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GROWTH OF SOIL FUNGI ON CULTURE MEDIA CONTAMINATED WITH SELECTED HERBICIDES

WZROST WYBRANYCH GRZYBÓW GLEBOWYCH NA PODŁOŻACH ZANIECZYSZCZONYCH WYBRANYMI HERBICYDAMI

Abstract: The study aimed at showing the effect of culture media contaminated with triazine herbicides and urea herbicide on the growth of selected soil fungi. Contamination of culture medium with Gesaprim 500 WP, Gesatop 50 WP, Gesagard 50 WP and Ustilan 70 WP herbicides has a significant effect on the growth of soil fungi examined. The magnitude of this effect depends on the quantity of introduced active ingredient contained in preparations and the species of soil fungi. Both stimulation and inhibition of the growth of surface mycelia was observed. Fungi of some species of the genus *Penicillium* as well as *Alternaria* sp., *Fusarium* sp., *Trichotecium roseum*, *Trichocladium asperum* and *Trichderma* sp. were most resistant to active ingredients contained in the herbicides under discussion, whereas *Penicillum claviforme* and *Mucor* sp. proved to be most sensitive to contamination of culture medium with herbicides.

Keywords: fungi, herbicides, contamination

Chemical substances introduced into soil in result of human activity are not meaningless for soil microflora [1–5]. Most frequently, these compounds are pesticides that are being used to reduce the occurrence of agrophages and to improve crop quantity and quality. Predominant position among them is taken by herbicides which constitute over 50 % of all crop protection chemicals used. Introduction of these chemicals into soil may have a remarkable effect on development of soil microflora, inhibiting or stimulating its growth and metabolic activity. Some substances may be a source of carbon or nitrogen for it, contributing to its growth.

There are many microorganisms known to live in environments that are heavily contaminated with crop protection chemicals [6–9]. Preparations being used in an

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uncontrolled manner in last years which can be retained for a long-time and then withdrawn from use and stored in tanks from which they can penetrate into soil and groundwater are unusually dangerous [10–13]. This generates a necessity of searching for standardised researches and evaluation criteria of harmfulness for such chemical compounds and their susceptibility to biodegradation [14].

The study aimed at determination of the growth of soil fungi on culture media heavily contaminated with selected herbicides.

Material and methods

In the experiment were used soil fungi cultures from the collection of the Department of Microbiology and Biotechnology of Environment, West Pomeranian University of Technology in Szczecin. In total, 12 different types and species were used, ie *Aspergillus niger*, *Penicillum claviforme*, *Alternaria* sp., *Penicillum* sp. (1), *Mucor* sp., *Trichotecium roseum*, *Penicillum allii*, *Penicillum* sp. (2), *Trichocladium asperum*, *Fusarium* sp., *Peaciliomyces* sp. and *Trichderma* sp., which were point inoculated on solid culture medium. Different herbicide quantities were introduced into a Martin medium [15], receiving the following doses: 1, 10, 100, 1000, and 10000 mg · dm^{-3}. The control was culture medium with no herbicide addition. In the experiment were used herbicides belonging to two groups. The first group was triazine herbicides with an active ingredient in the form of atrazine (Gesaprim 500 WP), simazine (Gesatop 50 WP), prometrine (Gesagard 50 WP), whereas the other one was urea herbicides (Ustilan 70 WP). All herbicides were within Toxicity Class IV. Triazine preparations contained 50 % of active ingredient, with a recommended dose per 1 hectare being 10 dm^3 or kg depending on the ingredient from. All these herbicides were a make of Novartis Crop Protectrion AG, Switzerland, whereas Ustilan 70WP, belonging to urea herbicides containing ethidimuron as an active ingredient, was a product of Bayer France Phytochim. The dose recommended by manufacturer for the latter is 10 kg · ha^{-1}.

Culture incubation was carried out at 25 ^{o}C. For 7 days, measurements of the growing colony were made (diameter, in cm).

Statistical analysis of the received results was made by calculating analysis of variance, with significance of differences being calculated with Duncan's test at $p = 0.05$.

