



Dissipation kinetics and harvest time residues of pyrazosulfuron-ethyl + pretilachlor in rice

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ABSTRACT

The study on dissipation and harvest time residues of pyrazosulfuron-ethyl 0.75% and pretilachlor 30% WG after foliar application at seedling stage in rice at two doses 2000 g/ha (15 + 600 g/ha) and 4000 g/ha (30.0 + 1200 g/ha) was conducted at Integrated Farming System Research Station, Kerala Agricultural University, Thiruvananthapuram, Kerala. The pyrazosulfuron-ethyl and pretilachlor residues were estimated using LCMS/MS. The mean initial deposit of pyrazosulfuron-ethyl at recommended and double the recommended doses were 0.18 and 0.48 mg/kg, respectively. The residue dissipated with time and reached below limit of quantification (LOQ) of 0.01 mg/kg after 5 days in the recommended dose and 7 days in double the recommended dose. The mean initial deposit of pretilachlor at recommended and double the recommended dose were 8.84 and 15.50 mg/kg, respectively. The residue dissipated with time and reached below LOQ of 0.05 mg/kg within 15 days both in the recommended and double the recommended dose. The half-life values (days) were 1.29 and 1.80 for pyrazosulfuron-ethyl and 1.88, 1.37 for pretilachlor at recommended and double the recommended doses, respectively. In addition, the harvest time residues of pyrazosulfuron-ethyl 0.75% + pretilachlor 30% WG were estimated in samples of rice grain, husk, straw and cropped soil and were found below LOQ.

INTRODUCTION

Pyrazosulfuron-ethyl (Ethyl 5-[(4,6-dimethoxypyrimidin-2-ylcarbonyl) sulfamoyl]-1-methylpyrazole-4-carboxylate) is a sulfonylurea herbicide for rice with excellent herbicidal activity in both pre- and post-emergence applications. It is different from other sulfonylurea herbicides in the substitutions on the pyrazole ring and does not include a triazinic and pyridinic ring (Sarmah and Sabadie 2002). Therefore, common degradation pathways occurring for sulfonylureas, such as O- and N-dealkylation of the group on the triazine ring or triazine ring opening to form a triuret does not take place in pyrazosulfuron-ethyl. Rajkhowa *et al.* (2006) reported that pyrazosulfuron-ethyl at 20 g/ha was as effective as butachlor 1250 g/ha in reducing weed growth and increasing grain yield of rice.

Pretilachlor (2-chloro-N-(2, 6-diethylphenyl)-N-(2-propoxyethyl) acetanilide) belongs to the chloroacetanilide group and is used as pre-emergence and early post-emergence herbicide for the control of annual grasses and some broad-leaved weeds such as

Echinochloa crus-galli and *Ischaemum rugosum* in both seeded and transplanted fields (Han and Hatzios 1991). Chauhan *et al.* (2014) reported that broad spectrum of weed flora can be easily managed by a lower dosage of pretilachlor in wet-seeded rice; however, the dose needs to be increased to 900 g/ha in order to decrease the weedy rice problem. Increased yield in rice was reported in pretilachlor treated rice as reported in Thailand (Allard *et al.* 2005), China (Shen *et al.* 2013), Vietnam (Chauhan *et al.* 2015). This response was observed mainly due to less crop-weed competition in the pretilachlor treated plots.

Pyrazosulfuron-ethyl 0.75% + pretilachlor 30% WG is the combination product for better weed management and having the highest weed control efficiency (Dibyendu *et al.* 2018). The studies on the dissipation of herbicide mixture, pyrazosulfuron-ethyl 0.75% + pretilachlor 30% WG in rice soil and water was studied by Ezhilarasi *et al.* (2018). In present study, the dissipation of these molecules was studied in rice plant and harvest time residues were also determined in rice grain, husk, straw and soil.

