Pol. J. Environ. Stud. Vol. 20, No. 5 (2011), 1313-1318

Original Research

Seeking Ways to Eradicate Potentially Pathogenic Fungi Isolated from Soil

Anna Szwedek-Trzaska, Anna Głowacka*

Department of Environmental Biology, Medical University of Łódź, Hallera 1, 90-647 Łódź, Poland

Received: 30 October 2010
Accepted: 15 March 2011

Abstract

Our study evaluates the susceptibility of 17 strains of mould isolated from the soil of allotments in Łódź, Poland to 6 types of herbicide: Roundup Ultra 170 SL, Mniszek 540 SL, Casoron 6.75 GR, Basta 150 SL, Fusilade Forte 150 EC, and Starane 250 EC. Of these herbicides, only Casoron 6.75 GR is used in agriculture on the root system, whereas all the others are used on the leaf surface. Isolated strains of fungi belonged to 9 species: *Fusarium aquaeductuum*, *Fusarium oxysporum*, *Mucor indicus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium purpurogeum*, *Penicillium rugulosum*, *Penicillium spinulosum*, and *Scedosporium prolificans*. The herbicide Basta 150 SL is the strongest inhibitor of the growth of the examined fungi strains, as well as being the fastest herbicidal agent (2-5 days after use), and can be used to eradicate mould fungi in soil.

Keywords: fungi, herbicides, eradication

Introduction

Soil is inhabited by many saprobiontic organisms such as bacteria, fungi, and small invertebrates, which are responsible for biochemical and biogeochemical transformations of nourishing ingredients (mineral and organic) in soil [1]. Of all the saprobionts occurring in the soil, fungi play an essential role, not only in initiating soil processes, but also in maintaining its physical and chemical quality and, together with vegetation, influencing the health and sanitary state of the soil. Saprobiontic fungi, especially moulds existing in ecosystems where imperfect forms can be etiologic factors of human and animal mycoses, have particular ecological and medical importance. Over time, the imperfect forms of the fungi can become infectious for living organisms after being inoculated into the tissues of the host [2]. The dynamic development of moulds can be promoted by global warming and increases of humidity such as the flood in Poland in 2010.

*e-mail: anna.glowacka1@umed.lodz.pl

The aim of our study was to evaluate the susceptibility of 17 mould fungi strains isolated from the soil of allotments in Łódź to 6 types of herbicides. These sites were chosen for two main reasons, firstly because of the occurrence of the Fusarium species among the isolated mould's strains in soil samples from allotments in Łódź and, secondly, because of the guidelines given by the European Commission in 2006 limiting the occurrence of *Fusarium* toxins in cereals and grains, as well as their prevention. The guidelines state that "the results of cooperation of the European Union countries on the field of the research of issues connected with the food have proven that *Fusarium* toxins are widely spread in the food chain in the EU" [3, 19].

Herbicides were chosen to be used in this study rather than fungicides because the moulds were isolated from the soil of allotments on which the owners cultivate vegetables (occasionally corn), and hence use herbicides such as: Roundup Ultra 170 SL, Mniszek 540 SL, Casoron 6.75 GR, Basta 150 SL, Fusilade Forte 150 EC, and Starane 250 EC. With the guidelines of the European Commission in

Table 1. Concentrations of herbicides used.

No.	Name of herbicide	Concentration [mg/ml]								
		1	2	3	4	5	6	7	8	9
1	Roundup Ultra 170 SL	17,000	8,500	4,250	2,125	1,062	0,531	-	-	-
2	Mniszek 540 SL	54,000	27,000	13,500	6,750	3,375	1,687	-	-	-
3	Casoron 6.75 GR	6,750	3,375	1,687	0,844	0,422	0,211	-	-	-
4	Basta 150 SL	15,000	7,500	3,750	1,875	0,9375	0,469	0,234	0,117	0,059
5	Fusilade Forte 150 EC	15,000	7,500	3,750	1,875	0,9375	0,469	-	-	-
6	Starane 250 EC	25,000	12,500	6,250	3,125	1,5625	0,7812	-	-	-

