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Research article

Dissipation behaviour and risk assessment of fipronil and its metabolites in paddy ecosystem using GC-ECD and confirmation by GC-MS/MS

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ABSTRACT

Fipronil -a broad spectrum phenylpyrazole insecticide has high level of toxicity towards environment. Therefore, an easy and reliable analytical method was developed for residue estimation of fipronil to ensure food and environmental safety. A modified QuEChERS technique was followed for estimation of fipronil (5% SC) in paddy ecosystem using GC-ECD and confirmation by GC-MS/MS. The initial residues (0.168–0.794 μg g^{-1}) of total fipronil *i.e.,* sum of fipronil and its metabolites (*viz.,* desulfinyl and sulfone) in leaf and soil were dissipated following first order kinetics. About 92–96% of fipronil residues were degraded after 15 days with half-life of 3.4–4.1 days and pre-harvest interval of 19.4–25.7 days in plant. Residues were below level of quantification (<0.005 μg g^{-1}) in plant and soil at harvest. The fipronil residues in rice grain present low dietary risk ($RQ_d < 1$) to human health. However, high risk ($RQ_d > 1$) was predicted for cattle health due to fipronil residues in paddy leaf up to 10 days. The residual level in soil was also at highrisk ($RQ_s > 1$) for soil ecological health.

1. Introduction

India has the largest area under rice (*Oryza sativa* L.) cultivation and ranked second in production in the world (FAO, 2018). Cultivation of paddy experiences severe pest infestation which causes reduction in production. Fipronil [(RS)-5-amino-1-{2,6-dichloro-4-(trifluoromethyl) phenyl}-4- (trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile)], a phenyl-pyrazole insecticide, is recommended and widely used as a broad spectrum insecticide for foliar application in wide number of crops (such as cabbage, chilli, cotton, grape, and sugarcane) including rice (DPPQS, 2020). It acts on the central nervous system of the target insect and block gamma amino-butyric acid (GABA) receptors causing impaired function of chloride ion uptake produced by neuronal stimulation (Ratra and Casida, 2001).

After field application of fipronil, it undergoes various environmental degradation processes involving oxidation, reduction, photodegradation, and hydrolysis which transforms the parent molecule into its metabolites *viz.,* sulfone, sulfide, desulfinyl, and amide, respectively (Cheng et al., 2014). Fipronil desulfinyl and sulfone have been reported as the principle metabolites which have significant mammalian toxicity than fipronil itself and higher environmental persistency compared to the other two metabolites (Mandal and Singh, 2014; Cheng et al., 2014). However, the rate and route of metabolism varies depending upon the type of soil, the nature of plant species and the prevailing agro-climatic conditions.

Bearing this in mind, our present investigation comprises the dissipation of fipronil along with two major metabolites *viz.,* desulfinyl and sulfone in paddy ecosystem. Dissipation of fipronil has been reported in various crops like brinjal (Gupta et al., 2007), cauliflower (Duhan et al., 2015), cotton (Chopra et al., 2011; Wu et al., 2017), cabbage (Bhardwaj et al., 2012), chilli pepper (Xavier et al., 2014), grapes (Mohapatra et al., 2010), rice (Kumar and Singh, 2013; Kumar et al., 2013), maize (Wang et al., 2014), okra (Hingmire et al., 2015), peanut (Li et al., 2015), sugarcane (Mandal and Singh, 2014; Biswas et al., 2019) and vegetables (Kaur et al., 2015). The persistence behavior of fipronil has also been studied in water (Thuyet et al., 2011; Verma et al., 2014) and soil (Saini et al., 2014) as well.

The method of analysis for quantification of fipronil residues in different commodities was described by several researchers using GLC

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(Chopra et al., 2011; Wang et al., 2014), GC-MS (Kumar et al., 2013), GC-MS/MS (Duhan et al., 2015), HPLC (Verma et al., 2014), LC-MS (Xavier et al., 2014; Hingmire et al., 2015), and LC-MS/MS (Li et al., 2015). However, no information is available on the dissipation pattern and associated risk of fipronil residues in paddy ecosystem in tropical agro-climatic conditions in West Bengal, India. Therefore, the present study was carried out to investigate the degradation dynamics of fipronil and its metabolites in paddy ecosystem by using an easy and reliable analytical method for residue estimation. In addition, the potential risk due to fipronil residues in paddy and soil were also evaluated.

2. Materials and method

2.1. Chemicals and reagents

The analytical standards of fipronil (purity of 97.9%) and its two metabolites desulfinyl (purity 97.8%) and sulfone (purity 99.7%) were purchased from Sigma-Aldrich, USA and the formulation of fipronil 5% SC (trade name: RULAR) was supplied by Krishi Rasayan Export Pvt. Ltd., New Delhi for field application in paddy. Ethyl acetate and acetonitrile (purity 99.9%, J.T. Baker, USA), millipore water (prepared from Milli-Q system, Millipore Corp., Billerica, MA), magnesium sulphate heptahydrate (ACS, Merck, India), sodium sulphate anhydrous (Merck, India), sodium acetate (SRL, India), acetic acid (Merck, India), primary secondary amine (PSA; 40 μm, Bondesil, Agilent, USA), C18 (ODS; Agilent, USA) and graphitized carbon black (GCB; UCT, Bellefonte, PA) were procured for use in residue analysis.