Results and discussion

The examined fungi were characterised by varied growth on the control medium without herbicide addition, with mycelium diameter ranging 1.4–6.9 cm. The largest growth was observed in case of fungi *Mucor* sp. (on average 5.8 cm) and *Trichoderma* sp. (on average 6.9 cm), whereas the least one in a fungi identified as *Penicillum claviforme* (on average 1.4 cm).

Based on the statistical analysis carried out, significant differences were found in the growth of surface mycelium after introduction of different herbicides into culture medium (Table 1).

Table 1

The effect of herbicides on the growth of surface mycelium [cm]

Fungus	Dose of herbicide [mg · dm^{-3}]					
	$LSD_{0.05}$	Control	10	100	1000	10 000
Gesatop 50 WP						
Aspergillus niger	0.744	4.1	4.1	3.5	5.3	0.8
Penicillium claviforme	0.198	1.7	2.0	1.8	1.4	0.1
Alternaria sp.	0.906	2.4	1.1	2.4	3.4	1.1
Penicillium sp. (1)	0.797	3.4	4.0	3.7	4.8	1.1
Mucor sp.	1.276	7.2	6.3	6.8	8.0	0.6
Trichotecium roseum	0.501	4.8	5.4	4.4	8.0	1.3
Penicillim allii	0.415	3.1	3.2	3.5	3.2	2.6
Penicilium sp. (2)	0.697	2.8	2.6	2.8	3.1	1.4
Trichocladium asperum	0.191	2.0	2.0	1.9	1.8	1.0
Fusarium sp.	1.117	1.9	2.1	2.4	1.5	0.8
Peacilliomyces sp.	1.288	4.1	3.8	4.1	4.0	1.7
Trichoderma sp.	1.918	6.7	6.7	6.1	5.4	5.2
Ustilan 70 WP						
Aspergillus niger	0.916	2.2	2.0	2.0	2.5	0.1
Penicillium claviforme	0.245	0.8	0.8	0.9	0.4	0.1
Alternaria sp.	0.335	1.5	1.4	1.5	1.4	0.4
Penicillium sp. (1)	0.913	2.7	2.3	2.3	1.7	0.5
Mucor sp.	0.602	4.0	3.5	3.2	3.3	1.2
Trichotecium roseum	0.506	3.7	3.5	3.3	0.9	0.1
Penicillim allii	0.237	1.6	1.7	1.8	1.5	0.2
Penicilium sp. (2)	0.157	1.7	1.5	1.5	2.1	0.3
Trichocladium asperum	0.216	1.2	1.1	1.0	0.9	0.2
Fusarium sp.	0.311	1.2	1.3	1.4	1.0	0.2
Peacilliomyces sp.	0.645	2.3	1.9	1.8	2.0	0.2
Trichoderma sp.	2.094	6.1	6.8	4.4	6.8	0.3
Gesaprim 500 WP						
Aspergillus niger	1.159	5.2	4.0	4.2	3.4	1.8
Penicillium claviforme	0.319	1.9	1.7	2.0	0.9	0.5
Alternaria sp.	0.810	2.4	2.6	2.8	2.5	1.5
Penicillium sp. (1)	1.186	3.5	4.2	3.4	3.3	1.5
Mucor sp.	1.634	6.8	6.7	5.3	4.3	1.7
Trichotecium roseum	0.396	5.5	4.7	5.4	4.0	3.0
Penicillim allii	1.155	3.9	3.1	3.0	2.8	1.6
Penicilium sp. (2)	1.296	2.6	3.0	3.0	2.9	1.6
Trichocladium asperum	0.562	2.1	2.0	2.0	1.5	1.5
Fusarium sp.	1.199	2.0	2.3	4.4	1.8	0.6
Peacilliomyces sp.	1.471	5.1	4.9	4.4	4.5	1.8
Trichoderma sp.	0.824	8.3	8.0	8.8	4.9	2.7

Table 1 contd.