mind, one intention of our study was to examine the possibility of using herbicides both to destroy plants and moulds, the latter of which are a worldwide health problem. In the future such a procedure could limit leaching into the soil (the environment) to only one chemical agent instead of two (fungicide and herbicide). It is worth remembering that while on the one hand the use of both fungicides and herbicides destroys fungi and unwanted plants, on the other it has a negative influence on the environment of all living organisms, including human beings. A search of available literature reveals no mention of either limiting the development of potentially pathogenic moulds appearing in the soil, nor their eradication, that is the complete removal, lack of occurrence (of the pathogen) in humans, animal organisms, and other elements of the environment. The lack of data dealing with the issue of using herbicides to eradicate moulds isolated from soil promoted us to conduct such research.

The aim of this study was to evaluate the *in vitro* activity of the chosen herbicides on moulds isolated from soil, with a view to possibly using them to eradicate fungal species. Six herbicides commonly used in Poland in agriculture and gardening, also by the owners of allotments in Łódź, were chosen:

– Roundup Ultra 170 SL
• 170 g/l glyphosate ((N-(phosphonomethyl)glycine) as an isopropylamine salt)
– Mniszek 540 SL:
• mecoprop (methylchlorophenoxypropionic acid) 300 g/l
• MCPA (2-methyl-4-chlorophenoxyacetic acid) 200 g/l as a potassium salt
• dicamba (3,6-dichloro-2-methoxybenzoic acid) 40 g/l as a potassium salt
– Casoron 6.75 GR
• dichlobenil (2,6-dichlorobenzonitrile) – 6.75%
– Basta 150 SL
• ammonium gluphosynate 150 g/l
– Fusilade Forte 150 EC
• fluazifop-P-butyl (aryloxyphenoxypropionate) 150 g/l
– Starane 250 EC
• fluroxypyr as a methyl ester (4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid) 250 g/l

Material and Methods

Seventeen fungal strains isolated from the soil of allotments in Łódź were chosen and used in the research. Isolated strains belonged to 9 species: *Fusarium aquaeductuum*, *Fusarium oxysporum*, *Mucor indicus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium purpurogeum*, *Penicillium rugulosum*, *Penicillium spinulosum*, and *Scedosporium prolificans*. Most commonly isolated were *Fusarium aquaeductuum* (4 strains), *Penicillium citrinum* (4 strains), and *Penicillium chrysogenum* (3 strains), whereas the species *Fusarium oxysporum*, *Mucor indicus*, *Penicillium purpurogeum*, *Penicillium rugulosum*, *Penicillium spinulosum*, and *Scedosporium prolificans* were represented by the one strain (Table 1) [4, 5]. The susceptibility of the strains was evaluated by agar-gel diffusion.

A 48-hour-old liquid culture on a Sabouraud medium was used in the study. 0.5 ml of this culture was added to the 5 ml of liquid Sabouraud medium. From that dilution, 1 ml of the culture was taken and cultured on a Petri plate (diameter 10 cm) containing 30 ml of 3% concentration of Sabouraud agar at pH=5.6. After 1 hour of incubation at 37ºC, rows of 10 cm diameter were cut with a sterile tool. Each row was numbered, and to each was added 0.1 ml of the particular dilution of each of 6 herbicides. The plates were incubated for 48 hours at 37ºC. The diameters of the inhibitory area of growth of the fungal strain around each row was then measured in mm; an average measurement of two diameters perpendicular to each other was taken.