MATERIALS AND METHODS

Chemicals and reagents

Certified reference materials ($\geq 95\%$ purity) of pyrazosulfuron-ethyl and pretilachlor and formulation of pyrazosulfuron-ethyl 0.75% + pretilachlor 30% WG (UPH-814) were received from M/s UPL Ltd, Mumbai. Standard solution of pyrazosulfuron-ethyl and pretilachlor prepared with HPLC grade acetonitrile and suitably diluted to obtain the working standards. Acetonitrile, hexane and methanol of LiChrosolv grade, sodium chloride, anhydrous sodium sulphate, and anhydrous magnesium sulphate of GR grade were purchased from Merck Specialities Private Limited, Mumbai and the solid reagents were activated before use. Primary secondary amine (PSA) sorbent was purchased from Agilent Technologies, USA. All the glass wares were thoroughly washed as per the standard operating procedure to avoid the interferences from any contaminants during analysis. The suitability of solvents and other chemicals were ensured by running reagent blanks before actual analysis.

Recovery experiment

Recovery studies were carried out in order to establish the reliability of the analytical methods and to know the efficiency of extraction and clean up step for the present study by fortifying rice separately with pyrazosulfuron ethyl and pretilachlor. For pyrazosulfuron-ethyl, recovery experiment was done at 0.01 mg/kg (limit of quantification- LOQ), 0.05 mg/kg (5 X LOQ) and 0.10 mg/kg (10 X LOQ) level and for pretilachlor at 0.05 mg/kg (LOQ), 0.25 mg/kg (5 X LOQ) and 0.50 mg/kg (10 X LOQ) level.

Field experiment

Persistence of herbicides: Rice (var. *Uma*) was raised at Integrated Farming System Research Station, Kerala Agricultural University, Karamana, Thiruvananthapuram, Kerala ($8^{\circ}28'54.41''\text{N}$ latitude and $76^{\circ}57'56.69''\text{E}$ longitude at an altitude of 25.22 m above mean sea level) adopting the package of practices recommendations of Kerala Agricultural University to conduct the studies on dissipation of pyrazosulfuron-ethyl and pretilachlor. The trial was laid out in randomized block design (RBD) replicated thrice with a plot size of 25 m² with three treatments, *i.e.* recommended (X), double the recommended dose (2X) and control. Pyrazosulfuron-ethyl 0.75% and pretilachlor 30% WG was sprayed in rice plants at two doses, at recommended dose 2000 g/ha (15 + 600 g/ha) and at double the recommended dose 4000 g/ha (30.0 + 1200.0 g/ha). Spraying was done once at seedling stage and the persistence of residues in rice

green foliage was carried out from 2 hrs after the application of herbicides. About 500 g samples of rice was collected at 0 (within 2 hrs), 1, 3, 5, 7, 10, 15 days after the application. Three samples were collected from each replication corresponding to each treatment. The harvest time residues of rice grain, straw, husk and soil were also estimated.

Harvest time residues: In order to find out the multi-location harvest time residues of pyrazosulfuron-ethyl 0.75% and pretilachlor 30% WG in rice grain, husk, straw and cropped soil, field trials were conducted at three different locations, *viz.* 1. G.B. Pant University of Agriculture & Technology (GBPUAT), Pantnagar; Uttarakhand 2. Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha and 3. National Institute of Biotic Stress Management (NIBSM), Raipur, Chhattisgarh, based on the agro-climatic zone variation. The lay out of the experiment and dosage of spraying and schedule was same as explained in persistence study. Harvested samples of rice grain, husk, straw and cropped soil were collected from the field along with untreated control (weedy check) in all location and received at AINP on Pesticide Residue, Kerala Agricultural University, College of Agriculture, Vellayani, Kerala under dry ice condition for analysis.

Extraction and clean-up

Green foliage and rice grain: 500 g each of green foliage and rice grain was blended and from which 25 g was taken, added 50 ml acetonitrile and homogenized at 14,000 rpm for 2 min. The samples were shaken for 4 min after adding 10 g sodium chloride. The samples were then centrifuged for 5 min at 2500 rpm. A 16 mL supernatant was transferred in to 50 mL centrifuge tube containing 6 g anhydrous Na₂SO₄ and mixed well using high speed vortex shaker for 2 min. 12 ml extract was transferred to a 15 mL centrifuge tube containing 0.2 ± 0.01 g PSA sorbent and 1.2 ± 0.01 g anhydrous MgSO₄, shaken and centrifuged for about 3 min at 2500 rpm. five ml of the extract was evaporated in a turbovap and made up to 2 ml using methanol for LC-MS/MS analysis.

