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ACTIVITY OF 5-CHLORO-1-METHYL-4-NITROIMIDAZOL AGAINST PINE WOOD SAP-STAIN FUNGI

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One of imidazol derivatives, well known from its activity against soft-, white- and brown-rot fungi, was studied with the use of fungi causing sap-stain in scots pine wood. For comparative purpouses sodium pentachlorophenoxide was used.

INTRODUCTION

Despite of long lasting studies and series described in literature chemicals and technologies of their use, protection of pine wood against sap-stain is further open problem. In the last years this problem is more actual mainly due to the sharpening of requirements for wood protection chemicals from the point of view their influence for homoiothermal organisms and environement protection.

Such example could be fluor compounds, their use – although good protective properties – are in some countries drastically limited [21]. The some situation is with the preparations containing chlorinated phenols and their derivatives, among which is sodium pentachlorphenolate well known from 1932 year from its excellent protective properties against sap-stain [10]. Pentachlorophenoxide due to the strong toxic activity of accompanying it dioxins [1, 13], in many countries is completely with drawn from the use in wood protection.

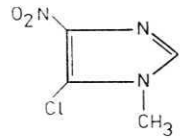
That is the cause of intense investigations on new, less noxious and harmful for men and animals compounds and chemical substances, showing biological activity against wood pests, in that fungi. The new fungicides, suitable for this purpouse are searched among others between plant protection chemicals or some biologically active compounds verified in medicine [18, 19, 20]. From till to this time conducted research studies appears, that some imidazol derivatives are characterized by such activity.

It was shown for instance, that 5-chloro-1-methyl-4-nitroimidazol used in proper doses efficiently protects wood against as well soft-rot fungi, as white or brown-rot fungi [5, 11].

Therefore it was intended to check efficiency of activity of above mentioned chemical compound in pine wood protection against fungi causing sap-stain.

MATERIALS AND INVESTIGATION METHODS

The scope of studies was 5-chloro-1-methyl-4-nitroimidazol (PCMNI) characterized by following properties:

chemical structure 

molecular mass – 161.1, melting temperature 147–148°C, easily dissolved in xylene, toluene, acetone, ethanol, hardly soluble in cold water. In studies there were used solutions of PCMNI in mixture of water with acetone in the ratio 1 : 1.

For comparative purpouses there was used water solution of sodium pentachlorophenoxide (PCP-Na) as the chemical in some time widely used in many countries for protection of wood against sap-stain [17].

In Polish sawmilling industry for this purpose there is used worked out in Wood Technology Institute in Poznań preparation consisting of sodium phenylo-phenoxide [9, 16]. Effectiveness of this compound is lower than PCP-Na [17].

In presented paper were applied threee methods of activity evaluation of mentioned chemicals in respect to fungi beeing cause of pine wood sap-stain. Each of them is characterised with series of advantages and also deficiencies. The main motive of methods selection was time of tests duration and their execution easines. Those conditions were fulfilled by:

- agar-substrate method,
- accelerated method,
- disc method.

Malt-agar culture test [7] allow for fast, aproximate determination of fungicidal concentration of tested chemical in artifical substrate. There was applied Czapek-Dox medium, into which tested chemicals were introduced in various concentration and after sterilisation were inoculated with following fungi species: *Ceratocystis minor* (Hedgc.) Hunt., *Aureobasidium pullulans* (de Bary) Arnaud., *Cladosporium herbarum* (Pers.) Link and *Sclerophoma pityophila* (Corda) Höhn.

Tests were made on Petri dishes of 100 mm diameter and they were placed in incubator at temperature 21 ± 1°C and relative moisture content of air 80 - 90%.

Observations and measurements were conducted during 10 days, measurements were made every day and diameter of developing mycelium on medium were made.

After that period final degree of covering of substrate surface was stated and toxic limit of tested chemical compounds in medium. For each concentration of tested preparation in substrate and test fungus species measurements were made on 5 dishes. On the basis of results of agar substrate method claser ranges of impregnation solution concentrations PCMNI and PCP-Na for the methods, where tests are carried out with the use of wood.