2.2. Field application and sampling

A supervised field experiment of fipronil 5% SC was conducted on short duration (90–110 days) variety of paddy *viz.,* Satabdi following Completely randomized block design (CRBD) at the Regional Research Sub-Station (RRSS, Chakdaha) of Bidhan Chandra Krishi Viswavidyalaya (BCKV) under new alluvial zone in the district Nadia, West Bengal, India. The meteorological conditions recorded during the study period were as follows: Av. Temperature (Min 17.5 °C, Max 37.9 °C); Av. Rainfall (Min 0.49 mm, max 11.4 mm) and Av. Humidity (Min 63.5%, Max 77.9%). Field soil is Gangetic alluvial under azonal soil order having physico-chemical characteristics as organic carbon = 8.2 kg ha^{-1}, pH = 6.9, CEC = 13.25 meq 100 g^{-1} soil, sand = 26.4% silt = 35.1%, and clay = 38.5%). All the experimental plots were measured at 18 m^2 for three treatment dosages *i.e.,* recommended (T_1 = 75 g a.i. ha^{-1}) and double the recommended (T_2 = 150 g a.i. ha^{-1}) along with respective untreated control plots (T_3 = nil application), each with three replicates. No experimental plots have been treated by fipronil in recent past. Fipronil 5% SC was applied twice, first at the tillering stage *i.e.,* 35 days after transplanting (DAT) and second at the milk grain stage *i.e.,* 66 DAT. After 2^{nd} application, the paddy green leaf (0.25 kg) and cropped soil (0.5 kg, within 6 cm from soil surface) samples were drawn from each plot at 0 (2 h after application), 1, 3, 5, 7, 10, 15, and 30 days by following a zig-zag pattern of standard sampling procedure. Paddy grain (0.5 kg) and straw (0.25 kg) samples were also collected at harvest (30 days after the 2^{nd} application). Collected samples were transported to the laboratory and stored at -18 °C until analysis.

2.3. Sample preparation

Collected samples were extracted by following the protocols of modified QuEChERS (quick, easy, cheap, effective, rugged and safe) techniques of residue analysis (Mondal et al., 2017; Saha et al., 2017). Paddy leaves and straws were cut and crushed into small pieces, whereas grains and husks were separated by using a mixer grinder (GX7, Bajaj). Cropped soil samples were air dried, ground, and screened through 60–80 mesh sieves.

Homogenized plant parts (5 g leaf, 5 g grain, 2 g husk, and 2 g straw) and soil (10 g) samples were taken separately in a 50 mL Teflon centrifuge tube with three replicates. All the matrices (moisture content <40%) were then hydrated by adding 10 mL of ice-cold millipore water acidified with 1% acetic acid and vortexed for 1 min and left for 10 min. After that, 10 mL acetonitrile, 4 g anhydrous sodium sulfate, and 1.5 g sodium acetate were added and vortexed again followed by shaking on a rotospin for 15 min at 50 rpm. The samples were then centrifuged at 5000 rpm for 5 min. After centrifugation, the upper solvent layer (1.5 mL) was collected in N-tubes and the solvent was exchanged to ethyl acetate (GC amenable solvent) using N-evaporator (Caliper Life Science, Hopkinton, MA) at 40 °C. For clean-up, supernatant (1.5 mL) was transferred into mini-centrifuge tube (2 mL) containing dispersive solid phase extraction (*d*-SPE) sorbents *viz.,* magnesium sulfate, PSA, and C18 in the proportion of 150, 40, and 25 mg, respectively. However, in case of pigmented extracts, GCB (15 mg) was added along with the above combination of *d*-SPE clean-up sorbents to get a clean extract and reduce matrix interferences on GC response. All the mini-centrifuge tubes were then vortexed and centrifuged for 5 min at 10000 rpm. Finally, the supernatants of clear extracts were filtered by a syringe filter (SGE Int. Pvt. Ltd.) using 13 mm, 0.22 μ nylon filter paper and transferred into the vials for analysis in GC-ECD (Thermo Fisher Scientific TRACE 1110, Auto injector 7683B Series; Mumbai, India) as well as in GC-MS/MS (Agilent Technologies 7890A GC system, 7000 GC/MS QQQ, 7693A Auto sampler; Lake Forest, CA).

2.4. Instrumentation

2.4.1. GC-ECD

The chromatographic analytical method for identification and quantification of fipronil and its metabolites were achieved by using a gas chromatograph (GC) equipped with electron capture detector (ECD) and fitted with TR-1701 (30 m × 0.25 mm × 0.25 μm) capillary column. The GC operating conditions were as follows: Injection temperature 275 °C; Oven initial temperature 240 °C (hold for 3 min) increased at 5 °*C* min^{-1} ramp up to 255 °C (2 min) again ramp up at 15 °*C* min^{-1} to 280 °C (hold for 5 min) with the flow rate of carrier gas (nitrogen, N_2) @ 1 mL min^{-1} through the column with splitless injection mode; Detector temperature was maintained at 300 °C with the makeup gas (N_2) flow @ 40 mLmin^{-1}. Standard solutions or cleaned up sample (2 μL) was injected manually. Pesticide molecules in samples were identified by comparing specific retention time and peak area of the respective pesticide in samples with that of the respective matrix-matched standard under identical operating conditions.

2.4.2. GC-MS/MS

Residues of fipronil and its metabolites in samples were confirmed using GC-MS/MS fitted with HP-5MS (30 m × 0.25 mm × 0.25μm) capillary column. Inlet temperature was set at 285 °C with splitless mode. Oven temperature gradually increased from 75 to 230 °C for 16.8 min. MS system includes positive electron ionization (+EI) energy mode at 70 eV energy with multiple reaction monitoring (MRM) scan mode. The source and quad temperature was maintained at 230 and 150 °C, respectively (Biswas et al., 2019). The specific mass transitions for fipronil, fipronil desulfinyl, and fipronil sulfone were tuned by imposing a collision energy trial which resulted an unique primary and secondary MS/MS transitions of the respective analytes with m/z values as follows: 368.70 > 215.00 and 366.80 > 213.00, 387.90 > 231.00 and 387.90 > 281.20, and 383.00 > 213.00 and 383.00 > 255.00, accordingly. Pesticides in samples were confirmed according to their specific retention time and transition ions (primary and secondary) with assistance of the inbuilt NIST (National Institute of Standards and Technology; Maryland, US) pesticide library.

