



Dissipation and fate of ready mix combination of pyrazosulfuron-ethyl and pretilachlor in rice field

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ABSTRACT

Application of ready-mix herbicides containing two active ingredients in rice to manage broad group of weeds is in practice currently. Among different ready mix formulations, one comprising pyrazosulfuron-ethyl and pretilachlor have been recently registered in India. Though, the persistence of each herbicide in soil under rice as single formulation was reported, the information on persistence of herbicides from ready-mix formulations is not reported under semi-arid tropical conditions of India. Hence, an experiment was conducted to study the dissipation and fate of these ready-mix formulation herbicides in rice soil, water and in rice grain. Pyrazosulfuron-ethyl and pretilachlor residues were determined using high performance liquid chromatograph (HPLC) and gas chromatograph (GC), respectively. The average recoveries of pyrazosulfuron-ethyl and pretilachlor from matrix ranged from 80.3-103.3% with less than 10% standard deviation and sensitivity up to 0.001 µg/g. Both herbicides showed rapid dissipation in rice field water than soil and the degradation followed first order reaction kinetics. While pyrazosulfuron-ethyl dissipated with a half-life of 2.17-5.45 and 0.77-0.79 days respectively in rice field soil and water, pretilachlor dissipated with a half-life of 5.18–6.68 days in soil and 2.59–3.00 days in field water. Pyrazosulfuron-ethyl has shorter half-life than pretilachlor and both the active ingredients dissipated rapidly in rice field soil than water. At harvest, the residues of both the herbicides in rice grain and straw were below the MRLs set by FSSAI.

INTRODUCTION

Rice (*Oryza sativa* L.) is the major food crop for one-third of world's population. About 90% of its production and consumption is contributed from Asia. India accounts for 19.3 to 22.9% of its global production (Mukherjee 2006, Janaki and Chinnusamy 2012). Weed infestation is the major cause for yield reduction in rice by competing crops with nutrients, moisture, light, air, space and other micro-environment factors (Chinnusamy *et al.* 2012). Consequently, providing timely control of weeds is vital to have productive use of growth factors. Weed management by mechanical and traditional methods is not a viable option owing to non-availability of weeders and scarcity of labour during crucial periods of requirement (Janaki *et al.* 2009). Hence, weed control through chemical methods using herbicides becomes inevitable to the farmers.

Pyrazosulfuron-ethyl {ethyl 5-[(4,6-dimethoxy-pyrimidin-2-ylcarbonyl)-sulfamoyl]-1-methyl-pyrazole-4-carboxylate} is a systemic herbicide belongs to sulfonylurea group. It can be applied as pre- or early post-emergence and has outstanding activity against a broad spectrum of annual and perennial weeds. Fate and persistence of sulfonylurea herbicides in soil are widely affected by the soil acidity, temperature, moisture content, soil microbial diversity and biochemical activity (Wang *et al.* 2013). It was almost unstable under acidic environment and hydrolysis of sulfonamide linkages the prime degradation path of pyrazosulfuron-ethyl in water (Singh and Singh 2013). It dissipates rapidly from puddled rice soil with a half-life 1.9 days in field water and 11 days in soil (Ishii *et al.* 2004).

Pretilachlor [2-chloro-2',6'-diethyl-N-(2-propoxyethyl) acetanilide] is a pre-emergence herbicide applied to control sedges and broadleaf weeds in rice fields (WSSA 2008). It quickly degrades with a half-life of 7 to 10 days in rice soil and 3.0 to 3.6 in field water (Fajardo *et al.* 2009) by following first order dissipation kinetics. Pretilachlor degradation in soil environment was chiefly depending on soil physico-chemistry, management practices and climatic conditions (Kaur *et al.* 2015). Pretilachlor might be toxic to aquatic organisms if the residues in crops, soil and water were above the maximum residue level (Sadeghi and Imanpoor 2013; Maryam *et al.* 2013).

In recent years a number of ready mix formulations having different herbicide combinations are registered in India due to their high efficiency at low application rate. To our knowledge, little work has been published on the dissipation behavior of herbicides in ready mix formulations. A range of herbicides including ready mix combinations are available for weed control in rice. Therefore, the present research was carried out with the objectives to assess to behavior and fate of ready mix formulation of pretilachlor and pyrozosulfuron-ethyl in rice soil, water, straw and grain.

