

Metsulfuron-methyl residues in soil and wheat under North-Western mid-hill conditions of Himalaya

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ABSTRACT

A field experiment was laid out in a randomized block design consisting of metsulfuron-methyl (a herbicide) treatments at 2, 4, 8 g/ha along with control during two consecutive years to study metsulfuron-methyl degradation in soil and terminal residues in wheat straw and grain. Soil samples were collected at 0 (4 hr), 1, 3, 7, 10, 15, 30, 45, 60, 75 and 90 days after herbicide application for degradation studies. Wheat straw and grain were collected at the time of harvest for terminal residues study. The herbicide residues were quantified using high performance liquid chromatography (HPLC) equipped with UV-Vis detector using C-18 column. The degradation data generated in the present investigation during both the years indicated that higher dose of metsulfuron methyl *i.e.* 8 g/ha persisted in soil upto 45 days after herbicide application. The degradation was rapid and more than 90% of applied metsulfuron-methyl in soil dissipated within 15 days of application. The logarithmic plots of herbicide concentration at different doses versus time fitted first order kinetics decay curves during both years. At the time of harvest, the metsulfuron-methyl residues were non-detectable level in wheat straw and grain. Thus, the use of metsulfuron-methyl in wheat could be considered safe.

INTRODUCTION

In India, wheat is the second major food crop next to rice in terms of its production and consumption. This crop is heavily infested with weeds and on an average reduced grain yield by 66% (Kumar *et al.* 2011). Nowadays, herbicides are commonly used in Indian agriculture so as to reduce yield loss and maintain the quality of crop produce. Sulfonylureas, a family of broad action spectrum herbicide is widely used in many parts of the world to control weeds (Zanardini *et al.* 2002). Among the sulfonylureas, metsulfuron-methyl (Methyl-2-(4-methoxy-6-methyl-1,3,5-triazin-2-yl carbamoyl sulfamoyl) benzoate) is used to control broad-leaf weeds in various plantation crops including the wheat due to its selective herbicidal activity (Singh and Singh 2002). It is biologically active at low application rates (2-100 g/ha). It acts as an inhibitor of acetolactate synthase (ALS), which retards the cell division in shoots and roots of the plant. ALS is the first enzyme involved in the biosynthesis of the branched chain amino acids valine, leucine and isoleucine in both plants and micro-organisms. Metsulfuron-methyl is rapidly taken up by the plants through roots and foliage, which alters the plant metabolism.

Metsulfuron-methyl is weak acid with pKa from 3 to 5 and in soils exists mainly in the ionized (anionic) form, repelled by negative charge of soil surfaces and exhibit low sorption to soils (Joshi *et al.* 1985). Metsulfuron-methyl is degraded both by chemical hydrolysis and soil micro-organisms. Herbicide-soil interaction depends on the chemical structure of the herbicide and the properties of soil. The degradation of herbicide is affected by several soil and environmental factors like pH, temperature, organic matter, soil moisture, microbial activity etc. The degradation rates of sulfonylureas are negatively correlated with soil pH. The chemical will degrade faster under acidic conditions, and in soils with higher moisture content at higher temperature. The chemical has higher mobility potential in alkaline soils than in acidic soils, as it is more soluble under alkaline conditions.

However, frequent use of metsulfuron-methyl herbicide to control the weeds in wheat field result in low efficacy and may lead to long term adverse effects in environment. It has been found that the extremely low levels of metsulfuron-methyl residues are phytotoxically sensitive to many crops in crop-rotation system (Li *et al.* 2005). Studies have also revealed that metsulfuron-methyl residues contaminate surface and ground water due to leaching and transport of

herbicides and have unintended side effects on non-target organisms (Perkova and Donkova 2003). Metsulfuron-methyl herbicide possesses high solubility in water which indicates its high potential for movement in soil. These toxic effects of herbicides in environment at certain level of doses can be controlled by implementing good agriculture practices. Thus, it is important to understand the environmental fate of metsulfuron-methyl in soil and determine impact of its residues. Taking these concerns into account, the study was conducted to determine the degradation behaviour of metsulfuron-methyl at different doses in soil, wheat straw and grain.