Accelerated method described by van Lenthe and Sutter [8] allows to obtain results in 3 weeks period. Its undiscutable advantage is use of wood in the tests,

that is substrate on which in natural conditions are developing fungi causing sap--stain. Samples with dimmensions $100 \times 50 \times 10$ mm^3 were out from pine sap wood (*Pinus silvestris* L.) and on one side various solutions of tested chemical were applied. After drying up each sample was cut accordingly to the scheme given on Fig. 1. Obtained smaller samples were placed into Petri dishes of filter paper. In sterile conditions filter paper dises were soaked with the use of 3 ml of sterile distillated water and than to each dish introduced 2 ml of water suspension of spores of *Aureobasidium pullulans* (de Bary) Arnaud., and *Sclerophoma pityophila* (Corda) Höhn. Dishes with samples were placed in poliethylene bag and kept in incubator in temperature 25 - 26°C. In 2 days intervals degree of fungi development was evaluated with the use of the scale elaborated by the authors of next applied method used in this investigations.

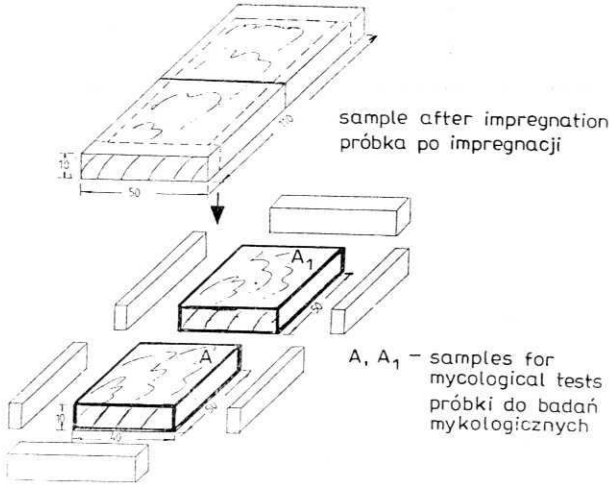


Fig. 1. Method of obtaining samples for tests accordingly to the accelarated test method [8]

Rys. 1. Sposób otrzymania próbek do badań metodą przyspieszoną [8]

The disc method described by Schulz [14]. Discs of diameter about 40 mm and thickness 100 mm cut from freshly cut and debarked pine sticks (*Pinus silvestris* L.) were used in this method. Those discs, diveded into two half discs, dried in temperature 105°C and immediately before use impregnated in lowered pressure in the "full cell" way with water solution of malt extract – concentration 1% to refill in wood nutrient carboksydrate constituents.

In such a way prepared samples were placed in twos in Kolle flasks and sterilised with vapour at 0.12 MPa pressure during 30 min. Then in sterile conditions samples were taken out from the flasks and soaked during 1 min. in solution of tested chemical. After draining of liquid surplus samples were put into the Kolle flasks. Next operation was introduction on samples about 1 ml of water solution of spores following fungi species causing pine wood sap-stain: *Ceratocystis minor* (Hedgc.) Hunt and *Aureobasidium pullulans* (de Bary) Arnaud and water suspension of spores beeing

mixture both mentioned species with spores *Cladosporium herbarum* (Pers.) Link., *Discula pinicola* (Naum.) Petrak and *Sclerophoma pityophila* (Corda) Höhn.

Flasks with samples were kept in incubator at temperature 20 - 22°C, evaluation degree of mycelium development on the samples at intervals of 2 days. For each concentration of tested chemical and species or mixture of fungi spores were used 4 pairs of half discs. Apropriate number of flasks was made with control (untreated) samples. Final degree of covering samples by test fungi and toxic limit of both chemicals was stated after 4 weeks, with the use of the scale given by the method authors [17]:

Degree of development of fungi on the samples	Surface with the sap-stain %
0	lack of blue-stain traces
1	below 5
2	6 - 15
3	16 - 25
4	26 - 50
5	51 - 75
6	above 75

In the year 1976 Lewandowski and Tarociński [9] have had published results of investigations of efficacy in wood protection against sap-stain of series protective preparations. In those tests newly worked out method of "plate samples" was applied. In this method sap wood samples of pine with dimmensions $100 \times 45 \times \times 10$ mm^3, after proper impregnation are exposed on the spores mixture of four various sap-stain fungi.