Straw/husk: 100 g of straw/husk taken from three treatments were powdered and from which 5 g was taken, added 40 ml distilled water containing 10g sodium chloride and kept for 1 hour, mixed well for uniform wetting and then 50ml acetonitrile was added. The samples were shaken for 10 min and were centrifuged for five min at 2500 rpm. A 25 mL supernatant was transferred in to 50 mL centrifuge tube containing 5 g anhyd. Na₂SO₄ and mixed well using high speed vortex shaker for 2 min, then

centrifuged for 3 min at 2500 rpm. 10 ml supernatant was transferred to a 15 mL centrifuge tube containing 0.125 ± 0.01 g PSA sorbent and 2.00 ± 0.01 g anhy. MgSO_4 . The sample was mixed well using high speed vortex shaker for 2 min and centrifuged for about 3 min at 2500 rpm. 5 ml of the extract was evaporated in a turbovap at 45°C and made up to 2 ml using methanol for LC-MS/MS analysis. Injected at LC-MS/MS with Atlantic dc-18 column, at 40°C using methanol-water mobile phase.

Soil: Analysis of soil was done by the method suggested by Asensio-Ramos *et al.* (2010) with slight modification. Soil samples (500 g) taken from three treatments were air dried and sieved through 2 mm sieve. Ten-gram soil sample was transferred to a 50 mL polypropylene tube to which 20 mL acetonitrile, 4 g MgSO_4 (activated) and 1 g NaCl were added and shaken vigorously for one minute. The contents were centrifuged at 3300 rpm for 4 min and 10 mL of the supernatant was transferred to another 15 mL polypropylene centrifuge tube containing 1.5 g of magnesium sulphate and 0.25 g of primary secondary amine (PSA). The contents were shaken for 30 seconds and then centrifuged for 10 min at 4400 rpm from which 4 mL aliquot of the supernatant was taken and evaporated to dryness using Turbovap at 40°C . The dry residue was reconstituted to 1 ml in methanol for LC-MS/MS analysis.

Instrumentation

Pyrazosulfuron-ethyl: Analytical grade (0.0101 g; 99.7%) pyrazosulfuron-ethyl was weighed and transferred to a 25 mL volumetric flask using the methanol. The volume was made up to the mark with methanol to give 400 mg/kg stock solution of pyrazosulfuron-ethyl and from this stock solution, 10 mg/kg intermediate standard was prepared. From 10 mg/L stock, 1.00, 0.50, 0.25, 0.10, 0.05, 0.025 and 0.01 mg/L were prepared.

Pretilachlor: Analytical grade (0.0100 g; 98.4%) pretilachlor was weighed and transferred to a 25 mL volumetric flask using the methanol. The volume was made up to the mark with methanol to give 400 mg/kg stock solution of pretilachlor. From this stock solution, 10 mg/kg intermediate standard was prepared. From this, 1, 0.5, 0.25, 0.10, 0.05 mg/kg concentrations were prepared. Calibration of the equipment was performed using pure analytical standard of the test material at concentration ranging from 0.025 to 1.0 mg/L and the response/area obtained was plotted against concentration. The response was found linear in the concentration tried (0.025 – 1.0 mg/kg) as evident from the calibration

curve attached in annexure. The correlation coefficient (r^2) value obtained was 0.9952 indicating perfect linearity.

Estimation of pyrazosulfuron-ethyl and pretilachlor in LC MS/MS

Analysis of pyrazosulfuron-ethyl and pretilachlor was carried out in LC-MS/MS (Applied Biosystems API-3200) triple quadrupole MS/MS with electro spray ionization (ESI) in the positive mode coupled to a Waters LC (Acquity UPLC™), which includes a binary pump, column oven and auto sampler.

Mass spectrometry parameters

The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reversed phase Atlantis d C-18 (2.1×100 mm, 5-micron particle size) column. A gradient system involving the following two-eluent components: A: 10% methanol in water + 0.1% formic acid + 5 mM ammonium acetate; B: 10% water in methanol + 0.1% formic acid + 5 mM ammonium acetate were used as mobile phase for the separation of residues. The flow rate remains constant at 0.8 mL/min and injection volume was 10 μL . The column temperature was maintained at 40°C . The effluent from the LC system was introduced into Triple quadrupole API 3200 MS/MS system equipped with an electrospray ionization interface (ESI), operating in the positive ion mode. The source parameters were temperature 600°C , ion gas (GS1) 50 psi, ion gas (GS2) 60 psi, ion spray voltage 5,500 V and curtain gas 13 psi. Under these operating conditions the retention time of pyrazosulfuron-ethyl and pretilachlor was found to be 0.383 and 0.528min, respectively.

