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Full Length Research Paper

Sulphonylureic herbicidal risk in the detection of soil fungi communities

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Cambic chernozem soil samples were collected from experimental field of plant breeding discipline, the U.S.A.M.V.B. department in Timişoara. The soil has been sifted through a sieve of 2 mm and placed in polyethylene bags. Thereafter, the soil was treated with two herbicides (tribenuron-methyl and nicosulfuron) in different doses. Samples were incubated for 7 days in a thermostat at 28°C and subsequently, quantitative and qualitative analysis of fungal colonies in soil samples was performed. The following fungi species were identified in the batch samples: *Fusarium* sp., *Chaetomium* sp., *Mucor* sp., *Humicola* sp., *Penicillium* sp., *Rhizopus* sp. and *Actinomucor* sp. Certain species were found in soil samples treated with tribenuron-methyl and nicosulfuron, using increased doses. Other species had reduced-growth due to their sensitivity to xenobiotics. Still, other species appear in treated variants: *Stachybotrys* sp., *Cladosporium* sp., *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. in every experimental variant presented an increasing resistance to the action of xenobiotic substances.

Key words: Sulphonylureic herbicide, fungi communities, xenobiotic.

INTRODUCTION

Herbicide usage in dry land, points out significant changes in soil micro population translated by a decreasing number of microorganisms sensitive to herbicide reaction, identifying microorganisms resistant to herbicide reaction, some leading to their degradation and also to degradation of their derivatives (Zarnea, 1994). Little is known of their effects on soil microorganisms whose activities may influence the productivity and sustainability of soils. Using ground-level sulfuronic, sulfonylurea and fenylurea herbicides, studies have been made in order to highlight the effect of microorganisms and their derivatives in degradation. Among soil micro-organisms, it seems that the most effective in herbicide degradation are the fungi.

Sulphonylureic herbicides are a group of post-emergent herbicide which contain in chemical structure sulphonylurea. Tribenuron-methyl and nicosulfuron are part of this group of herbicides. Discovery of

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sulphonylureic herbicides represent a turning point in the field, due to extremely low application rate, high selectivity and low rate of toxicity on mammals, fish and human, and the lack of unwanted effects on the environment (Alda, 2007). Sulphonylureic herbicides have three distinct parts in their molecule: a nitrogen heterocycles, a sulphonylurea bridge and an aryl group. Even if these herbicides have low toxicity on the environment, studies on different types of sulphonylureic herbicides showed that increasing doses of sulphonylureic herbicides causes significant decreases in soil microbial community (Ulea et al., 2002; 2010). Herbicides and their derivatives have negative effects on algae (Mostafa and Helling, 2001), invertebrates, ciliates, protozoa (Perrin-Ganier et al., 2001), fungi, actinomycetes and bacteria (Ayansina and Oso, 2006; Das and Dangar, 2008).

Consequently, polluted areas are detoxified by natural bioremediation strategies, such as the microflora. We mention here, active species frequently used in polluted soil. These fungi could be used to accelerate detoxification of polluted in areas (Bending et al., 2003). The advantage of fungi is to achieve herbicide metabolism could be explained by their slight capacity to metabolize environmental byproducts and their subsequent degradation by other organisms. Likewise, it is important to establish a connection between bacteria and fungi concerning herbicide degradation (Sorensen and Aamand, 2001; Sorensen et al., 2001, 2003).

The most difficult part in studying herbicides is related to criteria for assessing the aggressiveness of herbicide on the microflora. Actinomyces, bacteria and fungi have different sensitivity to most herbicides and this sensitivity is also influenced by other factors. Microscopic fungi are more sensitive to herbicide in lower doses than those required in agricultural practice (Eliade et al., 1983; Ghinea, 1997).

This paper summarizes the results of a laboratory experiment regarding the effects of two sulphonylureic herbicides (tribenuron-methyl and nicosulphuron) on soil fungi communities to determine potential risks posed by usage of these xenobiotics. Management of fungi species in herbicide-treated soil has a great importance for the rotational technique of crops.