Fungus	Dose of herbicide [mg · dm^{-3}]					
	$LSD_{0.05}$	Control	10	100	1000	10 000
Gesagard 50 WP						
Aspergillus niger	1.418	3.3	4.0	3.1	2.3	0.5
Penicillium claviforme	0.210	1.3	1.1	1.4	0.7	0.1
Alternaria sp.	1.089	2.1	2.2	2.5	3.0	1.8
Penicillium sp. (1)	0.721	2.8	4.7	3.2	2.7	1.0
Mucor sp.	0.545	5.2	8.1	4.2	4.5	0.5
Trichotecium roseum	1.507	5.1	6.3	3.8	3.1	1.3
Penicillim allii	1.974	3.8	2.3	3.9	3.2	1.5
Penicilium sp. (2)	1.292	3.6	2.5	3.3	3.0	1.4
Trichocladium asperum	0.436	2.0	1.3	1.5	1.4	1.0
Fusarium sp.	0.968	2.9	1.2	2.0	1.0	0.6
Peacilliomyces sp.	0.904	3.1	3.7	2.4	3.4	0.9
Trichoderma sp.	0.770	6.5	8.1	5.1	3.4	1.3

In certain soil fungi, eg of the genus *Penicillum* (strain 1), *Penicillum claviforme*, *Fusarium* sp., *Trichtecium roseum* and *Penicillum allii*, addition of Gesatop 50 WP preparation to culture medium in a dose contaminating it even considerably (1000 mg · dm^{-3}) stimulated the growth of surface mycelium, on average from several to even few dozen percent in relation to the control. In case of other fungi, a slight inhibition of growth was observed – from a few to several percent, whereas in case of *Alternaria* sp. a twice smaller growth was observed in relation to the control. The highest contamination with simazine-containing preparation in culture medium (10000 mg · dm^{-3}) induced a significant inhibition of the growth of all fungi, in particular of *Penicillum claviforme* and *Mucor* sp. The largest resistance to such a high contamination with the herbicide under discussion was demonstrated by fungi *Trichoderma* sp. and *Penicillum allii*. Jaworska and Dluzniewska [3] demonstrated in their study that herbicides had an effect on the growth and biological activity of fungi *Trichoderma harzianum* and *Trichoderma viride* but changes depended on the type of preparation and its concentration as well as fungus isolate. Nowak [16] reported intensification of the growth inhibition in a colony of *Trichoderma* spp. Wachowska [4] observed that herbicide Roundup tested under *in vitro* conditions demonstrated fungistatic activity in relation to *Trichoderma hamatum*, inhibiting the colony growth by 38 %. The varied effect of herbicides on the growth of *Trichoderma viride* was also observed by Klimach and Wieczorek [1].

Addition of herbicide Gesaprim 500 WP to culture medium also induced significant differences in the growth of surface mycelium of the fungi under examination (Table 1). In case of fungi *Alternaria* sp., *Penicillum* sp. (strain 1 and strain 2) as well as *Fusarium* sp., several percent stimulations were observed as affected by the presence of this herbicide in culture medium. The remaining fungi responded unfavourably, the result of which was smaller mycelium diameter. This effect was most visible after application of the highest contamination with herbicide Gesaprim 500WP.

In fungi such as *Aspergillus niger*, *Penicillum* sp. (strain 1), *Mucor* sp., *Trichotecium roseum* and *Peaciliomyces* sp., a stimulation of surface mycelium was observed as affected by an active ingredient contained in Gesagard 50 WP preparation added to culture medium in a least dose (10 mg · dm^{-3} of medium), on average by over 30 % in relation to the control. Larger concentration of active ingredient, a 10- and 100-fold one, stimulated only the growth of mycelium in *Alternaria* sp., *Penicillum* sp. (strain 1) and *Peacilliomyces* sp. in culture medium containing 1000 mg · dm^{-3} of active ingredient. The increase of contamination with this herbicide induced clear reduction in the growth of fungi, on average by almost 70 % when compared with the control.

The addition of herbicide Ustilan 70 WP induced significant differences in the growth of fungi, which also depended on the value of herbicide concentration in culture medium (Table 1). Introduction of this xenobiotic to a concentration of 1000 mg · dm^{-3} of culture medium induced stimulation of the growth of surface mycelium in some fungi, on average by several percent. An exception was fungi identified as *Penicillum* sp. (strain 1), *Mucor* sp., *Trichtecium roseum*, *Trichocladium asperum* and *Peacilliomyces* sp., in which inhibition of growth was observed in relation to the control, on average by over 30 %. Application of a ten-fold higher concentration of herbicide significantly decreased the growth of all fungi.

