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THE SIGNIFICANCE OF UREA PHOSPHATE IN PROTECTING MUSHROOM CULTIVATION

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Abstract

Introduction: Urea phosphate has been used with good results in animal feeding and preservation of wet corn and blood after slaughter that is destined for fodder use. Moreover, that compound, which is also found in poultry manure, shows fungicidal effect.
The aim of the present study was to examine disinfecting properties of urea phosphate in mushroom production.

Materials and methods: The investigation was carried out using the nutrient Mycobiol C® (containing urea phosphate) to assess the biocidal properties against selected mould fungi and *Basidiomycetes* fungi as well as its suitability for wood disinfection in mushroom cultivation rooms. Indication was done using the laboratory method on wood artificially infected by selected mould fungi (*Ascomycotina* and *Deuteromycotina*) as well as fungi decomposing wood (*Basidiomycotina*). Moreover, the effect of urea phosphate on the growth of selected bacteria such as *Pseudomonas aeruginosa*, *Pseudomonas tolaasii* and *Staphylococcus aureus* was also investigated.

Results and conclusions: The results indicate that urea phosphate shows fungicidal effect against the tested fungi and mycelium *Agaricus bisporus*, but not *Trichoderma viride*. The examined compound applied at 7.0 % concentration for 15 minutes showed bactericidal effect against *Pseudomonas aeruginosa* and *Pseudomonas tolaasii*.

Key words: Cultivated mushroom house; Urea phosphate; Protection; Prophylaxis; Cultivation

INTRODUCTION

Fungal and bacterial diseases may cause enormous losses in the cultivation, for example bacterial blotch alone decreased mushroom crop to 30% [1, 2]. Chlorine substances have been applied for cultivation protection and control against microbiological infection, however phenol derivatives have been used primarily for wood disinfection in mushroom cultivation rooms. In Poland, instead of commonly used Karbolina DNK, which contains phenol derivatives e.g. pentachlorophenol - PCP as biologically active substances, less toxic substances containing sodium phenate: Septyl, Lysotox or Rafasept have been frequently used. Moreover, the quaternary ammonium bases (QAC, Qant) and triazole compounds with modern disinfectant features (good effectiveness, wide range of ecological and hygienic properties) applied for general and wood disinfection in mushroom cultivation rooms were assessed in numerous studies before being introduced into practice in Poland. Application of those compounds (containing new biological substances) contributed to the development of general disinfection and protection of wood used by mushroom producers. Moreover, those substances have been found ecologically friendly [3-5], and are considered a new generation of preparations for wood disinfection [6]. Apart from those compounds, triazoles - biologically active substances are nowadays components of the most commonly used preparations for wood protection [7-9]. Since microorganisms produce mutants that are resistant to disinfecting chemicals the preparations used in food industry should be changed every 7-10 days. The economic aspect of the new disinfecting preparations should not be ignored. Apart from the already existing biologically active substances, peroxides and other new compounds such as urea phosphate, that have not been not used before in mushroom production, have recently been introduced.

Among the new trends in phosphorous compound technology, which are directed towards the production of fertilizing products, studies on urea phosphate have recently been conducted. The previous study has shown that

urea phosphate is potentially characterized by significant advantages as a fertilizer [11] that would be an extremely important source of nonprotein nitrogen [12-15]. The most recent studies have demonstrated that urea phosphate contained in the manure from the poultry also showed a strong fungicidal effect [16, 17].

The aim of the present study was to assess disinfecting properties of urea phosphate in mushroom cultivation production. The investigation was carried out using the nutrient Mycobiol C® (containing urea phosphate) to assess the biocidal properties against selected mould fungi and *Basidiomycetes* fungi as well as its suitability for wood disinfection in mushroom cultivation rooms. Indication was done using the laboratory method on wood artificially infected by selected mould fungi (*Ascomycotina* and *Deuteromycotina*) as well as fungi decomposing wood (*Basidiomycotina*). Moreover, the effect of urea phosphate on the growth of selected bacteria such as *Pseudomonas aeruginosa, Pseudomonas tolaasii* and *Staphylococcus aureus* was also investigated.