2.5. Method optimization parameters

The linearity (r^2) and sensitivity of the method was checked by plotting calibration curve (0.001–0.500 μg g^{-1}) of mixture standards of fipronil and its metabolites. The limit of detection (LOD) and quantification (LOQ) was calculated by dividing three and ten times respectively of the average standard deviation of the peak area of all calibration levels with the slope of the curve (DG-SANTE, 2017). Trueness and precision of the method has also been tested through recovery experiment which was carried out by spiking the respective control matrix at concentration levels of 0.005, 0.010, and 0.100 μg $g^{-1}$and processed by the above described method (Section 2.3). Precision was evaluated by calculating relative standard deviation (RSD) in terms of intra-day repeatability check. Matrix effect (ME) was evaluated using Eq. (1) depicted below. The negative and positive values of ME signify matrix-induced suppression and enhancement, respectively (Mondal et al., 2017).

$$\text{ME}\ (\%) = \frac{\text{Peak area of matrix standard} - \text{Peak area of solvent standard}}{\text{Peak area of solvent standard}} \times 100 \quad (1)$$

2.6. Dissipation study

Day wise residue data was subjected to first-order kinetics using Eq. (2) as follows,

$$C_t = C_0\ e^{-kt} \quad (2)$$

where, C_t denotes the concentration (μg g^{-1}) after time t (day); C_0 denotes the initial concentration and k denotes the rate constant (day^{-1}). A regression co-efficient (R^2) was used to represent the relationship between residual data with time and the residual half-life was calculated using Eq. (3) as shown below.

$$t_{1/2} = \ln 2/k \quad (3)$$

Pre-harvest interval (PHI) was computed from Eq. (4) outlined as follows.

$$\text{PHI (day)} = [\{\text{Log}\ (C_{0day}) - \text{Log (MRL)}\}/\text{Slope from regression equation}] \quad (4)$$

where, C_{0day} is initial residue detected (μg g^{-1}) and MRL is maximum residue limit (μg g^{-1}) of fipronil in paddy (Saha et al., 2017).

2.7. Risk calculation

Potential risk due to the presence of fipronil residues in paddy ecosystem was predicted for human, animal, and soil health by calculating risk quotient (RQ) as follows.

2.7.1. Human and animal health

Dietary risk quotient (RQ_d) for human and animal (Abbassy et al., 2017; Adeleye et al., 2019) was measured from Eqs. (5) and (6) as shown below.

$$RQ_d = EDI/\ (ADI \times \text{Body weight}) \quad (5)$$

$$EDI = \text{Fipronil residue (mg kg}^{-1}) \times \text{food or, feed intake (kg}^{-1}\ \text{capita}^{-1}\ \text{day}^{-1}) \quad (6)$$

where, EDI is estimated daily intake (mg kg^{-1} body weight); ADI is acceptable daily intake which in this case is 0.0002 (PPDB, 2017) and 0.0029 (EFSA, 2006) mg kg^{-1} body weight for human and animal, respectively. In India, an adult man weighing 56 kg of body weight (Shome et al., 2014) consume 0.30 kg of rice (Muthayya et al., 2012). In case of animal (considering cattle) the respective values are 350 kg (Sahu et al., 2016) and paddy leaf/straw of 14 kg (generally consumption of fodder is 1.4–4% of their body weight; Agri-news, 2018). Calculated values of RQ_d > and <1 indicates high and low dietary risk, respectively.

2.7.2. Soil health

Risk on soil health (RQ_s) has also been predicted by assessing risk quotient for soil biota (Ccanccapa et al., 2016; Biswas et al., 2019) using Eq. (7) as depicted below.

$$RQ_s = EC/PNEC \quad (7)$$

where, EC is the mean or maximum concentration (μg g^{-1}) of fipronil detected in soil; PNEC is predicted no effect concentration which has been calculated for measuring acute toxicity. PNEC is derived by dividing the EC_{50} or LC_{50} with an assessment factor of 1000 for soil algae (*Scenedemus subspicatus*), earthworms (*Eisenia fetida*), and macro-organism (Soil arthropods e.g., Collembola) and the respective values are 0.000068, 0.500, and 0.00032 μg g^{-1}, respectively (Biswas et al., 2019). RQ_s values > and <0.1 indicates high and low soil ecological health risk, respectively.

In all cases where the residual concentration of fipronil appeared below LOQ (*i.e.,* not detectable in samples), 1/2 of LOQ was allowed for calculating risk quotient that predicts potential risk as far practicable (USEPA, 2000; Wang et al., 2017).

3. Results and discussion

3.1. Method optimization

Quantization ability of the method was ascertained through linearity check and the obtained coefficient of determination (r^2) showed excellent linearity (≥0.99) between concentration and response across the matrix-matched calibration range of 0.001–0.500 μg g^{-1}. The chromatographic behavior of fipronil, fipronil sulfone, and fipronil desulfinyl in the GC-ECD and GC-MS/MS has been projected in Figures 1 and 2, respectively. The retention time for fipronil, fipronil desulfinyl, and fipronil sulfone in GC-ECD was at 6.83, 4.92, and 10.40 min, respectively (Figure 1), and in GC-MS/MS was at 13.13, 11.53, and 14.60 min, respectively (Figure 2). The limit of detection (LOD) and quantification (LOQ) of the method were optimized at 0.002 and 0.005 μg g^{-1}, respectively, which fulfilled the requirement of European Union, EU protocols (DG-SANTE, 2017) for method validation *i.e.,* LOQ ≤ maximum residue level, MRL. The current available MRLs of fipronil in rice are 0.010 (FSSAI, 2018) and 0.005 (EU, 2020) μg g^{-1} for India and European countries, accordingly. The present LOQ (0.005 μg g^{-1}) level was considerably improved over the LOQ (0.01 μg g^{-1}) of other methods that have been reported in different agricultural crops and soil matrices (Paramasivam and Chandrasekaran, 2012; Mandal and Singh, 2014; Kaur et al., 2015). Method accuracy was estimated by recovery experiment at three concentration levels (0.005, 0.01, and 0.10 μg g^{-1}) and the results have been summarized in Table 1. The recovery chromatograms of fipronil and its metabolites at LOQ (0.005 μg g^{-1}) level are presented in Figure 3. According to EU, the recovery capability of a multi-residue method should be in the range of 70–120% with RSD below 20% (DG-SANTE, 2017). The average recovery percentage of fipronil and its metabolites in paddy leaf, grain, husk, straw, and soil were in the range of 81.0–95.33%, 79.33–93.2%, 84.0–93.27%, 83.13–93.07%, and 83.67–97.97% with associated precision (relative standard deviation, RSD) of 2.3–7.71%, 2.09–14.74%, 2.84–10.82%, 0.33–7.96%, and 0.81–8.82%, respectively. Irrespective of matrices, a matrix-induced suppression effect has been