The minimal inhibitory concentration (MIC) was used as a measure of the antifungal activity of the herbicide, calculated from the curve of straight-line growth drawn in a semi-logarithmic scale according to the mathematical formula [6]:

$$\log \text{MIC} = \overline{\log C_1} + \frac{\overline{\log C_2} - \overline{\log C_1}}{\overline{N_2} - \overline{N_1}}\left(10 - \overline{N_1}\right)$$

$\overline{N_1}, \overline{N_2}$ – arithmetical average of the two groups of the diameters of the inhibitory area of growth of the fungal strain

$\overline{\log C_1}, \overline{\log C_2}$ – arithmetical average of logarithms of the two groups of the concentrations of the used herbicide

Results

The *in vitro* Minimal Inhibitory Concentrations of 17 strains of the fungi isolated from the soil of allotments in Łódź, were evaluated using different concentrations of herbicides (Table 1). Concentrations of the herbicide Roundup Ultra 170 SL were between 17.000 [mg/ml] and 0.531 [mg/ml]. Concentrations of Mniszek 540 SL were between 54.000 [mg/ml] and 1.687 [mg/ml]. Concentrations of Casoron 6,75 GR were between 6.75 [mg/ml] and 0.211 [mg/ml]. Concentrations of Basta 150 SL were between 15.000 [mg/ml] and 0.059 [mg/ml]. Concentrations of Fusilade Forte 150 EC were between 15.000 [mg/ml] and 0.469 [mg/ml]. Concentrations of Starane 250 EC were between 25.000 [mg/ml] and 0.7812 [mg/ml].

For Roundup Ultra 170 SL, MIC measurements were between the minimal value 114.02 mg/l = 0.114 mg/ml (strain No. 16 *Penicillium spinulosum*) and the maximum value 2801,56 mg/l = 2.801 mg/ml (strain No. 5 *Fusarium oxysporum*). For Mniszek 540 SL minimal inhibitory concentration measurements were between the minimal value 603.94 mg/l = 0.604 mg/ml (strain No. 16 *Penicillium spinulosum*) to the maximum value 8947,5 mg/l = 8.947 mg/ml (strain No. 5 *Fusarium oxysporum*). For Casoron 6.75 GR MIC measurements were between 393.9mg/l = 0.394 mg/ml (arithmetical average for the species *Penicillium chrysogenum*) and 2936,3 mg/l = 2.936 mg/ml (strain No. 14 *Penicillium purpurogeum*). For Basta 150 SL MIC measurements were between 23.51 mg/l = 0.024 mg/ml (strain No. 17 Scedosporium prolificans) and 316.23 mg/l = 0.316 mg/ml (strain No. 6 Mucor indicus). For Fusilade Forte 150 EC MIC measurements were between 4,786 mg/l = 0.0048 mg/ml (strain No. 16 *Penicillium spinulosum*) and 3101,54 mg/l = 3.102 mg/ml (arithmetical average for the species *Fusarium aquaeductuum*). For Starane 250 EC MIC measurements were between 207.54 mg/l = 0.2075 mg/ml (strain No. 16 *Penicillium spinulosum*) and 3148,91 mg/l = 3.149 mg/ml (arithmetical average for the species *Penicillium citrinum*).

The arithmetical average of the two groups of the diameters of the inhibitory area of growth of the fungal strain varied, resulting from 10.5 to 32.0 mm based on the concentration and type of herbicide. Fungal growth inhibition was most commonly observed in the sixth concentration of a herbicide. The largest areas of inhibition of fungal growth were observed after treatment with Basta 150 SL. By the sixth concentration, 0.469 mg/ml, growth of all the strains was still inhibited.

Discussion

Fungi, being plant parasites, are susceptible to a range of chemical agents used in agriculture, including most herbicides, with some stimulating and others inhibiting their growth.

Hodges compared the influence of a number of herbicides on the growth, development, and germination of *Drechslera sorokiniana* and *Drechslera teres* [7], and found that Aminopielik D and 2, 4 – D and dicamba demonstrate the least antifungal activity toward *Drechslera sorokiniana* in all used concentrations. Additionally, a study by Fabisiewicz and Mikołajska on two fungal species, *D. sorokiniana* and *D. teres*, using 4 herbicides (Chwastox liquid, Chwastox M, Chwastox D, and Aminopielik D in concentrations from 1 to 1000 μg/g) showed that higher concentrations of herbicides inhibit growth of the mycelium as well as germination of spores to a greater degree. The least effective herbicide in inhibiting the growth of *D. sorokiniana* and *D. teres* mycelium is Aminopielik D, whereas the most effective is Chwastox D [8].