MATERIALS AND METHODS

MATERIALS

Samples of the preparation containing urea phosphate: Mycobiol C (Corporacion Colombiana de Investig Agropecuaria, Colombia - CORPOICA) - were used in this study. The basic compound: urea phosphate was dissolved in water. The examined preparation contained 20% of the active component.

Experimental concentrations

The investigated preparation was put into the nutrient in the amount enabling to obtain the following degrees of concentration of the active component. The experimental concentrations were [ppm]: l0, 20, 50,100, 200, 500, 1 000, 2 000, 5 000, 10 000=1%.

The solution was extended with the following degrees of concentration [ppm]: 20 000, 50 000, 100 000 = 10% for the mould fungi and for *Chaetomium globosum* (to indicate LD value) [ppm] - 150000 = 15%

Tested fungi:

A. *Basidiomycetes* fungi (subdivision *Basidiomycotina*)

a) *Coniophora puteana* (Schum. ex Fr.) Karst, strain BAM Ebw. 15-mycelium

b) *Agaricus bisporus* (Lange) Imbach (delivered by the Institute of Vegetable Crops) - mycelium

B. Mould fungi (subdivision *Ascomycotina* and *Deuteromycotina*)

c) *Aspergillus niger* van Tieghem species SGGW 287 - mycelium with spores

d) *Trichoderma viride* Pers. ex Fr. species SGGW 118 - mycelium with spores

e) *Chaetomium globosum* Kunze species SGGW 11- mycelium with spores.

Pure fungi cultures (*Coniophora puteana, Aspergillus niger, Trichoderma viride* and *Chaetomium globosum*) came from the collection of Wood Protection Institute of Warsaw University of Life Sciences SGGW. Agar-maltose nutrient

of the following composition was used: maltose extract: 2% [weight] agar: 2% [weight] distilled water to total mass 1000 g. For *Agaricus bisporus* the nutrient was enriched with mineral components according to Czapek-Dox recipe.

METHODS

The study was conducted with the nutrient-screening method according to Ważny and Grzywacz [10], and Ważny and Thornton [6]. The preparation was put into sterile agar-maltose nutrient (liquid) at 60°C as indirect solutions. After thorough mixing of the preparation with the nutrient, the cultivation substrates were prepared in triplicate in sterile Petrie dishes of 90 mm diameter. When the nutrient cooled and solidified, the tested fungi of 5 mm in diameter centrally inoculated the substrates. Fungi incubation was conducted in the dark at the temperature of 24±1°C and relative air humidity 85±2%. The following values of ED_{50}, ED_{100} and LD (LD_{100}) were the criterion of biocidal proprieties of the preparation: ED_{50} - effective dose inhibiting growth of mycelium by 50%. The value of ED_{50} was determined by measuring the diameter of the tested fungi colonies: when the diameter of the examined colony (on nutrient without fungicide) completely covered the surface of the container. The approximate ED_{50} values were calculated by mathematical interpolation of the obtained results. ED_{100} was the effective dose inhibiting growth of mycelium in 100%. The value of ED_{100} was given as an interval value of the lowest fungicide concentrations, in which the inoculum did not reveal any signs of growth after 10 days from the LD indication - mortal dose (i.e. the dose causing mycelium death). The value of LD was obtained on the basis of inoculating onto substrates without fungicides to all inocula that did not grow on substrates with the fungicide. The interval of the lowest fungicide concentrations, in which the inoculum growth was not observable within 14 days after inoculation was considered as the level $LD = LD_{100}$.

Conditions of wood saturation

Time periods and temperature of wood soaking:

A. *Basidiomycetes* fungi (subdivision *Basidiomycotina*)
   - soaking time [min.]: l;
   - soaking temperature [°C]: 10, 20, 30;

B. Mould fungi (subdivision *Ascomycotina* and *Deuteromycotina*)
   - soaking time [min.]: 15, 60, 120;.
   - soaking temperature [°C] 10, 20, 30.

