Original scientific paper
UDC: 631.466:632.95
DOI: 10.7251/afts.2014.0611.077R
COBISS.RS-ID 4573720

EFFECTS OF METSULFURON-METHYL ON SOIL MICROBIAL ACITVITY

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ABSTRACT

Pesticide metsulfuron-methyl (chemical name: methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate) is a post-emergence sulfonylurea herbicide which controls most annual grass broadleaf weeds in cereals, and it has been used for a long time.

The effect of metsulfuron-methyl on microbiological activity in soil was investigated. Trials were set up in laboratory on chernozem soil (pH 7.0, organic matter 3.5%, sand 26%, silt 45%, clay 29%) at Surcin, Serbia. Metsulfuron-methyl was added at rates 1, 5, 25 i 50 mg/kg soil. Untreated soil served as control. Samples were collected for analysis 5, 20, 40 and 60 days after metsulfuron-methyl application. The effects were assessed on dehydrogenase activity, bacteria abundance and fungi abundance.

Metsulfuron-methyl was found to cause different effects on the soil microbial activity in soil and its influence depended on the rate of application and duration of activity. Metsulfuron-methyl applied at 1 and 5 mg/kg soil did not have any effect on microbial parameters. The higher herbicide doses (25 and 50 mg/kg) induced increasing activity from the 5th to 40th day. These experimental data indicated that metsulfuron-methylon affected soil microbial activity, but the effects were only detected at higher doses and they were slight and transitory.

Key words: *metsulfuron-methyl, dehydrogenase activity, bacteria abundance, fungi abundance,*

INTRODUCTION

Biological processes in soil are important to ecosystem function. Soil microbes are the driving force behind many soil processes including degradation of xenobiotics. On the other hand, microbes are very efficient indicators which able to respond immediately to environmental changes.

Number and biomass microorganisms, functional diversity and enzymatic activities are useful tools to evaluate the impact of xenobiotics on soil [1,2].

Modern agriculture depends on wide variety of synthetically produced chemicals, including pesticides. Pesticides are widely used in crop protection and are known to induce major environmental problems. With an increased pesticide use, questions are rising on potential effects regarding public health and environment. However, when applied to the field, pesticides not only control targeted organisms, but

may also have potential residual impact in soil. Pesticides pollute air, soil, water resources and contaminate the food chain. In this context, the influence of pesticides on the microbial activity of soil microorganisms has been studied by some investigators [3,4].

Sulfonylureas are class of pesticides characterized by high biochemical activity at low application rate. Depending on crop type and local legalisation, the application rate of these herbicides range from 2 g to 150 g a.i ha^{-1}. Sulfonylureas have high selectivity and very low acute and chronic animal toxicity. These compounds are readily degraded in soil and are not environmentally persistent [5,6,7,8].

Metsulfuron-methyl [chemical name: methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) benzoate] is a post-emergence sulfonylurea herbicide which controls most annual grass broadleaf weeds in cereals, and it has been used for a long time [9].

Several papers have been published on the effects of sulfonylurea herbicides on soil microorganisms, microbial community and activity [3,4,10,11], while less information is available for metsulfuron-methyl. Ismail et al. [12] reported that metsulfuron-methyl suppressed soil respiration and microbial biomass at a ten-fold field rate, although the effects were transient and there were no significant effects at the field rate.

The purpose of the present study was to examine how the herbicide metsulfuron-methyl at differecon centrations affects the microflora in soil. The interactions established between total number of bacteria, fungal population, dehydrogenase activity were determined.

MATERIALS AND METHODS

Metsulfuron-methyl [chemical name: methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) benzoate] tested in the experiment was a product of DuPont Company, Switzerland. Metsulfuron-methyl was applied at rates: 1, 5 25 and 50 mg/kg soil.