MATERIALS AND METHODS

Field experiment

Field experiment with rice as test crop was conducted during *Rabi* season at Tamil Nadu Agricultural University farm, Coimbatore, India in a randomized block design. Soil of the experimental field was clay loam in texture and has alkaline pH (8.18) and low EC (0.32dS/m) with CEC of 23.8 Cmol (p+)/kg soil. The field soil has low available N

(168 kg/ha), medium available P (18.5 kg/ha), high available K (503 kg/ha) and medium organic carbon (0.53%). The granular herbicides formulation containing pyrazosulfuron-ethyl 0.15% plus pretilachlor 6.0% was applied to the rice field as pre-emergence at three doses, *viz.* 10.0, 12.5, and 20.0 kg/ha along with control plot (without herbicide application). Each treatment was replicated thrice. Sides of the each plot were protected by bunds elevated to a level of 50 cm high and 30 cm width. Herbicide was applied by knapsack sprayer with a flat fan nozzle using the water spray volume of 500 l/ha and the control plots were sprayed with water alone. Weather variables prevailed over rice experimental period was documented (**Figure 1**).

The surface soil samples of 0-15 cm were taken at random from rice experimental field on 0 (2 hrs), 1, 3, 5, 7, 15, 30 and 45 days after herbicide application and at harvest. From each treatment plot, the soil samples were taken at 5-6 spots in net plot avoiding outer 30 cm from border using auger. The collected soils from each plot were pooled and stored at -10p C prior to analysis. The water samples were collected in plastic bottles at same interval as followed for soil sampling and stored at -4p °C for analysis. The rice grain and straw samples were taken at harvest from ready-mix herbicides formulation treated and untreated plots. The rice grains were powdered by blender and straw was sliced into tiny pieces prior to residue analysis.

Chemicals, reagents and soil

The certified standards of pyrazosulfuron-ethyl and pretilachlor (purity 98%) were purchased from Sigma-Aldrich and the test granular formulation of herbicide containing pyrazosulfuron-ethyl (0.15%) and pretilachlor (6%) was obtained from M/s United

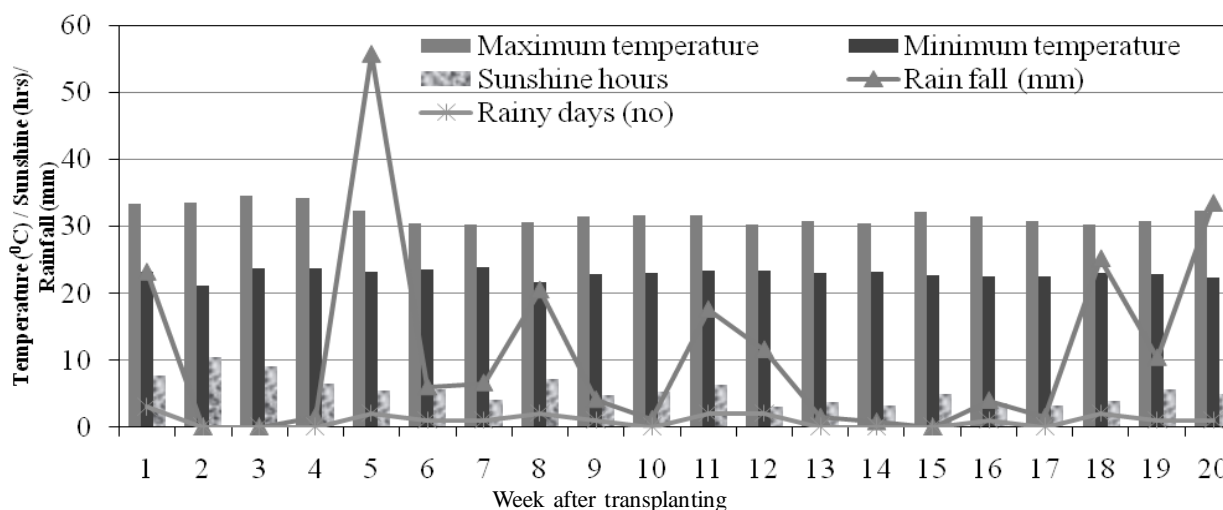


Figure 1. Weather conditions recorded during sampling period (May to September)

Phosphorus Limited, Mumbai. Analytical grade solvents and chemicals were purchased from S.D. fine chemicals, Mumbai were used for residue analysis. HPLC-grade acetonitrile, hexane and 0.2 µm filtered Milli-Q water was used for residue determination in HPLC / GC.

