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## Dissipation and persistence behaviour of fipronil and its metabolites in clay soil under laboratory condition

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**Abstract**

A laboratory experiment was conducted at Food Quality Testing Laboratory, Navsari Agricultural University, Navsari to study the dissipation behaviour of fipronil and its metabolites in clay soil under laboratory condition. In method verification, acetonitrile based extraction and dispersive clean-up approach adopted to quantify the residues of fipronil and its metabolites with gas chromatography (GC-ECD) from soil was accurate, precise and sensitive enough. The soils were spiked at the rate of 1.0 and 2.0 mg/kg level for single and double dose, respectively. Soil was extracted at 5, 10, 20, 30, 60, 90 and 120 days after incubation for residues and quantified on GC-ECD. The residues of total fipronil in soil were quantified upto 90 days after their incubation in both single and double dose. The DT<sub>50</sub> values of sum total of fipronil and its metabolites recorded at single and double dose were found to be 28.3 and 31.9 days, respectively.

**Keywords:** Dissipation, DT<sub>50</sub>, fipronil, metabolites, and soil

**Introduction**

Fipronil belong to phenyl pyrazole group of insecticide. It is a broad spectrum insecticides having novel mode of action that targets the  $\gamma$ -aminobutyric acid (GABA) receptor system of insects, resulted neural excitation, paralysis and ultimately death of insect. Fipronil controls a broad spectrum of damaging insects and can effectively delivered to the target pests *via* soil, foliar, bait or seed treatment and it is widely used to control many species of insects on various crops. It has also been recommended for use where the insects have developed resistance to conventional insecticides like pyrethroids, organophosphates and carbamates. The application of fipronil in pest management has been picked up the pace in India in recent past but simultaneously there are few reports which suggest that fipronil proved to be quite lethal to honey bees and aquatic organism *etc.* Henceforth, scientific community has focused on more thorough study on the environmental fate of fipronil.

Pesticides persistence in soil is governed by various loss mechanisms like microbial degradation, chemical hydrolysis, photolysis, volatility, leaching, and surface runoff (Wasim *et al.*, 2008) [11]. Soil conditions such as moisture, temperature, pH and organic matter affect the rate of degradation due to direct or indirect influence on microbial growth and activity. The duration over which the insecticide remains biologically active in the soil is one of the key factors that influence its toxicity.

After application of insecticides in soil it may convert into its metabolites and sometimes metabolites are more toxic for insect as compared to parent compound. Therefore, studies of insecticides metabolites also have a prime importance. In case of fipronil, after its application in soil it undergo through different pathway and convert into its metabolites like by reduction to sulfide (Ramesh and Balsubramanian, 1999) [8], oxidation to sulfone (Bobe *et al.*, 1998) [3], hydrolysis to amide (Bobe *et al.*, 1998) [3] and Ngim and Crosby, 2001) [7] and photolysis to desulfinyl (Hainzl and Casida, 1996) [4]. Persistence of biological activity does not mean the persistence of parent compound. Persistence of biological activity depends on the persistence and joint action of parent compounds and their metabolites. Laboratory studies are conducted to assess the contribution made by each of the loss mechanisms to the overall dissipation.

## Materials and Methods

**Chemicals and reagents:** Certified reference standards of fipronil (purity 99.2%), sulfone (purity 99.5%), sulfide (purity 99.9%), desulfinyl (purity 94.5%), and amide (99.9%) were supplied by Sigma Aldrich India limited. All reagents and solvents used were of analytical grade. Solvent like HPLC grade n-hexane, acetone and acetonitrile (purity  $\geq 99.9\%$ ) procured from Merck Life Science Private Limited. Primary Secondary Amine was from SUPELCO, Bellefonte, USA. Sodium chloride and anhydrous sodium sulfate were obtained from Fisher Scientific, UK. Fipronil (Regent 0.3GR) formulation used for field application was procured from the local market. The concentration of fipronil was found to be accurate with respect to its purity as claimed by the manufacturers.

**Instrumentation:** Thermo make gas chromatograph (model, Trace GC-Ultra) equipped with Electron Capture Detector (ECD) was used for the qualitative and quantitative estimation of residues of fipronil and its metabolites. The details descriptions are given below.

The details descriptions are given below.