LC- Separation: All LC separations were carried out using a reversed phase column, Atlantis d C₁₈ (2.1X100 mm) with 5 μm spherical porous particles. The elution was performed using gradient between methanol and water. Mobile phase A contained 5 milli molar ammonium acetate in water and B contained 5 milli molar ammonium acetate in methanol. Flow rate 0.80 mL/min, column temperature 40°C , sample temperature 5°C , and the injection volume 10 μL were used in all the estimation.

MS/MS: The MS/MS conditions were optimised using direct infusion in to ESI source in positive mode to provide the highest signal/noise ratio for the quantification ion of each analyte. Two MS/MS transitions were made in case of chemical interferences observed in the quantitation ion chromatogram and for qualitative purpose. The ion

source temperature was 550°C with ion spray voltage of 5500 V. Chromatographic elution zones were divided into appropriate number of time segments. In each segment, corresponding MS/MS transitions were monitored using multiple reactions–monitoring (MRM) mode.

Certified reference materials of pesticides and stock solutions were prepared using pesticide grade solvents. Single laboratory method validation was performed to establish the recovery of pesticides. Spiking solutions for measuring per cent recovery were prepared from stock solutions of concentration 1000 mg/L. Calibration was performed with six levels of serially diluted standard mixture, prepared from stock solutions. Calibration curves of working standards were used to evaluate the linearity of the gas chromatograph response in each day of analysis and pesticide residues were quantified based on these standards. The concentration of pesticide residue was calculated as given in Beevi *et al.* 2018

Studies on linearity check

Pyrazosulfuron-ethyl: Analytical grade (0.0101 g; 99.7 %) pyrazosulfuron-ethyl was weighed and transferred to a 25 mL volumetric flask using the methanol. The volume was made up to the mark with methanol to give 400 mg/L stock solution of pyrazosulfuron-ethyl. From this stock solution, 10 mg/L intermediate standard was prepared and 1, 0.5, 0.25, 0.10, 0.05, 0.025 and 0.01 mg/L concentrations were prepared from 10 mg/L.

Calibration curve of pyrazosulfuron-ethyl: Calibration of the equipment was performed using pure analytical standard of the test material at concentration ranging from 0.025 to 10 mg/L and the response/area obtained was plotted against concentration. The response was found linear in the concentration tried (0.025 – 10 mg/L). The

correlation coefficient (r^2) value obtained was 0.9925 indicating perfect linearity.

Pretilachlor: A linearity check study was carried out with the help of analytical standard of pretilachlor. In this study calibration curve was prepared by taking the areas corresponding to different concentrations of calibration standard, against which final quantification was done.

Analytical grade (0.0100 g; 98.40%) pretilachlor was weighed and was transfer to a 25 mL volumetric flask using the methanol. The volume was made up to the mark with methanol to give 400 mg/L stock solution of pretilachlor. From this stock solution 10 mg/L intermediate standard was prepared and from this, 1, 0.50, 0.25, 0.10, 0.05 mg/L concentrations were prepared.

Calibration curve of pretilachlor: Calibration of the equipment was performed using pure analytical standard of the test material at concentration ranging from 0.025 to 1.0 mg/kg and the response/ area obtained was plotted against concentration. The response was found linear in the concentration tried (0.025 – 1.0 mg/kg). The correlation coefficient (r^2) value obtained was 0.9952 indicating perfect linearity.