Instruments and operating conditions

For pyrazosulfuron-ethyl residue analysis, 1200 series liquid chromatography (Agilent Technologies, USA) equipped with diode array detector (DAD), binary pump and auto sampler was used. The computer enabled software EZChrom was used to record the chromatograms. The compound separation was achieved by Eclipse XDB – C 18 RP column with 5 µm, 4.6 x 150 mm dimension at the temperature of 30°C. The mobile phase of acetonitrile: water with 0.1% H₃PO₄ (70:30 v/v) in binary mode was used at a flow rate of 0.5 ml/min. The sample of 10 µl volume was injected and detection was performed at 236 nm.

For quantitatively analyzing the pretilachlor residue, Thermo (Chemito model GC8610) Gas Chromatograph equipped with ECD (⁶³Ni) and Computer enabled IRIS 32 software was used. The pretilachlor separation was performed using mega pore capillary column of 0.5 µm, 30 m x 0.5 mm at nitrogen gas flow rate of 10 ml/min. GC was operated in split-less mode with the sample injection volume of 0.5 µl. Temperature conditions of 210°C, 240°C and 260°C, respectively were maintained at oven, injector and detector.

Herbicides extraction

Homogenized rice grain, straw and soil samples were extracted separately for pyrazosulfuron-ethyl and pretilachlor by methanol and water (Singh *et al.* 2011; Janaki *et al.* 2012) using horizontal shaker for 1 hr. The supernatant was filtered through a Buchner funnel allowing the soil sediment to settle down. The process was repeated twice and the filtrate was combined to the same flask for partitioning and cleans up of each herbicide. The pyrazosulfuron-ethyl was partitioned using 10% NaCl and dichloromethane thrice and the pretilachlor was partitioned using 10% sodium chloride and hexane twice. The organic phase of each herbicide was dehydrated using anhydrous Na₂SO₄ and evaporated in a rotovap. The dried residues of pyrazosulfuron-ethyl and pretilachlor were re-dissolved in HPLC grade acetonitrile and hexane, respectively for chromatographic analysis.

Method validation and detection limits

Before proceeding to the main residue study, the extraction method was validated by fortifying control samples of rice grain, straw, husk and soil with known concentrations (1.00, 0.50, 0.10, 0.05 and 0.01 µg/g) of pyrazosulfuron-ethyl and pretilachlor separately as described by Singh *et al.* (2011) and Kaur *et al.* (2015). The extraction and cleanup of each herbicide was made as described in the methodology. The validated method was followed for the residue estimation in samples.

The instrument detection limit (IDL) or limit of detection (LOD) for each herbicide was assessed by repeated injections of a standard solution containing 0.005 to 1.0 µg/ml of working standards seven times. The IDL is calculated by 3:1 signal/noise ratio and replicate standard deviation. The method detection limit (MDL) or quantification limit (LOQ) was established at the signal/noise ratio of 10:1 using the fortified recovery studies.

Statistical analysis

The dissipation of both the herbicides in soil, were studied using the first-order kinetic equation

$$C = C_0 e^{-kt}$$

Where, C is the amount of herbicide recovered from soil at time t, C₀ is the amount of herbicide recovered at t = 0 interval, k is the degradation constant and t is the time in days.

The time taken by molecule for 50% dissipation (DT₅₀) from its initial concentration was calculated by the formula DT₅₀ = ln 2D / k.

