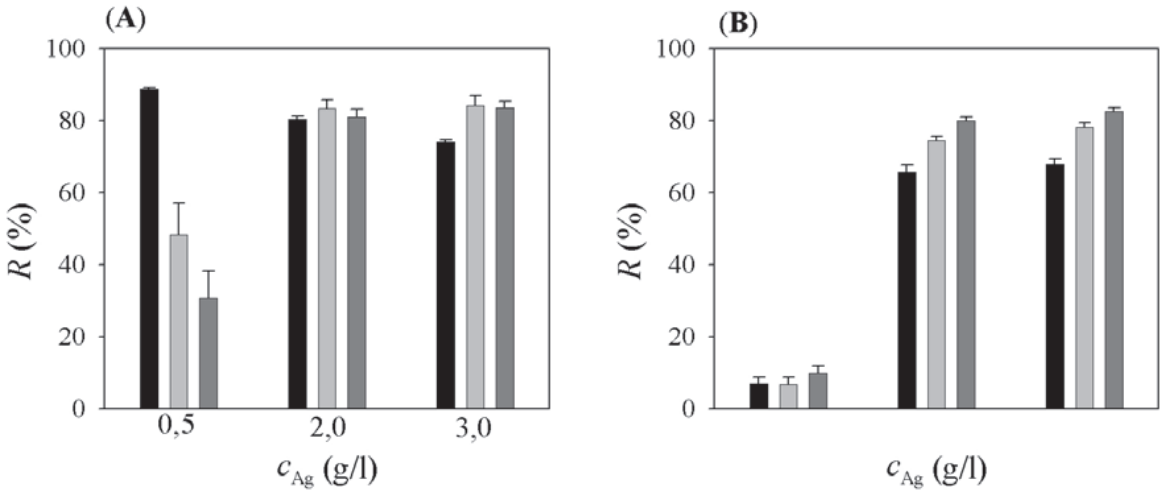

Figure 5: Degree of reduction, R, of fungi A. niger (A) and C. globosum (B) on SNA culture medium containing different concentrations of AG agent after certain time of incubation. Time of incubation: ■ 3 days, ■ 6 days, ■ 9 days.

the finished samples is well evident from Figure 3. These unexpected results raised the following questions: does the AG agent, for which excellent antibacterial action has already been determined [16], has no fungicidal action, is its concentration on the cotton fabric too low for efficient fungicidal action, or perhaps, is it possible that the results were influenced by the use of the highly nutritious MEA culture medium enriched by oat-meal, which is in accordance with the standard method for the tests with the A. niger fungus? Due to its high nutritional value, this medium could cause such aggressive growth of the fungi that even silver ions could not effectively inhibit it. In such a case, the MEA culture medium enriched by oat-meal would not be suitable for the determination of the fungicidal action of silver.

da se obe glivi nemoteno razraščata po SNA-gojišču, če v njem ni sredstva AG, in da uporabljeno apreturno sredstvo deluje toksično, če je prisotno v zadostni koncentraciji. V tem primeru je vrednost *R* v celotnem preučevanem časovnem območju višja od 65 %.

Fungicidni test po standardu DIN 53931 smo ponovili z uporabo SNA-gojišča. S slike 6 je razvidno, da je zamenjava gojišča na splošno zmanjšala intenziteto rasti gliv. Kljub temu sta obe preučevani glivi prerasli gojišče in površino vzorca neapretirane bombažne tkanine (sliki 6A in 6C), vzorec apretirane tkanine pa je ostal nepreraščen (sliki 6B in 6D). Izjema je bil vzorec v stiku z glivo *A. niger*, pri katerem smo na robovih opazili posamezne spore. Iz tega smo zaključili, da je srebro, prisotno na bombažnih vlaknih, delovalo fungicidno in učinkovito zavrlo rast obeh uporabljenih gliv. Po odstranitvi vzorcev z gojišča smo le-to preučili tudi pod mikroskopom. Mikroskopske slike so razkrile, da je gojišče v stiku z apretiranim vzorcem ostalo čisto le v primeru glive *C. globosum* (slika 7B), v primeru *A. niger* pa je micelij glive prerasel tudi

Table 1: Fungicidal activity of AG-RV coating on cotton fabric against fungi A. niger and C. globosum after 7 days of incubation at 29 °C according to the DIN 53931 Standard Method. (for sign explanation see chapter 2.4).