Our research included the herbicide Mniszek 540 SL, which has a similar chemical structure to 3 used herbicides: Chwastox (M, D and liquid) with the active agent dicamba, mecoprop, and MCPA. Mniszek 540 SL proved to be the least effective with respect to 8 of 9 examined fungal species: *Fusarium aquaeductuum*, *Fusarium oxysporum*, *Mucor indicus*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium purpurogeum*, *Penicillium rugulosum*, and *Scedosporium prolificans* (Table 2). *Penicillium spinulosum* was the species most susceptible to Mniszek 540 SL.

According to Klimek et al., concentrations up to 25 mM of the herbicide glyphosate (Roundup) stimulate the growth of *Penicillium chrysogenum* strains on minimal medium (agar). Glyphosate in the medium was the only nitrogen source for strains of *Penicillium chrysogenum*. The authors suggest that Roundup may enter the fungi cells by simple passive diffusion, or inducible carriers and products of glyphosate breakdown (aminoacids) are used by mycelium cells; this may be confirmed by the fact that amino compounds, such as aminoacids and ammonia, have been isolated from mycelium instead of herbicide [9]. Our research showed *Penicillium chrysogenum* to be the second most susceptible species of all nine fungal species to Roundup Ultra 170 SL.

Araujo, Monteiro and Abarkeli studied *in vitro* the effects of glyphosate (Roundup) on microbial activity in two typical Brazilian soils, one with and one without a previous history of glyphosate application. A solution of technical glyphosate (analytical standard) sufficient to give a final glyphosate concentration of 2.16 mg/kg was added to 75.0 g sub-samples of soil. Biodegradation of glyphosate was evaluated by carbon dioxide evolution and soil microbial activity was evaluated by measuring fluorescein diacetate (FDA) hydrolysis over a period of 32 days. After 32 days of incubation, the number of actinomycetes and fungi increased while the number of bacteria showed a slight reduction. In addition, CO_2 increased by10-15% and FDA hydrolysis increased by 9-19% in the presence of glyphosate when compared with the same type of soil that had never received glyphosate. An increase of 10-15% in the evolved CO_2 suggests that soil microorganisms are able to use glyphosate as a carbon source. The authors observed that glyphosate quickly decomposed within 10 days of incubation [10].

Table 2. Comparison of values of MIC [mg/l] for used herbicides on 17 strains of fungi isolated from soil.

No.	Fungi species	No. of strain	Roundup Ultra 170 SL		Mniszek 540 SL		Casoron 6.75 GR		Basta 150 SL		Fusilade Forte 150 EC		Starane 250 EC	
			MIC value [mg/l]											
				$\bar{x}$		$\bar{x}$		$\bar{x}$		$\bar{x}$		$\bar{x}$		$\bar{x}$
1.	*Fusarium aquaeductuum*	1	936.05		5,130.97		843.33		207.062		6,966.3		1,563.15	
		2	893.3		4,443.24		1,112.75		224.52		1,318.26		1,811.34	
		3	714.5		4,010.97		919.65		369.83		2,267.5		1808	
		4	921.51	866.34	6,745.36	5,082.6	843.33	929.77	240.66	260.52	1,862.1	3,103.54	543.25	1,431.43
2.	*Fusarium oxysporum*	5	2,801.56		8,947.5		1,002.88		176.2		467.73		1,043.28	
3.	*Mucor indicus*	6	921.51		5,722.68		1,475.7		316.23		117.48		835.6	
4.	*Penicillium chrysogenum*	7	182.39		2,137.96		289.3		72.99		659.17		514.28	
		8	274.79	652.86	4,355.12	2,843.1	357.2	393.9	120.53	86.5	959.4	804.3	341.19	371.67
		9	1,501.4		2,036.1		535.2		65.97		794.33		259.54	
5.	*Penicillium citrinum*	10	842.75		2,005.6		1,003		150.59		467.73		3,689.77	
		11	252.34		5,053.6		1,192.6		196.34		505.01		3,312.07	
		12	1,609.16		4,641.9		709.17		144.58		331.13		2,941.03	
		13	613.05		3,829.13		1,300.5		134.89		602.56		2,652.77	
6.	*Penicillium purpurogeum*	14	889.4	829.32	3,592.5	3,882.6	2,936.3	1,051.32	74.47	156.6	831.76	476.61	2,376.8	3,148.91
7.	*Penicillium rugulosum*	15	1062		6,018.66		1687		263		14.12		1,407.99	
8.	*Penicillium spinulosum*	16	114.02		603.94		817.52		218.2		4.786		207.54	
9.	*Scedosporium prolificans*	17	2,524.93		5,199.95		1,003		23.51		537.03		2,530.46	