MATERIALS AND METHODS

Field experimentation

A field experiment consisting metsulfuron-methyl treatments at 2,4,8 g/ha, along with control (water alone) was conducted at the Research Farm of Department of Agronomy, CSKHPKV, Palampur, India in a randomized block design with five replications during two years of study. Plots of 4.1 x 2.0 m were prepared and sown with wheat variety 'HPW155' in 22.5 cm row spacing for each treatment. During both the years, metsulfuron-methyl as Supergrip (WP) containing 20% active ingredient was sprayed in the plots.

Reagents and chemicals

All solvents used in study were of analytical grade and purchased from Merck India Pvt. Ltd. Metsulfuron-methyl and its three major metabolites *i.e.* Methyl-2-sulfonyl amino benzoate (compound II), Saccharin (compound III) and 2-amino-6-methoxy-4-methyl triazine (compound IV) were procured from Sigma Aldrich, India.

Sample collection

The soil samples were drawn randomly from 0-15 cm depth using a tube auger from 6-7 spots in each of treated and untreated plots. Soil samples were collected at 0 (2 h), 1, 3, 5, 7, 10, 15, 30, 45, 60, 75, 90 days after herbicide application and at harvest (180 days). These soil cores were mixed together, air dried, powdered and passed through a 2mm sieve to achieve uniform mixing. Soil samples were sealed in polythene bags and stored in desiccator and analysed for metsulfuron-methyl residues. Wheat straw and grain samples were collected at the maturity of crop for terminal residual analysis.

Preparation of standards

Metsulfuron-methyl (10 mg) was dissolved in HPLC grade methanol in 10 mL volumetric flask to prepare stock solution of 1000 µg/mL (1000 ppm).

From this stock solution, a working solution of 100 µg/mL (100 ppm) was prepared using methanol. The analytical standards were prepared in the range of 2, 1, 0.5, 0.1, 0.05 and 0.01 ppm in methanol by serial dilution technique. 20 µL volume of each solution was injected into HPLC for analysis. Calibration curve was prepared by plotting concentration vs average peak area. Solutions of compounds II, III, IV were also prepared and calibrated in similar way.

Sample processing and extraction for herbicide residue analysis

Extraction and clean-up for residue analysis was carried out in soil, wheat straw and grain as described by Sondhia (2008) with slight modification. Representative soil samples were taken in 250 mL flask and extracted by shaking for 1 hr. on a horizontal shaker with 50 mL of 80% methanol (in deionized water) containing 0.5% glacial acetic acid. The contents were filtered through Buchner funnel. The soil was subjected to the same extraction step for one more time. The combined filtrate was then concentrated and residues were dissolved in 5 mL of methanol for HPLC analysis. However, clean up step was not required in case of soil substrate.

Powdered samples of wheat grains and straw were dissolved in 80 mL of methanol and deionized water (80:20) containing 0.5 per cent glacial acetic acid. The contents were shaken on a horizontal shaker for 1 hr. and were filtered through Whatman filter paper no. 1 using activated charcoal. This step was repeated twice. The collected filtrate was partitioned with 50 mL of dichloromethane thrice. Dichloromethane layer was collected and solvent was concentrated to approximately 3 ml on a rotary vacuum evaporator. For clean-up, a mixture of florisol (2 g) and activated charcoal (0.5 g) was packed in the glass column (2 cm id 30 cm long) sandwiched by anhydrous sodium sulphate (4 g) on both the sides. After pre-washing of column with methanol the concentrated extract was added at the top and eluted with methanol. Eluents was collected and solvent was concentrated on a rotary vacuum evaporator to dryness and dissolved in 5 mL methanol for HPLC analysis. To ascertain the extraction efficiency of metsulfuron-methyl, recovery experiments were carried out in soil, wheat straw and wheat grain fortified with 0.25, 0.50 and 1.0 µg/g of metsulfuron-methyl.

