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EFFECT OF HERBICIDES ON CELLULOLYTIC ACTIVITY OF SOIL MICROMYCETES

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Abstract

This study examines the effect of different rates of 2,4-D and Paraquat herbicides under *in vitro* conditions on the cellulolytic activity of 20 strains of cellulolytic fungi isolated from the chernozem soil taken from Mount Fruška Gora. Results showed that high rates of 2,4-D (60, 120 and 240 μg/ml) inhibited the cellulolytic activity of *Fusarium aquaeductum* var. *dimerum, Fusarium solani var. argillaceum, Aspergillus candidus, Aspergillus ustus* and *Fusarium* sp. (L-7). Lower rates of 2,4-D (30 and 60 μg/ml) stimulated the cellulolytic activity of most *Penicillium* species. Paraquat exhibited higher toxicity to cellulolytic micromycetes and their cellulose degradation ability. Its inhibitory effect was observed at rates as low as those above 3μg/mL. Inhibition increased with increasing rates of the herbicide, with only eight fungi retaining at least some degree of cellulose degradation ability at 24 μg/mL Paraquat. Most species of the genera *Aspergillus* and *Penicillium* showed high sensitivity to Paraquat at rates as low as 6 μg/mL. At 24 μg/mL, none of them had the ability to degrade cellulose. A considerable degree of resistance to Paraquat was exhibited by *Fusarium* spp. (*Fusarium nivale* and *Fusarium solni var. argillaceum)* and *Hormodendrum sp.* (L-11), which retained their cellulolytic activity even at Paraquat rates of 6 μg/mL, whereas *Hormodendrum sp.* (L-10) retained its cellulolytic activity even at twofold higher rates of Paraquat (12 μg/mL).

Keywords: *cellulolytic fungi, 2,4-D, Paraquat*.

Introduction

The last six decades have been characterized by an abrupt expansion in the use of agrochemicals in crop production, with plant protection agents playing an important role in the process (Liu *et al*., 2002). Their absence has led to a decline in crop yield by 32-78% (Cai, 2008). However, in addition to their main role, once they enter the soil, pesticides may also have side-effects on the soil biological component and, indirectly, on the cycling of elements, as well as a range of other adverse health impacts (Djukic *et al*., 2007; Kalia and Gosal, 2011). These effects primarily refer to changes in the community composition, numbers and enzymatic activities of beneficial soil microorganisms, and alterations in the dynamics and direction of soil biological processes. Based on these considerations, herbicides can be classified into agrochemicals which have extremely variable impacts (Mandić *et al*., 2005; Riah, *et al*., 2014). Overall, low (recommended) rates of herbicides do not generally cause adverse effects on soil microorganisms. Initial depression is followed by the recovery of the microbial status of the soil. Microorganisms use herbicides as their source of energy, phosphorus, carbon and nitrogen, which often increases their biomass (Araujo *et al*., 2003; Zabaloy *et al*., 2008). However, most results show that herbicides have an adverse effect on soil microorganisms by reducing their numbers and enzymatic activities; therefore, these parameters are often used as bioindicators of the soil ecological status (Killham, 2002; Das

and Varma, 2011). The reduction in the numbers and activities of microorganisms is induced by changes in their biosynthetic mechanisms and cell membrane permeability, and disturbances in their enzymatic activities (Sannino and Gianfreda, 2001; Hussain *et al*., 2009). The degree of this impact is dependent on herbicide type, rate and application timing, duration of exposure, solubility, soil pH, soil organic content and cumulative co-metabolic effects (Zain *et al*., 2013; Mohiuddin and Khan, 2013). Herbicides have different effects on different types of microorganisms. The most common parameters of their active effect on the soil microbial status are the numbers and enzymatic activity of fungi (Zain *et al*., 2013), and their ability to degrade cellulose and other polymeric compounds in the soil (Lynd *et al*., 2002), even herbicides themselves (Zahid *et al*., 2016). Soil fungi are important cellulase producing microorganisms. However, their activity is heavily disturbed through direct or indirect exposure to herbicides (Smith and Mayfield, 1977).

The objective of this study was to examine the effect of 2,4-D and Paraquat herbicides under *in vitro* conditions on cellulose degradation by different species of micromycetes isolated from the chernozem soil taken from Mount Fruška Gora.

