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Soil enzymatic activity and pyroxasulfone residues under *in situ* paddy (*Oryza sativa*) straw management in wheat (*Triticum aestivum*)

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ABSTRACT

Managing paddy residues without burning and weeds particularly *Phalaris minor* have been foremost challenges in wheat (*Triticum aestivum* L.) production in irrigated rice (*Oryza sativa* L.)–wheat system in north-western India. Short wheat sowing window compels farmers to burn paddy residues, wasting a 'nutrient storehouse'. Hence, to overcome the challenge, different seeding machines (for straw incorporation and surface retention) were evaluated along with herbicide pyroxasulfone. While straw retention enhances soil organic matter, the potential of herbicide residues to negatively impact soil health necessitates the investigation. The current study was carried out to investigate the effect of *in situ* paddy residue management methods, wheat varieties and of pyroxasulfone herbicide on soil health via soil enzymatic activities during winter (*rabi*) season of 2021–22 and 2022–23 at Punjab Agricultural University, Ludhiana, Punjab. The two wheat varieties (namely PBW 869 and 766) were sown with four *in situ* paddy residue management machines (Smart seeder, PAU Happy seeder, Super and Mittar seeder) having variable paddy residue management scenarios and sprayed with pyroxasulfone 127.5 kg/ha and kept unsprayed as well. All paddy residue management methods and wheat varieties exhibited similar dehydrogenase, alkaline phosphatase and urease activities indicating seeding machines and varieties did not adversely affect soil microbial functions. Pyroxasulfone application temporarily inhibited the dehydrogenase (8.2%) and alkaline phosphatase activity (5.0%) at 60 days after herbicide application suggesting short term suppression of microbial response following herbicide mobilisation into soil solution whereas urease activity remained unaffected. Harvest-time pyroxasulfone residues in soil, wheat grain and wheat straw were all below the detectable limit (<0.01 μg/g). It can be concluded that integration of pyroxasulfone is an ecologically safe and effective tool for weed management in straw retention wheat production systems.

Keywords: Alkaline phosphatase, Dehydrogenase, Herbicide residues, Pyroxasulfone, Urease

Management of paddy straw has been among the foremost challenges in rice (*Oryza sativa* L.)–wheat (*Triticum aestivum* L.) system in north western India. In a combine harvested fields, leftover paddy residues make field preparation difficult which delays wheat sowing (Singh *et al.* 2017). For timely sowing of wheat, farmers in the region resort to *in situ* or *ex situ* methods of straw management while some farmers resort to burning of paddy straw in the field. *In situ* burning of paddy straw increase air pollution resulting in severe impacts on human and animal health in rice growing regions (Singh *et al.* 2010, Huang *et al.* 2012). Paddy straw is also not preferred as dry fodder due to high silica. From sustainability point of view, *in situ* management is the best option. Straw is either kept on surface as mulch or is incorporated. While Turbo Happy seeder and Super seeder machines are commonly used for surface retention and incorporation of residues, respectively, new innovations in this segment includes Smart seeder for surface retention and Mittar seeder for incorporation. All these machines manage paddy residue and sow wheat in a single pass.

Apart from residue management, the competition by weeds particularly *Phalaris minor* is another critical issue in the region, causing yield reductions of upto 95% (Chhokar and Malik 2002). It has developed herbicide resistance against various modes of action i.e. photosynthesis at photosystem II site A, acetolactate synthase and acetyl-coA carboxylase inhibition (Heap 2024). Pyroxasulfone, a pyrazole-based pre-emergence herbicide, controls weeds by inhibition of very-long-chain fatty acids biosynthesis (Tanetani *et al.* 2009), has recently been introduced to manage resistant *P. minor* (Kaur *et al.* 2019).

