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In vitro saprotrophic basidiomycetes tolerance to pendimethalin

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ABSTRACT

Pendimethalin is a dinitroaniline herbicide classified among the persistent-bioaccumulative toxics. In this paper, the tolerance to this herbicide has been studied in isolates of basidiomycetes (10 species including 9 wood-rotting and 1 litter fungi), collected in different areas of the Campania region (South Italy). The isolates were grown on two different agar media, rich and poor for the presence/absence of dextrose and NH_4NO_3, and amended with 0, 100 and 500 ppm herbicide. The mycelial growth was recorded daily, and statistical analysis of fungal growth rates, determined via linear regression, allowed us to compare the hyphal extension of various macrofungi in presence of herbicide. These data represent the adaptation capacity of the fungal organisms to the peculiar environmental situation. In amended agar, all fungi exhibited a certain tolerance to pendimethalin and, normally, the fungal growth decreased with the increasing of pollutant concentration. Nevertheless, in some cases, the difference of ground reflected the different agar media. In fact, the growth of *Agrocybe aegerita*, in rich agar decreased with the increase of herbicide dose, while in poor agar it increased with the pollutant. Among the examined fungi, *A. aegerita* was the species which resulted the most tolerant to herbicide, showing growth to about 70% of the control to the highest concentrations of pollutant and for the two different agar media.

Scientific relevance of the paper: No published report is available regarding pendimethalin herbicide biodegradation with basidiomycetes; therefore this work represented the first and preliminary investigation as regards. It's directed to determine the fungal tolerance to the pollutant.

1. Introduction

Environmental contamination through anthropogenic and industrial activities is a widespread and serious problem in many parts of the world. The most frequent contaminants through industrial activities are inorganic pollutants (heavy metals, metalloids, etc.), and organic compounds such as pesticides and herbicides (Stegmann et al., 2001).

The extensive and massive use of pesticides in agriculture has serious impacts on the environment, compromising soil and water quality and major concern has been to protect water resources (Younes and Galal-Gorchev, 2000). This is also true for the dinitroaniline herbicides, even though the levels of these compounds detected are generally low (Savage and Jordan, 1980; Larson et al., 1999; WHO, 2003).

Pendimethalin (*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine) is a dinitroaniline herbicide used, in various formulations, in terrestrial agro-ecosystem.

The US Environmental Protection Agency classified pendimethalin among the moderately persistent-bioaccumulative toxics (PBTs) and has lowered the Toxic Release Inventory (TRI) reporting the threshold of this compound.

Widespread use of this herbicide led to its detection as a contaminant in soil ($\geq$0.3 mg kg^{-1}) and water (0.1–6 $\mu g\, l^{-1}$) (Barbash and Resek, 1996; Capel et al., 1998; Larson et al., 1999) by means of evaporation, drift, leaching, and runoff (Strandberg and Scott-Fordsmand, 2004); moreover it was found in drinking-water at concentration below 0.1 $\mu g\, l^{-1}$ (Funari and Sampaolo, 1989; WHO, 2003).

For the terrestrial ecosystem, the highest concentrations of pendimethalin occur in agricultural soil immediately after application with an amount of about 13 mg kg^{-1} of soil (Strandberg and Scott-Fordsmand, 2004).

Some studies have shown that 10–20% of the herbicide vaporizes within 1 or 2 weeks from the application. The remainder can be dissipated via biological or chemical processes, with DT_{50} values varying between a few days and over 200 days (Walker and Bond, 1977; Oliver, 1979; Savage and Jordan, 1980; Zimdahl et al., 1984; Cooper et al., 1990; Schroll et al., 1999).

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Studies on phytotoxicity to crops and weeds 200 days after application confirmed that the dissipation time of pendimethalin is high enough to harm plants far beyond the period during which it is reported to be active. With this persistence, there are risks to both agriculture and the environment (Strandberg and Scott-Fordsmand, 2004).

