



## Behaviour of Fipronil in Paddy Field Ecosystem

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Under normal environmental conditions, fipronil insecticide degrades into a number of metabolites like fipronil sulfone, sulfide, desulfinyl, etc. They can persist in environment for longer duration and can have adverse effect on soil, water bodies along-with persistence of residuals in edibles and other plant by-products like straw. In order to evaluate the impact of using fipronil in paddy field ecosystem and to provide basic information for its degradation behavior, a field experiment was done by applying the insecticide @ 56 and 112 g a.i./ha following standard agronomic conditions. Soil and water samples were collected periodically. Paddy grain, husk and straw samples were collected at harvest. Fipronil residues were analyzed by GC-MS tandem mass spectrometry. In soil, more than 48 % residues degraded in 7 days and reached below detectable limit after 49 days with half-life period of 9.29 and 10.13 days at two doses showing degradation rate to be biphasic with first order kinetics. In field water, residues reached below detectable limit on 49th day at single dose with half-life period of 14.71 days where as at double dose application, 91.52 % fipronil was found dissipated on 49th day after application with half-life period of 14.31 days. In rice grains, husk and straw, fipronil residues were found below MRL at single dose.

**Keywords:** Fipronil, Residues, Paddy Field, Half-life, GC-MS tandem mass spectrometry.

### INTRODUCTION

Rice, *Oryza sativa* L. is feeding more than 80 % world population. Being second largest rice producer of the world, for the year 2015-16, India's total production was 104.32 million tonnes [1]. But there are many constraints affecting the total rice production in India among which attack of insect pests is the major one. Over 100 species of insects are responsible for crop infestation but brown plant hopper, *Nilaparvata lugens* and white backed plant hopper, *Sogatella furcifera* can damage the crop to severe extent [2]. Fipronil, (RS)-5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethyl-sulfinyl)-1H-pyrazole-3-carbonitrile, a phenyl pyrazole insecticides first synthesized in 1987 [3]. The other major insecticides groups like pyrethroids and organo-phosphates are sodium channel blockers which is the common biochemical pathways. On the other hand, the carbamates are cholinesterase inhibitors. These are classical insecticides and due to common mode of action, many insects have developed resistance against them [4]. Fipronil acts in a different way by inhibition of principal nerve transmitter of insect i.e. gamma aminobutyric acid (GABA receptors). Because of its different mode of action in comparison to classical insecticides fipronil also known as new generation insecticide [5].

Fipronil have excellent efficacy against lepidopterous and orthopterous pests on crops and coleopterous larvae in soil because of which it have extensive applications in paddy field especially in subtropical humid agro-climatic conditions of Haryana and Punjab states. In India, fipronil is marketed under various trade names like Termidor, Regent and Jump.

The literature shows that fipronil and its various metabolites have different mobility and fate in the environment. Some of its degradation products are even more toxic than the parent compound. Fipronil sulfone is 6.6 times and fipronil sulphide is 1.9 times more toxic than fipronil [6]. During last decade, considerable concern has been expressed to control the magnitude of pest chemicals left in food stuffs but still the risks associated with the consumption of pesticide-treated crops cannot be neglected because of the presence of toxic residues. Strict guidelines and restrictions for improving food standards as imposed by World Trade Organization and other food regulatory bodies demanded toxicologically acceptable quantities of residues in the commodities for international trade. Providing residues free food products along-with effective control of pests is a challenging task for farmers in modern agriculture. Ensuring the harvest time residues within permissible limit should be a mandatory practice before selling the crop produce in domestic as well as international trade. Therefore, to document the presence

of these compounds in the environment and food commodities, this study was undertaken to assess the persistence behaviour of fipronil in soil, paddy crop and in standing water of paddy field under subtropical agro-climatic conditions of Haryana state of India during crop season when temperature generally remains between 35-45 °C and average humidity between 70-95 %.