RESULTS AND DISCUSSION

LOD, recovery and LOQ

Under the standardized HPLC and GC conditions, the pyrazosulfuron-ethyl and pretilachlor was detected at 3.4 and 4.6 min respectively. The calibration curve of working standards of each herbicide was linear from 0.01 to 1.0 µg/ml and with correlation coefficient of >0.99. The LOD was 0.001 and 0.005 µg/ml for pyrazosulfuron-ethyl and pretilachlor, respectively. The per cent mean recovery of pyrazosulfuron-ethyl from fortified rice grain, straw, water and soil respectively varied from 82.1-88.3, 80.2-89.8 and 91.0-96.1 and for pretilachlor these were 83.2-85.1% and 84.5-88.8, 82.8-85.5, 86.4-97.5 and 82.5-91.6% respectively (**Table 1**). The LOQ for pyrazosulfuron-ethyl was established to

Table 1. Average recoveries of pyrazosulfuron-ethyl and pretilachlor from fortified rice grain, straw and soil

Matrix	Mean recovery (%)*±% S.D.				LOQ (µg/g or µg/ml)
	Concentration of herbicides fortified (µg/g)				
	0.01	0.05	0.10	0.50	
<i>Pyrazosulfuron-ethyl</i>					
Field soil	85.13± 1.32	83.19± 1.64	83.94± 1.27	84.80± 2.29	0.05
Field water	96.14± 1.70	94.04± 1.51	95.35± 1.07	91.05± 1.16	0.001
Rice straw	81.69± 3.98	80.21± 1.09	88.66± 1.13	89.75± 1.21	0.05
Rice grain	88.32± 3.92	82.12± 1.01	82.82± 2.85	82.50± 3.93	0.05
<i>Pretilachlor</i>					
Field soil	83.81± 1.90	82.48± 2.01	85.93± 1.76	91.57± 2.17	0.01
Field water	86.61± 1.28	89.35± 1.32	92.81± 1.86	97.50± 2.06	0.001
Rice straw	82.82± 1.03	85.49± 1.01	83.54± 2.98	85.45± 1.86	0.03
Rice grain	88.35± 2.52	85.42± 1.91	88.81± 2.79	84.50± 3.75	0.03

*Average of three replicates; ±S.D. – Standard deviation

be 0.05 µg/g for soil, rice grain and straw and 0.001 µg/ml for water. The LOQ for pretilachlor was found to be 0.001 µg/ml for water, 0.01 µg/g for soil and 0.03 µg/g for rice grain and straw.

Dissipation of pyrazosulfuron-ethyl in rice field soil and water

The ready-mix formulation consisting pyrazosulfuron-ethyl and pretilachlor were applied to transplanted rice as pre-emergence on 3rd day after transplanting at three rates, viz. 10, 12.5 and 20 kg/ha. Residues in rice soil were examined up to harvest after its application. Initial concentration of pyrazosulfuron-ethyl on 0th day (2 hr) was 0.134, 0.173 and 0.187 µg/g of soil at 10, 12.5 and 20 kg/ha, respectively (Table 2). Pyrazosulfuron-ethyl residues dissipated in soil within 7 days, and were below detectable level of 0.05 µg/g after 7 days at each applied rate. On day 1, pyrazosulfuron-ethyl dissipated rapidly from soil with the rate of 40.8, 34.3 and 21.8% at the applied doses of 10, 12.5 and 20 kg/ha. On day 5, dissipation of 85.4, 78.5 and 78.8% was observed at the application rates of 10, 12.5 and 20 kg/ha. Residues of pyrazosulfuron-ethyl were below the quantification limit in soil at harvest. In rice field water, initial concentration of pyrazosulfuron-ethyl on day 0 (2 hrs) was ranged from 0.0027 to 0.0656 µg/ml across different rates of application.

Table 2. Pyrazosulfuron-ethyl persistence and dissipation in rice soil and water under different application doses of ready-mix formulation

Days after herbicide application	Rice field soil (µg/g)			Rice field water (µg/ml)		
	10.0	12.5	20.0	10.0	12.5	20.0
	kg/ha	kg/ha	kg/ha	kg/ha	kg/ha	kg/ha
0 (2 hr)	0.134	0.173	0.187	0.003	0.012	0.066
1	0.111 (40.8)	0.114 (34.3)	0.146 (21.8)	0.001 (59.3)	0.005 (59.4)	0.027 (58.5)
3	0.058 (68.9)	0.093 (46.0)	0.071 (61.7)	0.001 (70.4)	0.001 (92.8)	0.015 (76.4)
5	0.027 (85.4)	0.037 (78.5)	0.039 (78.8)	<LOQ	<LOQ	0.003 (94.8)
7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

* Average of three replicates; SD: standard deviation; figures in parentheses indicate % dissipation

More than 50 and 90% of the initial pyrazosulfuron-ethyl residue dissipated from water on day 1 and 3, respectively. The rate of disappearance of pyrazosulfuron-ethyl in water was fast at lower dose of 10 kg/ha. On day 5, residue becomes below 0.01 µg/ml at all doses of application.