The bactericidal effect of urea phosphate (7% solution) dissolved in standard hard water (hardness equal to 300 ppm $CaCO_2$) with the addition of 0.03% albumin (Serva 11925) and of inactivator (broth with 3.0% Tween 80, 0.3% lecithin, 0.l% histidine, 0.5% sodium thiosulfate) was investigated in 15 and 30 minutes at the temperature of 20°C. Using the carriers method (the metal cylinder) [18] the following bacteria species were examined in this study: *Pseudomonas aeruginosa, Pseudomonas tolaasii* and *Staphylococcus aureus*.

RESULTS

Specific average values of linear growth of the given mycelium are presented in Tables 1 and 2. The applied concentrations (max. up to 15%) allowed determining all assumed toxic parameters of the fungicide (ED_{50}, ED_{100}, LD). A considerable differentiation between the ED_{100} and LD values was found for *Chaetomium globosum*. It might imply that spores of that fungus species are highly resistant to Mycobiol C. The lowest concentration of urea phosphate that showed fungicidal effects, and the optimal experimental conditions (temperature, soaking time) are presented in Table 3.

Table 1. Toxic value of urea phosphate (Mycobiol C) determined using the nutrient method.

Tested fungus	Toxic values [ppm] (The percentage of biologically active substance in medium)		
	ED_{50}	ED_{100}	LD
Coniophora puteana	≈4 000 (0.4%)	5 000 < ED_{100} <10 000 (0.5% < ED_{100} < 1.0%)	5 000 < LD <10 000 (0.5% < LD_{100} < 1.0%)
Agaricus bisporus	≈3 000 (0.3%)	2 000 < ED_{100} <5 000 (0.2% < ED_{100} < 0.5%)	2 000 < LD <5 000 (0.2% < LD < 0.5%)
Aspergillus niger	≈5 000 (0.5%)	20 000 < ED_{100} <50 000 (2.0% < ED_{100} < 5.0%)	20 000 < LD <50 000 (2.0% < LD < 5.0%)
Trichoderma viride	≈9 000 (0.9%)	20 000 < ED_{100} <50 000 (2.0% < ED_{100} < 5.0%)	50 000 < LD <100 000 (5.0% < LD < 10.0%)
Chaetomium globosum	≈1 200 (0.12%)	2 000 < ED_{100} <5 000 (0.2% < ED_{100} < 0.5%)	100 000 < LD <150 000 (10.0% < LD < 15.0%)

Table 2. The biocidal effect of urea phosphate on *Agaricus bisporus* in wood in mushroom cultivation.

Concentration of solution [%]	Temperature of solution [°C]	Soaking time [min.]	Effect
0.5	10	1	not tested
		15	**
		60	
	20	1	0
		15	0
		60	**
	60	1	0
		15	0
		60	**
1.0	10	1	0
		15	0
		60	**
	20	1	0
		15	0
		60	**
	60	1	0
		15	0
		60	**
2.0	10	1	0
		15	**
		60	**
	20	1	0
		15	**
		60	**
	60	1	0
		15	**
		60	**

Table 2. Continued.

Concentration of solution [%]	Temperature of solution [°C]	Soaking time [min.]	Effect
5.0	10	1	**
		15	**
		60	**
	20	1	**
		15	**
		60	**
	60	1	**
		15	**
		60	*
10.0	10	1	**
		15	0
		60	0
	20	1	**
		15	0
		60	0
	60	1	**
		15	0
		60	0
20.0	10	1	**
		15	0
		60	0
	20	1	0
		15	0
		60	0
	60	1	0
		15	0
		60	0

Legends: ** - slow growth of mycelium; * - moderate growth of mycelium;
0 - no growth of mycelium

Table 3. The fungicidal effect of urea phosphate (Mycobiol C) in wood in mushroom cultivation.