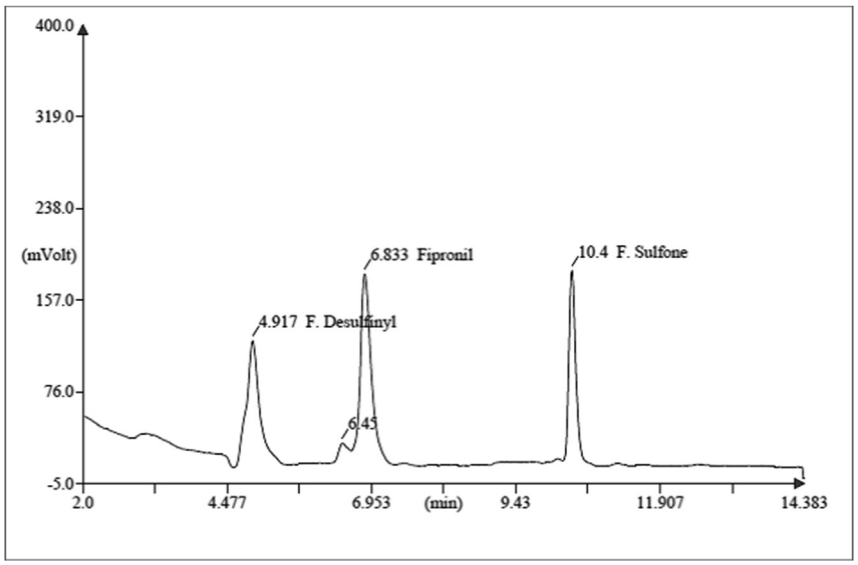

Figure 1. GC-ECD chromatogram of fipronil and its metabolites in pure standard solution of 0.005 μg mL^{-1}.

noticed for all the three analytes with the range of -2.54% to -12.43% which is under the threshold limit of ±20% (DG-SANTE, 2017). Contrary to above, matrix-induced enhancement effect (up to 24.5%) was showed by fipronil and its metabolites in cotton plant and soil (Wu et al., 2017).

The occurrence of residues of fipronil and its metabolites (sulfone and desulfinyl) detected and quantified by GC-ECD was subsequently

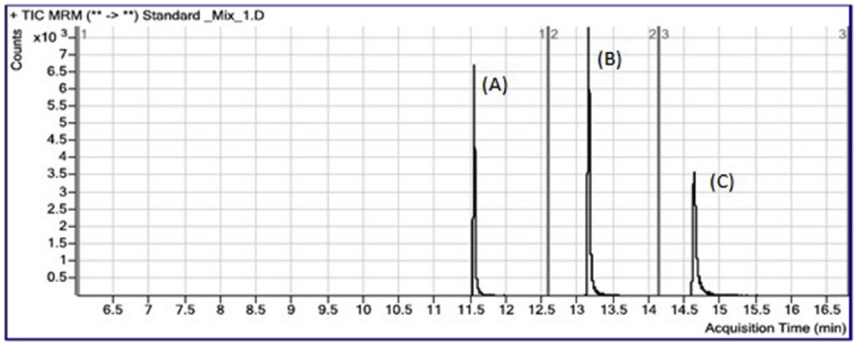

Figure 2. GC-MS/MS chromatogram of fipronil desulfinyl (A), fipronil (B), and fipronil sulfone (C) in pure standard solution of 0.005 μg mL^{-1}.

Table 1. Recovery results of fipronil and its metabolites in paddy plant and soil.

Matrix	Spiking level (μg g^{-1})	Fipronil			Fipronil desulfinyl			Fipronil sulfone		
		Mean recovery %	%RSD	%ME	Mean recovery %	%RSD	%ME	Mean recovery %	%RSD	%ME
Green leaf	0.005	83.3	7.7	-10.0	91.9	6.6	-10.7	81.0	5.3	-12.4
Grain		87.6	6.4	-8.3	79.3	14.7	-8.9	86.5	4.9	-10.6
Husk		87.3	10.8	-6.2	84.6	10.5	-6.6	84.0	4.7	-8.2
Straw		85.3	7.1	-6.7	83.1	6.1	-9.9	85.3	7.1	-9.6
Cropped soil		91.2	3.4	-4.1	83.6	6.8	-4.8	84.6	5.9	-5.3
Green leaf	0.010	87.0	2.3	-10.5	90.8	5.8	-9.4	85.3	5.5	-10.5
Grain		89.3	4.8	-7.1	88.2	6.2	-8.3	89.3	2.9	-9.1
Husk		93.0	2.8	-4.0	86.6	7.8	-4.9	93.0	2.8	-6.0
Straw		90.0	3.3	-5.1	83.1	7.9	-6.8	92.2	1.1	-7.1
Cropped soil		93.8	0.8	-3.1	87.3	8.8	-2.8	95.7	3.3	-4.0
Green leaf	0.100	87.3	3.7	-8.9	95.3	4.9	-11.3	88.1	2.9	-9.4
Grain		93.2	4.3	-8.8	93.2	2.0	-5.4	93.2	4.3	-9.6
Husk		93.2	6.3	-3.2	91.3	3.3	-4.8	85.8	6.9	-4.2
Straw		92.0	1.5	-2.5	86.8	2.2	-3.7	93.0	0.3	-4.5
Cropped soil		95.6	5.1	-2.7	93.0	2.7	-2.9	97.9	1.5	-3.5

Notation: Data of three replicates (n = 3); RSD = Relative Standard Deviation (intra-day repeatability check); ME = Matrix Effect.