GLC	:	Trace GC-Ultra
Auto sampler	:	Triplus AS
Column	:	DB-5, 30 m, 25 mm id, 0.25 $\mu\text{m}$ FT
Detector	:	GC-ECD
Carrier gas	:	Helium
Oven programming	:	180 $^{\circ}\text{C}$ 12 $^{\circ}\text{C}/\text{min}$ 270 $^{\circ}\text{C}$ (0.0 min) $\longrightarrow$ (2.0 min)
Column flow mode	:	Constant flow
Column flow	:	1.5 $\text{mL min}^{-1}$
Injection mode	:	Split
Split ratio	:	1:5
Injection volume	:	1.0 $\mu\text{L}$
Injector temp.	:	230 $^{\circ}\text{C}$
Detector temp.	:	330 $^{\circ}\text{C}$
Current	:	1.0 Amp
Makeup gas/ flow	:	Nitrogen/45 ml/min

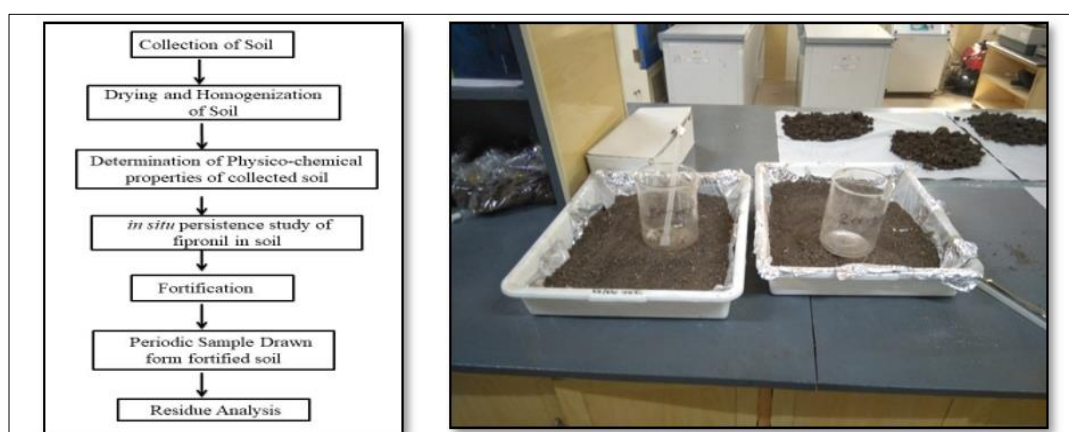
**Standard solution:** A traceable technical grade of fipronil and its metabolites standard was accurately weighed on Oahu's (maximum capacity 210 g and sensitivity 0.001 g) and transferred to 100 mL capacity volumetric flasks. The content was initially dissolved with n-hexane and final volume was made up with n-hexane: acetone (9:1, v/v). From the primary standards, a suitable aliquot was diluted with hexane: acetone

(9:1, v/v) in volumetric flask to prepare intermediate standard mixture of fipronil and its metabolites of 10.0  $\mu\text{g mL}^{-1}$ . The intermediate standard of fipronil and metabolites mixture was further diluted with n-hexane: acetone (9:1, v/v), to obtain final concentration of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 and 2.0  $\mu\text{g/mL}$ .

**Method verification:** A linearity study was performed to determine the performance of Electron Capture Detector. To establish the linearity seven different concentrations of the standards *viz.*, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0  $\mu\text{g mL}^{-1}$  were injected and their response (mV) was recorded. The volume of the standard used for the injection was 1.0  $\mu\text{L}$ . A correlation coefficient and equation was determined by using linear regression model. In order to ensure quality assurance information such as accuracy or trueness and precision of the analytical method, the recovery study was carried out from soil before taking up analysis of test sample. A representative soil samples were fortified with mixture of fipronil and metabolites mixture at 0.05, 0.1 and 0.5  $\text{mg kg}^{-1}$  level. The fortified samples were kept at room temperature for 2 hrs and residues were estimated. Prior to quantification of fungicide, the LOD and LOQ were worked out. This was carried out by injecting matrix-match standards in gas chromatograph to get signal to noise ratio 3:1 for LOD and 10:1 for LOQ.

## Experimental setup

Air dried, homogenized soil (approximately 8-10 kg) was brought from Main Sugarcane Research Station to Food Quality Testing Laboratory, Navsari Agricultural University, Navsari. The experimental soil was clay in texture having pH 7.34, EC 0.38  $\text{dS m}^{-1}$  and organic carbon 0.58%. The schematic illustration of experimental approach is given in Fig 1. The soil (1.9 kg) was transferred to three separate plastic trays for standard dose, double to standard dose and control which were covered with aluminium foil. The trays soils were spiked at the rate of 1.0 and 2.0  $\text{mg/kg}$  level for standard and double to standard dose. For the fortification, the 100 g soil was taken in a 250 mL capacity glass beaker from each tray. The beakers containing 100 g soil was fortified with retentive addition of fortification solution for respective dose. The soil was stirred with glass rod for homogeneous mixing. The soil was kept for few minutes until the solvents were not evaporated. Finally this, fortified soil for both doses was thoroughly added with the tray soil of respective doses. The soil of each tray was again thoroughly mixed with hands. The soil samples were drawn from the tray as per sampling schedule and analyzed for the residues of fipronil and its metabolites.



