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The Effect of Heavy Metal Ions and Their Complexions upon Growth, Sporulation and Pathogenicity of the Entomopathogenic Fungus *Paecilomyces farinosus*

D. Ropek[1]*, A. Para[2]

[1]Department of Environment Agricultural Protection, University of Agriculture, Al. Mickiewicza 21, 31-120 Kraków, Poland
[2]Department of Chemistry, University of Agriculture, Al. Mickiewicza 21, 31-120 Kraków, Poland

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Abstract

Heavy metal ions are harmful for entomopathogenic fungi. Derivatives of starch dialdehyde such as dihydrazone (DHZ), dithiosemicarbazone (DTSC), and dioxime (DOX) were used to form water-insoluble complexes with heavy metal ions. This phenomenon thus provided protection of entomopathogenic fungus *Paecilomyces farinosus* from the toxic effects of heavy metal ions. The fungus growth and the sporulation of mycelium was inhibited by heavy metal ions. This fungus grown on medium with heavy metal ions was also less pathogenic to tested insects. Because the metal complexes of DHZ, DTSC and DOX were non-toxic to *P. farinosus* these complexons could protect entomopathogenic fungi from the toxic effects of metal ions, given good approximation of empirical values.

Keywords: dihydrazone, dithiosemicarbazone, dioxime, *Paecilomyces farinosus,* sporulation, pathogenicity

Introduction

Contemporary human activity results in significant changes in natural distribution of minerals and organics of the environment. The environment suffers intoxication with industrial products and waste. Agricultural activity utilising artificial fertilisers, pesticides, and crop preservatives also contributes to unusual fluctuations in chemical composition of the environment, passing the limit of tolerance of many organisms in adapting to varying environmental conditions. Some heavy metals are the most harmful components of ecosystems. In some areas of Poland, mainly in Upper Silesia, their concentration exceeds officially accepted levels [1,2]. Heavy metal compounds may reside in ecosystems in immobile, insoluble as well as mobile, soluble forms. The latter might be particularly harmful for living components of the ecosystem by their direct effect as well as by entering the food chain. However, apparently insoluble metal compounds may enter a food chain being slightly and slowly accumulated by plants.

Certain areas are contaminated by heavy metals to such an extent that such land must be exempted from agricultural production. There have been several attempts to recover this land for agriculture. Metal ions can be immobilised in soil either by their conversion into insoluble forms or as trapped by complexons. Derivatives of starch dialdehyde [3] might be potentially useful as complexons

*Corresponding author

of various heavy metals. Certain complexons with heavy metal ions are biologically active [4,5]. These complexons could be used to protect certain soil microorganisms. Introducing these complexions into soil is a new approach in environmental protection.

Entomopathogenic *Hyphomycetes* fungi commonly reside in Polish soils. They have found their application in modern plant protection from pests. Their pathogenicity is controlled by several factors such as temperature, humidity, presence of antagonists [6,7].

Very little is known on the effect of xenobiotics on these fungi. Some studies [8-10] suggest that heavy metals influence their growth, amount of biomass, and enzymatic activity. Some heavy metal ions have a toxic effect on entomopathogenic fungi, whereas others do not influence them or even stimulate their activity [11,12].

This paper reports results of laboratory study of the influence of heavy metal ions and complexions on growth, sporulation, and pathogenicity of *Paecilomyces farinosus* belonging to the class of *Hyphomycetes*.

Experimental

Derivatives of low-oxidised (16%) dialdehyde starch prepared according to Para et al. [3] were used as complexing substances. Heavy metal ion solutions were prepared from the following salts: $CdCl_2$ -2.5 H_2O, $CuSO_4$, $Ni(NO_3)_2$ -8 H_2O, $Pb(NO_3)_2$, $ZnCl_2$ analytical grade were purchased from POCH Gliwice, Poland.

*Paecilomyces farinosus (*Polish strain from Professor Bajan's collection) was the tested fungus. Equal concentrations of heavy metal ions (300mg/dm^3) and complexing substances (DHZ, DTSC and DOX) (15g/dm^3) were applied. Heavy metal salts and complexing substance were added to the glucose-potato medium. Metal ions were used separately. The medium with metal ions and complexing substances was autoclaved (120°C). Sterile medium was applied to Petri dishes followed by inoculation with *P. farinosus*. The dishes with fungus growing on a medium devoid of heavy metal ions and complexing substances provided the control. The dishes were placed in a thermostat at 25°C and for two weeks in 48 h intervals the area of the fungus colony was checked. The *P. farinosus* sporulation was checked after 4 weeks. Using a cork borer a 0.758cm^2 area was marked out on the border of fungus colony growing on Petri dishes. Sporulating mycelium was collected with a scalpel and placed in a test tube with 10 cm^3 of distilled water. Spore concentrations in the initial solution were determined in a Bürker chamber according to methodology described by Lipa and Śliżyński [13]. *P. farinosus* pathogenicity was tested on G. *mellonella* caterpillars. Tested insects were placed on Petri dishes coated with filter paper and sprayed with the fungus spore suspension. The dishes were kept in a thermostat at 25°C. Tested insect mortality was checked for one week in 24 h intervals. All experiments were triplicated.

Results and Discussion

Heavy metal ions strongly inhibited surface growth of entomopathogenic fungi [8]. Their response to heavy metal ions depended not only on the examined ion but also on the species and strain of fungus [14,15]. However, some heavy metal ions, e.g. lead or zinc could stimulate growth of entomopathogenic fungi biomass [11].

