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DIRECT INDICATORS OF DETERMINATION OF GLYPHOSATE DECOMPOSITION BY FILAMENTOUS FUNGI

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Herbicides containing glyphosate undergo decomposition mainly by microorganisms. Monitoring this process requires an application of simple, quick and cheap instrumental methods. Thin layer chromatography was used in the present work to determine organic compounds in fermentation broth and absorption UV-Vis spectroscopy to determine orthophosphate ions (P_i). The obtained chromatograms of fermentation broth showed glyphosate biodegradation, but not with the use of commonly known route with the formation of glycin, aminomethylphosphonic acid (AMPA) and orthophosphate ions (P_i). This fact was confirmed by spectroscopic studies used for the parallel determination of concentrations of inorganic phosphorus (P_i), the levels of which were very low.

Key words: glyphosate, biodestruction, direct indicators

INTRODUCTION

Glyphosate is an active substance, of commonly used herbicide, so-called Roundup. Herbicide detoxification is possible first of all due to assessed activity of soil microorganisms (Gołębiowska and Grzybowska, 1991).

Generally assumed, and the mostly significant route of glyphosate degradation is decomposition of C-P bond, catalyzed by microbiol enzymes (C-P lyase, acidic and basic phosphatase) (La Nauze and others, 1970; Wacket and others, 1987). The final product of decomposition should be orthophosphate ion PO_4^{3-} (P_i), as well as organic by-products, that do not depend on the mechanism of decomposition. For the identification of glyphosate as well as organic compounds formed during its decomposition, high performance liquid chromatography (HPLC) is used (Gratzfeld-Husgen and Schuster, 1994). However, due to large costs and reduced access to these

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instruments there exists a need to employ cheaper analytical methods in these studies. One of them is thin layer chromatography (TLC) for determination of organic compounds. The second method used in determination of the concentration of inorganic phosphate (P_i) is method of absorption spectroscopy UV-Vis, in which the formation of colored heteropolyacids is used (Mejbaum-Katzenellenbogen and Mochnacka, 1969). This method is sensitive and universal, since enables to determine phosphorus in inorganic as well as in organic samples or in various types of minerals.

The selection of indicator methods is very significant since they must be quick and relatively cheap in order to be able to control biochemical processes while they occur. The aim of the present work was to employ the two mentioned methods to follow-up the transformations of glyphosate, influenced by strains of *Fusarium*.

MATERIAL AND INVESTIGATION METHODS

The studied material consisted of fermentation broth from the cultivation of 5 strains of *Fusarium*: *F.solani* (H_{38}, H_{39}), *F.oxysporum* (H_{41}), *F.moniliforme* (H_{40}) i *F.sporotrichioides* (H_{65}). The cultures were carried out on the synthetic growth medium with glyphosate as the sole source of phosphorus in the concentrations: 0.5 ($P_{0.5}$), 1.0 (P_1), 1.5 ($P_{1.5}$) and 2.0 (P_2) mmol/dm^3. The control was full synthetic medium (P_c) and medium containing no source of phosphorus (P_p). The cultures were carried out with shaking method for 3 weeks at the temperature 25 ^oC.

ANALYTICAL METHODS

The analysis of orthophsophate ions (P_i) in fermentation broths of the studied fungi were carried out with the use of Fiske-Subbarow method with absorption spectrometry.

The chromatographic analysis of organic compounds from fermentation broths was carried out on Kieselgel 60 F_{254} dishes from Merck in the developing system $CHCl_3$: CH_3OH : NH_3(aq) in the volumetric ratio 30 : 25 : 15. The chromatograms were produced with butanol solution of ninhydrin. The following standards were used: glyphosate (G), glycin (Gly) and aminomethylphosphonic acid (AMPA).

RESULTS AND DISCUSSION

Table 1 shows the results of determination of the concentration of orthophosphate ions (P_i) of the tested *Fusarium* strains depending on the concentration of glyphosate in fermentation broths. Chromatograms of fermentation broths of the studied strains of *Fusarium* fungi are shown in fig. 1.

Fiske-Subbarow method is very sensitive and could be used for determination of the concentration of orthophosphate ions on the level 0.01 mg/100 cm^3. When comparing with the initial concentrations (P_c), they are very low, that could reflect the

use of inorganic form of phosphorus in the growth of fungal biomass or other route of glyphosate decomposition in the presence of the studied fungi, as the result of which, inorganic phosphorus (P_i) was not released (Krzyśko-Łupicka and Sudoł, 2005).