Several studies have been carried out on the toxicity of pendimethalin in plant species (Skroch et al., 1991; Smith et al., 1995), the nodulation processes of *Rhizobium* bacteria (Pahwa et al., 1988; Gupta and Pandey, 1992) and vesicular-arbuscular mycorrhizal (VAM) (Siqueira et al., 1991). Furthermore, pendimethalin is not only toxic to plants and microorganisms but also fishes such as rainbow trout and bluegill sunfish which were sensitive to this herbicide, with LC_{50} values of 0.14 and 0.2 mg l^{-1}, respectively (Kidd and James, 1991).

In the last decade, several studies were performed to analyze the degradation processes of pendimethalin in the environment. Some field works were carried out to elucidate the effect of seasonal factors evidencing a reduced degradation during the winter months (Horst et al., 1996; Tsiropoulos and Miliadis, 1998; Kewat et al., 2001). Further laboratory studies demonstrated that the dissipation time increased with low temperatures and drought conditions in a modelled scenario running from 72 to 2094 days (Zimdahl et al., 1984, 1994). Moza et al. (1992) reported the decomposition of pendimethalin by photolysis in the presence of titanium dioxide.

Bacterial biodegradation of pendimethalin in soil was reported by some investigators under both aerobic and anaerobic conditions and in different types of soils (Shetty et al., 1996; Smith et al., 1997; Zheng and Cooper, 1996). Enhanced biodegradability of pendimethalin in contaminated soil was demonstrated after Fenton's treatment (Miller et al., 1996).

Recently, increasing numbers of studies have inclined to demonstrate that fungi are able to degrade a large number of pollutants. Furthermore, fungi grow by hyphal extension and thus can search for pollutants in the soil in ways that other organisms cannot.

As regards deuteromycetes, Barua et al. (1990) studied the degradation of the pendimethalin with *Aspergillus flavus*, *Aspergillus terreus*, *Fusarium solani*, *Fusarium oxysporum*, *Penicillium citrinum* and *Penicillium simplicissimum*. Interestingly, biodegradation by *F. solani* resulted in the isolation and identification of *N*-propyl-3-methyl-4-hydroxy-2,6-dinitroaniline, *N*-(1-ethylpropyl)-2-amino-6-nitro-3,4-xylidine and 2,6-dinitro-3,4-xylidine. On the contrary, despite their biological characteristics, no studies have been carried out with basidiomycetes on pendimethalin biodegradation. In fact, some species of basidiomycetes, especially wood-rotting fungi, have been shown to be very resistant and tolerant to high concentrations of polluting chemicals such as polycyclic aromatic hydrocarbons, chlorinated aromatic compounds, dyes, nitroaromatics and other environmental pollutants (Evans and Hedger, 2001).

In this context the aim of this work was to analyze the potential tolerance to the pendimethalin of various species of basidiomycetes.

2. Materials and methods

2.1. Species and fungal isolates

Basidiomycetes selected for tolerance to pendimethalin (Fig. 1) are reported in Table 1. There are nine species of wood-rotting fungi – collected in different areas of Campania region, in southern Italy (Roca et al., 2007) – and one species of litter fungi, *Agaricus bisporus* (J.E. Lange) Singer – coming from commercial cultivation.

These fungi play different ecological roles in forest ecosystems and many of them have been differently studied by literature: *Agrocybe aegerita* was studied for phenanthrene and pyrene biodegradation (Sack et al., 1997), *Armillaria mellea* for creosote tolerance (Richter et al., 2003), *Fistulina hepatica* for copper tolerance

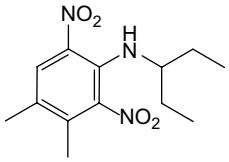

Fig. 1. Chemical structure of pendimethalin.

(Sierra-Alvarez, 2007), *Ganoderma lucidum* for decoloration of wood pulping and bleaching effluent (Wang et al., 1992), *Pleurotus ostreatus* for degradation of polychlorinated biphenyls (Zeddel et al., 1993), polycyclic aromatic hydrocarbons (Bogan et al., 1999; Bezabel et al., 1997), some herbicides such as atrazine, terbuthylazine and diuron (Bending et al., 2002), DDT (Gadd, 2001) and for industrial dye decolorization (Rodriguez et al., 1999), *Schizophyllum commune* for decoloration of bagasse-based pulping effluent (Belsare and Prasad, 1988), cleaning process of various bleachery effluent lignins (Bergbauer and Eggert, 1992) and for degradation of ferulic acid (Ghosh et al., 2005), *Agaricus* sp. for bioextraction of heavy metals from contaminated substrates (Garcia et al., 2005). Instead, white root fungi *Fomes fomentarius*, *Ganoderma australe* and *Polyporus varius* are today even understudied.