Material and methods

Under laboratory conditions (*in vitro*), different rates of 2,4 D (2,4-dichlorophenoxyacetic acid) and Gramoxone (Paraquat) (dichloro-1,1-dimethyl-4,4-bipyridine) were used to evaluate their effect on the cellulose degradation ability of 20 strains of cellulolytic fungi isolated from the chernozem soil sampled from Mount Fruška Gora (19°48'58" E and 45°09'25", altitude 507 m): *Aspergillus candidus, Aspergillus ustus, Penicillium sp. funiculosum, Penicillium nalgiovensis, Penicillium sp. purpurogenum, Penicillium piscarium, Penicillium* sp. (L-8), *Fusarium* sp. (L-7), *Fusarium* (tn-11), *Trichoderma* sp. (111), *Fusarium avenaceum* var. *herbarum, Fusarium solani var. argillaceum*, *Fusarium nivale*, *Hormodendrum* sp. (L-10), *Hormodendrum* sp. (L-11), *Nigrospora* sp., *Stachybotrys atra, Verticillium candelabrum, Mycelia sterilia* and *Fusarium aquaeductum* var. *dimerum*. The nutrient solution (3g $NaNO_3$, 1g K_2HPO_4, 0.5g $MgSO_4 \cdot 7H_2O$, 0.5g KCl, 0.03g rose bengal, 30μg streptomycin/1mL nutrient medium, 1L distilled water) was supplemented with 2% powdered cellulose (pure cellulose) and a single herbicide. The herbicide 2,4-D was used at 30, 60, 120 and 240 μg/mL, and Gramoxone (Paraquat) at 3, 6, 12 and 24 μg/mL, in three replications. The resulting solutions were inoculated with the spore suspensions of the tested micromycetes and incubated in a thermostat for 12 weeks at 28°C. Thereafter, the amount of cellulose (%) remaining in the nutrient solution was determined by potassium dichromate oxidation (Petkov, Markova, 1969). Cellulose residues were the result of difference in carbon content between cellulose-containing soil samples and soil samples without cellulose (control). The importance of differences in the level of cellulose degradation (%) for each individual fungus, as dependent on the herbicide application rate, was assessed by LSD test (Statistica SPSS 5).

Results and discussion

The results obtained in control treatments showed high variations in cellulolytic activity i.e. cellulose degradation ability across the micromycetes tested (Tab. 1, 2). The highest cellulolytic activity was exhibited by *Hormodendrum* sp. (L-11), *Fusarium aquaeductum* var. *dimerum, Mycelia sterilia* and *Fusarium* sp. (L-7). This result is in agreement with the findings of Tomas *et al*. (2011) reporting that *Hormodendrum* fungi had pronounced cellulolytic activity under optimal culture conditions. Similar data were observed for some species of *Fusarium* (Panagiotou *et al*., 2003) and *Mycelia sterilia* (Sunitha *et al*., 2013). The cellulolytic activity of some micromycetes was inhibited by the herbicide 2,4-D (Tab. 1), particularly when applied at high rates (60, 120 and 240 μg/mL) in *Fusarium aquaeductum* var. *dimerum, Fusarium solani var. argillaceum, Aspergillus candidus, Aspergillus ustus* and

Fusarium sp. (L-7). This result complies with the report of Joshi and Gupta (2008) on the significant inhibitory effect of 2,4-D on the growth and, hence, enzymatic activities of some *Fusarium* species and *Aspergillus ustus*. The low ability of these fungi to degrade this herbicide is another reason for its toxic effect (Vroumsia *et al*., 2005). In contrast, at almost all rates, this herbicide had no significant effect on the cellulolytic activity of *Fusarium avenaceum* var. *herbarum*. Low rates of 2,4-D (30 and 60 μg/mL) enhanced the cellulolytic activity of most *Penicillium* species examined. This may be associated with the marked ability of these micromycetes to rapidly mineralize 2,4-D and use it in their own metabolism (Vroumsia *et al*., 2005; Joshi and Gupta, 2008).