The soil and crop management practices have differential effect on soil health. Soil enzymes are valuable indicator for assessing these impacts (Trasar-Cepeda *et al.* 2000). Dehydrogenase enzyme is the most important indicator of overall soil microbial activity and is used to assess side effects of pesticides (Hanajik *et al.* 2017),

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alkaline phosphatase plays an important role in the P cycle while urease is vital for the nitrogen cycle and regulation of N supply after urea fertilisation (Makoi and Ndakidemi 2008). Residue retention adds organic matter, serving as a carbon source for these enzymes.

Pesticides can affect microbial dynamics (Baćmaga *et al.* 2014) and consequently, soil enzymatic activity (Baćmaga *et al.* 2012) and affects varies from positive, negative and neutral. Rising environmental concerns have highlighted the need to study the fate of these herbicides in soil under residue retention/incorporation conditions and their subsequent effect on enzymatic activity. The study aimed to investigate the effect of *in situ* paddy residue management, varieties and pyroxasulfone application on soil enzymatic activities and to determine whether induced changes are temporary or long-lasting. In addition, the study evaluated the harvest time residues of pyroxasulfone in soil, wheat grains and straw under variable *in situ* residue management condition.

MATERIALS AND METHODS

Experiment design and details: The present study was carried out during winter (*rabi*) season of 2021–22 and 2022–23 at Punjab Agricultural University, Ludhiana (30° 54'N latitude and 75° 46'E longitude), Punjab. The experimental soil was clay loam (Sand: 42%, silt: 20% and clay: 38%) in texture with moderate organic carbon (0.60%), available nitrogen (276.0 kg/ha) and available phosphorus (13.2 kg/ha) and having sufficient available potassium (140.0 kg/ha) with *p*H 7.55, before sowing in 2021–22. Final soil *p*H after harvesting of first and second year experiments varied between 7.56–7.57 and 7.55–7.56, respectively. Sowing of two wheat varieties namely PBW 869 and PBW 766 were done in standing rice stubbles employing four *in situ* paddy residue management machines, i.e. Smart seeder, PAU Happy seeder, Super seeder and Mittar seeder. The experiment was laid out in a split plot design (SPD) with both varieties and four machines were kept in main plots whereas pyroxasulfone and unsprayed control kept in sub-plots. PAU Happy seeder leaves residues as surface mulch, Smart seeder till narrow zone where seed is to be drilled (minimum tillage) with slight incorporation of residues while Super and Mittar seeder incorporates the rice straw completely. Sowing was done on 1st November 2021 and 10th November, 2022. The full dose of 75 kg P_2O_5/ha was applied through DAP at time of sowing. 135 kg N/ha through urea was applied in three splits, one split before sowing (for pre-plant application of pyroxasulfone in Smart and PAU Happy seeder and without pyroxasulfone in Super and Mittar seeders), 2nd and 3rd split just before the first and second irrigation. Pyroxasulfone at 127.5 g/ha was applied as pre-plant before sowing of wheat in Happy and Smart seeders plots along with urea and as pre-emergence herbicide after wheat sowing in Super and Mittar seeder plots as per treatments. First irrigation was given 25 days after sowing (DAS) of wheat. Total of four irrigations were given at an appropriate interval to each experiment depending upon rainfall during both years. On attaining maturity, crop was harvested on 9th April, 2022 during first year and 21st April, 2023 during second year.

Chemicals used for enzymatic activities and herbicide residues estimation: Stock solution (1000 µg/mL) of pyroxasulfone was prepared using HPLC grade acetonitrile (ACN). Working standards of herbicide was made from respective stock solutions in the range of 0.001–100 µg/mL. Commercial grade of herbicide was purchased from local market of Ludhiana, Punjab. HPLC grade acetonitrile and other chemicals used in the study were also procured.

Soil enzymatic activities: Dehydrogenase activity was determined by quantifying the release of triphenyl formazan (TPF) from triphenyl tetrazolium chloride (TTC) by following procedure as reported by Tabatabai (1994) whereas alkaline phosphatase activity was estimated from production of p-nitrophenol (PNP) from p-nitrophenyl phosphate disodium (Tabatabai and Bremner 1969). Urease activity was estimated from amount of urea remaining after hydrolysis from urea solution (Douglas and Bremner 1970).