## EXPERIMENTAL

Paddy (variety, HKR-47) was raised during June 2016 in fields of nearby village Dabra, Hisar city of Haryana state following recommended agronomic practices [7] in a randomized block design (RBD) with plot size of 23.5 × 6.8 m. After 50 days of transplantation, fipronil granular formulation (Regent 80 WG) was applied at single dose (T<sub>1</sub>: 56 g a.i./ha) and double dose (T<sub>2</sub>: 112 g a.i./ha) along-with a control plot where insecticide was not applied. Soil samples were collected periodically on 0 Day (1 h), 7, 14, 21, 28, 35, 42 and 49 days after insecticide application. Water samples were also collected on 1, 3, 7, 14, 28, 42 and 49 days after application in three replicates along with control. Paddy grain, husk and straw samples were collected at harvest.

The technical grade analytical standard of fipronil having 97.5 % purity and formulation (Regent 80 WG, used in field application) was supplied by M/s BASF India Ltd., Mumbai, India. In order to check the purity of analytical compound, acetone extract of the formulation was prepared which on analysis showed the presence of fipronil and neither of its metabolic products nor any impurity were having interfering peak in the vicinity of the retention time of fipronil. Other working solvents like acetone, hexane, dichloromethane and other chemicals were purchased from Merck, Darmstadt, Germany. All common solvents were redistilled before use in order to remove any impurity if present. The suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis. A standard stock solution of the parent fipronil was prepared in acetone. The working solutions required for calibration at 2.00, 1.50, 1.00, 0.50, 0.25, and 0.10 µg/mL were prepared from stock solution using serial dilutions with acetone. All standard and working solutions were stored before use at -10 °C in deep freezer.

### Extraction and clean-up

**Soil:** 0.5 mL ammonia solution was added to the well ground, sieved soil sample (15 g). The sample was kept for 0.5 h to ensure complete removal of ammoniacal smell. To the above mixture, 10 g anhydrous sodium sulphate, 0.3 g florisil, 0.3 g activated charcoal were added and mixed properly. The homogenous sample mixture was packed compactly by gentle tapping in a glass column (60 cm × 22 mm i.d.) sandwiched between two layers of anhydrous sodium sulphate. The column was eluted with 150 mL hexane:acetone (1:1 v/v). The elute was collected in a reagent bottle and concentrated to 5 mL on a Heidolph rotary vacuum evaporator at 35 °C followed by gas manifold evaporator to dryness. The concentrated extract was reconstituted to a final volume of 2 mL using *n*-hexane for analysis on GC-MS/MS.

**Water:** Samples collected at different time intervals were filtered through Whatman filter paper no.1 and extracted as per the method of Kumari *et al.* [8]. A portion of water (500 mL)

was taken in a separatory funnel, then analytical grade sodium chloride (15 g) was added to it and shaken gently. The residues of fipronil from water were extracted thrice using 50, 30 and 20 mL 15% dichloromethane in hexane by vigorous shaking. Organic phases were pooled together and concentrated on a rota-vapour. Dichloromethane from the extract was removed meticulously and the extract was reconstituted using 2 mL *n*-hexane for analysis on GCMS/MS.

**Grain, husk and straw:** Acetonitrile (100 mL) was added to the representative sample of coarse grain (20 g), husk (10 g) and straw (5 g) and shaken on a mechanical shaker for 1 h. Extract was filtered and concentrated to 50 mL using rota-vapour. The extract was taken in a 1 L separatory funnel and 50 mL of 10 % brine solution was added to it. The extract was partitioned thrice with hexane (75, 50 and 25 mL). The organic phases were pooled in a single flask each time and concentrated to about 5 mL using rotary vacuum evaporator at 40 °C and finally concentrated to dryness using a gas manifold evaporator. Each extract was reconstituted to a final volume of 2 mL using *n*-hexane before analysis. No clean-up was required for soil, water as well as grain, straw and husk samples as no interfering peaks were observed in GC-MS/MS analysis.