MATERIALS AND METHODS

Soil samples were collected from a cambic chernozem at depth ranges of 0 to 20 cm, from the experimental field of plant breeding discipline, the U.S.A.M.V.B. department in Timişoara. The collected soil samples have been brought to the laboratory and treated with two herbicides. The herbicides were represented by tribenuron-methyl and nicosulfuron. Treatments of soil samples were performed under laboratory conditions.

Tribenuron-methyl known as 2[4-methoxy-6-methyl-1,3, 5-triazin-2-yl methyl) carbamoysulfamoyl] benzoate is also present in herbicides from local markets under the trade name „Helmstar" (Tellurium Chemical, Romania). Nicosulfuron known as 2- (4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-N,. N-dimethyl- under the trade name Mistral" (ISK Biosciences Europe, Romania).

Soil treatment with herbicides

The soil was sifted through a sieve of 2 mm and placed in polyethylene bags. Different doses of herbicides have been prepared with distilled water and applied to the ground as a part of moisture in order to ensure 40% soil moisture. Herbicide application on dry soil was calculated assuming a uniform distribution of herbicides in the plow layer (Atlas et al., 1978).

An untreated sample was preserved, while experimental samples were performed with increasing doses of herbicide. The following experimental variants were obtained after applying the herbicides: group A - normal doses (ND) (A1 - 0.6 µg tribenuron-methyl, A2 - nicosulfuron 0.4 µg), group B - 2xND (B1 - 1.2 µg tribenuron-methyl, B2 - nicosulfuron 0.8 µg), group C - 3xND (C1 - 1.8 µg tribenuron-methyl, C2 - nicosulfuron 1.2 µg), group D - 5xND (D1 - 3 µg tribenuron-methyl, D2 - nicosulfuron 2 µg), group E - 7xND (E1 - 4.2 µg tribenuron-methyl, E2 - nicosulfuron 2.8 µg). The herbicides were applied separately in the above mentioned doses. Samples were incubated for 7 days in a thermostat at 28° C. Subsequently quantitative and qualitative analyses of fungal colonies were performed.

Total number of mold CFU (colony forming units) / 1 g soil

The culture medium used for fungi isolation was Potato-Glucose-Agar habitat (PGA) (Carl Roth GmbH, Romania). The chemical composition of the medium was: glucose - 20 g, peptone - 10 g, agar - 20 g, distilled water - 1000 ml. Sterilization was made by autoclaving for 15 min at a temperature of 121°C. Sterile medium was poured into Petri plates and after cooling the plates were seeded (1 ml inoculum / Petri plates) with the test samples (in 2 repetitions). The used seeding technique was Inoculum dissemination technique" (Stefanic, 2000). Inoculum dissemination was with the help of an "L shaped "rod, removing the excess and incubating the plates in a thermostat. Seeding plates were kept for 48 h at 28°C (Dragan-Bularda, 2000). Determination of mold colony was performed up to gender level using identification keys (Bergey's, 1984; Robert et al., 1988; Parvu, 1999).

Statistical data interpretation

Statistical data interpretation has been performed using Past Statistical Program version 2.12 Freeware. For graphics, Microsoft excel 2003 it was used.

RESULTS AND DISCUSSION

Effects of various concentrations of tribenuron-methyl on fungi communities are shown in Figure 1. In every soil sample treated with tribenuron-methyl species of *Penicillium* sp. and *Fusarium* sp. were observed which indicate a strong resistance of the molds. Regarding the effects of nicosulfuron the effects of this herbicide can be observed in Figure 2. In the batch sample, the following types of fungi were identified: *Fusarium* sp., *Chaetomium* sp., *Mucor* sp., *Humicola* sp., *Penicillium* sp., *Rhizopus* sp. and *Actinomucor* sp. *Aspergillus* sp. and *Penicillium* sp. are frequently found in soil samples (Adeniran and Abiose, 2009). Certain types of fungi were found in soil samples treated with increasing doses of herbicides while other fungi were reduced and still others disappear as a