Due to the preliminary character of this investigations, this method was not used in our investigations. We are of opinion that the preparation of samples in form of the discs from freshly cut sticks is more simple and less tiresome and less material consuming, and to the some extent for one serie is more uniform.

RESULTS OF INVESTIGATIONS

Comparison of PCMNI and PCP-Na action against sap-stain fungi in agar substrate method indicates for 10 times lower activity of tested imidazol derivative in respect to the sodium salt of pentachlorphenoxide. A little less differentation was found in case of methods, where as the substrate was pine sap-wood.

In the disc method for both tested compounds close values of concentrations were obtained, where hindering of test fungi was observed.

In the disc method PCMNI against mixture of sap-stain fungi appered to be effective in solution with concentration 1.8%, in accelerated method similar effect accured at concentration 1.6%. For PCP-Na in both methods preservative effect was stated for nearly identical concentrations that is 0.2 - 0.3%.

To compare obtained results with some other perparations, Table 2 was made. As it may be seen antiseptic bath or pine sawn wood spraying are made with the use of various preparations in solutions of concentration 0.5 - 5%. As it results from performed studies, PCMNI effectively protects pine sap-wood against sap-stain

Table 1

Activity of 5-chlor-1-methyl-4-nitroimidazol (PCMNI) and sodium pentochlorophenoxide (PCP-Na) against the sap-stain fungi, determinated with malt-agar culture test [7], accelerated method [8] and disc method [16]

Aktywność 5-chloro-1-metylo-4-nitroimidazolu (PCMNI) i pentachlorofenolanu sodowego (PCF-Na) wobec grzybów powodujących siniznę drewna sosnowego, oznaczona metodą pożywkowo-agarową [7], przyspieszoną [8] i krążkową [16]

Method Metoda	Test fungus Grzyb testowy	Efficacious concentration of preparation Skuteczne stężenie preparatu	
		PCMNI	PCP-Na
Malt-agar culture test Pożywkowo-agarowa	*Cladosporium herbarum* (Pers.) Link	0.06	0.005
	Aureobasidium pullulans (de Bary) Arnaud	0.06	0.005
	Ceratocystis minor (Hedgc.) Hunt	0.06	0.005
	Sclerophoma pityophila (Corda) Höhn	0.06	0.005
	Discula pinicola (Naum.) Petrak	0.05	0.005
Accelerated method Przyspieszona	Mixture of spores: Mieszanina zarodników: *Aureobasidium pullulans* (de Bary) Arnaud *Sclerophoma pityophila* (Corda) Höhn	1.6	0.2
Disc method Krążkowa	*Ceratocystis minor* (Hedgc.) Hunt	1.0	0.2
	Aureobasidium pullulans (de Bary) Arnaud	1.2	0.2
	Mixture of spores of blue-stain fungi Mieszanina zarodników grzybów siniżny	1.8	0.3

Table 2

Efficatious concentrations of some chemical preparations protecting wood against sap-stain

Skuteczne stężenia niektórych preparatów chemicznych zabezpieczających drewno przed siniżną

Preparation Preparat	Concentration Stężenie %	Source Źródło
Amical	1.5 - 2.0	[3]
Benlate	4.0	[3]
Borax	> 5.0	[3]
Wolmanit CB	5.0	[12]
Dinocap	> 2.0	[12]
Brestan	> 2.0	[12]
NF 44	> 2.0	[12]
Natrium-orthophenylo-phenoxide	2.0	[17]
Sinoxan	2.5	[17]
Improsol	5.0	[4]
Mycocid	5.0	[4]
Mertect	0.2 - 0.25	[4]
Hager Blu	6.0	[4]

at concentration in the range 1.6 - 1.8%. It can be stated, that this compound with activity is comparable with many other cited in the Table 2.

As it was stated at prior tests [11] PCMNI protects effectively wood against fungi from *Basidiomycetes* class. In comparison to pentachlorophenol its activity is a little lower, ratio of the toxic limit of PCMNI to PCP in solid wood block method was in dependence upon of wood and test fungus 0.3 - 2.5.