**Estimation:** Analysis of fipronil residues were carried in Agrochemicals Residues Testing Laboratory, Department of Agronomy, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India using an Agilent 7890A series GC-MS/MS. The instrument was conditioned and tuned for obtaining standard instrumental operating parameters before injection of standard of fipronil. The operating parameters constituted injection port temperature as 280 °C. HP-5 column having dimension 30 m × 0.32 mm i.d. × 0.25 µm film thickness was containing 5 % diphenyl and 95 % dimethyl polysiloxane. Oven temperature ramping used for the separation of analyte consisted of initial hold of 2 min at 70 °C followed by increase in compartment temperature @ 25 °C/min upto 150 °C without any hold to further increase @ 15 °C/min upto 200 °C without any hold and final increase @ 8 °C/min up-to 280 °C with 2 min hold. Mass detector Agilent 7000 was having detector parameters with source temperature as 230 °C; emission current as 35 µA; energy, -70 eV; repeller voltage as 11 V; ion body at 12 V; extractor with -7.2 V; ion focus at -7.4 V were used. Quadrupole one (MS<sup>1</sup>) and quadrupole two (MS<sup>2</sup>) temperature was maintained at 150 °C. Helium was used as carrier gas at a flow rate of 1 mL/min through column and 2.25 mL/min as quench flow and 1.15 mL/min in collision cell. Split ratio was 1:10; vacuum (high pressure) was 2.23 × 10<sup>-5</sup> torr followed by rough vacuum pressure as 1.51 × 10<sup>2</sup> torr. Injection volume was 2 µL. Under above operating conditions retention time of fipronil was 26.3 min. The confirmation and quantification of fipronil was achieved by developing a programming in SCAN (Fig. 1), product ion (Fig. 2) and multiple reaction monitoring (Fig. 3). Characteristic ions with high intensity and strong anti-turbulence were categorized and selected as qualifier and quantified ions for monitoring and quantitative analysis (Table-1).

## RESULTS AND DISCUSSION

A known chemical can be estimated in certain plant or soil matrix by developing a specific method of residue estimation.

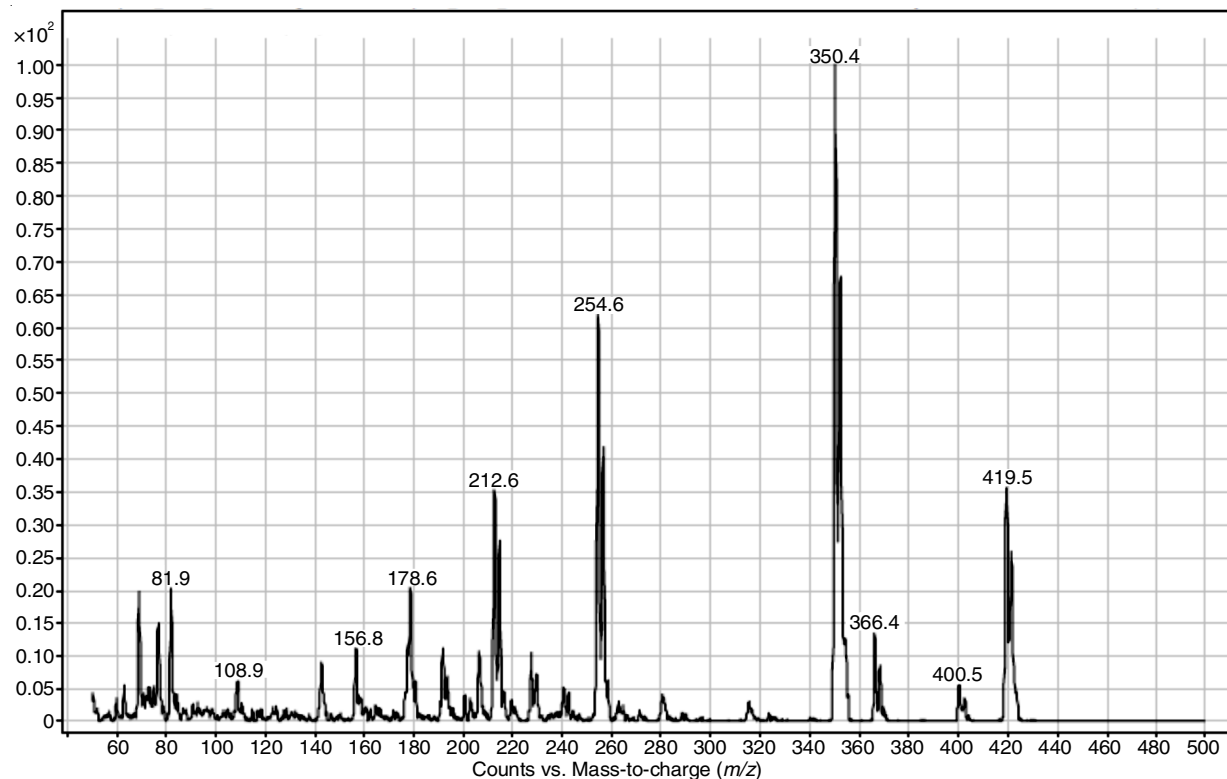


Fig. 1. Fipronil analysis in SCAN mode with ions at different mass-to-charge ( $m/z$ ) ratios and their relative abundance