Sample[1)]	Culture medium	Overgrowth of the sample		Spore intensity growth	
		A. niger	*C. globosum*	*A. niger*	*C. globosum*
U	MEA	5 (100)	5 (100)	+++	+++
	SNA	5 (100)	4 (> 75)	+++	+++
A	MEA	4 (> 75)	4 (> 75)	+++	+++
	SNA	1 (5)	[0] (0)	+	/

[1)] *U – untreated sample, A –Ag-RV coated sample.*

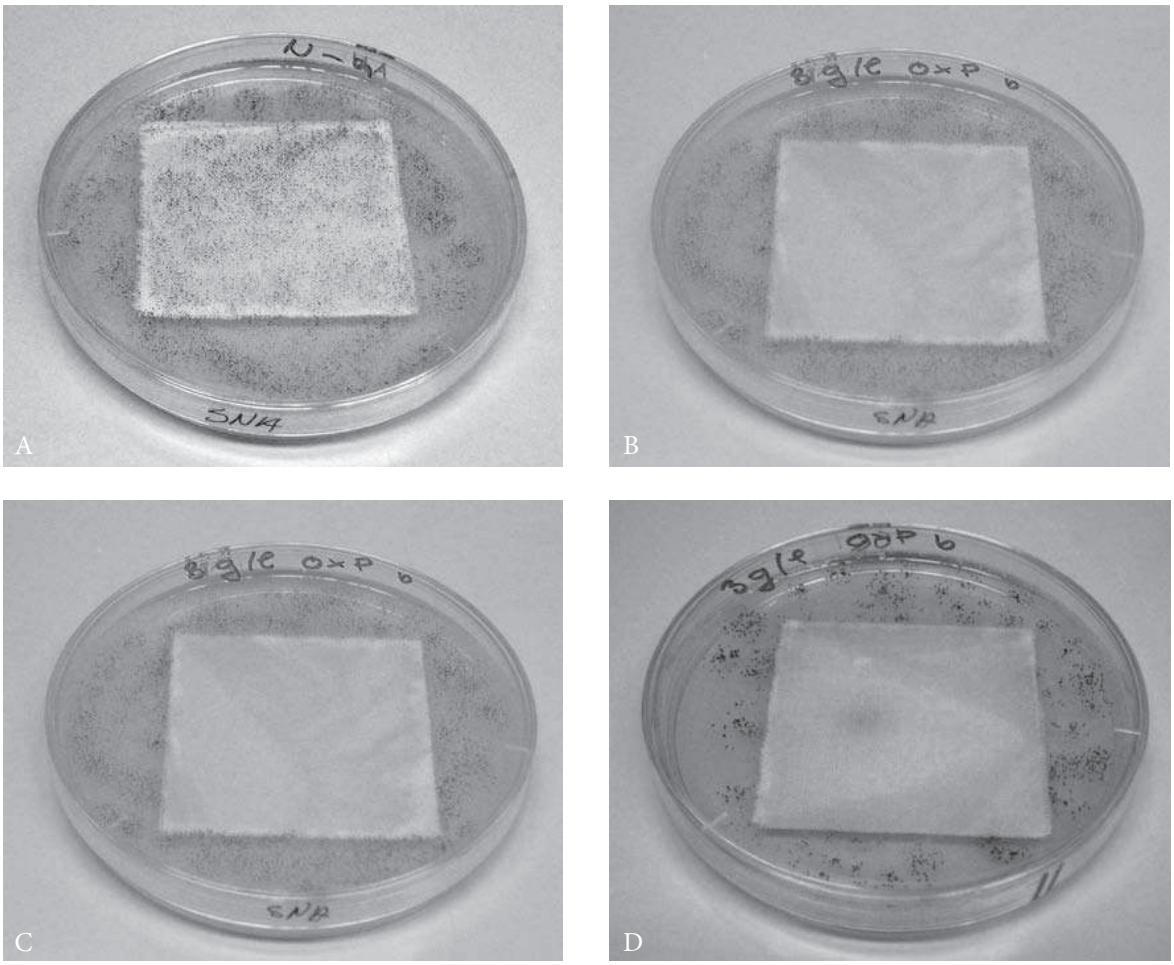

Figure 6: Growth of A. niger (A and B) and C. globosum (C and D) on SNA culture medium covered with the untreated cotton fabric sample (A and C) and Ag-RV coated cotton fabric (B and D) after 7 days of incubation.