RESULTS AND DISCUSSION

The mean recovery percentage of pyrazosulfuron-ethyl ranged between 92-100 in green foliage, 71-93 in grain, 88-101 in straw, 79-88 in husk and 72-118 in soil with relative standard deviation of repeatability (RSDr) between 3.10-12.30, 0.20-1.10, 0.70-1.90, 2.10-4.80 and 0-8.20%, respectively (**Table 1**), whereas the mean recovery percentage of pretilachlor ranged between 99-117 in green foliage, 75-88 in grain, 72-82 in straw, 73-88 in husk and 113-119 in soil with relative standard deviation of repeatability (RSDr) between 3.70-18.50, 0-8.90,

Table 1. Recovery of pyrazosulfuron-ethyl (%) in green foliage, rice grain, husk, straw and soil

Fortification (mg/ kg)	Recovery (%)					RSD (%)				
	Green foliage	Grain	Straw	Husk	Soil	Green foliage	Grain	Straw	Husk	Soil
0.01	100	71	101	83	72	12.3	0.20	1.90	4.80	1.40
0.05	99	93	100	79	118	3.10	1.10	0.80	2.90	0
0.10	92	72	88	88	109	11.3	0.70	0.70	2.10	8.20

LOQ (Limit of quantification) = 0.01 mg/kg

Table 2. Recovery of pretilachlor (%) in green foliage, rice grain, husk, straw and soil

Fortification (mg/ kg)	Recovery (%)					RSD (%)				
	Green foliage	Grain	Straw	Husk	Soil	Green foliage	Grain	Straw	Husk	Soil
0.05	113	88	72	88	119	18.50	0.70	1.40	4.40	0.10
0.25	117	84	82	82	118	3.70	0	0.60	1.70	2.40
0.50	99	75	75	73	113	8.20	8.90	0.70	2.20	0.70

LOQ (Limit of quantification) = 0.05 mg/kg

0.60-1.40, 1.70-4.40 and 0.10-2.40%, respectively (Table 2). The satisfactory recovery values indicated the accuracy and repeatability of the method and were within the accepted range for residue estimation

The mean initial deposit of pyrazosulfuron-ethyl at recommended and double the recommended doses were 0.18 and 0.48 mg/kg, respectively (Table 3). The residue dissipated with time and reached below limit of quantification of 0.01 mg/kg after 5 days in the recommended dose and 7 days in double the recommended dose. The dissipation of residues of pyrazosulfuron-ethyl recorded one day after spraying was 11% and three and five days of spraying were 88.88 and 94%, respectively in recommended dose, whereas the percentage dissipation of residue reported one, three, five and seven days after spraying were 35.42, 85.42, 95.83 and 97.92% in double the recommended dose (Figure 1).

The dissipation of pretilachlor was slower as compared to pyrazosulfuron-ethyl. The residue reached below quantification level of 0.05 mg/kg after 15 days both in the recommended and double the recommended dose. The mean initial deposit of pretilachlor at recommended and double the recommended dose were 8.84 and 15.50 mg/kg, respectively. The percentage dissipation of pretilachlor after 1, 3, 5, 7 and 10 days were 75.23, 97.51, 98.86, 98.52 and 98.98, respectively in recommended dose, whereas in double the recommended dose the corresponding values were 70.77, 91.61, 96.32, 98.19 and 99.16, respectively (Figure 1).

The pattern of dissipation of pyrazosulfuron-ethyl and pretilachlor in rice has been presented in several research works whereas the studies on the dissipation of combination product of these two herbicides are so meagre. Mukherjee *et al.* (2006) reported that the dissipation follows first order kinetics in both the alluvial and red lateritic soils under laboratory condition when applied 10 and 20 mg/kg of the active ingredient per gram of soil. About 80% of the initial concentration of the herbicide in soil was dissipated by 30 days and further increased to more than 95% by 60 days and the reported half-life of pyrazosulfuron-ethyl was 15 days in both soils. In present study, the half-lives reported were 1.293 and 1.795 days respectively in recommended and double the recommended dose in foliage. This shows the faster degradation of pyrazosulfuron-ethyl in foliage. The findings of the present study were in agreement with Singh *et al.* (2012) and Ezhilarasi *et al.* (2018). Singh *et al.* (2012) reported that the herbicide was the least stable under acidic conditions and the

predominant degradation route of pyrazosulfuron-ethyl in water is hydrolysis of sulphonamide linkage. Ezhilarasi *et al.* (2018) reported that both pyrazosulfuron-ethyl and pretilachlor showed rapid dissipation in rice field water than soil and the degradation followed first order reaction kinetics. The half life of both molecules in rice plant was more or less same, whereas the harvest time residue was found below LOQ in rice grain, straw, husk and cropped soil in the present study and it was in agreement with the study of Ezhilarasi *et al.* (2018).