The experiment was carried on chernozem soil soil (pH 7.0, organic matter 3.5%, sand 26%, silt 45%, clay 29%) at Surcin, Serbia. The soil chosen for the study had never been treated with pesticide before. Soils samples were collected from the upper layer (0-10 cm), carefully dried, sieved to pass 5 mm mesh, and stored at 4°C. Before using, the soils were air-dried at room temperature for 24h. Each herbicide concentration was pipetted to the surface of 1 kg of soil before homogenization on a rotating stirrer for 30 minutes. After homogenization by mixing the soil was portioned out in pots. Untreated soil served as control. The experiments were conducted with four replications. The pots were kept in a controlled-environment chamber at 20 ± 2°C temperature, 50% air humidity and 12/12 h day/night photoperiod throughout the experiment. Soil humidity was kept at 50% field capacity. Samples were collected for analysis 5, 20, 40 and 60 days after metsulfuron-methyl application.

Soil dehydrogenase activity was determined by method reported by Tabatabai [13]. The soil samples were prepared by incubation with triphenyltetrazolium chloride (TTC) under moist conditions at 37°C for 24h. Determination of triphenylformazan (TPF), which is derived from triphenyltetrazolium chloride (TTC) as a product of enzyme activity was done spectrophotometricaly. Measurements were done at 485 nm wavelength and enzyme activity given as μg TPF g^{-1} soil.

Total culturable microorganisms were counted by a soil dilution plate technique using tryptic soy agar for bacteria and Czapek agar for fungi. The inoculated agar plates (three replicates) were incubated at 28°C for 3 days for bacteria and 5 days for fungi, before the colonies were counted.

Statistical evaluation: the obtained data were analyzed using ANOVA and the means were separated by Duncan's multiple range test. In all analyses, the level of significance was at least $P< 0.05$.

RESULTS AND DISCUSSION

The effects of metsulfuron-methyl on soil microbiological and biochemical variables tested are shown in Tables 1-3. Metsulfuron-methyl was found to cause different effects on activity in soil and its influence depended on the rate of application and duration of activity.

Table 1 Dehydrogenase activity (µg TPF g^{-1} soil) in soils in the presence of metsulfuron-methyl

Metsulfuron-methyl (mg a.i.kg^{-1})	Days after application			
	5	20	40	60
control	55.2a	51.5a	57.4a	52.5a
1	58.6a	59.8a	55.4a	57.6a
5	50.1a	54.5a	53.0a	56.7a
25	60.4b	87.1b	72.7b	62.1a
50	62.2b	101.3c	81.9b	75.6b

a-means of variants were separated by Dankan's multiple range test (P<0.05)

Dehydrogenases exist as an integral part of intact cells and represent the oxidative activities of soil microbes and testing of soil dehydrogenase is necessary to evaluate the side-effects of pesticides on soil microorganisms [14,15,16]. Metsulfuron-methyl applied at 1 and 5 mg/kg soil did not have any effect on dehydrogenase activity. Dehydrogenase activity was found to be stimulated by 25 and 50 mg metsulfuron-methyl treatment in soil. The maximum increase value was 101.3 µg TPF g^{-1} soil (50.0 mg metsulfuron-methyl, 20 days after application) (Table 1). The experimental data are consistent with results reported by other authors on the effect of different pesticides on this enzyme. Dinelli et al. [17] and Accinelli et al. [15] reported that sulfonylurea herbicides at a dose up to 20 mgkg^{-1} stimilated dehydrogenase acitivity. The results Radivojević et al. [18] showed a changes activity of dehydrogenase under all nicosulfuron concentrations (0.3, 1.5 and 3.0 mg a.i. kg^{-1}). from day the 1st to the 30th day after nicosulfuron application.