**Fig 1:** Schematic diagram of persistence study under laboratory condition and fortified trays

**Sampling:** The representative soil sample (10 g) was drawn per treatment from the trays at different day's interval *i.e.* 5,10,20,30,45,60,90 and 120 days.

**Extraction and clean up:** The method followed for the multi-residue analysis from soils is popularly known as QuEChERS method (Asensio-Ramos *et al.* 2010) [1]. A representative 10 g of fine ground soil sample was transferred in 50 mL capacity centrifuge tube, to which 20 mL of acetonitrile was added and shaken it vigorously for 1 minute. After this 4 g MgSO<sub>4</sub> and 1 g NaCl were added in the tube and vortex followed by centrifuged at 3500 rpm for 2 minute. After it, 10 mL of supernatant solution was transferred in the 15 mL capacity centrifuge tube containing 1.5 g MgSO<sub>4</sub> and 0.250 g PSA (Primary Secondary Amine) and again centrifuged it for 2 minute at 3500 rpm. 4 mL of aliquot was transferred in test tube and evaporated it to dryness with TurboVap at 40°C. Finally the volume was made up to 1 ml with n-hexane: acetone (9:1, v/v) and quantified on GC-ECD.

**Dissipation kinetics:** The degradation kinetics of fipronil and its metabolites in soil samples was determined by plotting residue concentration against time, and the maximum squares of correlation coefficients found were used to determine the equations of best-fit curves. For all the studied samples, exponential relations were found to apply, corresponding to first-order rate equation. Confirmation of the first-order kinetics was further made graphically from the linearity of the plots of C against time. The rate equation was calculated from the first-order rate equation:  $C_t = C_0 e^{-kt}$ , where  $C_t$  represents the concentration of the pesticide residue (in milligrams per kilogram) at time  $t$  (in days),  $C_0$  represents the initial concentration (in milligrams per kilogram), and  $k$  is the first-order rate constant (per day) independent of  $C_t$  and  $C_0$ . The half-life ( $t_{1/2}$ ) was determined from the  $k$  value, being  $t_{1/2} = \ln 2/k$ .

## Results and discussion

**Method validation:** The linear dynamic range of fipronil and its metabolites *viz.* desulfynil, sulfide, sulfone and amide on GC-ECD was 0.05-1.0  $\mu\text{g mL}^{-1}$  with co-efficient of determination ( $R^2$ ) were  $\geq 0.99$ . The average % deviation due to back calculated amount of Fipronil and its metabolites on the basis regression model obtained in linearity study for fipronil and its metabolites over actual amount (0.25  $\mu\text{g mL}^{-1}$ ) obtained were in the range of 2.08-14.41% which were satisfactorily lower than the acceptance criterion *i.e.* <20%. The overall per cent recovery and RSD obtained for fipronil and its metabolites from soil at different individual or intra-

spiking level were found in the range of 55.40 $\pm$ 1.42 to 105.73 $\pm$ 2.63% whereas the % RSD varied between 0.69 to 12.19% at different fortification levels which are well within method validation criteria *i.e.* % recovery (70-120%) and % RSD ( $\geq 20\%$ ). The LOD worked out for fipronil and its metabolites from soil were in the range of 0.001-0.002  $\mu\text{g g}^{-1}$  while, LOQ worked out for different matrices were 0.003 to 0.005  $\mu\text{g g}^{-1}$ , respectively. On the basis of method validation studies, the analytical method applied for the residue analysis applied for the estimation of fipronil and its metabolites was found accurate (recovery, 70-120%), precise (RSD; <20%), sensitive (at lowest spiking level accuracy and precision parameters are satisfactory) and instrument's response is linear as average % deviation due to back calculated amount of was  $\leq 20$  as prescribed by SANTE (2017) [9] guidelines (Table 1).

**Persistence and dissipation:** The residues of fipronil (parent compound) recorded upto 90 days after the application at both doses and were BQL (<LOQ) on 120<sup>th</sup> day after application at either dose. The loss of residues of fipronil was faster up to 45 days at either dose as 87.0 and 81.1% of initial residues were either degraded or lost from the soil on 30 days after application Sulfone and amide metabolites of fipronil were quantifiable upto 10 and 30 days after the application at both rate of application. Desulfynil and sulfide metabolites were not detected on any sampling day at either rate of application. The DT<sub>50</sub> and DT<sub>90</sub> values recorded for sum total of all residues of fipronil and its metabolites at standard and double to the standard rate were found to be 28.3 and 31.9, and 94.0 and 106.1 days, respectively (Table 2).