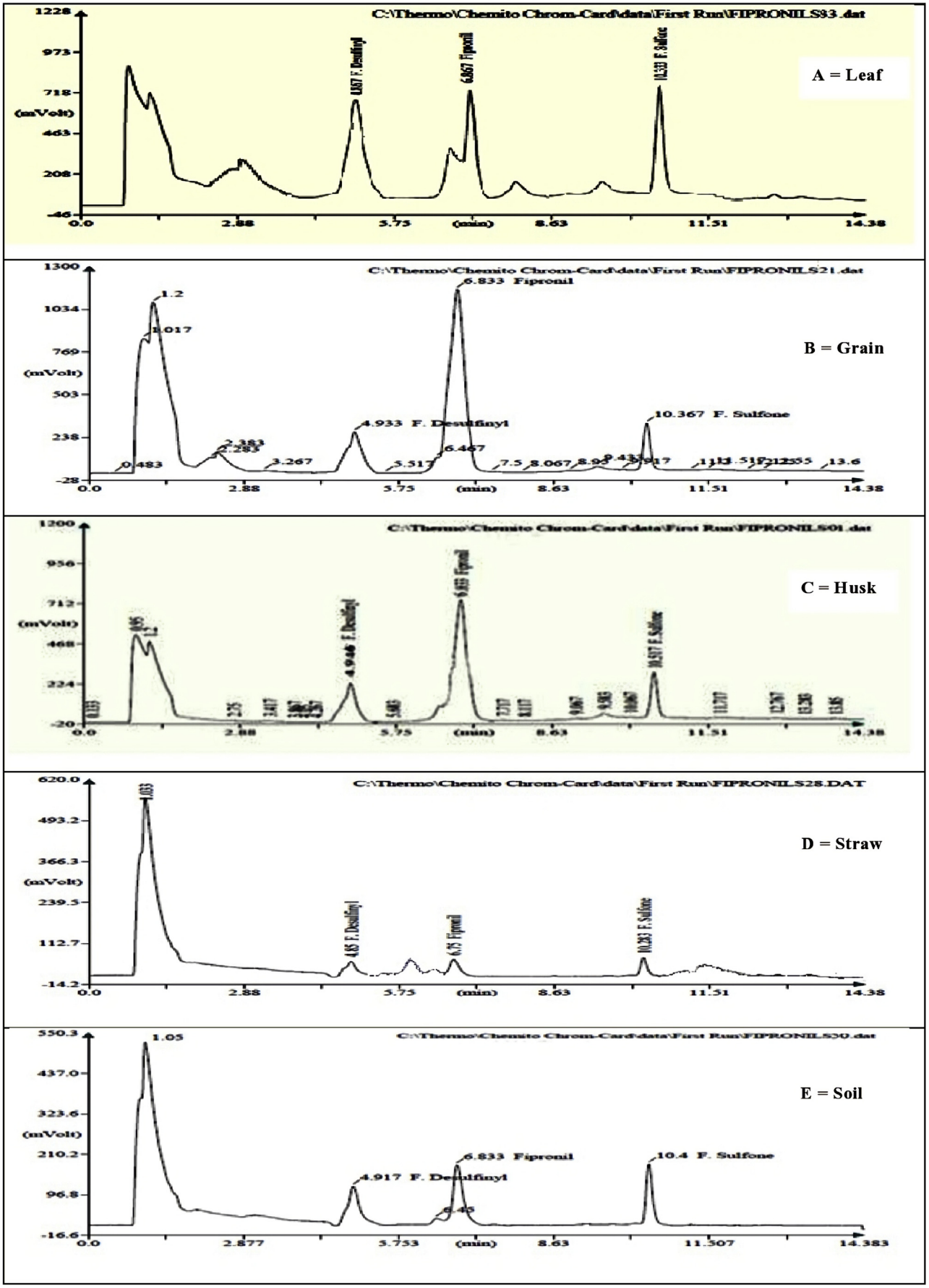

Figure 3. Recovery chromatogram of fipronil and its metabolites in paddy leaf (A), grain (B), husk (C), straw (D), and soil (E) @ LOQ level of 0.005 μg g^{-1}.

confirmed using GC-MS/MS. Therefore, overall performance of the analytical method satisfied the quality control procedures for pesticide residues analysis (DG-SANTE, 2017), indicated that the analytical method was efficient enough for analysis of fipronil and its metabolites in paddy field samples *viz.,* leaf, grain, husk, straw, and soil.

3.2. Dissipation performance

The residual dissipation of fipronil and its metabolites following second application were found to be dose dependant and it was observed up to a certain day in case of paddy leaf and soil only. Afterwards, the residual concentration went down to below limit of quantification, BLOQ (<LOQ = 0.005 μg g^{-1}). At harvest, no residues have been detected in any of the selected plant matrices (*viz.,* leaf, grain, husk, and straw) and soil. The residual dissipation data of fipronil and its metabolites in paddy leaf and soil has been presented in Table 2 and their dissipation pattern has been shown in Figure 4.

3.2.1. Fipronil

The initial (2 h after application) residual concentration of fipronil were found to be 0.430 μg g^{-1} and 0.656 μg g^{-1} in green leaf and 0.096 μg g^{-1} and 0.181 μg g^{-1} in soil for recommended (T_1) and double the recommended (T_2) doses, respectively (Table 2). Dissipation of the parent compound further increased with time and exceeded 94% on 15 DAA (days after application) in leaf at both the doses, while in soil the same has been occurred with 86% on 10 DAA at T_1 and with 92% on 15 DAA at T_2 doses. No residues were detected on the subsequent days. The nature of dissipation followed first order reaction kinetics as has also been observed by Kumar et al. (2013) in paddy and the regression coefficient (R^2) in both leaf and soil were ≥0.95 (Table 3 and Figure 4). At T_1 dose, the calculated residual half-life ($t_{1/2}$) of fipronil in leaf and soil were 3.58 and 3.76 days, respectively, whereas at T_2 doses the respective values were 3.76 and 4.12 days (Table 3). Therefore it signifies that the dissipation rate of fipronil in paddy plant was relatively faster in comparison to soil. The results are fairly in accordance with the findings of Duhan et al. (2015) who reported $t_{1/2}$ of 3.66 days in unprocessed cauliflower but in cropped soil it was 2.59 days at 56 g a.i. ha^{-1} dose. The estimated pre-harvest interval (PHI) of fipronil (Table 3) in leaf was 19.44 and 22.70 days at T_1 and T_2, respectively. Therefore, the harvested paddy straw following 30 days after 2nd application of fipronil may be considered safe for use as fodder.

3.2.2. Fipronil metabolites

The presence of metabolites in plant and soil collected on zero day (2 h), might come from the quick initiation of environmental conversion of parent compound under paddy ecosystem succeeded by physiological

Table 2. Persistence of fipronil and its metabolites in paddy leaf and soil.