Instrumentation

Shimadzu HPLC equipped with UV detector and Lichrosphere column C-18 (25 cm × 4.6 mm, 5 µm) column was used for the quantification of residues of metsulfuron-methyl. Detection was done at 230 nm

wavelength using methanol:water (70:30) containing 1% glacial acetic acid as a mobile phase with flow rate at 1 ml/min. Injection volume was 20 μ l. Retention time for metsulfuron-methyl was found to be 4.3 minute.

RESULTS AND DISCUSSION

Validation of HPLC method for metsulfuron-methyl analysis

HPLC is the most widely used analytical technique for micro level analysis. This technique offers the analysis with speed, accuracy, reproducibility and sensitivity and has been found to be useful in the quantitative determinations. Retention value of metsulfuron-methyl was found to be 4.3 minutes. The calibration curve was linear over the concentration ranged from 0.01 to 2 μ g/mL with a regression equation of $y=224055x+5015$ and regression coefficient $r^2=0.99$. This method has been adopted to analyze the herbicide residues. Metsulfuron-methyl recoveries in soil were 86.0, 83.0 and 84.2% for fortification at the level of 0.25, 0.50 and 1 μ g/g respectively. From spiked samples of wheat straw and wheat grain, the per cent recoveries of metsulfuron-methyl were 81.6, 83.5 and 80.0; and 80.6, 79.0 and 78.0 at the level of 0.25, 0.50 and 1 μ g/g, respectively (Table 1). The above per cent recoveries are well within acceptable limits and are in direct conformity with findings of other workers (Sondhia 2008, Sanyal *et al.* 2006).

Residues of metsulfuron-methyl under field conditions

Terminal residues of metsulfuron-methyl in wheat straw and grain were determined by estimating metsulfuron-methyl concentration at the maturity of the crop. It was found that residues of metsulfuron-methyl were below detectable level (≤ 0.02 μ g/g) in both straw and grain samples at harvest. The above

Table 1. Per cent recoveries of metsulfuron-methyl from different matrices fortified with known amount of herbicide

Herbicide	Amount added μ g/g	*Average amount recovered μ g/g	Average recovery (%)
Soil	0.25	0.215 \pm 0.005	86.0
	0.50	0.410 \pm 0.014	83.0
	1.00	0.841 \pm 0.018	84.2
Wheat straw	0.25	0.204 \pm 0.012	81.6
	0.50	0.418 \pm 0.011	83.5
	1.00	0.800 \pm 0.006	80.0
Wheat grain	0.25	0.202 \pm 0.014	80.6
	0.50	0.395 \pm 0.017	79.0
	1.00	0.780 \pm 0.019	78.0

*Values are mean of five determinations with standard deviation (\pm)

findings are in conformity with results of Sondhia (2008), Paul *et al.* (2009).

The data on degradation and per cent dissipation of metsulfuron-methyl in soil for two *Rabi* seasons at different doses have been presented in Table 2. Initial residues (0 day) of metsulfuron-methyl in soil immediately after application of metsulfuron-methyl applied at 2, 4 and 8 g/ha during first and second year of experiment were 0.112 and 0.119 μ g/g, 0.218 and 0.230 μ g/g and 0.402 and 0.448 μ g/g, respectively. After 1 day of herbicide application, residues reached to 0.083 and 0.087 μ g/g, 0.170 and 0.183 μ g/g, and 0.323 and 0.341 μ g/g, respectively at 2, 4 and 8 g/ha during first and second year. Approximately 40.2 and 41.1% at 2 g/ha, 33.5 and 34.8% at 4 g/ha, and 36.8 and 34.8 % at 8 g/ha of metsulfuron-methyl remained in soil on 5 days after herbicide application during both the years of study. The data on per cent dissipation of metsulfuron-methyl revealed that approximately 90% applied metsulfuron-methyl dissipated within 15 days of herbicide application at all the three levels of metsulfuron-methyl. During first year of study, on 30 days after herbicide application, the metsulfuron-methyl concentration was found to be 0.001 μ g/g when applied at 2 g/ha. Whereas, it was below detectable levels during second year indicated that applied metsulfuron-methyl has dissipated completely. At 45 days after herbicide application, per cent dissipation in first year and second year for metsulfuron 8 g/ha were 99.3% and 99.6% indicating that only less than 1 per cent of applied metsulfuron-methyl was left in the soil during both the years.

