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EFFECT OF CADMIUM ON MYCELIAL GROWTH AND MORPHOLOGY OF CHOSEN PHYLLOPLANE FUNGI

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Abstract: Cadmium was recognised as one of the major environmental and public health risk problem. The aim of this study was to assess the influence of cadmium on growth and morphology of phylloplane fungi. Discs of fungal cultures were placed on Cd amended PDA medium. *Alternaria alternata, Septoria tritici, Epicoccum purpurascens, Fusarium avenaceum, Bipolaris sorokiniana* were tested. Mycelial growth, morphology and sporulation were studied. All tested species differed in cadmium response. Retardation of radial growth of mycelium was observed. Changes of pigmentation, and inhibition or loss of sporulation was noted. The most sensitive to cadmium was *S. tritici*.

Key words: cadmium, phylloplane fungi, toxicity

INTRODUCTION

Studies of the interaction between heavy metals and phylloplane fungi can be complicated by physical and chemical nature of the environment (Gadd 1993). Studies using laboratory media and pure cultures mainly elucidated response of soil fungi and their relative sensitivity to heavy metals, or the influence on decomposition of organic matter (Babich and Stotzky 1977a; Płaza et.al. 1998). In the above studies cadmium (Cd) has been recognised as one of the major environmental and public health risk problem (Babich and Stotzky 1985). However much less is known about the response to heavy metals (cadmium) of phylloplane fungi, mainly those potentially plant pathogenic. The objective of this paper was to present a simplified test system and the results of assessment of the influence of cadmium on growth and morphology of chosen phylloplane fungi.

MATERIALS AND METHODS

Cultures of fungi used in this study were:
Alternaria alternata (Fr.) Keissler
Epicoccum purpurascens. Ehrenb.
Fusarium avenaceum (Fr.) Scc.
Bipolaris sorokiniana (Sacc.) Shoem.
Septoria tritici Rob. ex Desm.

The strains were originally isolated from diseased leaves of wheat and barley, grown on unpolluted fields. Stock cultures of fungi were grown on PDA medium pH 6.5 at 25°C for 7 do 21 days. Appropriate solutions of $CdSO_4$ containing differentiated amounts of Cd were autoclaved separately and then added to melted PDA, so that final concentration in the medium ranged from 0 to 100 mg/l. Agar discs with actively growing mycelium were cut out with sterile cork borer and placed on central point of Petri dishes. Four replicate plates were inoculated for each Cd concentration.

The plates were incubated at 25°C and, after appropriate time intervals (24 to 48 hours) two diagonal measurements of mycelial growth were made on each plate. The description of mycelial morphology was performed every 24 hours. The duration of incubation depended on a specific fungus species. Final measurements were taken after 9 to 18 days when fungal growth reached the periphery of Petri dishes. After the test has been completed from each plate 1 cm^2 discs of mycelium were cut out, homogenised and the number of spores were counted using a hemotocymeter.

RESULTS

All tested species differed in cadmium response. In the case of *A. alternata* and *E. purpurascens* linear growth of mycelium was gradually inhibited accordingly to cadmium concentration increase (Tabs. 1, 2). Morphological change of mycelium was noticed at 2nd observation day. Cultures grown on medium amended with 20 mg Cd/l produced hyaline and delicate hyphae. At 50 mg Cd/l radial growth was strongly inhibited and unusually sparse mycelium was produced. In the case of *A. alternata* from 5th day of observation at all Cd concentrations the fungus produced usual in shape and pigmentation mycelium, only radial growth was inhibited. Also sporulation was inhibited up to 30% at 100 mg Cd/l (Tab. 6). *E.*

Table 1.The influence of cadmium on *Alternaria alternata* mycelium radial growth

Cd concentration (mg/l)	Mean radial growth (mm)								
	Observation day								
	1	2	3	4	5	6	7	8	9
5	9.63 a*	1638 a	23.31 a	33.13 a	37.56 a	44.88 a	51.69 a	59.00 a	67.88 a
10	9.50 a	16.00 a	23.19 a	30.44 ab	37.13 a	43.06 ab	48.44 a	55.31 ab	61.88 ab
20	8.56 ab	14.69 a	21.13 a	27.44 b	33.25 a	39.75 b	45.25 a	50.06 b	55.63 b
50	7.88 abc	10.78 b	13.56 b	16.50 c	19.56 b	22.31 c	25.44 b	27.94 c	30.88 c
100	6.88 c	9.81 b	11.25 b	12.81 c	14.00 c	16.00 d	17.50 c	18.06 d	19.88 d
0	11.06 d	18.81 c	27.50 c	35.44 a	43.88 d	52.31 c	60.06 d	68.44 e	75.50 e