Matrix	Dose	DAA	Fipronil		Fipronil sulfone		Fipronil desulfinyl		Total Fipronil	
			Residue (μg g^{-1})	% RSD	Residue (μg g^{-1})	% RSD	Residue (μg g^{-1})	% RSD	Residue (μg g^{-1})	% Dissipation
Leaf	T_1	0	0.430	4.0	0.028	12.1	0.019	5.1	0.477	-
		1	0.255	7.1	0.121	5.3	0.056	6.9	0.431	9.5
		3	0.140	9.3	0.069	4.6	0.079	8.2	0.288	39.6
		5	0.118	5.3	0.07	12.5	0.047	5.1	0.235	50.6
		7	0.059	6.7	0.042	6.2	0.022	12.0	0.123	74.0
		10	0.038	5.5	0.023	6.0	0.012	10.2	0.073	84.6
		15	0.022	7.8	0.011	5.7	0.006	8.4	0.038	91.8
		30	BLOQ	-	BLOQ	-	BLOQ	-	BLOQ	-
	T_2	0	0.655	2.6	0.084	9.2	0.055	10.0	0.794	-
		1	0.385	2.4	0.232	1.5	0.119	12.5	0.736	7.3
		3	0.225	2.9	0.142	5.4	0.109	9.6	0.476	40.0
		5	0.228	6.4	0.125	4.1	0.086	12.9	0.439	44.6
		7	0.184	5.3	0.084	6.2	0.035	7.1	0.303	61.8
		10	0.076	7.1	0.043	3.7	0.016	10.4	0.135	82.9
		15	0.033	5.4	0.019	13.0	0.013	10.7	0.065	91.7
		30	BLOQ	-	BLOQ	-	BLOQ	-	BLOQ	-
Soil	T_1	0	0.096	5.9	0.038	6.8	0.035	15.5	0.168	-
		1	0.074	0.8	0.033	8.1	0.042	4.7	0.149	11.3
		3	0.055	4.8	0.017	3.2	0.029	15.2	0.102	39.4
		5	0.044	4.7	0.011	6.1	0.019	5.3	0.074	56.1
		7	0.031	4.9	BLOQ	-	0.012	7.0	0.042	74.8
		10	0.013	8.9	BLOQ	-	BLOQ	-	0.013	92.0
		15	BLOQ	-	BLOQ	-	BLOQ	-	BLOQ	-
		30	BLOQ	-	BLOQ	-	BLOQ	-	BLOQ	-
	T_2	0	0.181	10.8	0.048	3.2	0.079	2.5	0.309	-
		1	0.137	4.7	0.046	1.4	0.089	1.1	0.272	11.7
		3	0.092	6.9	0.026	7.0	0.066	5.1	0.184	40.4
		5	0.070	4.3	0.016	5.3	0.050	1.5	0.136	55.9
		7	0.045	8.9	0.011	1.0	0.032	7.7	0.088	71.5
		10	0.034	7.3	BLOQ	-	0.021	9.1	0.055	82.1
		15	0.013	11.9	BLOQ	-	BLOQ	-	0.013	95.6
		30	BLOQ	-	BLOQ	-	BLOQ	-	BLOQ	-

Notation: Data of three replicates (n = 3); T_1&T_2 = 75 & 150 g a.i. ha^{-1}, respectively; DAA = Days after 2nd application; RSD = Relative Standard Deviation (variability of data); BLOQ = Below Limit of Quantification (LOQ = 0.005 μg g^{-1}); Total Fipronil = Fipronil + Sulfone + Desulfinyl.

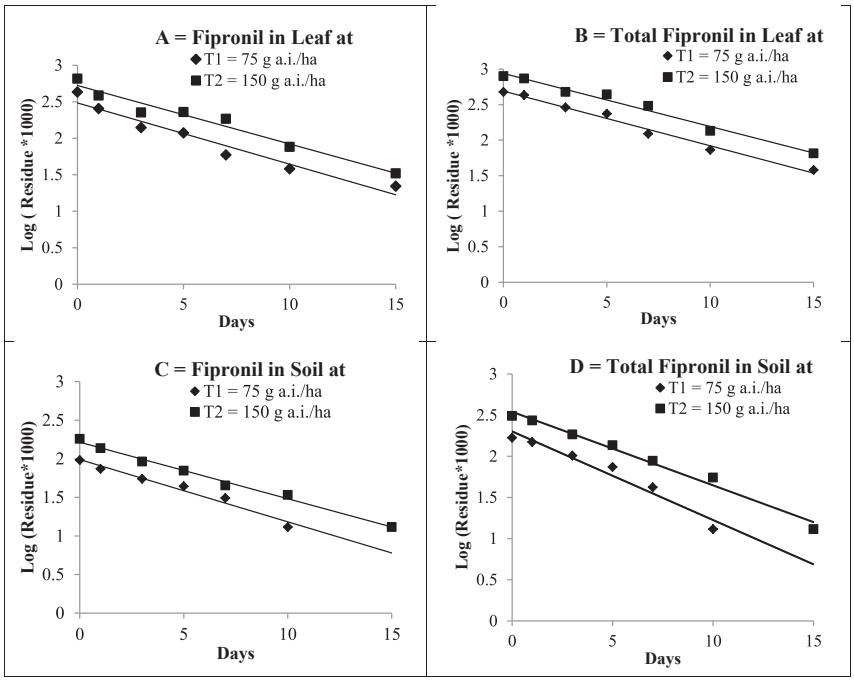

Figure 4. Linear plot for 1st order reaction kinetics of fipronil and total fipronil dissipation in leaf (A and B) and soil (C and D) following application of fipronil@ T_1: 75 g a.i. ha^{-1} & T_2: 150 g a.i. ha^{-1}.

Table 3. Dissipation kinetics of fipronil in paddy leaf and soil.

