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Persistence of the herbicides florasulam and halauxifen-methyl in alluvial and saline alluvial soils, and their effects on microbial indicators of soil quality

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ABSTRACT

The persistence of florasulam and halauxifen-methyl in combination was investigated in an alluvial (AL) and saline alluvial (SAL) soils under laboratory condition at field recommended (FR) dose (i.e. 12.76 g a.i. ha^{-1}), 10 FR and 0FR (control) throughout the experiment. Effect of these herbicides on soil microbial biomass carbon (MBC), basal soil respiration (BSR), substrate induced soil respiration (SIR), hydrolysis of fluorescein diacetate (FDHA) and β-glucosidase activities was also measured under same conditions. The residues of florasulam and haluaxifen-methyl were extracted with acetonitrile using modified QuChERS method, followed by quantification using liquid chromatography-mass spectrometry (LC-MS/MS). Half-lives of florasulam and halauxifen-methyl were in the range of 12.65–16.82 days and 7.13–10.24 days in AL respectively and the same for SAL was 15.28–17.60 days and 7.25–8.88 days. Herbicide treatment inhibited MBC, BSR, SIR, and microbial metabolic quotient (qCO_2) up to 30 days of incubation at the applied doses in both the soils. At the end of incubation, FR treated soils became statistically at par with control. However, the FDHA and β-glucosidase activities were affected in both the soils at the applied doses of the herbicide up to 15 days. Thereafter, the FR treated soils were statistically at par with control. Data revealed that, recommended dose of this herbicide formulation did not ultimately impair microbial components of the studied soils.

1. Introduction

Recent strategies for maximizing agricultural productivity is primarily dependent on the use of high yielding seeds, application of fertilizer and synthetically produced pesticides. Herbicides, in particular, are the important components to protect crops from weed infestation, and those are directly applied to soil. Soil acts as a large sink of applied pesticides where most of the transformation of pesticides occurs. Soil types have a profound influence in this respect. Soils vary with respect to their physical, chemical and microbiological properties. All these properties lead to transformation processes [1]. Of those, microbiological transformations are the predominant [2].

A good pesticide is one that dissipates immediately after performing their assigned role. However, this being rarely the case, they may remain into the soil and affect soil microbial activities [3,4], resulting in alteration of usual functions of terrestrial ecosystem [5]. Thus, it is prudent to evaluate the fate and impact of new herbicides on soil microorganisms prior to their introduction in a particular soil and climate.

Sulfonylurea is a well known herbicide for weed killing through inhibition of acetolactate synthase (ALS). A number of studies related to their impact on non-target sites of these herbicides have been published [6–8]. To overcome the shortfall of sulfonylurea herbicides [9], after late '90s a new group of ALS inhibitors, triazolopyridine sulfonamide, have been marketed. Florasulam (N-[2,6-difluorophenyl]-8-fluoro-5-methoxy [1,2,4] triazolo [1,5-c] pyrimidine-2-sulfonamide) belongings to this group commonly used as mixtures with other herbicides of different mode of action [9]. Halauxifen-methyl (methyl 4-amino-3-chloro-6-[4-chloro-2-

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fluoro-3-methoxyphenyl]) pyridine-2-carboxylate) is the first member of a new synthetic auxin herbicides, the arylpicolinates. Together with florasulam it can provide a new option for controlling broadleaf weeds in food and forage crops. It is likely to be introduced within short time in tropical climates like India. Information is available only on the persistence of this herbicide mixture under field condition [10,11]. It is prudent to understand the fate of these herbicides on their persistence pattern and their impact on microbial and biochemical components in different types of soils.

Microbial biomass carbon (MBC) is an important attribute of soil quality [12], and they give an early warning of soil disturbances due to natural and anthropogenic stresses [13]. The MBC has been used as a tool of determining pesticide toxicity [7,14,15]. Both basal and substrate induced soil respirations (BSR and SIR respectively), and enzyme activities are the long standing methods in deciding microbial activities in soil. Changes in soil respirations have been used as an important parameter for pesticide toxicity estimation [16]. The microbial metabolic quotient (qCO_2) [17] has been widely used as a measure for detection of the effect of xenobiotic compounds on soil microflora. Effect of pesticides on soil enzyme activity has been recorded [18]. The β-glucosidase is an important enzyme in terrestrial carbon cycle producing glucose, which constitutes important energy source for microbial biomass [19]. Thus determination of β-glucosidase has been suggested as a good indicator of soil quality among other hydrolytic enzyme activities [20] and is not confounded by any soil input [21]. Pesticide influence on β-glucosidase activity has been reported [22,23]. The fluorescein diacetate hydrolysis is a broad spectrum enzyme assay because such hydrolysis requires the simultaneous mediation of protease, lipase, and esterase activities [24,25] and this activity occurs within a numbers of primary decomposers like bacteria and fungi [26]. This enzyme assay is a better choice from soil quality perspective than individual soil enzyme assay [21] and has been used in pesticide toxicity studies.

The aim of this study was to evaluate the persistence behavior of the mixture formulation containing florasulam and halauxifen-methyl in tropical alluvial and saline alluvial soils when applied at FR (field recommended) and 10 FR doses, and to find out the correlation of dynamics of the herbicides with that of microbial components and enzyme activities. To determine the effects on microbial components, MBC, BSR and SIR were measured and microbial metabolic quotient (qCO_2) was calculated. Soil enzyme activities (hydrolysis of fluorescein di-acetate and β-glucosidase) were also carried out and specific hydrolytic activity (qFDHA) was evaluated. Such information for this herbicide mixture is lacking.