Applied complexons had no adverse effect on the surface growth of *P. farinosus* mycelium (Tab. 1). Surface growth of the tested *P. farinosus* strain was strongly inhibited by the heavy metal ions. The ions of lead, cadmium, zinc, and copper were particularly toxic. Some authors [12] pointed to a resistance of *P. farinosus* to high concentrations of copper ions. High sensitivity of *P. farinosus* to Cu ions found in this experiment might result from the application of another, more sensitive strain. Complexions added to the medium faintly with heavy metal ions had diverse effect on the surface growth of *P. farinosus.* DHZ supplement did not affect *P. farinosus* growth, whereas DOX reduced the negative effect of the Pb(ll) ions and DTSC admixed to the medium together with the Pb(ll) ions inhibited the mycelium growth to a higher degree than Pb(ll) ions applied separately.

DHZ effectively limited the toxic effect of the Cd ions, whereas the other complexons did not influence the effect of these ions on the *P. farinosus* growth. A strong toxic activity of the Cu(ll) ions was best limited by DHZ and DOX. Only DHZ removed the toxic effect of the Zn ions on the mycelium growth. In the media with the Pb+DTSC, Zn+DTSC and Ni+DTSC combinations the fungus growth was more inhibited than in the medium with the same complexon used separately or with individual ion. DTSC complexes with ions are more toxic than the ions themselves.

P. farinosus sporulation was similar in both control medium and containing additional complexions (Tab. 1). Not all examined ions limited sporulation of *P. farinosus.* The lead and cadmium ions limited sporulation to the highest extent. The copper ions which had a very strong toxic effect on surface growth did not effect sporulation. DHZ and DTSC totally cancelled the toxic effect of the Pb ion on sporulation. On the other hand, DOX, which most effectively controls the toxic effect of Pb on mycelium growth, did not influence sporulation. None of the tested complexons limited effectively the toxic effect of Cd on sporulation. Toxic activity of Zn was annihilated by DHZ, DTSC and DOX. DHZ seemed to be the most effective complexion limiting toxic effect of Ni on sporulation.

P. farinosus caused total mortality of G. *mellonella* caterpillars within a week from spraying them with a spore suspension (Tab. 1). The complexons used separately did not affect *P. farinosus* pathogenicity to the tested insects. Its pathogenicity was most limited by the Cd and Pb ions. Cu, Zn, and Ni ions only weakly influ-

Table 1. Effect of heavy metal ions and complexons upon the growth, sporulation, and the pathogenicity of *P. farinosus.*

Heavy metal	Complexon	Colony area [cm^2]	Spore concentration [milions/1 cm^3]	Insect test mortality [%]
Pb	DHZ	18.3b	72.0c	100c
	DTSC	11. 3a	111. Od	100c
	DOX	28.3c	10.0b	80b
	-	19.6b	3.7a	60a
None - Control	-	40. 3d	66.7c	100c
Cd	DHZ	29.2b	22.2c	80c
	DTSC	20.9a	7.7b	60b
	DOX	21.2a	6.2b	50b
	-	18.3a	2.7a	30a
None - Control	-	40.3c	66.7d	100d
Cu	DHZ	5.0c	18.2a	70a
	DTSC	1.8b	89.0c	100b
	DOX	6.1c	83.2bc	100b
	-	0.8a	80.0bc	80a
None - Control	-	40.3d	66.7b	100b
Zn	DHZ	23.7c	53.0b	100b
	DTSC	8.2a	64.2c	100b
	DOX	15.9b	62.5c	100b
	-	15.9b	12.5a	80a
None - Control	-	40.3d	66.7c	100b
Ni	DHZ	13.6b	67.7d	100a
	DTSC	1.1a	33.7c	100a
	DOX	18.6bc	11. 5a	100a
	-	23.7c	22.5b	90a
None - Control	-	40.3d	66.7d	100a
	DHZ	35.4abc	70.4a	100a
	DTSC	31. 2a	60.2a	100a
	DOX	38.4bc	64. 3a	100a
None - Control	-	40.3bc	66.7a	100a

*Means in columns marked with identical characters arę not different (p=0.05) according to Duncan multiple test.

enced pathogenicity of the tested fungi. DHZ, DTSC and DOX efficiently reduced the toxic effect of the Pb and Cd ions on *P. farinosus* pathogenicity.

The results implied that the Cu, Pb and Cd ions were the most toxic for the investigated *P. farinosus* strain. The tested heavy metal ions affected growth, sporulation and pathogenicity of *P. farinosus* differently. The Cu ions, which nad a very strong toxic effect on the surface growth of the fungus, influenced neither sporulation nor pathogenicity. On the other hand, the Pb and Cd ions proved toxic for growth, sporulation, and pathogenicity of *P. farinosus.* None of the investigated complexions protected fungus from all tested heavy metal ions. Their activity were selective. DTSC coupled with heavy metal ions was more toxic to *P. farinosus* growth than the separate ions. Such toxic effects of certain complexions with heavy metal ions on microorganisms was found also by other authors [4,5,16]. The investigated complexes might be utilised for some pathogen control.

Conclusions

Starch dialdehyde derivatives are suitable agents for protection of soil microorganisms from heavy metal ions. Complexions, which efficiently immobilise heavy metal ions making them inaccessible for living organisms, may be used in protection of soil microorganisms against toxic

effects of the metal ions. Because of their origin, tested complexions readily undergo biodegradation and do not pose any hazard to the environment.

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