Table 1. Mean values of percent cellulose degradation by cellulolytic fungi (%) as dependent on 2,4-D rate (30, 60, 120, 240 μg /mL)

Strains of fungi	2,4-D, μg /mL				
	0	30	60	120	240
	Cellulose degradation, %				
Aspergillus candidus	20.3a	19.7a	18.6ab	15.3b	10.4c
Aspergillus ustus	81.2a	82.4a	66.5b	60.8c	49.7d
Penicillium sp. funiculosum	74.2ab	73.7ab	76.9a	72.1c	69.8c
Penicillium nalgiovensis	79.6c	86.1b	89.4a	80.4c	81.5c
Penicillium sp. purpurogenum	76.5b	83.7ab	87.5a	71.4c	50.2d
Penicillium piscarium	49.7a	50.7a	39.8b	35.4b	27.9c
Penicillium sp. (L-8)	60.9a	64.6a	63.8a	62.7a	53.1b
Fusarium sp. (L-7)	83.4a	68.2b	63.5b	61.4b	59.8b
Fusarium (tn-11)	79.8a	65.3b	57.4b	50.2c	40.1d
Fusarium aquaeductum var. *dimerum*	87.4a	73.6b	32.4c	– d	– d
Fusarium avenaceum var. *herbarum*	61.2b	64.8ab	63.2ab	66.5ab	68.4a
Fusarium solani var. argillaceum	75.3a	76.4a	59.7b	39.5c	17.2d
Fusarium nivale	38.4a	39.7a	29.3b	27.4b	26.5b
Hormodendrum sp. (L-10)	39.6a	41.4a	35.3c	37.6b	30.9d
Hormodendrum sp. (L-11)	90.4a	85.3b	80.7bc	82.5bc	78.8c
Nigrospora sp.	25.7a	25.6a	27.2a	21.3b	19.5b
Stachybotrys atra	47.3bc	50.4ab	56.2a	49.1bc	44.3c
Verticillium candelabrum	43.7a	41.5ab	34.8b	24.7c	18.4c
Mycelia sterilia	86.7a	71.2b	54.6c	39.7d	30.3d
Trichoderma sp. (111)	80.3a	79.5a	77.4ab	71.5b	53.2c

Means followed by the same lowercase letters in rows are not significantly different ($p > 0.01$) according to LSD test

As opposed to 2,4 D, Paraquat exhibited higher toxicity to the cellulolytic micromycetes and their cellulose degradation ability (Tab. 2). Its inhibitory effect was observed at rates as low as those above 3μg/mL. The degree of inhibition increases with increasing rates of Paraquat. At the highest rate (24 μg/mL), only eight fungi retained some degree of cellulose degradation ability. Most species of *Aspergillus* and *Penicillium* were highly sensitive to Paraquat at a rate as low as 6 μg/mL. At 24 μg/mL, none of them showed the ability to degrade cellulose. This result is consistent with Smith and Lyon (1976) who observed a linear decline in the growth and germination of *Aspergillus* and even some *Penicilluim* species as the Paraquat application rate was increased from 500 to 2000 mg/L, which consequently affected their enzymatic activity. The inhibitory effect of Paraquat on fungal growth and cellulose degradation was also reported elsewhere (Smith and Mayfield, 1977). As found by the authors, the application

of Paraquat above its recommended label rate reduced cellulose degradation and soil respiration by 39-58%. The degree of inhibition decreases with increasing content of organic matter in the soil i.e. its increasing sorption (Bromilov, 2003). In this regard, many authors have observed that the inhibitory effect of Paraquat on cellulolytic fungi is more pronounced under *in vitro* conditions than under natural soil conditions, even at rates twice lower than the recommended label rate (Zain *et al*., 2013).

Table 2. Mean values of percent cellulose degradation by cellulolytic fungi as dependent on Paraquat rate (3, 6, 12, 24 μg /mL)