When analysing the results of experiment with herbicides Gesaprim 500 WP, Gesatop 50 WP, Gesagard 50 WP and Ustilan 70 WP, a tendency was observed for the growth of some surface mycelia on solid culture media with smaller quantity of active ingredient contained in the preparation under examination which depended on the introduced dose. Most frequently observed stimulation was found in fungi as affected by herbicides Gesatop 50 WP and Gesaprim 500WP, while a smaller one in result of introduction of Ustilan 70 WP preparation. After introduction of larger herbicide concentrations, a clear inhibition of the growth of surface mycelium was observed. The strongest inhibition of mycelium growth was observed after addition of herbicide Ustilan 70 WP and herbicide Gesagard 50WP. The smallest inhibition of growth was found in result of the effect of active ingredient contained in Gesatop 50 WP preparation.

The fungi most resistant to active ingredients contained in the herbicides discussed above were as follows: *Penicillium* sp. (strain 1) and *Penicillium allii*, *Alternaria* sp., *Fusarium* sp., *Trichotecium roseum*, *Trichocladium asperum* and *Trichderma* sp. in culture medium contaminated with Gesatop 50 WP preparation. The fungus most sensitive to contamination of culture medium with the herbicides under discussion proved to be *Penicillum claviforme* and also frequently *Mucor* sp.

Most herbicides in small doses had no significant effect on the growth and development of fungi and other soil microorganisms, whereas a varied response of these microorganisms was observed after application of larger doses of preparation consisting in inhibition or stimulation of their growth and proliferation [5, 17–20].

Conclusions

Contamination of culture medium with herbicides Gesaprim 500 WP, Gesatop 50 WP, Gesagard 50 WP and Ustilan 70 WP had a significant effect on the growth of the

soil fungi under examination. The magnitude of this effect depends on the quantity of introduced active ingredient contained in preparations and the type or species of soil fungi. On solid culture media with addition of the smallest quantity of active ingredient from preparations Gesatop 50 WP and Gesaprim 500 WP a tendency was observed for the growth of some surface mycelia and a considerably smaller stimulation in result of introduction of Ustilan 70 WP preparation. The strongest inhibition of mycelium growth was observed after addition of herbicide Ustilan 70 WP and herbicide Gesagard 50WP. The smallest inhibition of growth was found in result of the effect of active ingredient contained in Gesatop 50 WP preparation.

The fungi most resistant to active ingredients contained in the herbicides discussed above were those of the genus *Penicillium* strain 1 and *Penicillium allii*, *Alternaria*, *Fusarium*, *Trichotecium roseum*, *Trichocladium asperum* and *Trichderma* sp. in culture medium contaminated with Gesatop 50 WP preparation. The fungus most sensitive to contamination of culture medium with the herbicides under discussion proved to be *Penicillum claviforme* and also frequently *Mucor* sp.

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WZROST WYBRANYCH GRZYBÓW GLEBOWYCH NA PODŁOŻACH ZANIECZYSZCZONYCH WYBRANYMI HERBICYDAMI

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Abstrakt: Celem badań było wykazanie wpływu zanieczyszczenia herbicydami triazynowymi oraz herbicydem mocznikowym podłoży hodowlanych na wzrost wybranych grzybów glebowych. Zanieczyszczenie podłoża herbicydami Gesaprim 500 WP, Gesatop 50 WP, Gesagard 50 WP i Ustilan 70 WP wpływa znacznie na wzrost badanych grzybów glebowych. Wielkość tego wpływu zależy o ilości wprowadzonej substancji aktywnej zawartej w preparatach oraz rodzaju czy gatunku grzybów glebowych. Stwierdzono zarówno stymulacje, jak i hamowanie wzrostu grzybni powierzchniowych. Do najbardziej odpornych grzybów na substancje aktywne zawarte w powyżej omawianych herbicydach były grzyby z niektórych gatunków z rodzaju *Penicillium*, a także *Alternaria* sp., *Fusarium* sp., *Trichotecium roseum*, *Trichocladium asperum* oraz *Trichderma* sp. Najbardziej wrażliwym grzybem na zanieczyszczenie podłoża omawianymi herbicydami okazał się *Penicillum claviforme* i *Mucor* sp.

Słowa kluczowe: grzyby, herbicydy, zanieczyszczenie