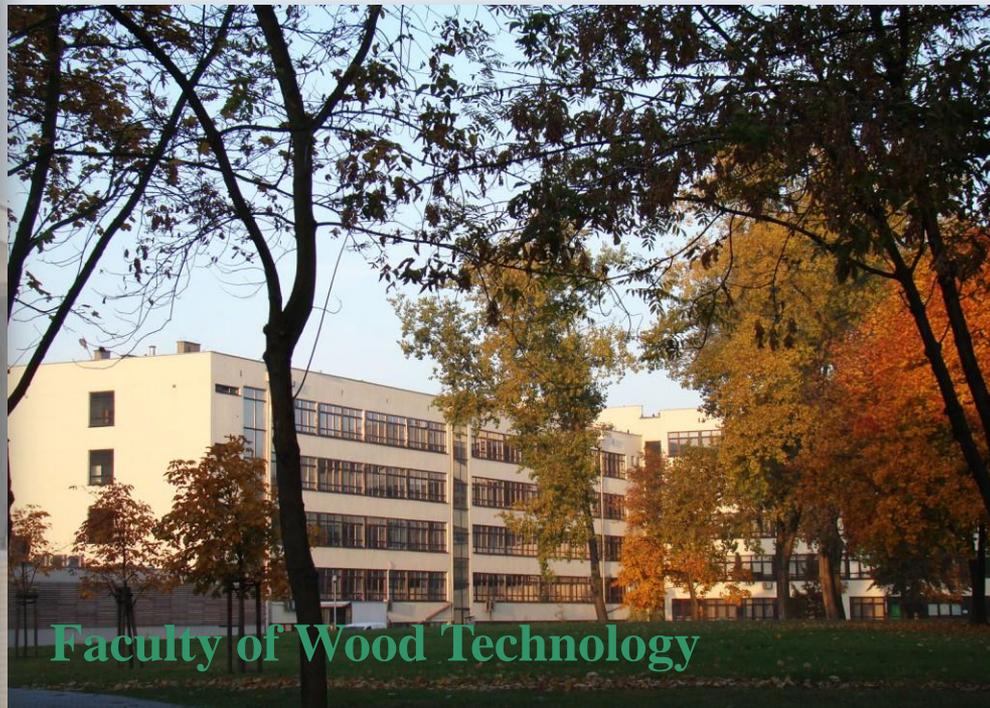


BIOASSAY OF SELECTED FUNGICIDE ON THIN LAYER CHROMATOGRAPHY AGAINST MOULDS

Patrycja Kwasniewska, Anna Komasa, Grzegorz Cofta, Bartłomiej Mazela



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Globally, the most common and most dangerous biological factor in constructions and in architectural monuments are **decaying fungi** and **moulds**.

The efficiency and stability of the formulations for preservatives lignocellulosic materials depend mainly on active ingredients - **BIOCIDES**.