Matrix	Dose	Parameter	Fipronil	Total Fipronil
Leaf	T_1	Regression Equation	y = 0.084x + 2.484	y = -0.076x + 2.690
		R^2 value	0.949	0.986
		$t_{1/2}$	3.58	3.96
		PHI	19.44	22.08
	T_2	Regression Equation	y = -0.080x + 2.723	y = -0.074x + 2.938
		R^2 value	0.962	0.982
		$t_{1/2}$	3.76	4.07
		PHI	22.70	25.67
Soil	T_1	Regression Equation	y = -0.080x + 1.989	y = -0.107x + 2.302
		R^2 value	0.970	0.957
		$t_{1/2}$	3.76	3.81
	T_2	Regression Equation	y = -0.073x + 2.215	y = -0.089x + 2.540
		R^2 value	0.991	0.984
		$t_{1/2}$	4.12	3.38

Notation: T_1& T_2 = 75 & 150 g a.i. ha^{-1}, respectively; R^2 = Regression coefficient; $t_{1/2}$ = Half-life; PHI = Pre-Harvest Interval.

transmission of metabolites to soil system. Maximum residues of fipronil and its metabolites have also been reported in PAU201 paddy plant (19.85 μg g^{-1}) collected after 7 days after spraying of Regent 0.3G at 180 g a.i. ha^{-1} by Kumar and Singh (2013) and also in fresh chilli pepper (0.69 μg g^{-1}) collected on zero day after spraying of fipronil 80% WG at 40 g a.i. ha^{-1} by Xavier et al., (2014). Here, the initial concentration of fipronil sulfone and desulfinyl in paddy leaf were recorded as 0.028–0.084 μg g^{-1} and 0.019–0.055 μg g^{-1}, respectively, which were found to be higher up to second days (0.121–0.232 μg g^{-1} and 0.079–0.119 μg g^{-1}, respectively) followed by further decrease to reach BLOQ (<0.005 μg g^{-1}) on 30 DAA (Table 2). The residual concentration data indicated that the formation of sulfone metabolite was favored in paddy plant rather than desulfinyl metabolite (Figure 5). Oxidative formation of fipronil sulfone (4.91–7.70%) over desulfinyl (0.89–1.02%) has also been reported in basmati paddy (Basmati 386) ecosystem (Kumar et al., 2013). In case of soil, the maximum residues (0.038–0.048 μg g^{-1}) of fipronil sulfone was detected initially which decreased with time to achieve BLOQ during 7–10 days (Table 2). Whereas in case of desulfinyl, the initial residual concentration (0.035–0.079 μg g^{-1}) increased up to first day (0.042–0.089 μg g^{-1}) and then reduced with time to reach BLOQ after 10–15 days. Unlike paddy leaf, the residual level of desulfinyl metabolite was appeared to be higher in soil than sulfone and that may be due to photochemical degradation of fipronil in soil preferred over aerobic oxidation (Figure 5). Quite similar incident has been observed in field soil (Saini et al., 2014) and in paddy water (Thuyet et al., 2011).

3.2.3. *Total fipronil*

The residues of total fipronil (*i.e.,* fipronil + sulfone + desulfinyl) on zero day in plant was found as 0.477–0.794 μg g^{-1} and in soil it was 0.168–0.309 μg g^{-1} (Table 2). More than 45% of total residue dissipated after 5 day in both plant leaf and soil followed by further increased to

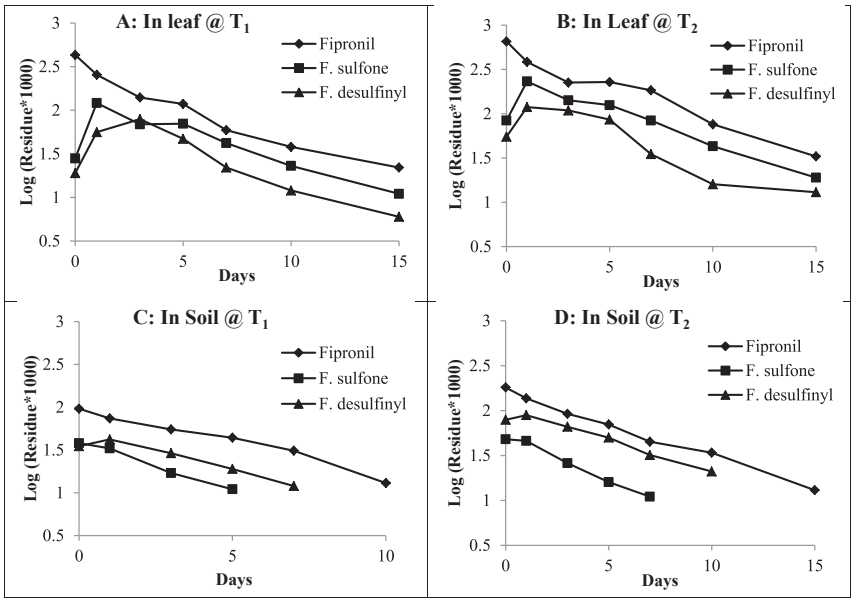

Figure 5. Degradation behavior of fipronil with its metabolites in paddy leaf @ T_1 (A) and T_2 (B) and in soil @ T_1 (C) and T_2 (D) [T_1: 75 g a.i. ha^{-1}; T_2: 150 g a.i. ha^{-1}].

more than 83% in plant and more than 92% in soil after 10 days. Residues reached below LOQ after 15–30 days in plant and soil. The total residues of fipronil in rice field were reached below the detectable limit after 60 and 120 days following application of 45 and 180 g a.i. ha^{-1}, respectively (Kumar and Singh, 2013). The half-life of total fipronil in plant was 3.96–4.07 days while in soil it was 3.38–3.81 days (Table 3). Comparable half-life values of total fipronil have been reported in brinjal (2.3–3.5 days; Gupta et al., 2007), in cabbage (3.2–3.4 days; Bhardwaj et al., 2012), in okra (2.5 days; Hingmire et al., 2015), and in sugarcane (3.7–6.0 days; Biswas et al., 2019). However, a higher half-life value of total fipronil was reported in maize (9.9–10.34 days; Wang et al., 2014), in grape (13.6–20.1 days; Mohapatra et al., 2010) and in cotton soil it was 23.3–24.3 days (Chopra et al., 2011).