Sampling strategy: For enzymatic activity assessment, soil samples (0–15 cm depth) were collected from all plots at various intervals, 0 (3 h), 30, 60 and 90 days after herbicide application (DAHA) and finally at harvest. To assess herbicide residues in both soil and wheat, samples were gathered at harvest. This included soil (0–15 cm depth), along with grain and straw samples. All collected soil and produce samples were immediately stored at -4ºC in a deep freezer to prevent any degradation of herbicide residues or enzymatic activity.

Herbicide residues extraction: The extraction of pyroxasulfone from soil, wheat grain and straw followed the method described by Westra *et al.* (2014).

Extraction from soil: 10 g of soil was combined with 10 mL of water and 10 mL of water-saturated toluene.

From wheat grain/straw extraction: For wheat grain, 10 g of finely ground material was mixed with 20 mL of water and 20 mL of water-saturated toluene. For straw, 5 g was mixed with the same solvent volumes.

Samples were shaken for 2 h and then centrifuged at 1000× for 10 min. The syringe was used to remove supernatant containing organic layer and solvent was evaporated to dryness using rotary vacuum evaporator at 45 ± 2ºC. The resulting residues were reconstituted in 2 mL of acetonitrile prior to quantification.

Quantification of herbicide residues: Pyroxasulfone residues at harvest were quantified using high-performance liquid chromatography (HPLC) equipped with a UV detector. Separation was achieved using a C18 column (5 µm; 4.6×250 mm). The mobile phase consisted of acetonitrile:water (80:20) at a flow rate of 0.8 mL/min. The UV detector wavelength was set at 230 nm and the retention time for pyroxasulfone was 2.360 min (Fig. 1).

Statistical analysis: Statistical analysis was done as per standard statistical procedures for split plot design (SPD) as per Gomez and Gomez (1984). Fisher's least significant difference (LSD) test (p=0.05) was used to

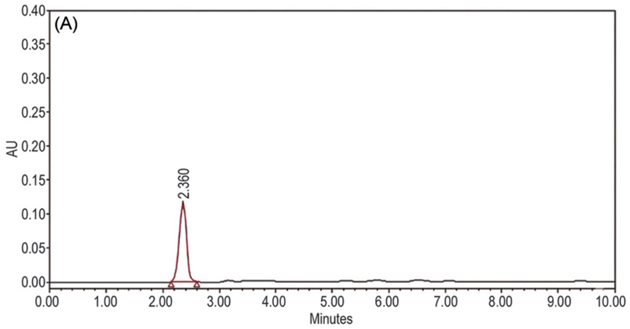

Fig. 1 HPLC chromatogram of pyroxasulfone.

assess the difference among the mean values. The data for year 2021–22 and 2022–23 were pooled as similar trends in results were observed during both the years.

RESULTS AND DISCUSSION

Dehydrogenase and alkaline phosphatase activities: The effect of different factors i.e. *in situ* paddy residue management and wheat varieties on dehydrogenase activity (DHA) and Alkaline phosphatase activity (APA) at various stages of crop growth have been presented in Table 1. DHA for Smart, PAU Happy, Super and Mittar seeders varied from 47.1–49.4, 47.3–49.1, 46.5–49.0 and 46.6–48.8 µg of TPF/g soil/h, respectively (Table 1). The corresponding values for APA ranged from 22.7–23.6, 22.6–23.6, 22.4–23.3 and 22.8–23.7 µg of PNP/g soil/h. Among various paddy residue management methods, there has been slight increase in DHA and APA with advancement in growth till harvest (Table 1). Non-significant differences between various mechanical solutions indicate that *in situ* straw retention creates broadly uniform micro-environmental conditions under conditions of present study.