The residues of metsulfuron-methyl exhibited a declining pattern as a function of time. It could be seen from the data that the rate of dissipation was very rapid during initial 10 days and thereafter becomes slow. The residues were decreased gradually with passage of time and below detectable level on 60 days after herbicide application during both years of experiment. Almost similar observations have been reported by Sanyal *et al.* (2006). It is quite likely that faster dissipation of metsulfuron-methyl in soil may be due to chemical and microbial dissipation mechanism. In acidic soils (pH less than 7) initial step in the degradation of parent herbicide is chemical hydrolysis however, further degradation of parent compound and its metabolites to CO₂ requires microbial activity. The chemical hydrolysis of metsulfuron-methyl in soil involved cleavage of sulfonylurea bridge, O-demethylation as well as third mechanism, triazine ring opening (Sarmah and Sabadie 2002). Microbial breakdown depends upon the type of microorganisms present in the soil that resulted into rapid disappearance of herbicide. The demethylation of triazine ring and

Table 2. Residues of metsulfuron-methyl and per cent dissipation in soil (0-15 cm) treated at different doses

Days after herbicide application	Residues ($\mu\text{g/g}$)					
	At dose 2 g/ha		At dose 4 g/ha		At dose 8 g/ha	
	First year	Second year	First year	Second year	First year	Second year
0	0.112 \pm 0.017(0.0)	0.119 \pm 0.021(0.0)	0.218 \pm 0.018(0.0)	0.230 \pm 0.023(0.0)	0.402 \pm 0.026(0.0)	0.448 \pm 0.019(0.0)
1	0.083 \pm 0.006(25.9)	0.087 \pm 0.013(26.9)	0.170 \pm 0.015(22.0)	0.183 \pm 0.018(20.4)	0.323 \pm 0.023(19.7)	0.341 \pm 0.015(23.9)
3	0.062 \pm 0.008(44.6)	0.065 \pm 0.006(45.4)	0.123 \pm 0.016(43.6)	0.160 \pm 0.011(30.4)	0.234 \pm 0.017(41.8)	0.250 \pm 0.011(44.2)
5	0.045 \pm 0.010(59.8)	0.049 \pm 0.009(58.8)	0.073 \pm 0.006(66.5)	0.080 \pm 0.009(65.2)	0.148 \pm 0.008(63.2)	0.156 \pm 0.009(65.2)
7	0.032 \pm 0.011(71.4)	0.034 \pm 0.008(71.4)	0.054 \pm 0.003(75.2)	0.059 \pm 0.010(74.3)	0.099 \pm 0.005(75.4)	0.115 \pm 0.005(74.3)
10	0.014 \pm 0.002(87.5)	0.019 \pm 0.004(84.0)	0.047 \pm 0.004(78.4)	0.048 \pm 0.007(79.1)	0.087 \pm 0.007(78.4)	0.093 \pm 0.006(79.2)
15	0.006 \pm 0.005(94.6)	0.007 \pm 0.002(94.1)	0.019 \pm 0.007(93.3)	0.024 \pm 0.006(89.9)	0.027 \pm 0.004(91.3)	0.045 \pm 0.002(89.6)
30	0.001 \pm 0.002(99.1)	BDL	0.003 \pm 0.002(98.6)	0.004 \pm 0.005(98.3)	0.008 \pm 0.010(98.0)	0.009 \pm 0.003(97.9)
45	BDL	BDL	0.001 \pm 0.002(99.5)	BDL	0.003 \pm 0.002(99.3)	0.002 \pm 0.004(99.6)