In order to answer all of the questions above, the highly nutritious MEA culture medium, which includes malt extract and oat-meal, was replaced by a less nutritious synthetic nutrient agar (SNA) culture medium [18]. During its preparation, the AG agent was mixed with the ingredients at various concentrations. The SNA agar plates were separately inoculated with A. niger or C. globosum. After various periods of incubation (3, 6 and 9 days) at 29 °C, the mycelium growth gain of the individual fungus was determined (Figure 4). By comparing the growth of the mycelium of the fungi on the culture media with and without the AG agent, the degree of the reduction, R, was calculated from the equation 1.

Where M is the mycelium growth gain in mm on the SNA culture medium which contained

gojišče pod apretiranim vzorcem (slika 7A). Rezultate fungicidnega testa, dobljene na obeh gojiščih – MEA z dodanimi ovsenimi kosmiči in SNA, smo ovrednotili po standardu DIN 53931 in jih zbrali v preglednici 1.

Lastnosti gojišča so vplivale na rezultate fungicidnega testa. Medtem ko je na MEA-gojišču, obogatenem z ovsenimi kosmiči, toksičnost apreture AG-RV komaj zaznavna in je preraščenost apretiranih vzorcev za obe glivi večja od 75 %, je v primeru SNA-gojišča toksičnost apreture veliko bolj opazna. Ocena preraščenosti vzorca se je znižala s 5 na 1 za glivo *A. niger* in na [0] za glivo *C. globosum*. Pri tem je bil neapretirani vzorec ne glede na gojišče in preučevano glivo skoraj v celoti preraščen. Iz tega smo zaključili, da je SNA-gojišče primernejše za preučevanje fungicidne učinkovitosti apreture AG-RV na bombažni tkanini kot po standardu predpisano MEA-gojišče z dodanimi ovsenimi kosmiči. Apretura AG-RV na bombažni tkanini je bila učinkovitejša pri zaviranju rasti glive *C. globosum* kot *A. niger*. Pri slednji rast micelija ni bila zavrta v celoti.

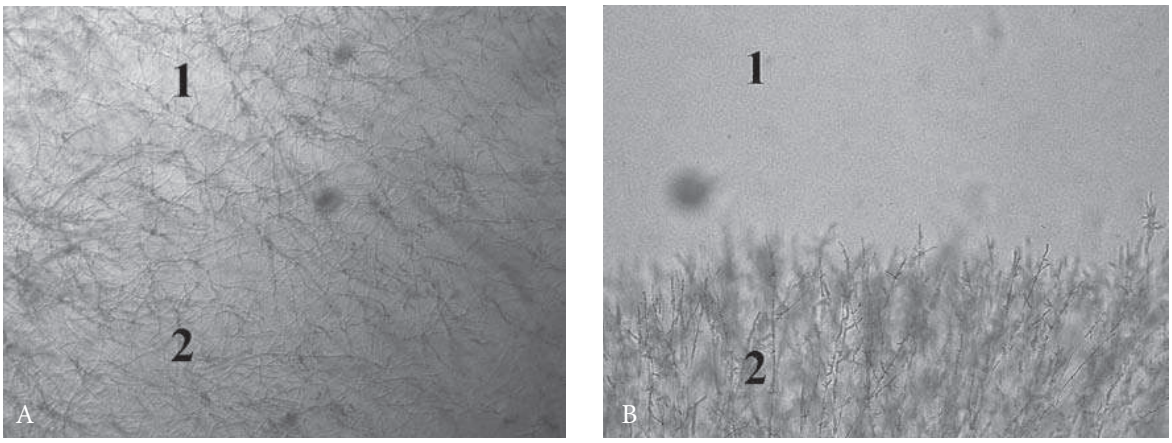

Figure 7: Microscopic picture of A. niger (A) and C. globosum (B) mycelium on SNA culture medium after removment of coated cotton sample. 1 – medium under the sample, 2 – medium on the outer edge of the sample.

the AG agent at the determined concentration, and N is the mycelium growth gain on the SNA culture medium whereas no AG agent was present. The R values for both studied fungi are presented in Figure 5. From the results, it is evident that both fungi smoothly grew on the SNA culture medium if there was no AG agent present and that the finishing agent acted toxic when present at a sufficient concentration. In this case, the R value was higher than 65% for the hole studied time period.