The results obtained in studies reflects that that fipronil degrades slowly on loamy soil when exposed to light, with a DT<sub>50</sub> of 34 days (USEPA, 1996 and Mulrooney and Goli., 1998) [10, 6] while DT<sub>50</sub> of 37.63 days reported by the Mandal and Singh (2015) [5] in clay loam soil which is in the agreement of the findings of present study where DT<sub>50</sub> for total residues were in the range of 28.3-31.9 days. The finding of present investigation is also quite close to study of Belayneh *et al.* (1998) [2] where sulfone and amide metabolites were formed and detected.

## Conclusion

The acetonitrile based extraction and dispersive clean-up approach adopted to quantify the residues of fipronil and its metabolites with gas chromatography (GC-ECD) from soil was accurate, precise and sensitive enough. The DT<sub>50</sub> values of sum total of fipronil and its metabolites recorded at single and double dose were found to be 28.3 and 31.9, respectively.

**Table 1:** Method validation parameters of fipronil and its metabolites from soil

Criterion	Varification parameter	Bench mark	Range of method performance				
			Compounds				
			Fipronil	Fip-desufinyl	Fip-sulfide	Fip-sulfone	Fip-amide
Deviation of back calculated concentration from true concentration	Linearity/sensitivity	$\leq \pm 20\%$	2.08	3.45	3.71	14.41	9.19
Accuracy or trueness	Recovery at 0.5 ( $\mu\text{g/g}$ ) level	70-120%	86.64 $\pm$ 0.60	79.49 $\pm$ 3.67	74.87 $\pm$ 1.16	105.73 $\pm$ 2.63	88.32 $\pm$ 4.62
Precision (Repeatability)	(% RSDr) through intra-spiking level	$\leq 20\%$	0.69	4.62	1.55	2.49	5.23
Robustness (Within-laboratory reproducibility, derived from on-going method validation verification)	Precision (RSDwR) through inter-spiking levels <i>i.e.</i> 0.1,0.5 and 1.0 $\mu\text{g/g}$ level	70-120%	76.27 $\pm$ 10.84	68.39 $\pm$ 9.98	63.85 $\pm$ 9.04	90.16 $\pm$ 18.01	78.20 $\pm$ 8.47
		$\leq 20\%$	14.21	44.59	14.16	19.98	10.83

**Table 2:** Residues and dissipation pattern of total fipronil in soil under laboratory condition

Days after application	Residues (mg/kg)													
	Dose (1.0 mg/kg)						Double dose (2.0 mg/kg)							
	Fipronil	Desulfinyl	Sulfide	sulfone	Amide	ΣFipronil	% loss over initial	Fipronil	Desulfinyl	Sulfide	Sulfone	Amide	ΣFipronil	% loss over initial
5	0.423 <sup>#</sup>	*BQL	BQL	0.166	1.03	1.623		0.671	BQL	BQL	0.218	0.63	1.515	
10	0.321	BQL	BQL	0.142	0.78	1.243	23.41	0.449	BQL	BQL	0.034	0.53	1.009	33.40
20	0.075	BQL	BQL	BQL	0.69	0.761	53.11	0.184	BQL	BQL	BQL	0.47	0.656	56.70
30	0.055	BQL	BQL	BQL	0.31	0.361	77.76	0.127	BQL	BQL	BQL	0.38	0.502	66.86
45	0.035	BQL	BQL	BQL	BQL	0.035	97.84	0.102	BQL	BQL	BQL	BQL	0.102	93.27
60	0.029	BQL	BQL	BQL	BQL	0.029	98.21	0.052	BQL	BQL	BQL	BQL	0.052	96.57
90	0.026	BQL	BQL	BQL	BQL	0.026	98.40	0.029	BQL	BQL	BQL	BQL	0.029	98.09
120	BDL	BQL	BQL	BQL	BQL	BQL	-	BDL	BQL	BQL	BQL	BQL	BQL	-
LOQ (mg/kg)	0.003	0.003	0.005	0.003	0.005	-	-	0.003	0.003	0.005	0.003	0.005	-	-
Reg. eq. (R <sup>2</sup> )	y = -0.0245x + 2.2188 (R <sup>2</sup> = 0.84)							y = -0.0217x + 2.2176 (R <sup>2</sup> = 0.94)						
DT50 (days)	28.3							31.9						
DT90 (days)	94.0							106.1						
# Mean of three replicates, *BQL Below Quantification Limit i.e.<LOQ														

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