*means followed by the same letter do not differ at $p>0.05$ (Tukey test)

Table 2. The influence of cadmium on *Epicoccum purpurascens* mycelium radial growth

Cd concentration (mg/l)	Mean radial growth (mm)								
	Observation day								
	2	4	6	8	10	12	14	16	18
5	11.06 a	19.44 a	27.27 a	35.75 a	43.50 a	51.94 a	58.50 a	65.25 a	69.25 a
10	10.88 ab	18.81 a	27.13 a	34.63ab	42.75 a	50.25 a	57.00 a	63.81 a	67.88 a
20	10.69 ab	19.00 a	27.38 a	34.56ab	42.50 a	49.94 a	56.25 a	62.13 a	65.50 a
50	9.63 ab	17.06ab	24.00 b	30.56 b	36.00 b	41.88 b	46.13 b	50.88 b	53.63 b
100	9.13 b	14.19 b	18.38 c	22.38 b	26.25 c	30.69 c	32.63 c	36.75 c	38.50 c
0	11.25 a	20.00 a	28.5 a	36.50 a	44.40 a	53.06 a	59.63 a	65.75 a	69.13 a

Explanation – see table 1

purpurascens cultures showed a similar reaction to Cd concentration but retardation of pigmentation of medium was observed. At 10 mg Cd/l pigmentation of medium was brown instead usual yellow. On plates amended with Cd sporulation was absent.

F. avenaceum and *B. sorokiniana* linear growth significantly decreased starting from 4th observation day (Tabs. 3, 4). Up to that time all cultures were unchanged, with usual morphology. Then change in pigmentation was observed. Cultures became darker. Radial growth was inhibited as compared to plates without Cd ammendment. The most intensive change was noted at 100 mg Cd/l. Sporulation was inhibited up to 50% at the mentioned Cd concentration.

Table 3. The influence of cadmium on *Fusarium avenaceum* mycelium radial growth

Cd concentration (mg/l)	Mean radial growth (mm)								
	Observation day								
	1	2	3	4	5	6	7	8	9
5	5.38 a	14.00 a	12.8 ab	17.75ab	31.62ab	27.63ab	32.44ab	37.31ab	41.87ab
10	5.50 a	16.50 a	16.25 a	24.25 a	53.62 a	43.63 a	53.69 a	64.25 a	73.62 a
20	5.25 a	14.00 a	13.56 a	17.44ab	29.50ab	24.31 b	29.12 b	31.00 b	36.00bc
50	5.00 a	8.13 ab	8.25 ab	9.75 b	14.75 b	13.50 b	14.81 b	16.25 b	17.37bc
100	5.00 a	5.00 b	5.00 b	5.25 b	7.62 b	6.75 b	7.37 b	7.62 cb	7.87 c
0	5.75 a	17.50 a	17.75 a	25.56 a	53.75 a	44.81 a	54.00 a	63.87 a	74.88 a

Explanation – see table 1

Table 4. The influence of cadmium on *Bipolaris sorokiniana* mycelium radial growth

Cd concentration (mg/l)	Mean radial growth (mm)								
	Observation day								
	1	2	3	4	5	6	7	8	9
5	9.31 a	16.25 a	22.50 a	31.44 a	39.50 a	47.31 a	53.60 a	57.38 a	63.06 a
10	9.19 a	15.06 b	19.44ab	23.00 b	27.00 b	30.94 b	33.44 b	35.13 b	36.69 b
20	8.63 a	14.00ab	17.56ab	21.89 b	24.69bc	28.81 b	32.31 b	34.63 b	37.63 b
50	7.25 ab	11.31 b	13.63 c	15.38 c	15.88 c	16.63 c	16.88 c	17.88 c	19.13 c
100	5.25 b	8.94 b	11.00 c	11.75 c	12.13 c	12.56 c	12.68 c	12.81 c	12.81 c
0	9.00 a	13.44 a	21.56ab	28.38ab	34.63ab	40.13ab	9.21 ab	46.00ab	50.00ab

Explanation – see table 1

S. tritici was also sensitive to cadmium. But because this fungus formed delicate and slow growing mycelium, changes in linear growth were difficult to determine. After statistic calculation we assumed that cultures grown on Cd amended medium were smaller than on unamended medium, but differences were not significant (Tab. 5). All cultures grown on PDA with Cd had normal morphology. Fungus produced hyaline and formed nearly cream colonies with feathery morphology. At 100 mg Cd/l PDA *S. tritici* cultures lost their vitality. On plates with lower concentration of Cd in medium two from investigated cultures also lost their vitality too. Sporulation or picnidia formation was absent on plates amended with Cd.