BDL = Below detection limit, Conc. of Metsulfuron-methyl residues at 60, 75, 90 days post herbicide application was BDL. Values in parentheses indicates percent dissipation of metsulfuron-methyl at different doses

cleavage of the sulfonilureic bridge are the two different pathways identified for metsulfuron-methyl degradation by microbial activity (Zanardini *et al.* 2002). In the soil, increase in microbial activity is directly proportional to the decrease in concentration of herbicide in soil, thus increasing rate of dissipation (Morrica *et al.* 2001).

Another important contributing factor in present study for faster degradation of metsulfuron-methyl in soil might be acidic nature (pH 5.2) of experiment field. Bayer *et al.* (1988) reported that adsorption of sulfonylureas decreases on increase in soil pH due to increase amount of anionic species. Similar results of faster degradation of metsulfuron-methyl under acidic conditions and in soils with higher moisture content at higher temperature has been reported by Smith (1986). The logarithmic plots of herbicides residues versus time for first and second year of study have been presented in **Figure 1**. The plots indicated that the dissipation of metsulfuron-methyl at all levels of application, *viz.* 2, 4, 8g/ha fitted first order kinetics decay curve during both years. The half-life values were 3.54, 4.93, 6.40 days during first year of metsulfuron-methyl treatment at 2, 4, 8 g/ha respectively. Half-life values were 3.76, 5.19, 5.90 days during second year at 2, 4, 8 g/ha of metsulfuron-methyl respectively.

During second year of study (*Rabi* 2014-15), a HPLC method was developed for simultaneous identification of metsulfuron-methyl (I) and its three major metabolites *i.e.* Methyl-2-sulfonyl amino benzoate (II), 2-amino-6-methoxy-4-methyl triazine (III) and saccharin (IV). The retention values for metsulfuron-methyl and its three major metabolites *i.e.* Methyl-2-sulfonyl amino benzoate, 2-amino-6-methoxy-4-methyl triazine and saccharin were found to be 4.17 min, 3.19 min, 2.68 min and 3.75 min respectively as shown in **Figure 2**.

Wheat field soil samples of metsulfuron-methyl applied at recommended dose *i.e.* metsulfuron-methyl 4 g/ha were analysed at 15 days intervals for the identification of degradation products. HPLC analysis of field samples indicated the presence of metsulfuron-methyl in soil samples collected at zero and 15 days after herbicide application. At zero day, there were no traces of metabolites. However, at 15 days after herbicide application, metabolites *i.e.* 2-amino-6-methoxy-4-methyl triazine and methyl-2-sulfonyl amino benzoate were identified indicative of formation of these metabolites. At 30 days after herbicide application, metabolites 2-amino-6-methoxy-4-methyl triazine and methyl-2-sulfonyl amino benzoate were found along with third metabolite saccharin. However, there was no trace of