The fungicidal test in accordance with the DIN 53931 standard was repeated with the use of the SNA culture medium. From Figure 6 it is evident that the replacement of the culture medium reduced the intensity of the fungal growth in general. Both studied fungi overgrew the culture medium and the surface of the unfinished cotton sample (Figures 6A and 6C), while there was no growth on the finished samples (Figures 6B and 6D). The exception was only the sample which was in contact with A. niger, where some individual spores were observed on the edges. Nevertheless, it was concluded that the silver present on the cotton fabric acted fungicidal and effectively inhibited the growth of both fungi used. After removing the samples from the culture medium, the medium was observed microscopically. The culture medium remained free of C. globosum (Figure 7A) but it was completely overgrown by A. niger mycelium (Figure 7B). The results of the fungicidal test performed on MEA and on SNA culture media were eval-

4 Zaključki

Iz rezultatov raziskave lahko zaključimo:
- da je za preučevanje fungicidnega delovanja AgCl na celuloznih vlaknih treba izbrati ustrezno standardno metodo; uporaba standarda DIN 53931 je bila neustrezna, saj je intenzivna rast gliv *A. niger* in *C. globosum* po visoko hranilnem MEA-gojišču z dodanimi ovsenimi kosmiči onemogočila oceno toksičnosti apreture;
- modifikacija standardne metode z zamenjavo MEA-gojišča z ovsenimi kosmiči z manj hranilnim SNA-gojiščem je omogočila uspešno izvedbo fungicidnega testa;
- protimikrobna apretura, pripravljena iz AgCl v kombinaciji z reaktivnim vezivom na podlagi silicijeve spojine, je na celuloznih vlaknih delovala toksično za glivi *A. niger* in *C. globosum*;
- koncentracija AgCl na vlaknih, ki je znašala približno 130 ppm, je bila dovolj visoka za njegovo fungicidno delovanje, ki je bilo učinkovitejše za glivo *C. globosum* kot *A. niger*.

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uated in accordance with the DIN 53931 standard and summed up in Table 1.

From Table 1 it is evident that the characteristics of the culture medium significantly affected the results of the fungicidal test. While the toxicity of the AG-RV coating was hardly detectable on MEA enriched by oat-meal, i.e. the overgrowth of the samples was higher than 75% for both fungi, it was much more noticeable when SNA was used. The mycelial growth on the AG-RV coated sample was reduced from 5 to 1 for A. niger and from 5 to [0] for C. globosum, while unfinished samples were always overgrown entirely. Therefore, it was concluded that in comparison to MEA enriched by oat-meal, SNA is more suitable for the study of the fungicidal activity of the AG-RV coating on cotton fabric. From Table 1, it is also evident that the AG-RV coating on the cotton fabric more effectively inhibited the growth of C. globosum than A. niger. The growth of the latter mycelium was not completely inhibited.

4 Conclusions

- *For the study of the antifungal activity of silver ions on cotton fibres, appropriate agar media must be selected. The use of MEA of the DIN 53931 standard is inapropriate because it allows mycelial growth of A. niger and C. globosum also in the presence of antimicrobial finish.*
- *Modification of the standard method by replacing MEA enriched by oat-meal with the low nutrient SNA enabled successful performance of the fungicidal test.*
- *Antimicrobial AgCl coating when combined with the reactive silica binder is toxic to the standard tester strains of A. niger and C. globosum on cellulose fibres.*
- *The AgCl concentration on cotton fibres in the amount of approximately 130 ppm, was high enough for the fungicidal activity of the coating, which was more effective against fungus C. globosum than A. niger.*

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