In our *in vitro* study, Roundup Ultra 170 SL proved to be the agent most strongly inhibiting the growth of *Penicillium spinulosum*, and to a lesser degree the growth of *Fusarium oxysporum* (Table 1).

Roundup Ultra 170 SL and Basta 150 SL are based on *aminophosphate*. Roundup consists of N-(phosphonomethyl)glycine) as an isopropylamine salt, and Basta consists of ammonium gluphosynate. Despite the fact that both agents consist of *aminophosphate*, Basta 150 SL proved to be the more effective fungistatic agent against 8 of 9 fungal species, as well as being the most effective of all 6 herbicides.

The herbicide glufosinate-ammonium (GA) [butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-ammonium salt] was tested at concentrations from 2 to 2,000 g per ml for activity against growth and aflatoxin B1 (AFB) production by the mycotoxigenic fungus *Aspergillus flavus Link:Fr* by Tubajika and Damann. The highest concentration (2,000 mg per ml) reduced the colony diameter of *A. flavus* strain AF13 by 80%. AFB production was inhibited by 90% at this concentration. Reduction in mycelial dry weight and AFB1 production in response to GA application ranged from 17.2 to 97.1% and from 39.1% to 90.1%, respectively. Results indicate that GA has an inhibitory effect on growth and AFB1 production by *Aspergillus flavus* [11].

El-Hissy and Abdel-Kader tested the effect of three doses of 5 pesticides: Ceresan and Orthocid (fungicides), VCS-438 (herbicide), Durban and Dipterex (insecticides) on the mycelial dry weight of *Aspergillus fumigatus*, *Fusarium moniliforme*, *Penicillium italicum,* and *Sclerotium cepivorum* after 2, 4, 6, 8, and 10 days of treatment. Four pesticides (Ceresan, Orthocid, Durban, and Dipterex) induced inhibition of mycelial growth; low doses of VCS-438 were stimulatory to *Fusarium moniliforme* after 4 and 6 days, and to *Penicillium italicum* after 4 days. According to the authors, the rate of inhibition was influenced by the type of fungus, the age of the mycelium, and the dose of the pesticide [12].

El-Said, Abdel-Hafez, and Saleem examined the effect of using two herbicides, Herbizid and Touchdown, on the mycelial growth and production of extracellular enzymes by fungal strains isolated from Egyptian soil. The authors treated *in vitro* fungal strains, *Fusarium oxysporum*, *Mucor hiemalis*, and *Penicillium chrysogenum*, with different concentrations of herbicides Herbizid and Touchdown at concentrations from 0.019 to 0.152 mg/kg soil. Lower doses of herbicides occasionally promoted extracellular enzyme production and mycelial growth of some fungi. The incorporation of 50 ppm of Herbizid and Touchdown significantly activated amylase production by tested fungal strains. Treating the fungal strains *Fusarium oxysporum*, *Mucor hiemalis*, and *Penicillium chrysogenum* with "Herbizid" in concentrations of 50, 100, and 200 ppm increased Cx-cellulase production only in *Penicillium chrysogenum*. Lipase and protease production was always lower in the treated cultures than in the controls [13].