The pyrazosulfuron-ethyl persistence in both soil and water follows first order reaction kinetics and accordingly the disappearance factors, viz. the degradation constant (k) and half-life (T_{1/2}) were

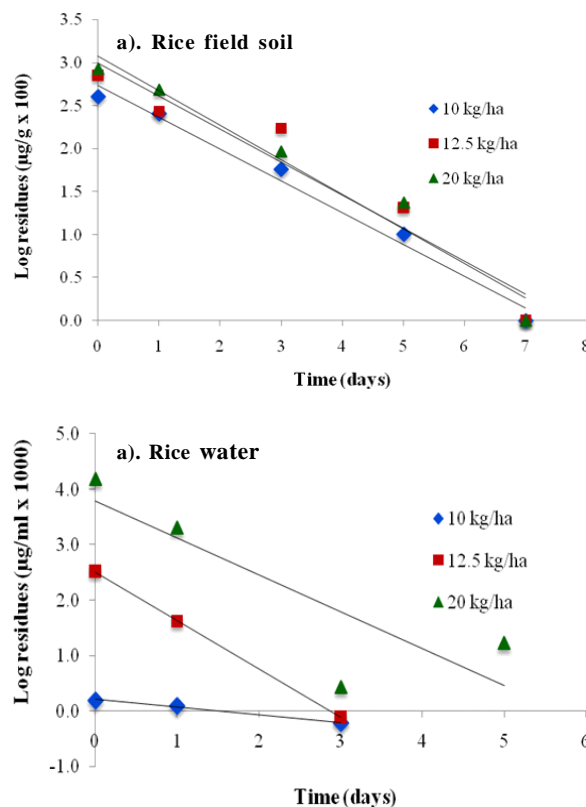


Figure 2. Linear plots of pyrazosulfuron-ethyl dissipation kinetics in rice field soil and water

calculated. The logarithmic concentration of pyrazosulfuron-ethyl against time showed good linearity in both rice field soil and water and signifying first order rate of dissipation (**Figure 2**). The calculated DT₅₀ of pyrazosulfuron-ethyl in rice field soil and water ranged between 2.17-5.45 and 0.77-0.79 days, respectively (**Table 3**).

The present study revealed that the pyrazosulfuron-ethyl dissipation was fast and the observed half-life in rice soil and water was in accordance with the range reported by Singh *et al.* (2011) who reported a half-life of 0.9 and 5.4 days, respectively for water and soil in rice fields. The present findings are differing to the Ishii *et al.* (2004) who observed half-lives of 11.0 days in soil and 1.9 days in water while applied as formulation of 0.3% pyrazosulfuron-ethyl and 10% mefenacet. This could be attributed to the properties of the soil and herbicides or formulation used in the present study and climatic conditions. Higher rainfall received during the first week of herbicide application (**Figure 1**) might have augmented its hydrolysis and dissipation from soil and field water. It dissipated fast in the present study from field water with the mean half-life of 0.70 days since pH of the field water sample was above 8.0. Zheng *et al.* (2008) found that the pyrazosulfuron-ethyl hydrolysis was faster in acidic and basic aqueous solution than in neutral solution. Similarly, shorter half-life in soil could be attributed to the high soil pH (8.25) and low organic carbon (0.56%) of the experimental field.