Table 5.The influence of cadmium on *Septoria tritici* mycelium radial growth

Cd concentration (mg/l)	Mean radial growth (mm)								
	Observation day								
	2	4	6	8	10	12	14	16	18
5	5.50 a	5.75 a	6.00 a	6.25 a	6.50 a	7.06 a	7.88 a	9.19 a	10.31 a
10	5.50 a	5.50 a	5.50 a	5.69 a	5.81 a	5.88 ab	6.19 ab	6.31 ab	6.75 ab
20	5.50 a	5.50 a	5.50 a	6.06 a	6.44 a	6.63 ab	7.13 ab	7.50 ab	7.75 ab
50	5.38 a	5.38 a	5.38 a	5.38 a	5.63 a	5.63 ab	5.69 ab	5.75 ab	5.81 ab
100	5.63 a	5.63 a	5.63 a	5.63 a	5.63 a	5.63 ab	5.63 ab	5.63 ab	5.63 ab
0	5.63 a	6.06 a	6.81 a	7.56 a	8.88 a	10.69 a	11.84 a	13.06 a	15.13 a

Explanation – see table 1

Table 6.The influence of cadmium concentration on sporulation of fungi

Cd concentration (mg/l	Spore formation (%)		
	A. alternata	*F. avenaceum*	*B. sorokiniana*
5	98	100	80
10	76	98	76
20	50	75	68
50	51	62	59
100	30	50	50
0	100	100	100

DISCUSSION

A wide spectrum of potentially toxic interactions between metals and fungi in almost every aspect of their metabolism, growth and differentiation may change depending of the organism, metal, species and concentration and physico-chemical factors (Tobin et al. 1994; Gadd 1993; Turnau 1991; Babich and Stotzky 1977c). High concentration of Cd could usually occur in industry polluted area. As the quantity of mycoses caused by *Fusarium* spp. increased and were severe on industry polluted area (data unpublished) it is of biological and ecological interest to explain the influence of Cd and other industrial toxicants on the distribution of fungal pathogens and their pathogenicity.

Thus in our toxicity experiments with different concentration of Cd added to agar medium, growth inhibition and morphology changes examined after subsequent incubation, revealed marked differences between studied fungi. Considering the results obtained by other scientists we tried to explain our observations.

Babich and Stotzky (1977a) grouped microscopic fungi into Cd-sensitive categories. The first category consists of fungi capable of growing in the presence of up to 10 mg of Cd/l, the second category consists of fungi capable of growing in the presence of up to 100 mg of Cd/l. In our study *S. tritici* could probably be included into the first category, while the rest (*A. alternata, B. sorokinina, E. purpurascens*) into the second category. In different investigations (Bagy et. al. 1981) the second group was recognised as the most common consisting mainly of saprophytic fungi. One investigated species *S. tritici* is known as cereal pathogenic fungus. This could be linked to its high sensitivity to Cd concentration in medium.

In our tests there was no correlation between the class of fungus and sensitivity to Cd. This was also recognised by Babich and Stotzky (1977c). However all tested cultures were in their imperfect stage and that present a possibility that the stage of growth could also be important in their sensitivity to Cd concentration. *Fungi Imperfecti* are also potential *Ascomycetes* and this could explain above mentioned suggestion. In a test conducted by Płaza et al. (1988) statistical differences were demonstrated in Cd growth inhibition only between *Zygomycetes* and *Ascomycetes*.