The degradation dynamics of fipronil in different crops usually leads to the formation of four common metabolites *viz.,* sulfone, desulfinyl, sulphide, and amide among which the major metabolite in each crop ecosystem varied widely (Bhardwaj et al., 2012; Mandal and Singh, 2014). In the present investigation, the nature of dissipation of the fipronil followed biphasic first order reaction kinetics where the immediate conversion of parent compound to its metabolites (*viz.,* sulfone and desufinyl) seemed to be taken place simultaneously through oxidation and photolytic degradation processes after application (Figure 5). However, across the dissipation period, the residual concentration levels of fipronil were found to be comparatively higher rather than its metabolites and this may imply that the greater environmental stability of the parent compound

Table 4. Dietary and ecological risk quotient (RQ) of total fipronil residues.

Dose	DAA	Human			Cattle			Soil			
		Total residue in grain	EDI	RQ_d	Total residue in leaf	EDI	RQ_d	Total residue in soil	RQ_s		
									Earth worms	Macro-organism	Algae
T_1 = 75 g a.i. ha^{-1}	0	-	-	-	0.477	6.678	6.579	0.096	0.192	300.0	1411.7
	1	-	-	-	0.431	6.034	5.945	0.074	0.148	231.2	1088.2
	3	-	-	-	0.288	4.032	3.972	0.055	0.110	171.8	808.8
	5	-	-	-	0.235	3.290	3.241	0.044	0.088	137.5	647.0
	7	-	-	-	0.123	1.722	1.697	0.031	0.062	96.8	455.8
	10	-	-	-	0.073	1.022	1.007	0.013	0.026	40.6	191.1
	15	-	-	-	0.038	0.532	0.524	0.0025	0.005	7.8	36.7
	30	0.0025	0.00075	0.067	0.0025	0.035	0.034	0.0025	0.005	7.8	36.7
T_2 = 150 g a.i. ha^{-1}	0	-	-	-	0.794	11.116	10.952	0.181	0.362	565.6	2661.7
	1	-	-	-	0.736	10.304	10.152	0.137	0.274	428.1	2014.7
	3	-	-	-	0.476	6.664	6.566	0.092	0.184	287.5	1352.9
	5	-	-	-	0.439	6.146	6.055	0.070	0.140	218.7	1029.4
	7	-	-	-	0.303	4.242	4.179	0.045	0.090	140.6	661.7
	10	-	-	-	0.135	1.890	1.862	0.034	0.068	106.2	500.0
	15	-	-	-	0.065	0.910	0.897	0.013	0.026	40.6	191.1
	30	0.0025	0.00075	0.067	0.0025	0.035	0.034	0.0025	0.005	7.8	36.7

Notation: DAA = Days after application; Total residue (μg g^{-1}) = Detected concentration value [in case where the residue was not detectable, ½ LOQ value (*i.e.,* 0.0025 μg g^{-1}) of fipronil and its metabolites was considered]; EDI = Estimated Daily Intake (mg kg^{-1} body weight).

under paddy ecosystem. Biphasic dissipation nature of fipronil has also been observed earlier in other paddy ecosystem (Kumari, 2008; Kumar and Singh, 2013). An overall rapid dissipation of fipronil under paddy ecosystem might be due to the fact that the congenial agro-climatic conditions (high temperature and humidity) that have been required for cultivation of summer paddy, catalyzed the degradation processes of parent compound which was applied in form of fipronil 5% SC on paddy plant surface (foliar application). The effect of similar climatic condition on fipronil degradation has also been investigated by Chopra et al. (2011) in cotton and soil under tropical climatic conditions.

3.3. Risk prediction

Since no residues of fipronil and its metabolites were detected in paddy grain on harvest, ½ LOQ level *i.e.,* 0.0025 μg g^{-1} was considered for prediction of dietary risk on human health USEPA, 2000; Wang et al. (2017). It has been observed that the calculated value (0.067) of dietary risk quotient (RQ_d) for human was appeared to be at low risk ($RQ_d < 1$) (Table 4).

Health risk on cattle due to fipronil residues in paddy leaf (considered as fodder) was assessed in terms of RQ_d for both the doses. The results indicated that the deposited residue levels of total fipronil in paddy leaf might have high risk ($RQ_d > 1$) for both the doses up to 10 DAA (Table 4). Moreover, it can also be assumed that if the leaves of 0 (2 h after application) to 10 DAA are supposed to be fed to cattle, there is a possible transfer of fipronil residues from feed to milk and thereby enter into the food chain (Faouder et al., 2007). However, leaves of the following days were found to be safe as cattle feed ($RQ_d < 1$).

The risk on soil ecology due to the application of fipronil in paddy field was also assessed by evaluating soil risk quotient (RQ_s) for different soil organisms which are important for maintaining soil health (Table 4). The residue levels of fipronil in soil were appeared to be at low risk ($RQ_s < 1$) for earthworms (0.005–0.362), but in contrary high to extreme risk ($RQ_s >> 1$) can be assumed for soil algae (36.7–2661.7) and macro-organisms (7.8–565.6) for both the treatment doses.

4. Conclusion

The present analytical method using GC-ECD (for quantification) and GC-MS/MS (for confirmation) is capable enough for residual analysis of fipronil along with its metabolites in paddy ecosystem. Fipronil is rapidly dissipated (more than 90% during 10–15 DAA) under paddy ecosystem and degraded into two major metabolites *viz.,* sulfone and desulfinyl following biphasic first order kinetics in plant and soil system with residual half-life of 3.4–4.1 days. Greater oxidation rather than photo degradation has been occurred in paddy leaf whereas the converse has been noticed in paddy soil. Fipronil was found to be stable in comparison with its metabolites. Pre-harvest interval for paddy leaf as fodder was 19.44–25.67 days. From health safety point of view, the residual risk of fipronil was appeared to be lower in human health after harvest, but higher in case of cattle (up to 10 DAA) and soil ecological health (macro-organism and algae).

Declarations

Author contribution statement

Ayan Mukherjee: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rahul Mondal: Analyzed and interpreted the data; Wrote the paper.

Subrata Biswas; Sabyasachi Ghosh: Contributed reagents, materials, analysis tools or data.

Soumen Saha: Performed the experiments; Analyzed and interpreted the data.

Ramen Kumar Kole: Conceived and designed the experiments.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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