Singh and Singh (2013) reported that half-life of pyrazosulfuron-ethyl varied from 2.6 days (pH 4) to 19.4 days (pH 7) and half-life in distilled water was comparable to half-life at pH 7 buffer. Yu *et al.* (2019) established a simple and reliable QuEChERS method coupled with HPLC-MS/MS and GC-MS methods was to determine pyrazosulfuron-ethyl, residues in rice cropping systems.

Table 3. Persistence of pyrazosulfuron-ethyl and pretilachlor in rice plant at different intervals (days)

Days after treatment	Residues of herbicides (mg/ kg)			
	T1 – Recommended dose 2000 g/ha		T2 – Double the recommended dose 4000 g/ha	
	Pyrazo-sulfuron-ethyl	Pretilachlor	Pyrazo-sulfuron-ethyl	Pretilachlor
0	0.18	8.84	0.48	15.50
1	0.16	2.19	0.31	4.53
3	0.02	0.22	0.07	1.30
5	<LOQ	0.10	0.02	0.57
7	<LOQ	0.13	<LOQ	0.28
10	<LOQ	0.09	<LOQ	0.13
15	<LOQ	<LOQ	<LOQ	<LOQ
Half life (days)	1.293	1.877	1.795	1.372

LOQ (Limit of quantification) of pyrazosulfuron-ethyl -0.01 mg/kg, LOQ of pretilachlor -0.05 mg/kg

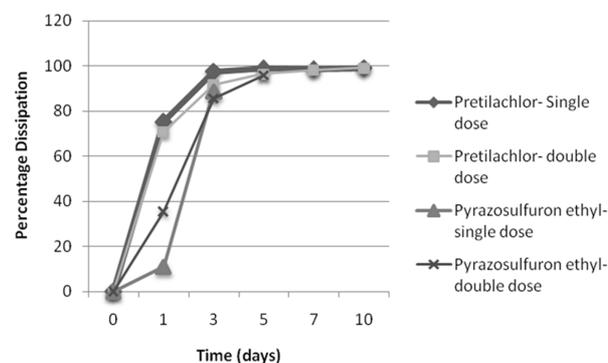


Figure 1. Dissipation pattern of pyrazosulfuron-ethyl and pretilachlor in rice

Dharumarajan *et al.* (2012) revealed that 0.75 kg/ha of pretilachlor dissipated to below detectable limit at 30 days after application, while 1.5 kg/ha persisted up to 60 days after application in rice plant. The difference in dissipation rate of pretilachlor in various studies may be due to the diversity in the agro climatic situation prevailed in experimental locations. Kaur *et al.* (2015) found that the dissipation rate of pretilachlor in paddy field soil and paddy field water followed first-order kinetics with decrease in pretilachlor residues as a function of time. Faster dissipation of pretilachlor was observed in paddy field water than in paddy field soil with half life of 1.89-2.97 days and 7.52-9.58 days, respectively. At harvest, the residues of pretilachlor in the paddy soil and paddy crop samples were below the detection limit and this is in agreement with present study.

Residues of pyrazosulfuron-ethyl and pretilachlor was below quantification level of 0.01 mg/kg in pyrazosulfuron-ethyl and 0.05 mg/kg in pretilachlor in rice grain, straw, husk and cropped soil collected at the time of harvest received from three locations. The result of the present study is in agreement with studies conducted by Rana *et al.* (2018) and they reported that residues of pyrazosulfuron-ethyl in grain and straw at the time of harvest were below quantification level.

A quick, easy, cheap, rugged, safe (QuEChERS) extraction method, coupled with LC MS/MS analysis was developed to determine the dissipation dynamics and residue of pyrazosulfuron-ethyl and pretilachlor in rice. The study could be concluded that the dissipation of pyrazosulfuron-ethyl 0.75% + pretilachlor 30% WG in green leaf ranged from 3-10 days and the harvest time residue was below limit of quantification in rice grain, straw, husk and soil and the result revealed the safety of the combination product to the end users.

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