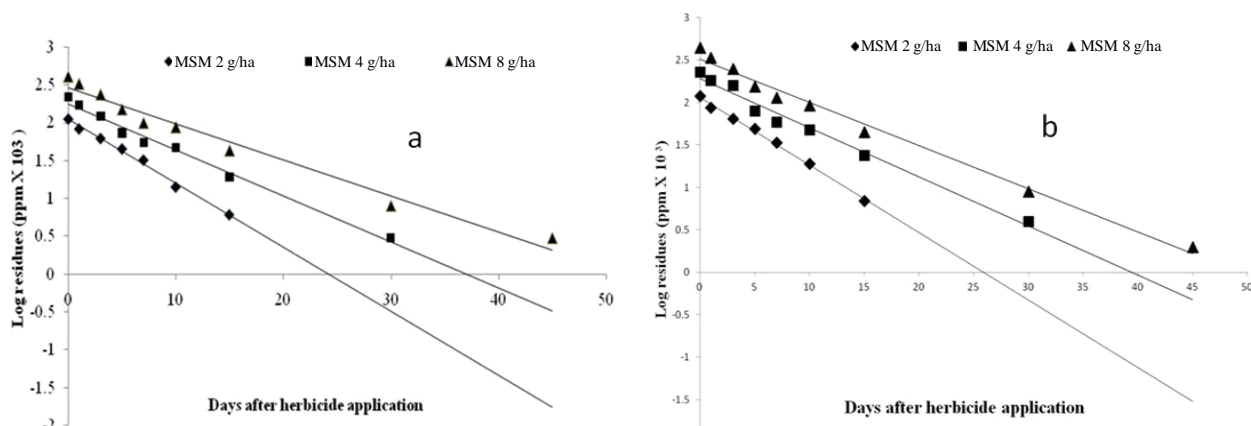


Figure 1. First order degradation behaviour of metsulfuron-methyl at 2, 4, 8 g/ha in soil a) First year, b) Second year

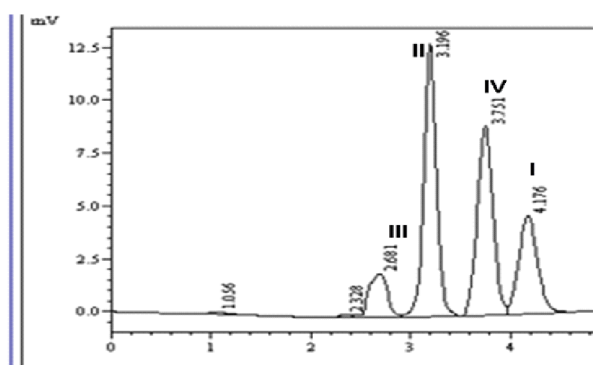


Figure 2. Chromatogram showing retention value of metsulfuron-methyl along with three major metabolites

parent compound metsulfuron-methyl on 30 days after herbicide application. Neither parent compound metsulfuron-methyl nor its metabolites *i.e.* 2-amino-6-methoxy-4-methyl triazine; methyl-2-sulfonyl amino benzoate and saccharin could be identified in soil samples collected at 45 days after metsulfuron-methyl 4 g/ha application. These results clearly indicated that at recommended dose of metsulfuron-methyl, residues persisted only upto 30 days and do not leave any residues in soil.

In the present study, the experimental field is acidic in nature (pH 5.2) therefore both the processes (chemical hydrolysis and microbial) occurs simultaneously for degradation of parent compound. Parent compound (metsulfuron-methyl) undergo chemical hydrolysis in initial step and do not require presence of microorganisms for the formation of three major products *i.e.* 2-amino-6-methoxy-4-methyl triazine, methyl-2-sulfonyl amino benzoate and saccharin that includes breakdown of sulfonylurea bridge, O-demethylation of methoxy triazine and triazine ring opening. The further degradation of saccharin to CO₂ carried out in the presence of viable microorganisms (Anderson and Dulka 1985).

An almost similar result was obtained by Paul *et al.* (2008). In their study, they reported presence of metsulfuron-methyl in 0 and 7 day samples under field conditions. Initiation of two metabolites *i.e.* 2-amino-6-methoxy-4-methyl triazine, methyl-2-sulfonyl amino benzoate was found on 15th day. These two metabolites were found in major quantity on 20th day along with third metabolite saccharin. However, parent herbicide completely disappeared from soil surface on 20th day of herbicide application. They reported that formation of these compounds were mainly due to photodegradation of metsulfuron-methyl.

Thus, it may be concluded that post-emergence application of metsulfuron-methyl 4 g/ha for weed

management could be considered as safe, as the residues were below maximum residue limits.

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