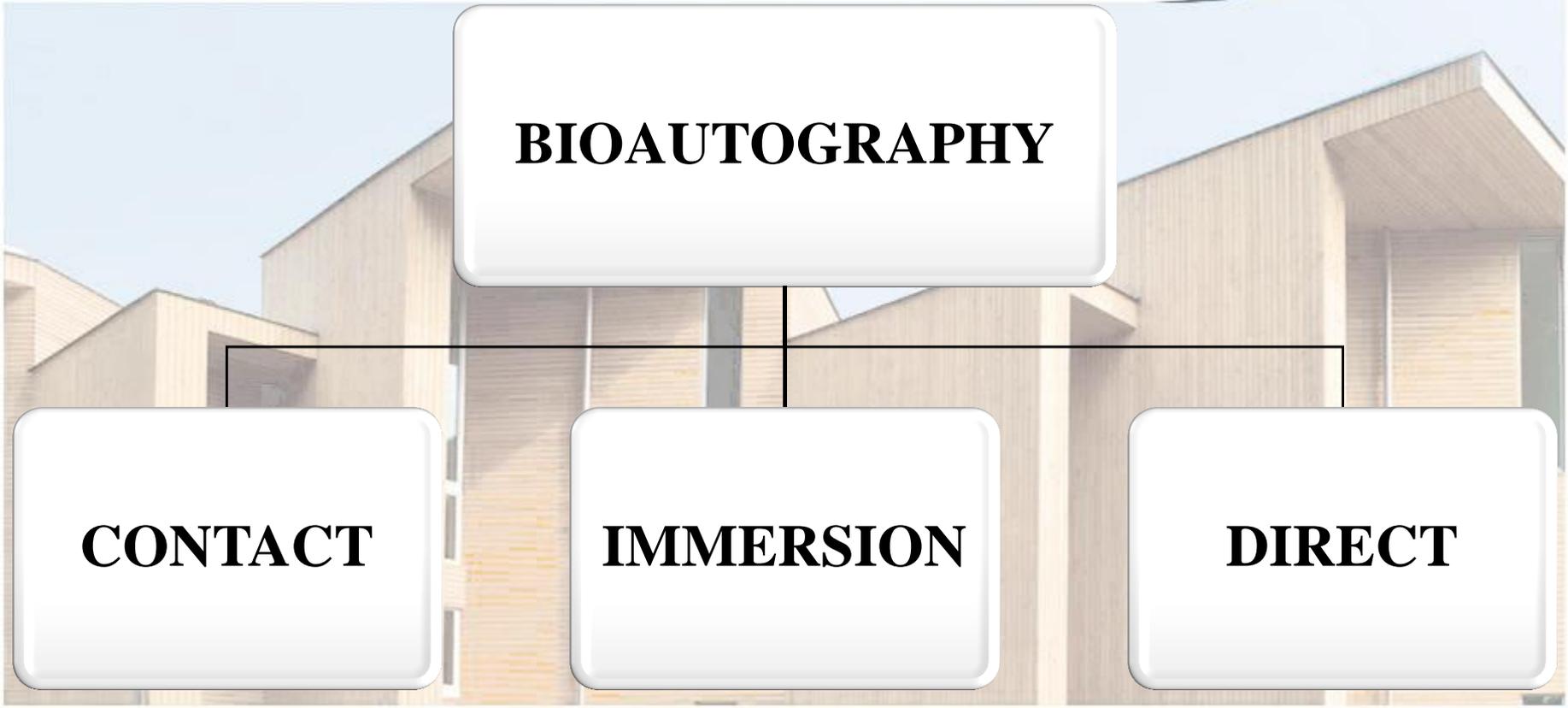


BIOAUTOGRAPHY

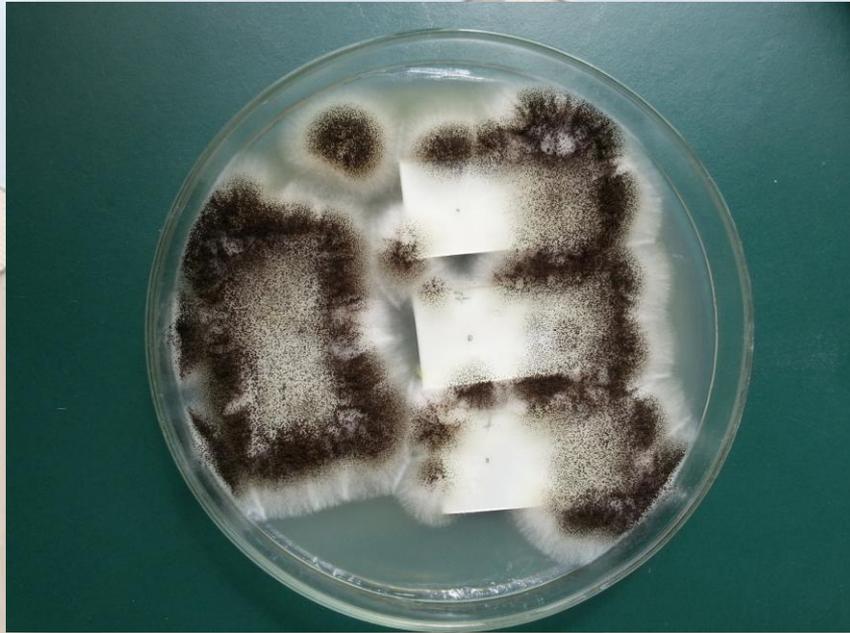
CONTACT

IMMERSION

DIRECT

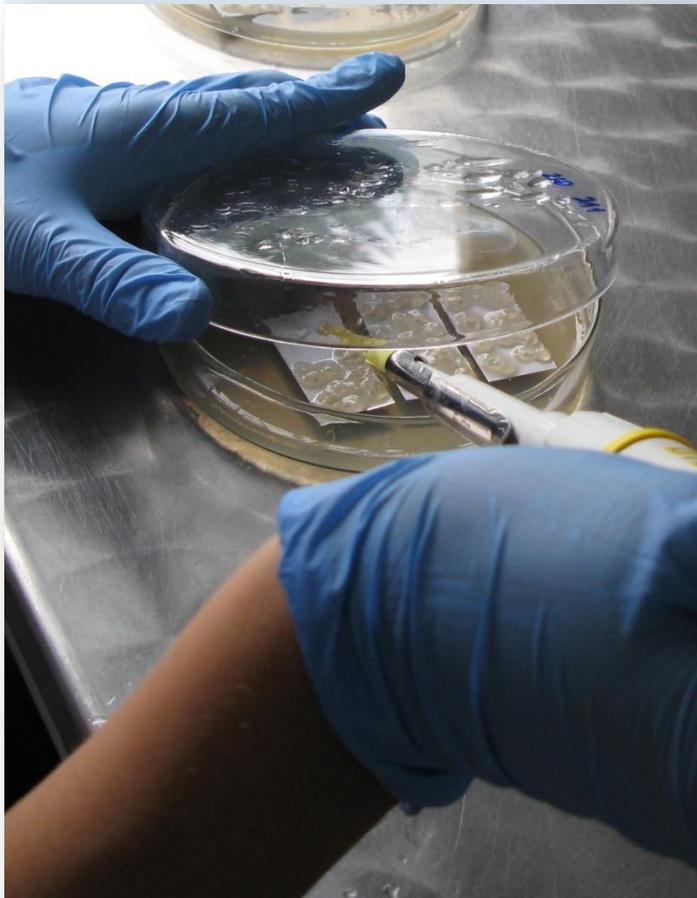


BIOAUTOGRAPHY-TLC



DURAWOOD

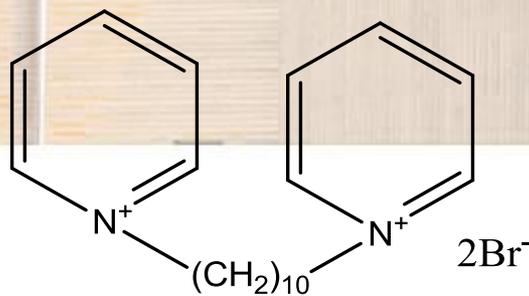
How it works?



BIOASSAY OF SELECTED FUNGICIDE

The aim of the present work was to analyze the minimal inhibitory concentration (MIC) of selected fungicides:

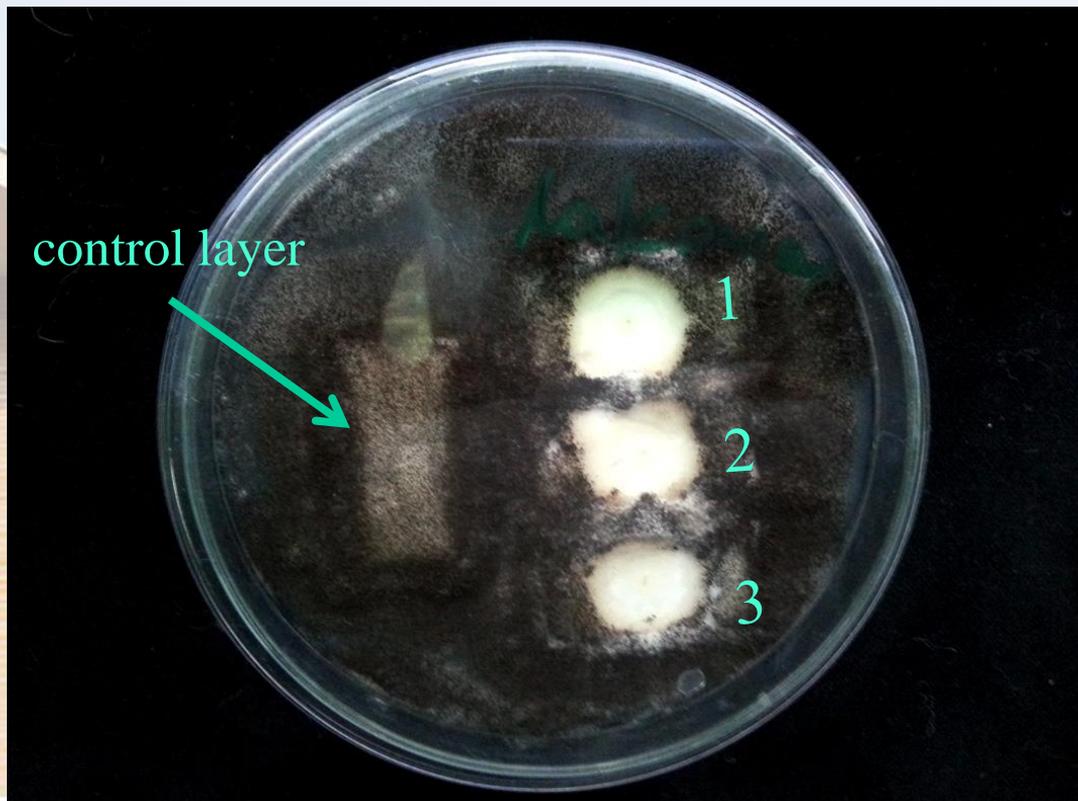
- 4,5-dichloro-2-octyl-2H-isothiazol-3-one,
 - dichloro-2-n-octyl-4-isothiazolin-3-one with 2-octyl-2H-isothiazol-3-one,
 - n-alkyl(C12-18)-N,N-dimethyl-N-benzylammonium chloride)
- and new synthetic compound using bioautography-TLC method
- (1,10-di(3-hydroxymethylpyridinium) decane dibromide



BIOASSAY OF SELECTED FUNGICIDE

- Microorganisms: *Aspergillus niger* van Tiegen, a human fungal pathogen.
- Each substance was prepared at concentrations of
 - a) 1%
 - b) 0.5%
 - c) 0.1%
 - d) 0.01%
 - e) 0.001%
 - f) 0.0001%
- TLC-layers were incubated at 28°C and above 95 RH in darkness.

Fungal growth was
evaluated macroscopically
for 4 days



RATING-SCALE OF FUNGAL GROWTH

Visual evaluation of microfungi growth on samples was held by the four-point scale of intensity mycelium growth of *Aspergillus niger* set out in table 1.