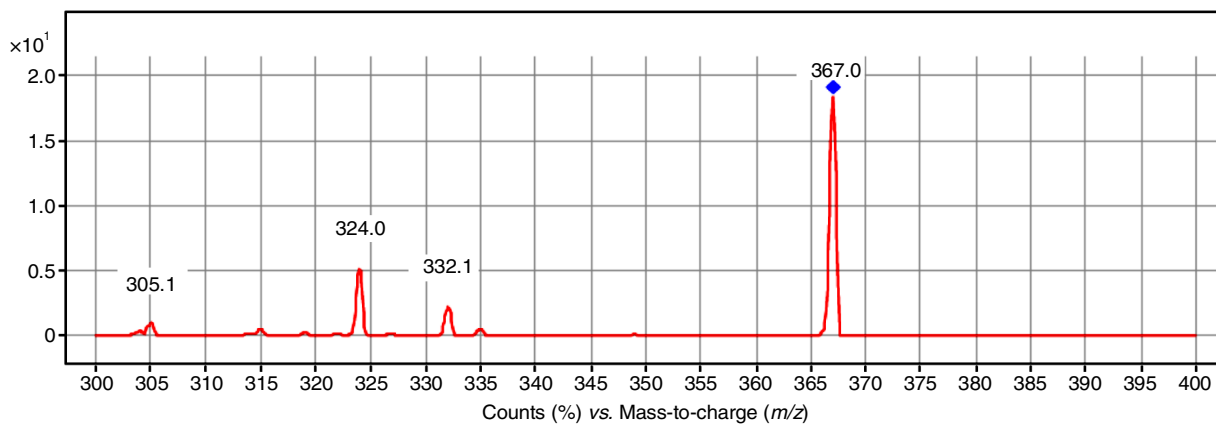
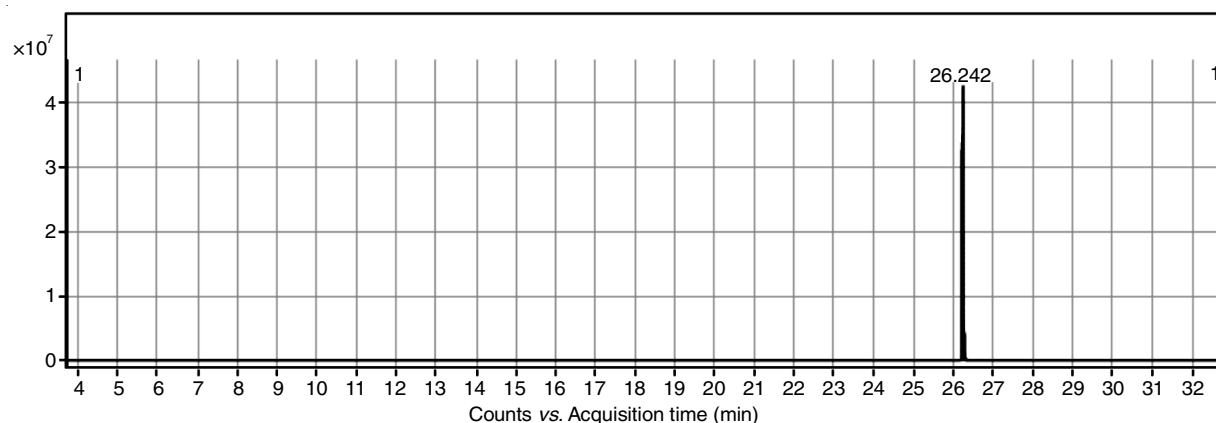


Fig. 2. Fipronil analysis in PI mode with ions at different mass-to-charge ( $m/z$ ) ratios and their relative abundance

TABLE-1  
PROGRAMMING PARAMETERS FOR MULTIPLE REACTION MONITORING (MRM)