Gigliotti and Allievi evaluated and compared the toxicity of Bensulfuron and Cinosulfuron on soil microbes at the normal field application concentration and 100 times higher. In both cases, sulfonylureas had no effect on the total number of bacteria and nitrifiers, or on the respiration activity in the soil, but they did decrease nitrification activity. *In vitro* toxicity tests carried out on representative soil microbial strains using Bensulfuron at 50 mg/l showed some inhibition in 3 of the 17 bacterial strains and strong inhibition of almost all the 12 fungal strains. Cinosulfuron was found to have no effect on any of these strains in a previous study. Bensulfuron is potentially more toxic on soil heterotrophic microorganisms, but only at very high concentrations that are nearly impossible to reach though the usual agricultural applications [14].

Larson et al. tested the effect of glyphosate application on disease severity with certain isolates of *Fusarium* and *Rhizoctonia* in glyphosate-resistant sugar beet. Glyphosate at a concentration of 400 $\mu g \cdot mL^{-1}$ inhibited the growth of tested strains, but only by 17% and 11%. This indicates that *F. oxysporum* tolerates glyphosate [15].

Korzycko-Lupicka and Sudol investigated the survival of autochthonous fungi in soil treated with 1 mM aqueous solution of glyphosate. It was shown that *Fusarium* strains have a high tolerance against the applied doses of glyphosate (0.5-2.0 mM). Glyphosate is used as a sole source of phosphorus by tested fungi and, at concentrations 1.0-1.5 mM significantly increases the dry mass of tested fungi [16].

Vinther et al. examined the differences in microbial activity and soil characteristics due to the mineralization and sorption of two pesticides, glyphosate and metribuzin, in samples of sandy soil from Denmark. They focused on the clay and organic carbon content as well as soil respiratory and enzymatic processes and microbial biomass. The results indicated that the spatial variation of the soil parameters, particularly the content of organic C, had a major influence on the variability of the microbial parameters and on sorption and pesticide mineralization in the soil. For glyphosate, with a co-metabolic pathway for degradation, the mineralization was increased in soils with high microbial activity [17].

Evangelista, Cooper, and Yargeau examined the influence of the structure and presence of another source of carbon on the degradation of chlorphenoxyacids, such as mecoprop by microorganisms (including *Aspergillus niger*). Mecoprop was easily decomposed by microorganisms and, similar to our observations, the authors concluded that it doesn't inhibit the fungi growth [18].

Due to our research and analyzed literature of different authors, the issue of destroying soil fungi potentially pathogenic for humans is still current and worldwide. It is worth continuing research and seeking new herbicides whose low concentrations can have fungistatic potential and can be useful in agriculture.

Conclusions

1. Basta 150 SL proved to be the most effective of 5 herbicides used in agriculture applied to leaf surface

(Roundup Ultra 170 SL, Mniszek 540 SL, Basta 150 SL, Fusilade Forte 150 EC, Starane 250 EC) in inhibiting the growth of examined strains of mould fungi that are potentially pathogenic for humans.
2. The strongest inhibiting effect of Basta 150 SL, in contrast to the other herbicides used on the leaf surface, is connected with the presence of ammonium gluphosynate, which inhibits the synthesis of aminoacids such as glutamine.
3. Basta 150 SL is most effective in inhibiting the growth of examined strains of mould fungi as well as being the quickest-acting herbicide (2-5 days from applying); it is the only herbicide of the five tested that can be used to eradicate soil fungi.
4. Casoron 6.75 GR proved to be the weakest inhibitor of growth of the fungi mycelium, even though it was the only herbicide used on a plant's root system.
5. *Penicillium spinulosum* proved to be the most susceptible fungi species to 4 of 6 examined herbicides, with the lowest MIC values after using Roundup Ultra 170 SL, Mniszek 540 SL, Fusilade Forte 150 EC, and Starane 250 EC.