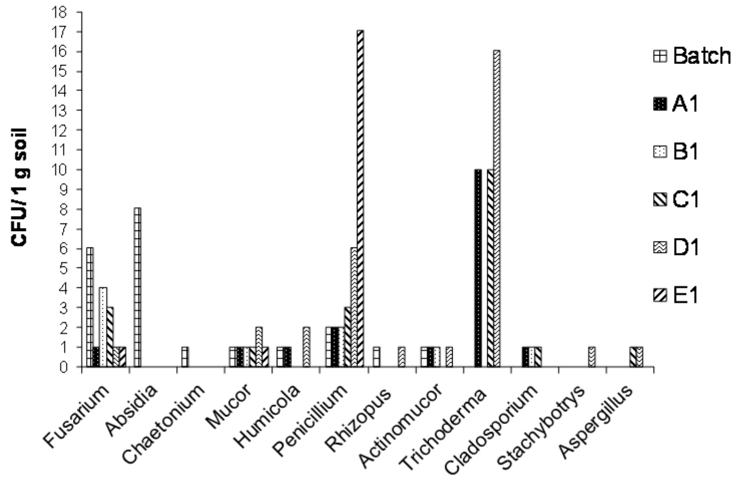

Figure 1. Effects of tribenuron-methyl on fungi communities.

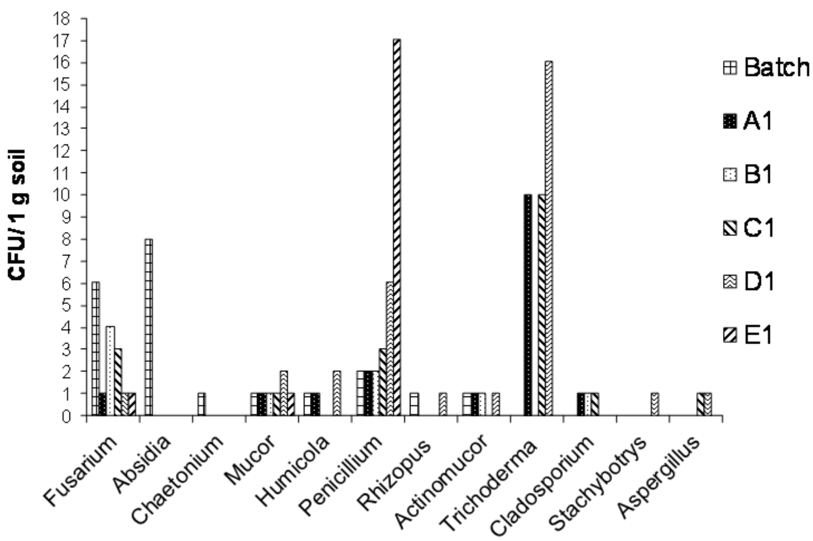

Figure 2. Effects of nicosulfuron on fungi communities.

result of their sensitivity to xenobiotics, and others species appear (*Stachybotrys* sp., *Cladosporium* sp. and *Aspergillus* sp).

Different types of mold treated with tribenuron-methyl and nicosulfuron have been also found in other studies made to determine the effect of xenobiotic substances on fungi communities. Molds decrease with the increasing usage of herbicide (Ulea et al., 2002, 2010; Sebai et al., 2004; Das and Dangar, 2008).

Fusarium sp. and *Penicillium sp.* are present in a large number in experimental variants D1 and E1. These suggest an increased resistance to the action of tribenuron-methyl and nicosulfuron.

Studying the herbicide effect on fungus colonies, we got to the conclusion that the *Aspergillus* sp. and *Actinomucor* sp. are the most poorly represented species. The species called *Actinomucor* sp. were found in the batch sample and also at variants treated with niosulfuron in normal dose (A2), two times normal dose (B2) and five times normal dose (D2). The *Aspergillus* sp. are found at variants treated with tribenuron-methyl three times normal dose (C1) (Figure 1.) and with nicosulfuron five times normal dose (D2) and three times normal dose (Figure 2).

Comparing the results obtained at experimental variants treated with tribenuron-methyl to the experimental variants treated with nicosulfuron it can be seen that in soil samples treated with nicosulfuron species of *Cladosporium* sp., *Stachybotrys* sp. and *Aspergillus* sp. does not appear which could indicate that these molds are sensible to the effect of nicosulfuron.