Rating	Fungal growth on surfaces
0	no visible growth under the microscope
1	invisible growth with the naked eye but are clearly visible under the microscope
2	visible growth with the naked eye, growth of hyphae without spores
3	visible growth with the naked eye, sporulation mycelium
4	intensive growth, covering the entire surface of the test

RESULTS

C [%]	A				B				C				New synthetic substance			
	1d	2d	3d	4d	1d	2d	3d	4d	1d	2d	3d	4d	1d	2d	3d	4d
1	0	0	0	0	0	0	0	0,33	0	0	0	0,33	0	0	0	0
0,5	0	0	0	0	0	0	0	0,66	0	0	0	0,33	1	1	2	4
0,1	0	0	0	0,33	0	0	0,67	1,67	0	0,33	1,33	2,67	1	1	2,33	4
0,01	0	0,33	1	1,33	1	3	3,67	4	1	3	3	4	1	2,67	3	4
0,001	0,67	1,67	1,67	2	2	4	4	4	2	4	4	4	1,67	3,67	4	4
0,0001	1	3	3,33	4	2	4	4	4	2	4	4	4	2	4	4	4
control	2	4	4	4	2	4	4	4	2	4	4	4	2	4	4	4

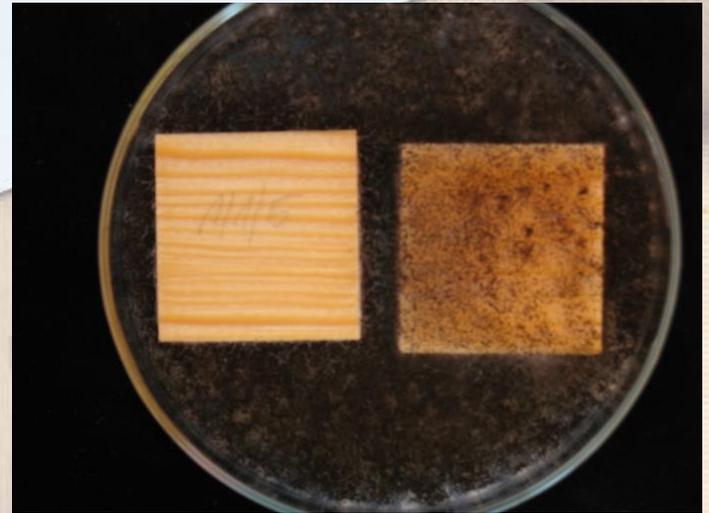
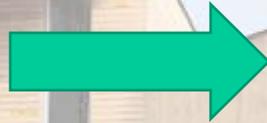
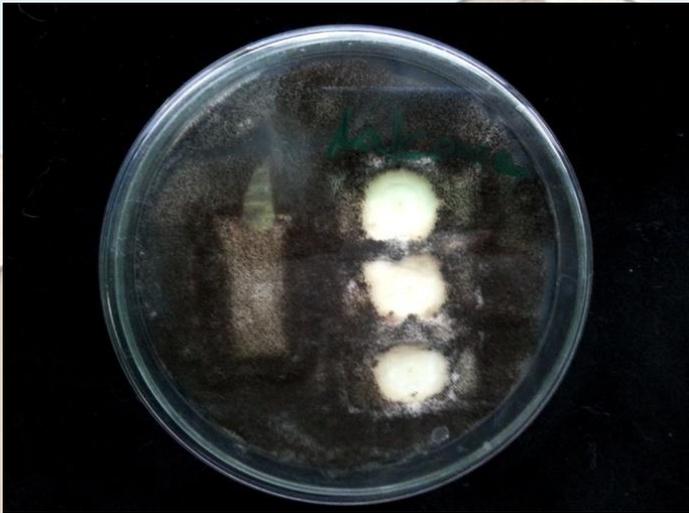
A. 4,5-dichloro-2-octyl-2H-isothiazol-3-one

B. dichloro-2-n-octyl-4-isothiazolin-3-one with 2-octyl-2H-isothiazol-3-one

C. n-alkyl(C12-18)-N,N-dimethyl-N-benzylammonium chloride

DURAWOOD

THE NEXT STEP OF TEST



BIOASSAY OF SELECTED FUNGICIDE ON WOOD

C [%]	A				B				C				New fungicide			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	0	0	0	0	0	0	0	0,2	0	0	0	0,2	0	0	0	1
0,5	0	0	0	0,2	0	0	0	0,6	0	0	0	0,6	2	3	4	4
0,1	0	0	1	2	0	0	0,6	1,6	0	0,4	1,2	2,6	2	3	4	4
0,01	0	1,2	2,6	4	1,6	3	3,6	4	1	3	3	4	3	4	4	4
0,001	0,8	2	3	4	2	4	4	4	2	4	4	4	3	4	4	4
0,0001	3	4	4	4	2	4	4	4	2	4	4	4	4	4	4	4
control	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Rating scale:

- 0z** no growth of fungi on the specimen, inhibition zone on the medium
- 0** no growth of fungi on the specimen
- 1** less than 10 % of the specimen area covered by fungi
- 2** less than 30 % of the specimen area covered by fungi
- 3** less than 60 % of the specimen area covered by fungi
- 4** specimen totally overgrown by fungi

COMPARISON OF RESULTS

C [%]	A				B				C				New synthetic substance			
	1d	2d	3d	4d	1d	2d	3d	4d	1d	2d	3d	4d	1d	2d	3d	4d
1	0	0	0	0	0	0	0	0,33	0	0	0	0,33	0	0	0	0
0,5	0	0	0	0	0	0	0	0,66	0	0	0	0,33	1	1	2	4
0,1	0	0	0	0,33	0	0	0,67	1,67	0	0,33	1,33	2,67	1	1	2,33	4
0,01	0	0,33	1	1,33	1	3	3,67	4	1	3	3	4	1	2,67	3	4
0,001	0,67	1,67	1,67	2	2	4	4	4	2	4	4	4	1,67	3,67	4	4
0,0001	1	3	3,33	4	2	4	4	4	2	4	4	4	2	4	4	4
control	2	4	4	4	2	4	4	4	2	4	4	4	2	4	4	4

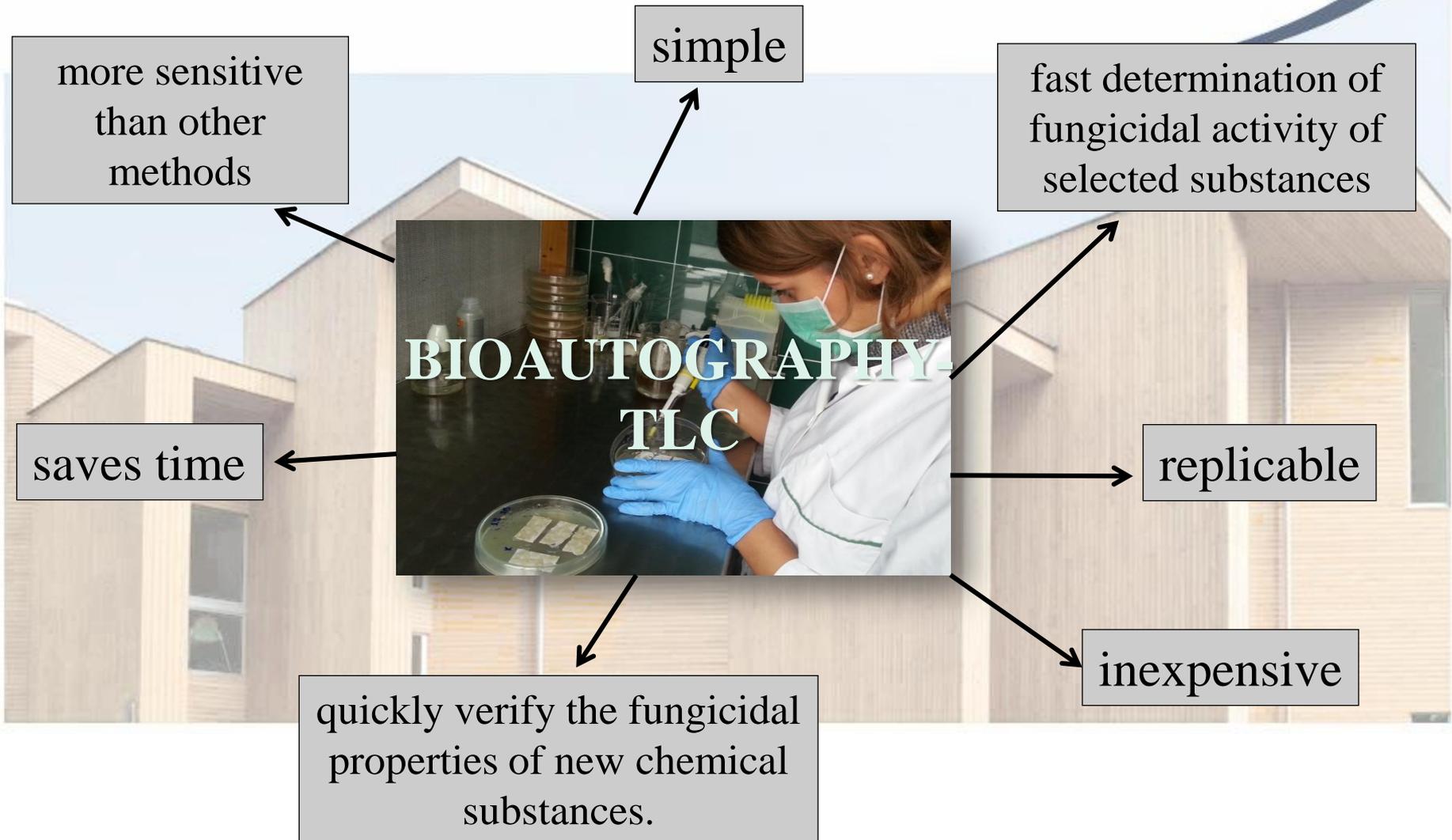
C [%]	A				B				C				New fungicide			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	0	0	0	0	0	0	0	0,2	0	0	0	0,2	0	0	0	1
0,5	0	0	0	0,2	0	0	0	0,6	0	0	0	0,6	2	3	4	4
0,1	0	0	1	2	0	0	0,6	1,6	0	0,4	1,2	2,6	2	3	4	4
0,01	0	1,2	2,6	4	1,6	3	3,6	4	1	3	3	4	3	4	4	4
0,001	0,8	2	3	4	2	4	4	4	2	4	4	4	3	4	4	4
0,0001	3	4	4	4	2	4	4	4	2	4	4	4	4	4	4	4
control	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

A. 4,5-dichloro-2-octyl-2H-isothiazol-3-one