References

1. KOŁODYŃSKI J. Macroresults of microorganisms' actions - all bout bacterial surfaces. Aura, **5**, 22, **1995**.
2. KURNATOWSKA A. (Ed.). Ecology. Its connections with different fields of medical knowledge, PWN, Warsaw, **2001**.
3. BURZAWA A., SZWEDEK A. Saprobiontic fungi potentially pathogenic for human, isolated from soil of recreation area of Łódź. XLIII Polish and I International Conference of Students Scientific Association and Young Doctors, Annales Universitatis Medicae Lodzensis, Łódź, **46**, 66, **2005**.
4. DE HOOG G.S., GUARRO J. Atlas of clinical fungi. Universitat Rovira i Virgili Reus, Spain, **1995**.
5. SKIRGIEŁŁO A., ZADARA M., ŁAWRYNOWICZ M. Fungi (Mycota),Vol. X, Warsaw-Cracow, **1979**.
6. KADŁUBOWSKI R. Approximate methods in analysis of dose/response curve. Abstracts of VII International Congress of Chemotherapy, Praha, A – **12/28**, **1971**.
7. HODGES C. F. Postemergent herbicides and the biology of *Drechslera sorokiniana*: effects on conidial germination, vegetative growth and reproduction. Mycologia, **6**, 1083, **1977**.
8. FABISIEWICZ W., MIKOŁAJSKA J. Effect of some herbicides on the growth of two *Drechslera* Species, Acta Mycologica, **XXVII**, (1), 105, **1991-1992**.
9. KLIMEK M., LEJCZAK B., KAFARSKI P., FORLANI G. Metabolism of the phosphonate herbicide glyphosate by a non-nitrate-utilizing strain of *Penicillium chrysogenum*, Pest Management Science, Sept. **57**, (9), 815, **2001**.
10. ARAUJO A. S. F., MONTEIRO R. T. R., ABARKELI R.B. Effect of glyphosate on the microbial activity of two Brazilian soils, Chemosphere, August **52**, (5), 799, **2003**.
11. TUBAJIKA K. M., DAMANN K. E. Glufosinate – Ammonium Reduces Growth and Aflatoxin B1 production by *Aspergillus flavus*, Journal of Food Protection, **65**, 9, 1483, **2002**.
12. EL-HISSY F. T., ABDEL-KADER M. I. A. Effect of five pesticides on the mycelial growth of some soil and pathogenic fungi, Zeitschrift für Allgemeine Mikrobiologie, **20**, (4), 257, **1980**.
13. EL-SAID A. H., ABDEL-HAFEZ S. I., SALEEM A. Effect of Herbizid and touchdown herbicides on soil fungi and on production of some extracellular enzymes, Acta Microbiologica et Immunologica Hungarica, **52**, (1), 105, **2005**.
14. GIGLIOTTI C., ALLIEVI L. Differential effects of the herbicides Bensulfuron and Cinosulfuron on soil microorganisms, Journal of Environmental Science and Health, **36**, (6), 775, **2001**.
15. LARSON R. L, HILL A. L., FENWICK A., KNISS A. R., HANSON L. E., MILLER S. D. Influence of glyphosate on *Rhizoctonia* and *Fusarium* root rot in sugar beet, Pest Management Science, **62**, 1182, **2006**.
16. KRZYSKO-LUPICKA T., SUDOL T. Interactions between glyphosate and autochthonous soil fungi surviving in aqueous solution of glyphosate, Chemosphere **71**, 1386, **2008**.
17. VINTHER F. P., BRINCH U. C., ELSGAARD L., FREDSLUND L.,IVERSEN B.V., TORP S, JACOBSEN C.S. Field – scale variation in microbial activity and soil properties in relation to mineralization and sorption of pesticides in a sandy soil, Journal of environmental quality, Aug 8, **37**, (5), 1710, **2008**.
18. EVANGELISTA S. COOPER D.G., YARGEAU V. The effect of structure and a secondary carbon source on the microbial degradationof chlorophenoxy acids, Chemosphere **79**, (11), 1084, **2010**.
19. Guidelines of the European Commission 2006/583/WE from 29. 08. **2006**.