The scope of laboratory tests was also the activity PCMNI in respect to the soft rot fungi and occurence of moulds [5].

It was stated in them, that effective toxic concentration of this compound, determined with the use of agar substrate method is about 1000 ppm. That is the proof of biological activity PCMNI also in respect to this group of fungi.

This evaluating more widely activity of 5-chloro-1-methyl-4-nitroimidazol in respect to various fungi developing on the wood, it can be stated that this compound is characterised by the features of fungicide with wide spectrum.

Toxical properties of this compound against homoiothermal organisms are to be stressed. Acute oral toxicity determined on rats [15] allows to classify PCMNI to the noxious substances of IV class accordingly to classification of Hodge and Sterner [6]. The LD_{50} was determined on the level 660 mg/kg. It is to be stressed, that it concerns pure compound, which in ready for use impregnation mixture is in limited quantity. With the assumption, that other components of protective preparation are of low toxicity it can be expected that toxicity of whole composition will be lower from active component. In this place it is to be stressed, that the used till to this time in wood protection against sap-stain. PCP-Na is classified to the II toxicity class [2], that accordingly to mentioned classification it is poison. Some of preparations listed in the Table 2 belong even to the I class of toxicity.

Other problem is usable form, in which 5-chloro-1-methyl-4-nitroimidazol could be applied in practice. Hardly it could be imagined antiseptic bath of sawn-wood or its spraying with solutions of PCMNI in mixture of water and acetone. As well economical as safety factors are to be taken into account. In respect to the usable forms of protective chemicals with the PCMNI solutions known in plant protection practice are to be adopted. There insoluble in water fungicides are used in form of emulsions, suspensions etc. It demands special additional investigations.

CONCLUSIONS

1. 5-chloro-1-methyl-4-nitroimidazol is chemical compound effectively protecting pine sap-wood against sap-stain fungi in solution of water-acetone mixture below 2%.
2. Activity of this compound against sap-stain fungi is several times greater from sodium pentachlorophenoxide activity.
3. Due to lower than PCP-Na toxicity against homoiothermal organisms, tested imidazol derivative can be assumed as valuable, potential fungicide in compositions of preparations protecting wood against sap-stain.
4. It is indispensable to carry out investigations on the usable form of protective preparation with the use of PCMNI in form of mixture or emulsion, without share of easily inflammable, volatile and costly organic solvents.

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AKTYWNOŚĆ 5-CHLORO-1-METYLO-4-NITROIMIDAZOLU WOBEC GRZYBÓW POWODUJĄCYCH SINIZNĘ DREWNA SOSNOWEGO

Streszczenie

5-chloro-1-metylo-4-nitroimidazol poddano badaniom laboratoryjnym, określając za pomocą trzech różnych metod jego działanie na grzyby powodujące siniznę drewna sosny (*Pinus silvestris* L.). Stwierdzono 6- do 7-krotnie niższą aktywność badanej pochodnej imidazolu od pentachlorofenolanu sodowego. Ze względu na niższą toksyczność 5-chloro-1-metylo-4-nitroimidazolu w stosunku do organizmów stałocieplnych, związek ten można uznać za potencjalny fungicyd w komponowaniu preparatów chemicznych chroniących drewno sosny przed siniznę.

АКТИВНОСТЬ 5-ХЛОР-1-МЕТИЛ-4-НИТРОИМИДАЗОЛА ПО ОТНОШЕНИЮ К ГРИБАМ, ВЫЗЫВАЮЩИМ СИНЕВУ СОСНОВОЙ ДРЕВЕСИНЫ

Резюме

5-хлор-1-метил-4-нитроимидазол был подвергнут лабораторным исследованиям, чтобы при помощи трех различных методов определить его действие на грибы, вызывающие синеву сосновой древесины (*Pinus silvestris* L.). Была обнаружена в 6 - 8 раз более низкая активность исследуемого производного имидазола, чем соснового пентахлорфенолятя. В связи с более низкой токсичностью 5-хлор-1-метил-4-нитроимидазола по отношению к теплокровным организмам это соединение можно считать потенциальным фунгицидом при компоновке химических соединений для защиты сосновой древесины от синевы.

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