Wheat varieties had non-significant effect on DHA and APA across various growth stages till harvest indicating that selected enzymatic activities were not influenced by the varietal differences (Table 1). The dehydrogenase activity varied from 46.8–49.0 µg of TPF/g soil/h for PBW 869 and from 46.9–48.9 µg of TPF/g soil/h for PBW 766 (Table 1). The corresponding APA values ranged from 22.8 to 23.6 µg of PNP/g soil/h for PBW 869 and 22.5 to 23.5 µg of PNP/g soil/h for PBW 766 (Table 1). The values observed for both varieties were within a narrow range, suggesting similar trends in rhizospheric enzyme activity.

There has been slight increase in both DHA and APA irrespective of residue management method and wheat variety. Increase in activities of both enzymes might be due to retention/incorporation of paddy residues as these residues after decomposition, might have acted as substrate for soil microorganisms which play a key role in producing dehydrogenase and alkaline phosphatase enzymes. Similar positive effects of residue retention on enzyme activities like dehydrogenase, phosphatase were also reported by Bera *et al.* (2018).

Between weed control treatments, unsprayed plots showed non-significant increase in DHA till harvest right

Table 1 Effect of *in situ* residue management and wheat varieties on dehydrogenase, alkaline phosphatase and urease activities (pooled data of year 2021–22 and 2022–23)

Treatment	Dehydrogenase activity (µg of TPF/g soil/h)					Alkaline phosphatase activity (µg of PNP/g/soil/h)					Urease activity (µg of urea hydrolyzed/g soil/min)				
	0 DAHA	30 DAHA	60 DAHA	90 DAHA	At harvest	0 DAHA	30 DAHA	60 DAHA	90 DAHA	At harvest	0 DAHA	30 DAHA	60 DAHA	90 DAHA	At harvest
Residue management															
Smart seeder	48.1	49.4	47.1	48.4	49.1	22.7	23.6	23.2	23.6	23.3	3.02	3.31	3.10	3.00	2.88
PAU Happy seeder	48.0	48.8	47.3	48.9	49.1	22.6	23.6	23.2	23.6	23.3	2.95	3.31	3.09	2.98	2.89
Super seeder	47.8	48.4	46.5	48.1	49.0	22.4	23.3	23.0	23.3	23.0	2.97	3.30	3.12	3.04	2.92
Mittar seeder	47.4	48.8	46.6	48.6	48.5	22.8	23.7	23.3	23.7	23.3	2.99	3.31	3.11	3.00	2.89
LSD (p=0.05)								NS							
Varieties															
PBW 869	48.1	49.0	46.8	48.5	48.9	22.8	23.6	23.3	23.6	23.3	2.98	3.30	3.11	3.00	2.89
PBW 766	47.5	48.6	46.9	48.6	48.9	22.5	23.5	23.1	23.5	23.2	2.99	3.31	3.11	3.01	2.90
LSD (p=0.05)								NS							

DAHA, Days after herbicide application; TPF, Triphenyl formazan; PNP, p-nitrophenol; LSD, Least significant difference; NS, Non-significant.
Day of wheat sowing/herbicide application was same.

from sowing, indicating natural weed growth did not produce a measurable effect on dehydrogenase activity. Similar observations have been reported by Fouad *et al.* (2023). Pyroxasulfone application didn't affect the DHA at 0 and 30 DAHA. Later at 60 DAHA, significantly lower DHA (44.9 µg of TPF/g soil/h) was observed as compared to unsprayed check (48.9 µg of TPF/g soil/h) (Fig. 2A) and later activity increased comparable to unsprayed check and stabilised at its normal levels at harvest (Fig. 2A). A similar temporary reduction and recovery pattern was observed for APA. This trend may be associated with transient effects of the herbicide followed by its dissipation over time, as suggested in earlier studies by Mueller and Steckel (2011). Pyroxasulfone, with low water solubility (3.49 mg/L at 20°C), remained soil adsorbed in clay loam soils (heavy-textured) as reported by Westra *et al.* (2014) also, pyroxasulfone entered the soil solution upon irrigation, resulting in an 8.2% inhibition in DHA (Fig. 2A) and 5.0% inhibition in APA at 60 DAHA (Fig. 2B), compared to untreated control. The increase in enzymatic activity after 60 DAHA may be associated with reduced pyroxasulfone concentration, potentially due to degradation and leaching. The inhibition at 60 DAHA was likely due to herbicide-induced stress due to adverse effect on microorganism's physiological processes leading to cell lysis and membrane changes, which alter soil enzymatic activity (Romero *et al.* 2010). Similar temporary decrease in enzymatic activity due to adverse effect of herbicide application on soil microbes followed by microbial adaptation/recovery has also been reported by Filimon *et al.* (2021) and Vasic *et al.* (2022) .