Tested fungus	Efficient activity of the preparation at the minimum concentration of solution		
	Min. concentration of solution [%]	Temperature of solution [°C]	Soaking time [min.]
Coniophora puteana	20	10	60
Agaricus bisporus	10	10	13
Aspergillus niger	20	≥10	120
Trichoderma viride	No efficiency result		
Chaetomium globosum	20	30	60

Table 4. Antibacterial effect of urea phosphate (Mycobiol C) determined by the carrier method.

Tested organisms	Growth of microorganisms											
	Number of tested tube										Control	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**a**	**b**
Staphylococcus aureus NCTC 416	-	-	-	-	-	-	-	-	-	+	+	+
	-	-	-	-	+	+	-	-	-	-	+	+
	+	-	-	-	-	-	+	-	-	-	+	+
Pseudomonas aeruginosa NCTC 6749	-	+	-	-	-	-	-	-	-	-	+	+
	-	-	-	-	-	-	-	-	-	-	+	+
Pseudomonas tolaasii	-	-	-	-	-	-	-	-	-	-	+	+
	-	-	-	-	-	-	-	-	-	-	+	+
		-	-									
	-			-	-	-	-	-	-	-	+	+
	-	-	-	-	-	-	-	-	-	-	+	+

a) control of growth - one carrier infected with the tested bacteria was placed in 10 mL medium culture

b) control of bactericidal effect of preparation in culture medium: one sterile carrier was exposed for 10 minutes in preparation solution and then in the inactivator and placed in a test tube containing culture medium. In the same tube one carrier infected with tested bacteria was placed [11].

The tested solution of urea phosphate showed the antibacterial action on *Pseudomonas aeruginosa* and *Pseudomonas tolaasii*. No bactericidal effect of the examined compound on *Staphylococcus aureus* was observed after 15 minutes or when the time of investigation was prolonged to approximately

30 minutes (Table 4). The possibility of using the preparation for disinfection of objects, which can be immersed was taken into consideration.

CONCLUSIONS

1. Urea phosphate is characterized by fungicidal properties against all of the tested fungi and mycelium *Agaricus bisporus* but not *Trichoderma viride*.
2. Urea phosphate used at the concentration of 7.0% for 15 minutes shows bactericidal effect against *Pseudomonas aeruginosa* and *Pseudomonas tolaasii*.

LITERATURE

1. Fordyce C. Microorganisms associated with marked deterioration of the cultivated mushroom *Agaricus bisporus*, Plant Diseases Report 1968; 52: 712-714.
2. Szymański J. Zastosowanie środków dezynfekcyjnych przy produkcji grzybni pieczarki. Biul Warz 1991; 37: 227-235.
3. Domagk G. Eine neue Klasse Desinfectionsmitteln. Dtsch Med Wascht 1935; 61: 828.
4. Chen G. In situ thermal condensation of glucose–diammonium phosphate in wood for fire and fungal decay protection. Wood and Fiber Science, April 2009; 41: 105-115.
5. Krzyśko-Łupicka T, Grata K. The assessment of environmental fungi sensitivity on urea phosphate. Roczniki PZH, 2004; 55: 243-248.
6. Ważny J, Thornton JD. Comparative laboratory testing of strain of the dry rot fungus *Serpula lacrymans* (Schum. eX Fr.) S.F. Gray, II The action of some wood preservation in agar. Holzforchung 1986; 40: 386-388.
7. Urbanik E, Fojutowski A. Proekologiczne zabezpieczenia drewna środkami przeciwgrzybowymi W: Mat. Sympozjum "Czwartorzędowe sole amoniowe i obszary ich zastosowania w gospodarce", ITD, Poznań. 1996; 5-26.