Table 1. The concentration of inorganic phosphorus (P_i) in fermentation broths of the tested fungi of *Fusarium* species depending on the concentration of glyphosate after three weeks of incubation, in mg/100 cm^3

strain	Pc (K)	Pp (K_1)	$P_{0.5}$	$P_{1.0}$	$P_{1.5}$	$P_{2.0}$
F.sporotrichioides H65	20.91	0.06	0.04	0.00	0.00	0.00
F. moniliforme H40	20.91	0.06	0.06	0.08	0.06	0.15
F. solani H39	18.30	0.08	0.06	0.06	0.15	0.02
F. solani H38	22.48	0.10	0.12	0.10	0.12	0.06
F. oxysporum H41	20.91	0.10	0.15	0.15	0.01	0.08

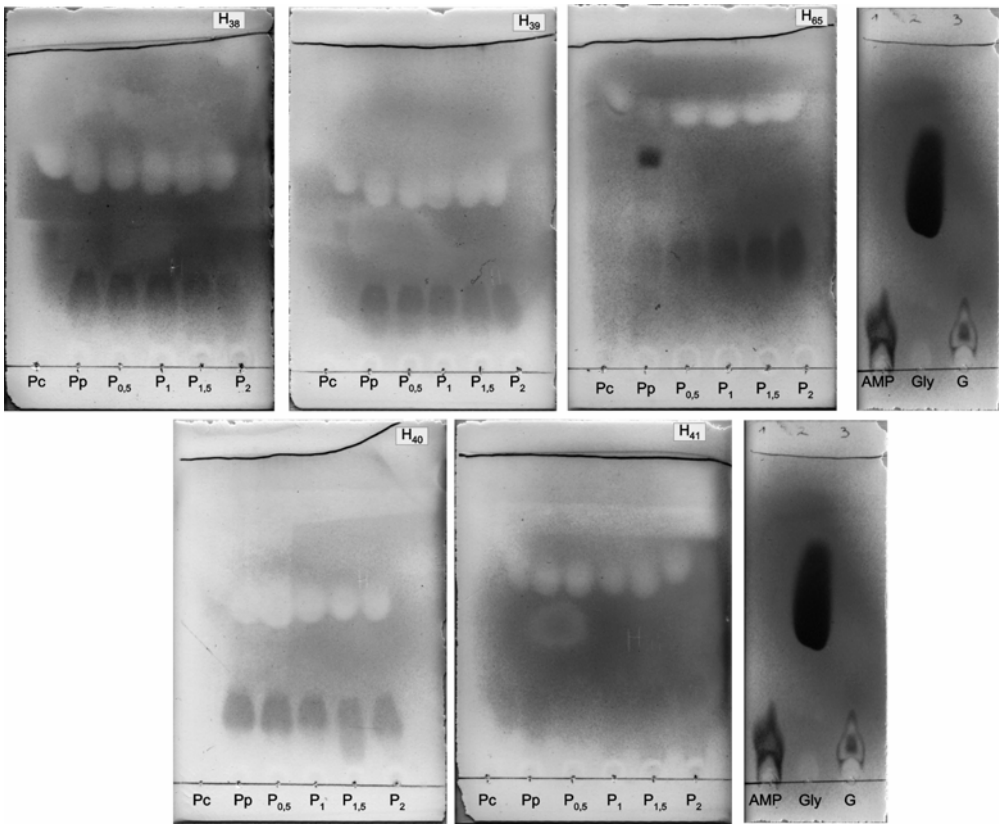

Fig. 1. The TLC chromatograms of fermentation broths of the tested fungi of *Fusarium* species after three weeks of incubation. Used standards: AMPA– aminomethylphosphonic acid, Gly–glycin and G-glyphosate. Tested strains: H_{38}, H_{39} – *F. solani*, H_{40} – *F. moniliforme*, H_{41} – *F. oxysporum*, H_{65} – *F. sporotrichioides*

Basing on the results obtained only by this method, it was impossible to uniquely assess the mechanism of glyphosate degradation.