Penicillium sp., *Trichoderma* sp., *Fusarium* sp. and

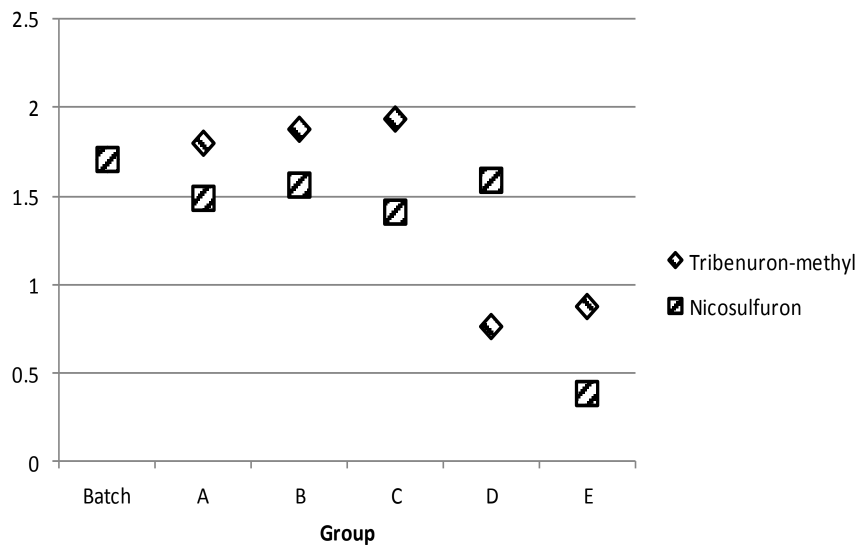

Figure 3. Shannon's diversity index.

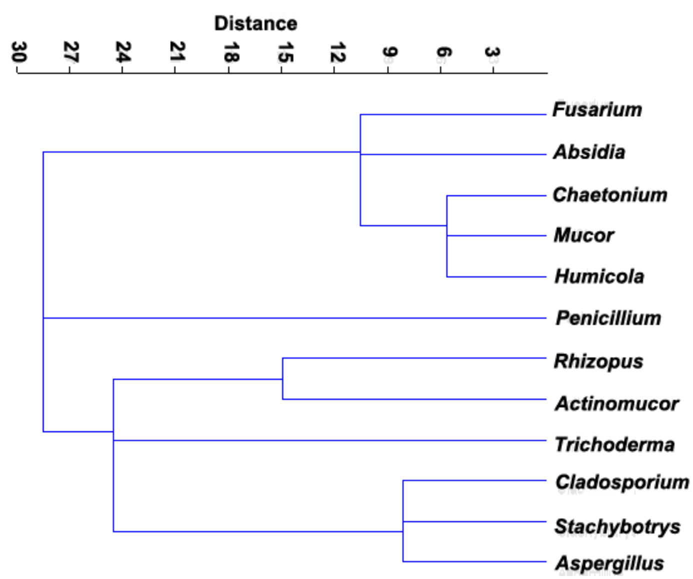

Figure 4. Cluster representation.

Rhizopus sp. species are well represented numerically. The *Penicillium* sp. is present in all experimental variants. Similar findings have been established in other studies, by other researchers: the *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Trichoderma* sp. were most frequently isolated fungi from herbicide treated soils (Romero et al., 2005; Ayansina and Oso, 2006; Romero et al., 2009). Among fungi species, *Penicillium* sp. has been proved to be the most efficient in order to increase degradation of certain sulphonylureic herbicides in soil (He et al., 2006).

Identified fungi species from the experimental variants were used to determine the Shannon's diversity index expressed in a logarithmic form. Shannon's diversity index shows the maximum value of 1.935 in the experimental variant C1 followed by B1 with 1.877 and A1 with a value of 1.798 (Figure 3). At high concentrations of herbicides the Shannon's index shows a decrease which indicate that high concentration of Tribenuron-methyl and nicosulfuron reduces the diversity of fungi communities in soil.

Data was transposed before analysis, taking into consideration the nearest neighbor we got to the conclusion that *Humicola* sp., *Mucor* sp., and *Chaetonium* sp. have a similar behavior (Figure 4). The herbicides should be used in doses recommended by specialized literature or even adoubledose. The effects of these xenobiotic substances are disruption of soil biology and microbiology, negatively influencing the microorganism quality and quantity in soil communities.

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