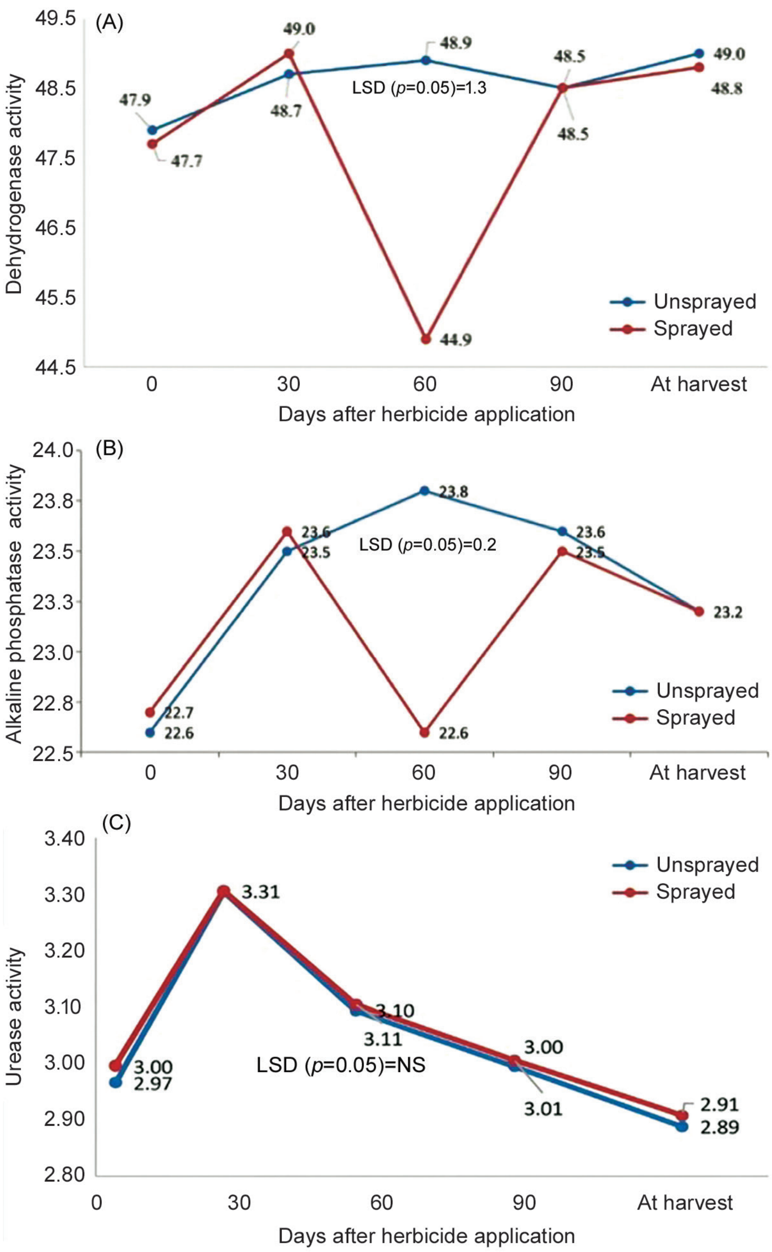

Fig. 2 Effect of pyroxasulfone on (A) dehydrogenase, (B) alkaline phosphatase and (C) urease activity (pooled data of year 2021–22 and 2022–23).