Chromatographic analysis of fermentation broths of the tested fungi, cultivated in the presence of glyphosate, or in the absence of inorganic phosphorus showed the presence of compounds that were not observed in the full growth medium (P_c). The two classes of compounds formed (the first class (with the higher values of R_f) did not possess free amino group and the second (with the lower values of R_f) contained free amino group (colored spots as the result of reaction with ninhydrin)) might reflect glyphosate transformations. However, the presence of neither glycin nor aminomethylphosphonate as the potential products of glyphosate degradation with the commonly suggested route, with inorganic phosphorus (P_i) as the final product was not detected. The results of analyses of the both analytical methods used in the present study might show that the studied strain of *Fusarium* fungi possessed other systems of glyphosate biodegradation. In the literature, there were found earlier reports in this area, also suggesting different mechanism of glyphosate utilization by various microorganisms (Pipke and Arnhein, 1988; Obojska and others, 1999).

CONCLUSIONS

In the studies on glyphosate transformations, thin layer chromatography (TLC) and UV-Vis spectroscopy were used as indicative methods. The obtained results showed that these methods can be considered as quick and cheap, sufficient to show biochemical transformations of glyphosate by microbiological route. However, an understanding of all the products of its degradation require the application of other instrumental methods, i.e. HPLC i GC/MS after preliminary derivatization of the studied samples.

REFERENCES

GOŁĘBIOWSKA D, GRZYB – MIKLASZEWSKA J., 1991, *Kompleksy humus – enzym,* Postępy Nauk Rolniczych, 4,5,6, 105 – 127.

GRATZFELD – HUSGEN A., SCHUSTER R., 1994, *HPLC for Environmental Analysis,* Hewlett – Packard, 82 – 83.

KRZYŚKO - ŁUPICKA T., SUDOŁ T., (wysłane do druku 2004), *The ability of selected Fusarium fungi to growth in the presence of different glyphosate concentrations,* Polish J. of Chem. Tech.

MAJBAUM – KATZENELLENBOGEN W., MOCHNACKA I., 1969, *Kurs praktyczny z biochemii,* PWN Warszawa, 198 – 201.

LA NAUZE I., ROSSENBERG H., SHAW P.C., 1970, *The enzymic cleavage of the carbon – phosphorus bond: puryfication and properties of phpsphonates,* Biochim. Biophys. Acta, 212, 332 – 350.

OBOJSKA A., LEJCZAK B., KUBRAK M., 1999, *Degradation of phosphonates by streptomycete isolates,* Appl. Microbiol. Biotechnol., 51, 872 – 876.

PIPKE R., AMRHEIN N., 1988, *Isolation and characterisation of a mutant Arthrobacter sp. strain GLP-1 which utilizes the herbicide glyphosate as its sole source of phosphorus and nitrogen,* Appl. Environ. Microbiol., 54, 2868 – 2870.

WACKETT L.P., SKAMES S., VENDITTI C., WALSH C., 1987, *Bacterial carbon – phosphorus lyase. Products, rates and regulation of phosphonic acid metabolism,* J. Bacteriol., 169, (2), 710 – 717.

SUDOŁ T., KRZYŚKO-ŁUPICKA T., *Pośrednie wskaźniki oznaczania destrukcji glifozatu przez grzyby strzępkowe,* Physicochemical Problems of Mineral Processing, 39 (2005) 257-261 (w jęz. ang)

Herbicydowe środki ochrony roślin, zawierające w swoim składzie glifozate, ulegają destrukcji przez mikroorganizmy. Monitorowanie tego procesu wymaga wykorzystania prostych, szybkich i tanich metod instrumentalnych. W pracy do tego celu wykorzystano chromatografię cienkowarstwową (TLC) do oznaczania związków organicznych w hodowlach i spektroskopię absorpcyjną UV-Vis do oznaczania jonów ortofosforanowych (P_i). Otrzymane chromatogramy płynów pohodowlanych wskazują na biodegradację glifozatu, ale nie na powszechnie uznawanej drodze z utworzeniem glicyny, aminometylofosfonianu (AMP) i jonów ortofosforanowych (P_i). Fakt ten potwierdzają badania spektroskopowe wykorzystane do równoległych oznaczeń stężeń fosforu nieorganicznego (P_i), których wielkości były bardzo małe.