The fleshy sporophores of Agaricaceae, Bolbitiaceae, Marasmiaceae and Pleurotaceae were broken open and small pieces of the inner tissues, in the anatomical part placed between pileus and stipe, removed and transferred on Potato Dextrose Agar Petri plates (PDA plates), amended with chloramphenicol 50 mg l^{-1}, streptomycin 15 mg l^{-1}; the PDA plates were incubated at 25 °C (Gams et al., 1987).

For sporophores of Ganodermataceae, Polyporaceae, Schizophyllaceae and Fistulinaceae, small anatomical fragments of basidiomes, just above the hymenophore, were removed, sterilized – by immersion in H_2O_2 30% for 1 min and rewashed with sterilized water – and transferred on 2% Malt Extract Agar Petri plates (2MEA plates), amended with chloramphenicol 50 mg l^{-1}, streptomycin 15 mg l^{-1}, benomyl 4 mg l^{-1}. The plates were then incubated at 25 °C (Maloy, 1974; Vizzini et al., 2007).

Purity of storage culture was verified by microscopic observation. Having obtained purified cultures, the isolates were stored on PDA plates at approximately 5 °C.

Fungal isolates were preserved at the Laboratory of Botany of Department of Life Sciences (Second University of Naples) and catalogued in collection as ER strains.

2.2. Tolerance tests

Fungal isolates were transferred on PDA plates and incubated at 25 °C for 1 week to supply "*mycelial inoculum*" for the realization of tolerance tests.

All fungi screened for pendimethalin tolerance were tested in Dextrose Nitrogen Mineral Agar (DNMA) and in Mineral Agar (MA) whose composition was differentiated for the presence/absence of dextrose (10 g l^{-1}) and NH_4NO_3 (0.05 g l^{-1}); the common mineral components consisted of the following: 2 g l^{-1} KH_2PO_4; 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$; 0.1 g l^{-1} $CaCl_2{\cdot}2H_2O$; 0.1 mg l^{-1} $FeSO_4{\cdot}7H_2O$; 0.02 mg l^{-1} $CuSO_4{\cdot}5H_2O$; 0.01 mg l^{-1} $ZnSO_4{\cdot}7H_2O$; 0.01 mg l^{-1} $MnSO_4$.

Dimethyl sulfoxide (DMSO) was used to dissolve the herbicide in the agar media; in particular 100 and 500 mg of pendimethalin were dissolved in the final volume of 10 ml of DMSO, to obtain, respectively, 100 and 500 ppm "pollutant solutions". Successively, the culture media were obtained with "pollutant solution" added in 990 ml of DNMA an MA.

Uniform dispersion and distribution of pollutant was obtained by constantly mixing agar media during the plate realization phase; 16 ml of different agar media were, respectively, added in each 90 mm diameter Petri plate.

Samples of inoculum were performed with 4 mm diameter of mycelium–agar plugs, cut with a sterile cork borer from the active margin of a previous predisposed "*mycelial inoculum*". A single mycelium–agar plug was placed with mycelium-side down on agar surface of each plate. All plates were sealed with Parafilm, to prevent contamination and dehydration, stored upside-down to reduce condensation and incubated at 25 °C, for 7 days.

For each species the experimental design was performed with three replicates for each of the two culture media and for each treatment (0, 100 and 500 ppm). A total of 18 sample plates were obtained for each species.

2.3. Fungal growth rates and statistical analysis

Mycelial growth from the edge of the inoculum was recorded as mm/day for a period of 7 days; two perpendicular measurements of mycelial mat ("colony diameters") were taken for each plate and the average values were calculated for each day. Successively, the growth rate was performed as linear regression in mm/day.

Data reported as growth rates of the different fungal species and as a percent of the control (% of fungal growth to 0 ppm pendimethalin) were analyzed for significant differences between separate treatments, using one-way ANOVA and post hoc tests.