Dissipation of pretilachlor in rice field soil and water

The dissipation behaviour of pretilachlor from ready-mix formulation in rice grown soil at all three different application doses was studied. The mean pretilachlor recovered from the rice soil and water is presented in **Table 4**. The quantity of pretilachlor residue detected on 0th day (2 hrs after application) was ranged from 0.104 – 0.247 µg/g in rice soil and

0.0122-0.0130 µg/ml in rice water at different doses of ready-mix application. The pretilachlor residue concentration at different time intervals was influenced by the doses and increased with increase in application dose. More than 50% of the initial pretilachlor concentration recovered on day 0, degraded from the water and soil, respectively on 3rd and 5th days irrespective of applied doses. This showed that the pretilachlor dissipation was fast in rice water than soil. The dissipation rate was slow at higher rate of 20 kg/ha specifically during the later period (on day 15 for soil and 5 for field water) in field water and *vice versa* in soil. On day 30 after application of ready-mix formulation, more than 90% of the initial concentration of pretilachlor dissipated from soil and degraded to below quantification limit on 45th day. However in field water, 68.5 to 73.8% of pretilachlor residue dissipation occurred on 5th day across different doses of application and then degraded to below quantification limit of 0.001µg/g.

The pretilachlor dissipation rate in rice soil and water followed first order degradation kinetics at all doses of application and observed a good linear fit of pretilachlor concentration at different intervals against time (**Figure 3**). The first order degradation rate constant, coefficient of determination (R²) and half-lives of pretilachlor in rice field soil and field water at three rates of ready-mix application are given in **Table 4**. Similar way it degraded from the field water and observed a significant correlation coefficient of 0.968 to 0.972 across the three doses of application (**Table 3**). The half-lives of pretilachlor calculated using linear equations was ranged from 5.18-6.68 days in soil and 2.59-3.00 days in field water. Similar first order kinetics for pretilachlor in rice soil was reported by Kaur *et al.* (2015) who stated that the soil physico-chemistry, management practices and climatic conditions largely influenced the pretilachlor dissipation in rice soil and water.

Table 3. Regression equation, correlation coefficient (R²) and half-life (t_{1/2}) of herbicides in rice soil and water different application doses of ready-mix formulation

Dose (commercial formulation)	Rice field soil			Rice field water		
	Regression equation	R ²	Half-life (days)	Regression equation	R ²	Half-life (days)
<i>Pyrazosulfuron-ethyl</i>						
10.0 kg/ha	y = 2.737 - 0.370x	0.984	2.17	y = 0.780 - 0.370x	0.801	0.77
12.5 kg/ha	y = 2.990 - 0.382x	0.903	2.26	y = 2.507 - 0.875x	0.999	0.77
20.0 kg/ha	y = 3.074 - 0.401x	0.964	5.45	y = 4.101 - 0.550x	0.963	0.79
<i>Pretilachlor</i>						
10.0 kg/ha	y = 2.257 - 0.129x	0.994	5.18	y = 2.522 - 0.284x	0.972	2.59
12.5 kg/ha	y = 2.582 - 0.099x	0.936	6.42	y = 2.565 - 0.250x	0.968	2.91
20.0 kg/ha	y = 2.888 - 0.091x	0.818	6.68	y = 2.544 - 0.237x	0.985	3.00

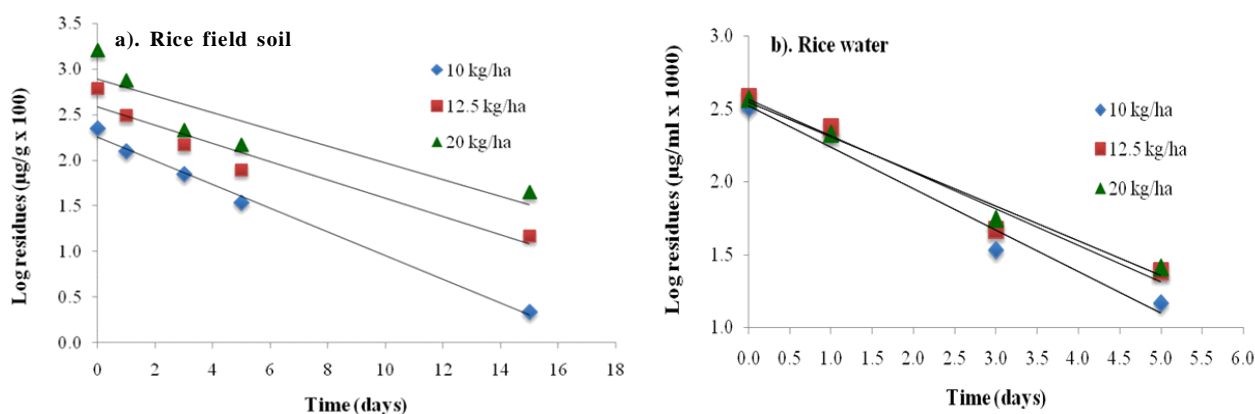


Figure 3. Linear plots of pretilachlor dissipation kinetics in rice field soil and water