Strains of fungi	Paraquat μg /ml				
	0	3	6	12	24
	Cellulose degradation, %				
Aspergillus candidus	20.3a	16.4b	11.7c	8.3d	– e
Aspergillus ustus	81.2a	67.2b	50.3c	17.2d	– e
Penicillium sp. funiculosum	74.2a	72.4a	53.5b	26.3c	– d
Penicillium nalgiovensis	79.6a	83.2a	42.1b	30.7c	– d
Penicillium sp. purpurogenum	76.5a	66.3b	31.5c	12.0d	- e
Penicillium piscarium	49.7a	53.4a	47.2b	14.8c	– d
Penicillium sp. (L-8)	60.9a	57.2a	43.4b	19.6c	– d
Fusarium sp. (L-7)	83.4a	73.4b	71.3b	48.2c	19.6d
Fusarium (tn-11)	79.8a	65.6b	60.3b	39.7c	10.2d
Fusarium aquaeductum var. *dimerum*	87.4a	76.4b	71.2b	60.3c	50.4d
Fusarium avenaceum var. *herbarum*	61.2a	55.6ab	47.4b	31.5c	– d
Fusarium solani var. argillaceum	75.3a	75.4a	73.4ab	67.5b	59.4c
Fusarium nivale	38.4a	40.2a	31.5a	18.7b	– c
Hormodendrum sp. (L-10)	39.6a	42.4a	40.5a	39.6a	18.7b
Hormodendrum sp. (L-11)	90.4a	90.3a	75.7b	29.6c	15.2d
Nigrospora sp.	25.7a	22.3a	15.2b	13.1bc	10.9c
Stachybotrys atra	47.3a	41.5b	36.4b	18.4c	– d
Verticillium candelabrum	43.7a	35.1b	24.9c	11.6d	– e
Mycelia sterilia	86.7a	82.3a	42.5b	11.4c	9.3c
Trichoderma sp. (111)	80.3a	75.4ab	72.1b	37.5c	– d

Means followed by the same lowercase letters in rows are not significantly different ($p > 0.01$) according to LSD test

This research (Tab. 2) showed that the cellulolytic activity of some species of *Fusarium* (*Fusarium nivale* and *Fusarium solni var. argillaceum)* and *Hormodendrum sp.* (L-11) was retained even at a Paraquat rate of 6 μg/mL, and that of *Hormodendrum sp.* (L-10) even at a rate twice as high (12 μg/mL). These results are supported by the findings of other authors who reported a decrease in the cellulolytic activity of some micromycetes in the soil only after the Paraquat application rate reached 500-1000 mg kg^{-1} soil i.e. above 12.5 mg l^{-1} under *in vitro* conditions (Bozarth *et al*., 1965; Wilkinson and Lucas, 1969).

The fact is that the cellulolytic activity of the micromycetes belonging to *Fusarium* and *Horodendrum* as important plant pathogens is resistant to Paraquat. This suggests that, under particular conditions, the use of this herbicide can exert a negative selection pressure on soil microorganisms for the dominance of the types of fungi which cause serious crop diseases.

Conclusion

The tested micromycetes exhibited various degrees of cellulolytic activity i.e. cellulose degradation ability. The highest cellulolytic activity was found in *Hormodendrum* sp. (L-11), *Fusarium aquaeductum* var. *dimerum, Mycelia sterilia* and *Fusarium* sp. (L-7). High rates of 2,4-D (60, 120 and 240 μg/ml) inhibited the cellulolytic activity of *Fusarium aquaeductum* var. *dimerum, Fusarium solani var. argillaceum, Aspergillus candidus, Aspergillus ustus* and *Fusarium* sp. (L-7). This herbicide had no significant effect on the cellulolytic activity of *Fusarium avenaceum* var. *herbarum*. Lower rates of 2,4-D (30 and 60 μg/ml) stimulated the cellulose degradation ability of most *Penicillium* species tested.

Paraquat showed higher toxicity to cellulolytic micromycetes and their cellulose degradation ability. Its inhibitory effect was observed at rates as low as those above 3μg/mL. Inhibition increased with increasing rates of the herbicide, with only eight fungi retaining at least some degree of cellulose degradation ability at 24 μg/mL Paraquat. Most species of the genera *Aspergillus* and *Penicillium* were highly sensitive to Paraquat at rates as low as 6 μg/mL. At 24 μg/mL, none of them had the ability to degrade cellulose

A considerable degree of resistance to Paraquat was observed in *Fusarium* spp. (*Fusarium nivale* and *Fusarium solni var. argillaceum)* and *Hormodendrum sp.* (L-11), which retained their cellulolytic activity even at Paraquat rates of 6 μg/mL, whereas *Hormodendrum sp.* (L-10) retained its cellulolytic activity even at twofold higher rates (12 μg/mL).

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