B. dichloro-2-n-octyl-4-isothiazolin-3-one with 2-octyl-2H-isothiazol-3-one

C. n-alkyl(C12-18)-N,N-dimethyl-N-benzylammonium chloride

CONCLUSION



Thank you for your attention

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References

- CHOMA I.M., **2005**: The Use of Thin-Layer Chromatography with Direct Bioautography for Antimicrobial Analysis, LC-GC EUROPE 18; 9, 482-489
- CHOMA I.M., GRZELAK E.M., **2011**: Bioautography detection in thin-layer chromatography, Journal of Chromatography A. 1218, 2684–2691
- HOMANS, A. L., FUCHS A., **1970**: Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances, Journal of Chromatography A. 51; 327–329
- JESIONEK W., GRZELAK E.M., MAJER-DZIEDZIC B., CHOMA I.M., **2013**: Thin-Layer Chromatography – Direct Bioautography for the Screening of Antimicrobial Properties of Plant Extracts, Journal of Planar Chromatography 26; 2, 109–113
- MUNOZ-OLIVAS R., **2004**: Screening analysis: an overview of methods applied to environmental, clinical and food analyses, Trends Anal. Chem. 23, 203-216
- RIOS J.L., RECIO M.C., VILLAR A., **1988**: Screening methods for natural products with antimicrobial activity: A review of the literature, J. Ethnopharmacol., 23, 127–149