Compounds	Molar mass	Precursor ion ( $m/z$ )	Collisions energies	Monitoring ions ( $m/z$ ) and relative abundance (in brackets)
Fipronil	436	368	30, 20, 25, 30	213 (419.4) and 282 (748.6)

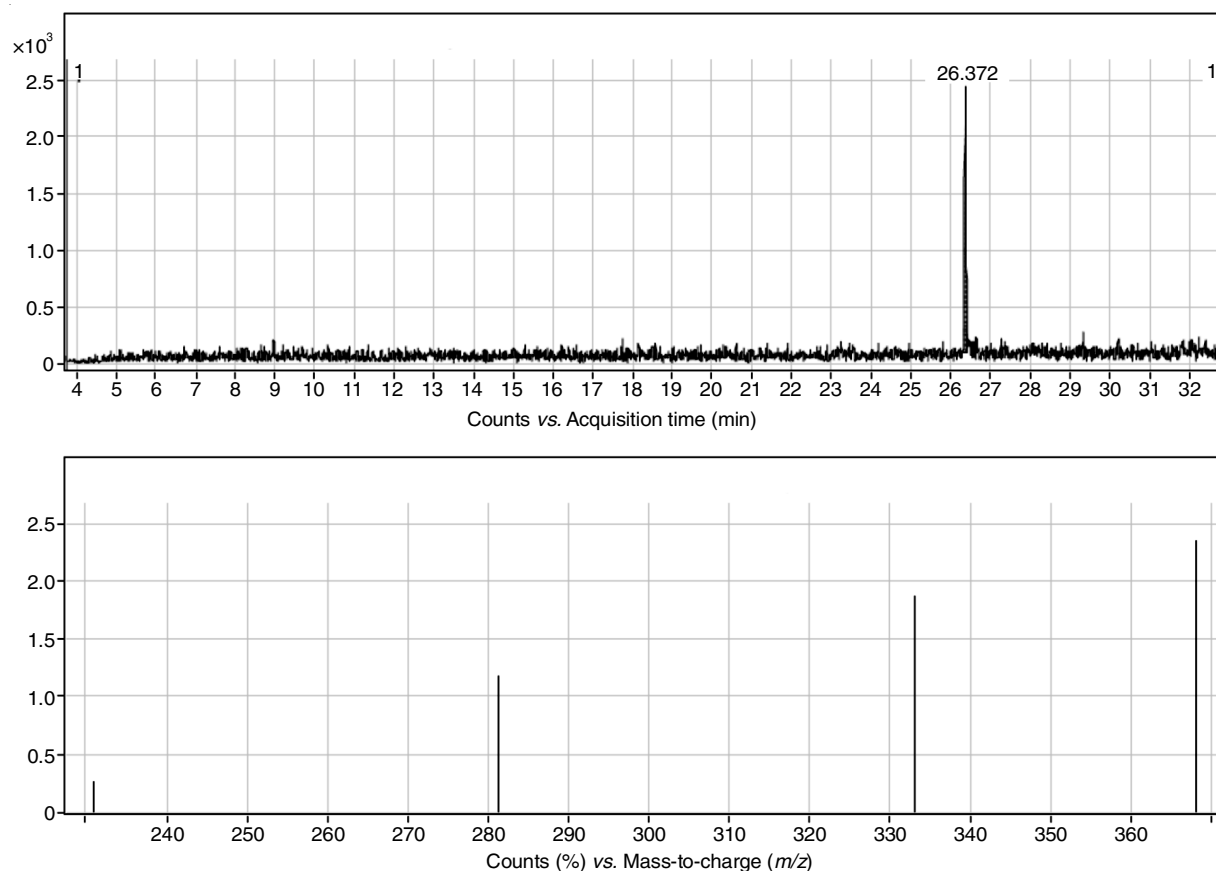


Fig. 3. Fipronil analysis in MRM mode with ions at different mass-to-charge ( $m/z$ ) ratios and their relative abundance