Urease activity: The urease activity for paddy residue management methods varied from 2.88–3.31 µg of urea hydrolysed/g soil/min (Table 1). No variable influence of different wheat varieties PBW 869 and PBW 766 was observed on urease activity and it varied from 2.89–3.30 and 2.90–3.31 µg of urea hydrolysed/g soil/min for PBW 869 and PBW 766, respectively (Table 1).

Urease activity was not significantly affected by pyroxasulfone application and the corresponding values for pyroxasulfone sprayed and unsprayed plots varied from 2.91–3.31 and 2.89–3.31 µg of urea hydrolysed/g soil/min (Fig. 2C). Perusal of data showed that urease activity peaked at 30 DAHA and slightly decreases after that across all growth stages till harvest with no significant variations between pyroxasulfone sprayed and unsprayed control treatments. Urea application along with first irrigation led to immediate increase in urease activity at 30 DAHA due to sufficient substrate availability in both treatments. Similar findings by Boyd and Mortland (1985) report that hydrolysis rate increases with the increasing substrate till saturation of urease enzyme. They also found that substantial percentage of urease enzyme gets adsorbed on clay complexes by special bonding which results in enzymatic activity as high as that in solution and enzymes immobilised on clay and in solution behave similarly. Pyroxasulfone failed to inhibit microbial processes related to urea hydrolysis might be due to the binding of urease enzyme on natural clay-organo complexes in clay loam soil. Neutral effect on urease activity to environmental impact also reported by Sannino and Gianfreda (2004) and Yao *et al.* (2006). Recently, Kaur *et al.* (2024) also reported pyroxasulfone's short time transitory inhibition of DHA and APA and

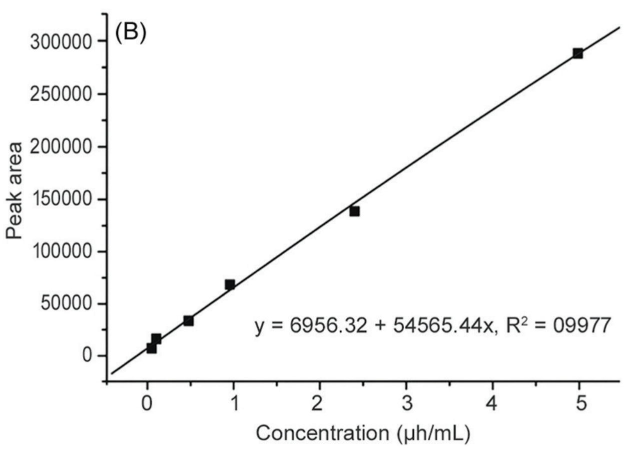

Fig. 3 Calibration curve of pyroxasulfone.

non-significant effect on urease activity.

Estimation of pyroxasulfone herbicide residues

Analytical method validation: The calibration curve of pyroxasulfone was linear with correlation coefficient R^2>0.99 (Fig. 3). The Limit of Quantification (LOQ) and Limit of Detection (LOD) of pyroxasulfone were 0.01 and 0.003 µg/g, respectively. The mean percent recoveries of pyroxasulfone from soil, wheat grain and wheat straw samples at fortification levels of 0.5, 0.1 and 0.01 µg/g ranged from 81.5 ± 3.99 to 89.1 ± 2.34, 74.9 ± 2.77 to 82.4 ± 3.55 and 72.3 ± 3.09 to 82.2 ± 4.23%, respectively (Table 2). Interday precision (% RSD_R) and intraday precision (percent RSD_r) of herbicides were assessed by repeating experiment three times a day and on three different days, respectively and were <10% in all matrices.