Between investigated fungi very special group are species of *Fusarium*. Ngu et. al. (1993) demonstrated that *Fusarium* spp. were highly tolerant of many metals in the soil, making it more difficult to find selective inhibitors for controlling these fungi. Also Gadd (1993) suggested that *Fusarium* pathogens of cereals were able to tolerate and grow in the presence of heavy metals but Cd seems to be the most toxic of the metals examined. In the presented study *F. avenaceum* was also very tolerant to Cd. After few days of observation *F. avenaceum* produced usual mycelium. We also observed a similar reaction in the case *B. sorokiniana*. It is difficult to explain this reaction, because there is no evidence about this species in literature. In the case of most investigated fungi we observed not only changes in hyphal morphology but also in pigmentation of mycelium or medium. According to Gadd (1993) and Babich and Stotzky (1985) the presence of toxic metal in growth medium alter cell wall composition, sometimes resulting in production of melanin and increase metal binding capacity. This may present the way of fungal detoxification of environment. We observed such reaction in the case *E. purpurascens*. *F. avenaceum*. *B. sorokiniana* also produced darker or brown cultures. It could also be concluded that *S. tritici* was more Cd sensitive species because it probably couldn't reduce toxicity by melanin mechanism.

In our investigation sporulation was more sensitive to Cd than mycelial proliferation. Spore formation by *E. purpurascens* was inhibited at 5mg Cd concentration. This concentration was not inhibitory to mycelial growth. Babich and Stotzky (1977b; c), Płaza et al. (1998) recognised this phenomenon and proposed to distinguish between concentration that are more inhibitory to spore formation or to mycelial proliferation.

Presented results also explain field observations (unpublished data). The adverse effect of Cd on fungal reproductive potential (e.g. reduction in growth rates and inhibition of fungal sporulation) could also influence the establishment, population dynamics and interactions, and general ecology of microbes in natural habitats. It could also be important in agroecology especially on fields polluted by industry.

REFERENCES

Babich H., Stotzky G. 1977a. Sensitivity of various bacteria, including *Acitnomycetes* and *Fungi* to cadmium and the influence of pH sensitivity. Appl. Environ. Microbiol., 33: 681–698.

Babich H., Stotzky G. 1977b. Reductors of the toxicity of cadmium to microorganisms by clay minerals. Appl. Microbiol., 38: 696–705.

Babich H., Stotzky G. 1977c. Effect of cadmium on fungi on interaction between fungi and bacteria in soil. Influence of clay minerals on pH. Appl. Environ. Microbiol., 33: 1058–1066.

Babich H., Stotzky G. 1985. Heavy metal toxicity to microbe-mediated ecologic processes: A review and potential application to regulatory policies. Environmental Research 36: 111–137.

Bagy M.M.K., El-Shorany H.M.M., El-Shanawary A.A. 1991. Effect of pH and organic matter on the toxicity of heavy metals to growth of some fungi. Folia Microbiol., 34 (4): 367–374.

Gadd G.M. 1993. Transley Review No.47. Interaction of fungi with toxic metals. New Phytol., 124: 24–60.

Ngu M., Moya E., Magan N. 1988. Tolerance and uptake of cadmium, arsenic and lead by *Fusarium* pathogens of cereals. International Biodeterioration Biodegradation 42: 55–60.

Płaza G., Łukasik W., Ulfig K. 1998. Effect of cadmium on growth of potentially pathogenic soil fungi. Mycopathologia 141: 98–100.

Tobin J.M., White C., Gadd G.M. 1994. Metal accumulation by fungi: applications in environmental biotechnology. Journal of Industrail Microbiol., 13: 126–130.

Turnau K. 1991. The influence of cadmium dust fungi in a Pino-Querectum forest. Ekologia Polska 39: 39–57.

POLISH SUMMARY

WPŁYW KADMU NA WZROST I MORFOLOGIĘ GRZYBNI WYBRANYCH GATUNKÓW FYLOPLANOWYCH

Wśród metali ciężkich kadm jest uważany za najgroźniejszy z pierwiastków zanieczyszczających środowisko i o istotnym znaczeniu dla zdrowia ludzi i zwierząt. Ponieważ mało wiadomo o wpływie tego pierwiastka na grzyby zasiedlające liście, celem niniejszej pracy była ocena zmian w rozwoju, morfologii i zarodnikowaniu grzybów. Wykonano doświadczenia szalkowe. Na podłoże PDA z dodatkiem kadmu wykładano krążki aktywnie rosnącej grzybni gatunków: *Alternaria alternata*, *Epicoccum purpurascens*, *Fusarium avenaceum*, *Bipolaris sorokiniana* i *Septoria tritici*. Każdy z badanych grzybów reagował odmiennie na zanieczyszczenie podłoża kadmem. W badaniach obserwowano ograniczenie wzrostu liniowego grzybni. Następowały zmiany w pigmentacji grzybni oraz podłoża, a także ograniczenie lub brak zarodnikowania. Najbardziej wrażliwymi na kadm okazał się gatunek *S. tritici*.