To detect trace amount of residues remaining on the treated commodities, the analytical method so developed should be sufficiently sensitive and highly repeatable. Samples preparation of the analytical procedure can be so efficient that all possibilities of having interferences present in the samples should be nullified. In order to check the reliability and validity of analytical method and to know the efficiency of extraction and clean-up procedures, recovery experiments were carried out at different fortification. The control samples of soil, water, grain, husk and straw were spiked with standard fipronil at 0.25 and 0.50 mg/kg and samples were processed using standard methodology. Mean recoveries of fipronil were observed from 81.44-96.53% (Table-2). The average recovery values from the fortified samples were greater than 80 % and therefore, the results have been presented as such without applying any correction factor. After adequate testing the tuning parameters of the instrument, the sensitivity parameters like limit of detection (LOD), limit of quantification (LOQ), precision and accuracy were derived as per the guidelines [9]. Accordingly, the limit of quantification (LOQ) and limit of detection (LOD) was found to be 0.001 and 0.0003 mg/kg, respectively.

At single and double application dose of 56 and 112 g a.i./ha, initial residue deposit of fipronil was 0.027 and 0.055

mg/kg in soil under paddy crop (Table-3). During initial stage, the dissipation was quite rapid and after 7 days of application, residues reached to 0.014 and 0.030 mg/kg with percent dissipation of 48.14 and 45.45 at single and double doses, respectively. The dissipation was little slow but continuous after 7th day and only 96.29 and 94.54 % of fipronil residues get dissipated upto 42 days after application. At the time of harvest, residues dissipated to below detectable level (BDL) of 0.001 mg/kg at both single and double dose. The half-life was observed to be 9.29 and 10.13 days at single and double dose respectively, following first order dissipation kinetics (Fig. 4). Statistical analysis carried out using ANOVA revealed that the residues decreased significantly with increase in duration. Higher dose showed significantly more residues (0.017 mg/kg) as compared to lower dose where average residues remained as 0.008 mg/kg ( $CD = 0.002$ ;  $p \leq 0.05$ ). With increase in number of days after application, the residue level reduced significantly ( $CD = 0.003$ ;  $p \leq 0.05$ ). Interaction between dose and duration were found significant ( $CD = 0.004$ ;  $p \leq 0.05$ ). The data thus, revealed that residues level were significantly less at single dose as compared to double dose and with duration there was a significant reduction in residues at both application doses. Mohammad *et al.* [10]

TABLE-2  
RECOVERY OF FIPRONIL FROM SOIL, WATER, PADDY GRAIN, HUSK AND STRAW

Fortification level (mg/kg and mg L <sup>-1</sup> )	Recovery (%) (average of three replicates)				
	Soil	Water	Grain	Husk	Straw
0.25	86.12 ± 0.9	92.34 ± 1.3	90.07 ± 3.8	82.68 ± 1.4	82.44 ± 0.6
0.50	84.06 ± 0.7	96.53 ± 2.5	86.97 ± 2.5	84.54 ± 3.6	81.44 ± 1.4

TABLE-3  
RESIDUES (mg/kg)\* OF FIPRONIL IN SOIL

Days after treatment	Dose (56 g a.i./ha)		Dose (112 g a.i./ha)		Mean days
	Average residues $\pm$ SD	Dissipation (%)	Average residues $\pm$ SD	Dissipation (%)	
0 (1 h)	0.027 $\pm$ 0.002	—	0.055 $\pm$ 0.003	—	0.041
7	0.014 $\pm$ 0.002	48.14	0.030 $\pm$ 0.002	45.45	0.022
14	0.010 $\pm$ 0.004	62.96	0.021 $\pm$ 0.003	61.81	0.016
21	0.005 $\pm$ 0.002	81.48	0.012 $\pm$ 0.003	78.18	0.009
28	0.003 $\pm$ 0.001	88.88	0.007 $\pm$ 0.002	87.27	0.005
35	0.002 $\pm$ 0.001	92.59	0.005 $\pm$ 0.002	90.90	0.004
42	0.001 $\pm$ 0.0008	96.29	0.003 $\pm$ 0.002	94.54	0.002
49	BDL	100.00	BDL	100.00	0.001
Mean dose	0.008		0.017		
	Correlation coefficient $r = -0.9967$ Regression equation $y = 1.4109 - 0.0324x$ $t_{1/2} = 9.29d$		Correlation coefficient $r = -0.9982$ Regression equation $y = 1.7153 - 0.0297x$ $t_{1/2} = 10.13d$		