8. Ważny J. Czwartorzędowe związki amoniowe - nowa generacja środków ochrony drewna W: Ochrona Drewna XVII Sympozjum. Dział Wyd. SGGW Warszawa. 1961; 1-21.
9. Weitzman I. Cutaneous and Subcutaneous Infection in the Immuno-compromised Host. Arch Dermatol. 1986; 122: 1161-1168.
10. Ważny J, Grzywacz A. Wartości toksyczne fungicydów w stosunku do grzybów wywołujących brunatny, biały i szary rozkład drewna. Zeszyty Problemowe Postępów Nauk Rolnicz 1976; 178: 1821-1826.
11. Bremner J M and Douglas LA. Decomposition of Urea Phosphate in Soils. Soil Sci Soc Am J 1971; 35:575-578.
12. Hoffmann, J. Nowe kierunki wykorzystania fosforanu mocznika w technologii związków fosforowych. Prace Naukowe Instytutu Technologii Nieorganicznej i Nawozów Mineralnych Politechniki Wrocławskiej. Monografie. 2001, 49: 164
13. Krzebietke S, Benedycka Z. Wpływ fosforanu mocznika na właściwości fizykochemiczne substratu torfowego w warunkach laboratoryjnych. Acta Agrophysica, 2006, 7: 969-976.
14. Oltjen RR, Slyter LL,. Kozak AS and. Williams EE, Jr**.** Evaluation of Urea, Biuret, Urea Phosphate and Uric Acid as NPN Sources for Cattle. J Nutrition. 1968; 94: 193-202.
15. Romano N, Russo Alesi D, La Licata R, Tolone G. Effects of urea phosphate, ammonium ions and pH on Ureaplasma ATP synthesis. Microbiologica. 1986; 9: 405-413.
16. Grata K. Ocena skuteczności mikostatycznej fosforanu mocznika i siarczanu mocznika z bentonitem w nawozie drobiowym. Ochrona Środowiska, 2008; 30: 45-49.
17. Grata K. Effect of urea phosphate on the Bacillus sp. population in soil and antifungal activity of selected Bacillus species on Fusarium sp. Proceedings of EC Opole 2008; 2: 315-319.
18. Krzywicka H, Janowska J, Tadeusiak B, Zarzycka E. Metoda określania stężeń użytkowych preparatów dezynfekcyjnych. Metoda nośnikowa. Wyd Met PZH, Warszawa. 1993.

ZNACZENIE FOSFORANU MOCZNIKA W OCHRONIE PRODUKCJI GRZYBÓW UPRAWNYCH

Streszczenie

Wprowadzenie: Fosforan mocznika jest stosowany z dobrymi wynikami w żywieniu zwierząt oraz do konserwowania wilgotnego zboża i krwi poubojowej, przeznaczonych na cele paszowe. Ponadto związek ten obecny w nawozie kurzym wykazuje działanie grzybobójcze.
Celem pracy było zbadanie właściwości dezynfekcyjnych fosforanu mocznika w uprawie grzybów.
Materiały i metody: Do badań wykorzystano preparat Mycobiol C® (zawierający fosforan mocznika) oceniając jego właściwości biobójcze przeciwko wybranym grzybom pleśniowym i grzybom rodzaju *Basidiomycetes*, a także jego przydatność do dezynfekcji drewna w pieczarkarniach. Badanie zostało przeprowadzone w warunkach laboratoryjnych na drewnie sztucznie zainfekowanym przez wybrane grzyby pleśniowe (*Ascomycotina* i *Deuteromycotina*) jak również grzyby rozkładające drewno (*Basidiomycotina*). Ponadto zbadano również wpływ fosforanu mocznika na wzrost wybranych bakterii takich jak *Pseudomonas aeruginosa*, *Pseudomonas tolaasii* oraz *Staphylococcus aureus*.
Wyniki i wnioski: Uzyskane wyniki wskazują, że fosforan mocznika wywiera działanie grzybobójcze na grzyby patogenne i grzybnię pieczarki *Agaricus bisporus*, ale nie niszczy *Trichoderma viride*. Badany związek stosowany w stężeniu 7.0% przez 15 minut działa bakteriobójczo na *Pseudomonas aeruginosa* i *Pseudomonas tolaasii*.

Słowa kluczowe: Pieczarkarnia; Fosforan mocznika; Ochrona; Profilaktyka; Uprawa.

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