Table 4. Pretilachlor persistence and dissipation in ricesoil and water under different application of doses of ready-mix formulation

Days after herbicide application	Rice field soil ($\mu\text{g/g}$)			Rice field water ($\mu\text{g/ml}$)		
	10.0 kg/ha	12.5 kg/ha	20.0 kg/ha	10.0 kg/ha	12.5 kg/ha	20.0 kg/ha
0 (2 hrs)	0.104	0.163	0.247	0.0122	0.0131	0.0130
1	0.082 (21.9)	0.121(25.8)	0.178(27.7)	0.0103 (15.5)	0.0107 (18.1)	0.0102 (21.3)
3	0.064 (39.1)	0.088(46.2)	0.103 (58.1)	0.0046 (62.2)	0.0053 (59.6)	0.0057 (56.2)
5	0.047 (55.3)	0.067(59.1)	0.088(64.5)	0.0032 (73.8)	0.0040 (69.5)	0.0041 (68.5)
15	0.015 (86.6)	0.032(80.2)	0.052 (78.9)	<LOQ	<LOQ	<LOQ
30	0.007 (93.6)	0.009(94.8)	0.010(96.1)	<LOQ	<LOQ	<LOQ
45	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

The half-lives of pretilachlor calculated in rice soil (5.18-5.45 days) and water (2.59-3.00 days) were in line with the findings of Fajardo *et al.* (2009); Dharumarajan *et al.* (2011) and Kaur *et al.* (2015). The longer persistence of pretilachlor in soil and water than pyrazosulfuron-ethyl could be attributed to its higher sorption to soil clay particles and dissolved organic matter (Braschi *et al.* 2003). Pretilachlor half-lives obtained in the present study were lower than that recorded by Vidotto *et al.* (2004). This could be ascribed to photo-decomposition and enhanced soil microbial degradation by favorable day temperature (30.3 to 34.6 °C) and soil pH during the cropping period.

Harvest residue studies in soil, rice grain and straw

The pyrazosulfuron-ethyl and pretilachlor residues in soil at crop harvest (110 days after transplanting) was analyzed and found the concentration was below detection limit of 0.001 and 0.005 $\mu\text{g/g}$, respectively. The high temperature, clayey soil texture and high rainfall and its even distribution during the crop growing period (Figure 3) might have enhanced the chemical and microbial degradation of pretilachlor to below 0.005 $\mu\text{g/g}$. Similar results were reported by Kaur *et al.* (2015)

for pretilachlor where the residue was below 0.01 mg/kg in rice grain. This could be due to the formation of inactive pretilachlor derivative in rice plant by its conjugation with reduced glutathione accomplished by glutathione-S-transferase enzymes (Scarponi *et al.* 2003). The maximum residue limits (MRL) of both pyrazosulfuron-ethyl and pretilachlor residues in rice grain have not been set by European Union (EU), USDA, WHO/FAO, FFCR, Japan and PMRA, Canada, however the MRL of 0.01 and 0.05 mg/kg respectively in rice grain was set by FSSAI (2017). The detection of pyrazosulfuron-ethyl residues below MRL set by FSSAI showed that, it doesn't transported to the rice plant significantly. Rapid metabolic inactivation of the parent pyrazosulfuron-ethyl through demethylation, hydroxylation, cleavage of sulfonamide linkage etc., may have contributed to low residue level below the detection limit (Zheng *et al.* 2008). Singh and Singh (2011) studied the translocation of pyrazosulfuron-ethyl to rice aerial portions through ^{14}C activity and found that its concentration never exceeds 1% of the initial activity over a period of 25 days. Since the residue of both the herbicides in the rice grain was below MRLs set by FSSAI, ready-mix formulation

containing pyrazosulfuron-ethyl and pretilachlor is considered to be non-toxic to rice crop and environment. However the indiscriminate and continuous use of these herbicides formulation in rice growing environment needs to be monitored to avoid bioaccumulation.

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