C.D. (P = 0.05) for days = 0.003, for dose = 0.002, for days x for dose = 0.004; \*Average of three replicates; BDL=0.001 mg/kg; ND: Not detected

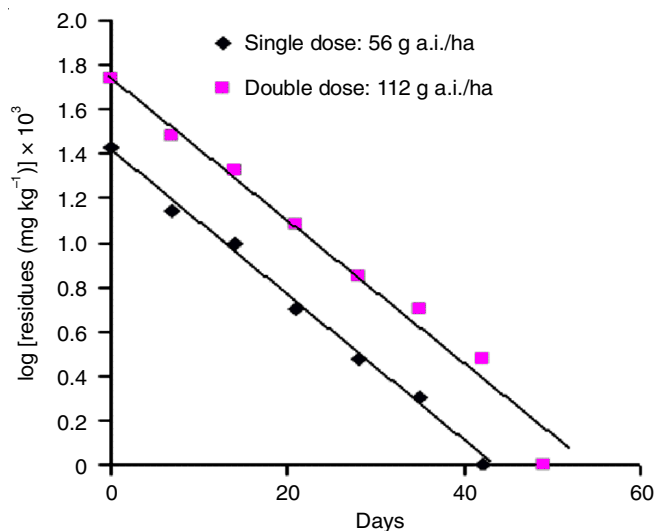


Fig. 4. Linear plot for first order kinetics of fipronil dissipation in soil

observed that in soil, the residues of fipronil dissipated from 0.032 to < 0.009 mg/kg from next day of application to 14 days and reduced to BDL after 28 days. Mohapatra *et al.* [11] reported degradation of fipronil on grape leaves to BDL at harvest time with half life of 9.6 days and 18.32 days at single and double dose, respectively.

The results for fipronil residues determined in water under paddy fields revealed that dissipation was fast upto 14th day

(Table-4). The average initial deposit of fipronil in water under paddy field, declined from 0.038 to 0.005 mg/L in single dose and from 0.059 to 0.005 mg/L from 1 to 49 day after application. In single dose, average initial residues of 0.038 mg/L dissipated to 0.034, 0.013 and 0.009 mg/L on 3rd, 14th and 28th day showing dissipation on corresponding days as 10.52, 65.78 and 76.31%, respectively. The residues level was below detectable level (BDL) on 49th day in single dose application. The dissipation followed a first order kinetics (Fig. 5) with half-life period of 14.71 days in  $T_1$  treatment. In water sample treated with double dose ( $T_2$ ), average initial deposit of 0.059 mg/L reached to the levels of 0.053, 0.021 and 0.015 mg/L after 3rd, 14th and 28th day. Percent dissipation during this period was observed to be 10.16, 64.40 and 74.57. After 49th days, residues of fipronil dissipated to a level of 0.005 mg/L showing dissipation of 91.52 %. The dissipation behaviour at both the doses were found significant (CD = 0.003;  $p \leq 0.05$ ). In double dose ( $T_2$ ), half-life period of 14.31 days was observed, following first order dissipation kinetics (Fig. 5). At the end of study period, the fipronil residues were significantly higher at double dose (0.029 mg/L) in comparison to the persistence of residues at single dose (0.017 mg/L) with CD = 0.002;  $p \leq 0.05$ . Interaction between application doses and dissipation duration was also found to be significant with CD = 0.004;  $p \leq 0.05$ , which suggested significantly less residues at each duration of single dose in comparison to double dose application. Feung and Yenne [12] studied the aquatic metabolism