2. Materials and methods

2.1. Collection of soil samples

The AL (Typic udifluvent) and SAL (Typic endoaquept) were used for this present study. The AL and SAL (0–15 cm) were collected in the month of January, 2014 from the agricultural fields of Bidhan Chandra Krishi Viswavidyalaya Experimental Farm at Mohanpur, India (N 22°56′ E 88°31′) and the Agricultural Experimental Farm of Central Soil Salinity Research Institute, Canning Town, India (N 22°15′ E 88°40′) respectively. The selected sites had no history of pesticide treatment in the recent past. Collected soil samples were sieved (2 mm) and maintained at 60% water-holding capacity of soil by adding the required amount of distilled water. The soil samples were incubated at 28 ± 2 °C in dark for 7 days before the commencement of dynamics of herbicides, microbiological and biochemical analyses. The physico–chemical parameters of the soils were determined by standard procedures [27] using air dried soil samples.

The AL was a clay loam containing 33.2% sand, 30.2% silt and 36.6% clay and the pH, EC_e (dS m^{-1}), organic carbon (g kg^{-1}) and total nitrogen (g kg^{-1}) were 6.85, 0.87, 10.3 and 0.98 respectively. The SAL was also a clay loam containing 25.7% sand, 45.3% silt and 29.0% clay and the pH, EC_e (dS m^{-1}), organic carbon (g kg^{-1}) and total nitrogen (g kg^{-1}) were 7.50, 5.60, 9.3 and 0.91 respectively.

2.2. Experimental plan

Altogether seventy-five pots (twenty-five each for 0FR, FR and 10 FR) each containing 250 g of soil were set up for the persistence study as well as microbiological and biochemical parameters for 5, 10, 15, 30 and 45 days. For persistence study at 0 day another fifteen pots (five each for 0FR, FR and 10FR) were set up and the soil samples were collected 2 h after herbicide treatment. The pots were covered with perforated polypropylene sheets and incubated at 28 ± 2 °C in dark. The formulation (florasulam 10% + halauxifen-methyl 10.4% WG, Dow Agro-Sciences, India) was dissolved in distilled water and applied to soils at FR (12.76 g a.i. ha^{-1}) and 10 FR (127.6 g a.i. ha^{-1}). The final concentration of the applied doses were 5.7 μg kg^{-1} (FR) and 57 μg kg^{-1} (10 FR) obtained assuming bulk density of soil was 1.5 g cm^{-3} and even distribution of applied herbicide. The control (0FR) soils received only equal volumes of distilled water. The 10 FR dose is recommended for ecotoxicological experiments in laboratory incubation study to testify the side effect of pesticide on soil microflora [28,29]. Desired moisture content of the soil samples was maintained by adding requisite quantity of distilled water periodically. Five replicate soil samples were drawn at 5, 10, 15, 30 and 45 days for microbiological and biochemical analyses. Persistence study of the herbicides was carried out with five replicate soil samples drawn at 0, 5, 10, 15, 30 and 45 days.

2.3. Residue analysis of florasulam and halauxifen-methyl

The soil sample (10 g) was taken in a 50 mL fluorinated ethylene propylene (FEP) centrifuge tube (Nalgene, Rochester, NY). 10 mL milli-Q water was added and samples were acidified with 0.1 mL acetic acid (Merck, India). It was then vortexed for 1 min for proper incorporation of the acidified water into sample matrix. After 15 min, 10 ml acetonitrile (J. T. Baker, HPLC grade) was added and shaken vigorously for 1 min. Then 6 g $MgSO_4$ and 1.5 g NaCl (SRL, India) was added to it and again vortexes for 2 min followed by 15 min vertical shaking. Then the sample was centrifuged for 5 min at 5000 rpm. 2 mL of the supernatant extract was cleaned up with 25 mg primary secondary amine (PSA: Varian, Harbor City, CA; 40 mm particle size) and 25 mg florisil (60–100 mesh; Acros, Geel, Belgium).

Quantification of florasulam and halauxifen-methyl residue was done by High performance Liquid Chromatography (HPLC) coupled with tandem mass spectrometry. The HPLC separation was performed by Alliance 2695 separation module liquid chromatograph (Waters, Milford, MA, USA) equipped with a quaternary solvent delivery system via auto sampler on a reversed phase Symmetry C_{18} (5 μm; 2.1 × 100 mm) column (Waters, USA). Micromass (Manchester, UK) Quattro Micro triple-quadruple spectrometer equipped with an electrospray source (ESI) was used for detection and quantification. Injection volume was20 μl and the analysis performed with a flow rate of 0.3 ml min^{-1}. The mobile phase was composed of (A) water, 5 mM ammonium acetate and 0.1% acetic acid and (B) methanol, 5 mm ammonium acetate and 0.1% acetic acid. Gradient: 0.0–2.0 min – 5.0% B to 95%B, 2.0–8.0 min; back to the initial condition of 5% B, at 10.0 min, it ends with 5% B. Quantification of the residue was performed in multiple reaction monitoring (MRM) mode. Ion transitions were 359.87 > 128.90, 359.87 > 81.60 for florasulam and 344.82 > 250.10, 344.82 > 285.00