Table 2 Number of total bacteria (10^6) in soils in the presence of metsulfuron-methyl

Metsulfuron-methyl (mg a.i.kg^{-1})	Days after application			
	5	20	40	60
control	84.2a	95.3a	87.1a	80.6a
1	75.5a	88.2a	90.4a	85.1a
5	80.1a	86.7a	88.0a	79.3a
25	104.7b	115.8b	126.2	93.4a
50	71.3a	121.4b	120.6	88.5a

a-means of variants were separated by Dankan's multiple range test (P<0.05)

Under the various experimental conditions changes in number of total bacteria content varies throughout the experiment and were depended on rates of application and exposure time (Table 2). In our conditions no significant effects were observed for concentrations 1 and 5 mg metsulfuron-methyl. The maximum increase was found at the highest application rate 25 and 50 mg metsulfuron-methyl. The maximum increase value was $121.4x10^6$ (50 mg metsulfuron-methyl, 20 days after application). However, these effects were transitory, because all the variables tested showed a tendency to the controls values. There have been other reports also on the activity of different pesticides in relation to number of total bacteria. Perucci and Sacrponi [11], for example, found that the effect of rimsulfuron and imazethapyr depended on soil moisture. Under reduced moisture, the activity of rimsulfuron lasted 36 hours, but as long as 72 hours under high moisture. Similar findings were reported also by Wardle and Parkinson [19], as well as Rath et al. [20], in their experiments investigating 2,4-D and glyphosate. Startton and Stewart [21] recorded effects of glyphosate on soil bacteria and respiration in

Canadian coniferous forests. Finally, Radivojević et al. [22] observed transitory effects of atrazine on soil bacteria.

Table 3 Number of fungi (10^4) in soils in the presence of metsulfuron-methyl

Metsulfuron-methyl (mg a.i.kg^{-1})	Days after application			
	5	20	40	60
control	33.4a	31.8a	34.7a	40.2a
1	38.1a	29.4a	40.5a	45.3a
5	30.2a	27.5a	38.9a	49.6a
25	45.3a	55.7b	66.3b	61.1b
50	43.8a	68.2b	60.4b	47.4a

a-means of variants were separated by Dankan's multiple range test ($P<0.05$)

Soil fungus is important organisms among the soil microbial group. Fungi su known to be extremely adaptable in different environments due to their ability to breakdown many complex substrates including herbicides [1,2]. The fungal populations were significantly increased in the presence 25 and 50 mg of metsulfuron-methyl and the degree of the change increased as the concentration of the herbicide increased (Table 3). Similar results had been reported by Ahtiainen et al. [23] and Zabalou et al. [24]. Ratcliff et al. [25] recorded minor changes in bacterial community after application 100x field rate of glyphosate. However, the effects obtained in our studies may be influenced by the degree of soil disruption and also by the species of the fungi present in the soil. Several studies [11,21,25,26] on soil microflora have shown that soil characteristics may modify the effect of pesticides on microbial numbers and their biological activity .

The lack of interference with soil biological processes would suggest that sulfonylurea herbicides has little or no harmful effect on soil microbial when it is applied at lower rate in soil. In agreement with the reports of Perucci and Scarponi [11], this finding can be attributed to the low toxicity of sulfonylurea herbicides on soil microbes or to soil processes, such as the adsorption of small amounts of herbicide on clay or soil organic matter, which limit the agrochemicals effects on the soil microbial biomass. This phenomenon is of primary importance especially when small amounts of a herbicide are employed in soil weed control [27]. In addition, the low persistence time makes it difficult to determine any indirect effects on soil microflora mainly for herbicides, such as herbicide s, applied at very low field rates [14,15,26].

CONCLUSIONS

Under laboratory conditions soil biological activities were affected by the tested metsulfuron-methyl when it is applied at different rates.

The response to the pesticide treatment of the microbiological parameters tested evidence that metsulfuron-methyl has no adverse effect on soil microorganisms. Changes of those parameters were observed particularly at the higher dosage but they were slight and transitory. Our study stresses the role herbicide dose and exposure time in determining the influence of changes on soil microorganisms.

This work was financially supported by the Serbian Ministry of Science and Technological Development under the projects TR31043 and III 46008.

(Received 01. September 2014, accepted 09. September 2014)

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