TABLE-4  
RESIDUES (mg/L)\* OF FIPRONIL IN FIELD WATER

Days after treatment	Dose (56 g a.i./ha)		Dose (112 g a.i./ha)		Mean days
	Average residues $\pm$ SD	Dissipation (%)	Average residues $\pm$ SD	Dissipation (%)	
1	0.038 $\pm$ 0.002	—	0.059 $\pm$ 0.004	—	0.049
3	0.034 $\pm$ 0.004	10.52	0.053 $\pm$ 0.004	10.16	0.044
7	0.024 $\pm$ 0.002	36.84	0.038 $\pm$ 0.002	35.59	0.031
14	0.013 $\pm$ 0.002	65.78	0.021 $\pm$ 0.003	64.40	0.017
28	0.009 $\pm$ 0.004	76.31	0.015 $\pm$ 0.001	74.57	0.012
42	0.005 $\pm$ 0.006	86.84	0.008 $\pm$ 0.002	86.84	0.007
49	BDL	100.00	0.005 $\pm$ 0.003	91.52	0.003
Mean dose	0.017		0.029		
	Correlation Coefficient $r = -0.9817$ Regression Equation $y = 1.7331 - 0.020454x$ $t_{1/2} = 14.71d$		Correlation Coefficient $r = -0.9885$ Regression Equation $y = 1.7431 - 0.02102x$ $t_{1/2} = 14.31d$		

C.D. (P = 0.05) for days = 0.003, for dose = 0.002, for days x for dose = 0.004; \*Average of three replicates; BDL = 0.001 mg/L; ND: Not detected



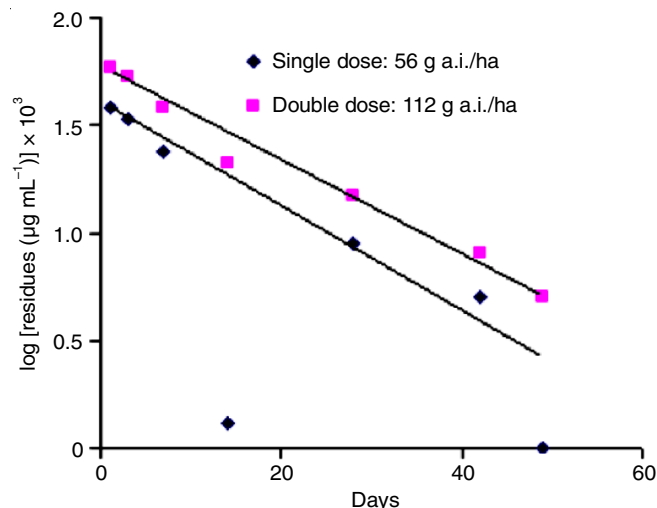


Fig. 5. Linear plot for first order kinetics of fipronil dissipation in water

of radioactively  $^{14}\text{C}$  fipronil in sandy loam soil having 8 % organic matter content with pH = 5.80 and reported half-life for fipronil under aerobic aquatic conditions to be 14.5 days. Almost similar result in water from rice field has been reported by Mohammad *et al.* [10].

In paddy grains, the average residues were below detectable level at single dose and 0.005 mg/kg (below MRL value of 0.04 mg/kg) at double dose. The residues (0.007 and 0.009 mg/kg) in husk and straw were also found below MRL (0.010 mg/kg) at single dose. At double dose 0.018 and 0.016 mg/kg residues were observed in husk and straw, respectively. These values at double dose application are slightly higher than MRL (Table-5). Kumari [13] also observed fipronil residues to below detectable levels in paddy grains and straw at the time of harvest.

TABLE-5  
RESIDUES (mg/kg)\* OF FIPRONIL IN GRAIN,  
HUSK AND STRAW AT HARVEST

Commodity	Average Residues $\pm$ SD	
	Single dose (56 g a.i./ha)	Double dose (112 g a.i./ha)
Paddy grain	BDL $\pm$ 0.0	0.005 $\pm$ 0.001
Husk	0.007 $\pm$ 0.001	0.018 $\pm$ 0.002
Straw	0.009 $\pm$ 0.002	0.016 $\pm$ 0.002

\*Average of three replicates; BDL: 0.005 mg/kg; MRL: 0.04 mg/kg for grains; 0.010 mg/kg for straw.

## Conclusion

Fipronil has an outstanding long term efficacy against lipid-opterous, orthopterous and coleopterous pests affecting the paddy crop. Half-life of fipronil at recommended and double the recommended dose in paddy field were observed to be 9 to 14 days in soil and water. At harvest time, the residues in the soil and water were below detectable level. Further, in grains, the fipronil residues were observed to below MRL value at both application doses. In husk and straw, the residues were found below MRL at single dose and near MRL at double dose application. Therefore, recommended dose application of fipronil against insect pest management of paddy field is quite safe for crop protection and prevention of environmental contamination.

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