Harvest time residues of pyroxasulfone in soil and crop produce (grain and straw): The harvest time residues of pyroxasulfone in soil, wheat grain and wheat straw were below the detectable limit (<0.01 µg/g) as evident from different chromatograms of pyroxasufone in soil (Fig. 4A), grain (Fig. 4B) and straw (Fig. 4C) as pyroxasulfone residues would have degraded by crop harvest hence its residues were not detected in soil and crop produce at the time of harvest due to longer time interval (more than 150 days) between herbicide application and crop harvest.

All paddy residue management methods and wheat varieties had non-variable effects on dehydrogenase, alkaline phosphatase and urease activities. Pyroxasulfone

Table 2 Mean percent recoveries of pyroxasulfone from soil, wheat grain and straw

Matrix	Recovery (%)		
	0.5 µg/g	0.1 µg/g	0.01 µg/g
Soil	89.1 ± 2.34	85.6 ± 1.81	81.5 ± 3.99
Wheat grain	82.4 ± 3.55	78.9 ± 2.90	74.9 ± 2.77
Wheat straw	82.2 ± 4.23	79.0 ± 1.24	72.3 ± 3.09

±, Standard deviation.

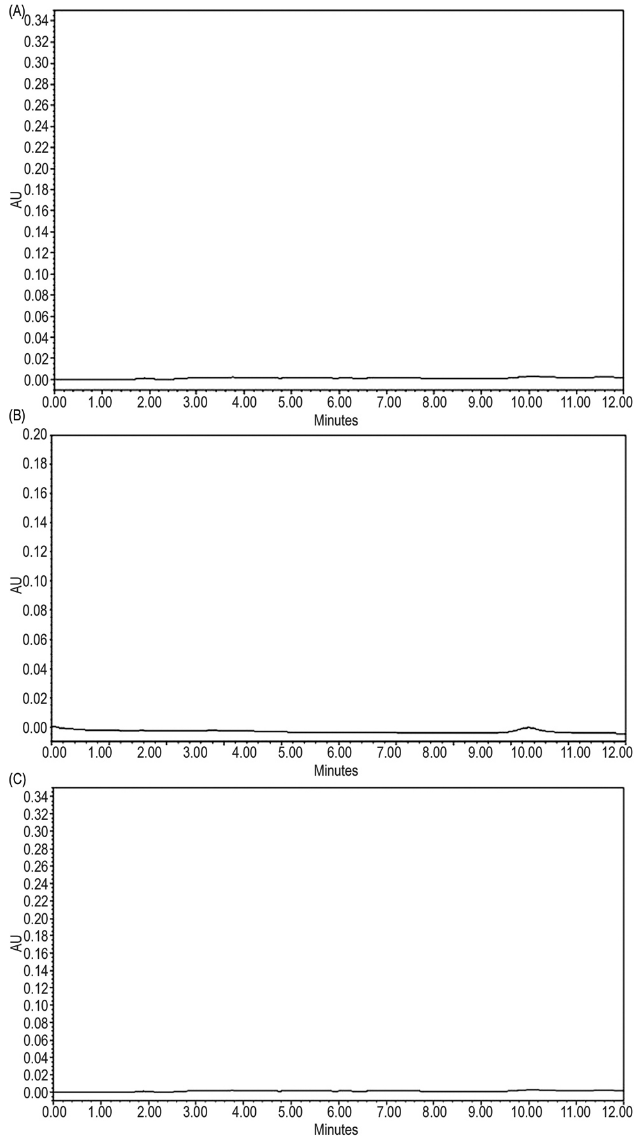

Fig. 4 Chromatograms of pyroxasulfone in (A) soil (B) wheat grain and (C) wheat straw.

has temporary inhibitory effect on soil activities of dehydrogenase and alkaline phosphatase enzymes only during initial stages which later recovered and stabilised to normal levels and neutral towards urease activity. Harvest time residues of pyroxasulfone in soil and crop produce were below detectable limits (<0.01 µg/g) which might be attributed to sufficient time gap between pyroxasulfone application and wheat harvest. Thus, it can be stated that pyroxasulfone, can be safely used by farmers even against herbicide resistant *Phalaris minor* in wheat particularly in clay loam soils.

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