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Persistence and dissipation behaviour of fipronil and its metabolites in sugarcane grown soil of South Gujarat

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Abstract
A field experiment was conducted at Main Sugarcane Research Station, Navsari Agricultural University, Navsari to study the dissipation behaviour of fipronil and its metabolites in sugarcane grown soil. The fipronil 0.3 GR was applied at standard and double dose *i.e.* 100 and 200 g ai ha^{-1} in gross plot when sugarcane crop was four month old. The soil sampling was done at 0 (2 hrs.), 1, 3, 5, 10, 20, 30, 60 and90 days after application of fipronil. 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. Persistence study indicated that residues of fipronil progressively dissipated and reached BQL (<LOQ) at 90 day after application at both doses. The dissipation rate of fipronil was faster in first phase *i.e.* upto 20 days with respect to second phase (20-60 days). The DT_{50} and DT_{90} values of sum total of fipronil and its metabolites was 23.7 and 32.9 days, respectively for single dose and 78.8 and 109.2 days, respectively for double dose.

Keywords: Agrochemicals, pesticides, health and well-being, environment health, food production

Introduction
Fipronil is a broad spectrum insecticide of phenyl pyrazole group having novel mode of action that targets the γ- aminobutyric acid (GABA) receptor system of insects, causing neural excitation, paralysis and death. This compound 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.

Persistence of pesticides in soil is governed by various loss mechanisms like microbial degradation, chemical hydrolysis, photolysis, volatility, leaching, and surface runoff (Wasim *et al.*, 2008) [7]. 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 soil and climatic conditions of the sugarcane growing areas vary widely across the country and ultimately influence the persistence of soil-applied granular insecticides to a greater extent. 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) [5], oxidation to sulfone (Bobe *et al.*,1998) [2], Hydrolysis to amide (Bobe *et al*., 1998 and Ngim and Crosby, 2001) [2, 4] and photolysis to desulfinyl (Hainzl and Casida, 1996) [3].

Materials and Methods

The field experiment was conducted at Navsari Agricultural University, Navsari, Gujarat. The climatic condition of this region is typically tropical, having warm and humid monsoon with quite cold winter and fairly hot summer. The average annual rainfall of the Navsari is about 1500 mm. The soil of experimental field was clay in texture having pH 7.34, EC 0.38 dS m^{-1} and organic carbon 0.58%.

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 ≥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 µm FT
Detector	:	GC-ECD
Carrier gas	:	Helium
Oven programming	:	180 ºC 12 ºC/min 270 ºC (0.0 min) → (2.0 min)
Column flow mode	:	Constant flow
Column flow	:	1.5 mL min^{-1}
Injection mode	:	Split
Split ratio	:	1:5
Injection volume	:	1.0 µL
Injector temp.	:	230 ºC
Detector temp.	:	330 ºC
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 µg 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 µg/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 µg mL^{-1} were injected and their response (mV) was recorded. The volume of the standard used for the injection was 1.0 µL. 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 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.

Application of fipronil

The fipronil insecticide (Fipronil 0.3 GR) was applied at standard and double standard dose i.e. 100 and 200 g ai ha^{-1} when sugarcane crop was almost 4 month old. The required quantity of fipronil was mixed thoroughly with dry sand of very fine texture and uniformly distribute in the gross plot.

Sampling

Approximately 150-200 g soil samples were taken from five to six points from each plot in zig-zag manner with standard sampling procedure at a depth of 15 cm. These collected soil samples were composited, properly labelled and brought to the laboratory. The soil samples were drawn from the field at different days 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 $MgSO_4$ 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 $MgSO_4$ 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: $Ct=C_0e^{-kt}$, where Ct 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.* desulfinyl, sulfide, sulfone and amide on GC-ECD was 0.05-1.0 µg mL^{-1} with co-efficient of determination (R^2) were ≥ 0.99. The average % deviation due to back calculated amount of Fipronil and its metabolits on the basis regression model obtained in linearity study for fipronil and its metabolites over actual amount (0.25 µg 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±1.42 to 105.73±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 (≥20%.). The LOD worked out for fipronil and its metabolites from soil were in the range of 0.001-0.002 µg g^{-1} while, LOQ worked out for different matrices were 0.003 to 0.005 µg 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 ≤20 as prescribed by SANTE (2017) [6] guidelines (Tabe 1).

Persistence and dissipation

The parent compound *i.e.* fipronil was quantifiable and detected upto 60 days after the application where sulfide, sulfone and amide metabolites were not detected on any sampling days. However, desulfinyl metabolites of fipronil were detected from soil samples collected on 20 to 60 days after the treatment and were BQL in soil samples collected on 90 days after application. The DT_{50} and DT_{90} values of sum total of fipronil and its metabolites recorded at single and double dose were found to be 28.3 and 94.0, and 32.9 and 109.2 days, respectively (Table 2).

Ying and Kookana (2002) [9] also studied the degradation of fipronil in a soil under laboratory and field conditions. Three metabolites of fipronil (desulfinyl, sulphide and sulfone derivatives) were identified from soils after treatment. The desulfinyl derivative, was formed by photodecomposition of fipronil on the soil surface under sunlight. The half-life of the total toxic component (fipronil and its metabolites) in field soil was 188 days on average while Wu *et al.* (2017) [8] reported DT_{50} of 21.7 days for fipronil in soil when adopted single first order kinetics.

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_{50} and DT_{90} values of sum total of fipronil and its metabolites recorded at single and double dose were found to be 28.3 and 94.0, and 32.9 and 109.2 days, respectively

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

Criterion	Validation 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/senstivity	≤±20%	2.08	3.45	3.71	14.41	9.19
Accuracy or trueness	Recovery at 0.5 (µg/g) level	70-120%	86.64±0.60	79.49±3.67	74.87±1.16	105.73±2.63	88.32± 4.62
Precision (Repeatability)	(% RSDr) through intra-spiking level	≤ 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.e. 0.1,0.5 and 1.0 µg/g level	70-120%	76.27±10.84	68.39±9.98	63.85±9.04	90.16±18.01	78.20± 8.47
		≤ 20%	14.21	44.59	14.16	19.98	10.83

Tables 2: Persistence and dissipation pattern of fipronil in sugarcane grown soil

Days after application	Residues (mg/kg)													
	Standard (100 g a.i./ha)							Double (200 g a.i./ha)						
	Fipronil	Desulfinyl	Sulfide	sulfone	Amide	ΣFipronil	% loss over initial	Fipronil	Desulfinyl	Sulfide	Sulfone	Amide	ΣFipronil	% loss over initial
5	0.168	BQL	BQL	BQL	BQL	0.168	-	0.195	BQL	BQL	BQL	BQL	0.195	-
10	0.150	BQL	BQL	BQL	BQL	0.150	10.71	0.101	BQL	BQL	BQL	BQL	0.101	48.21
20	0.027	0.003	BQL	BQL	BQL	0.030	82.14	0.013	0.004	BQL	BQL	BQL	0.017	91.28
30	0.018	0.013	BQL	BQL	BQL	0.031	81.55	0.011	0.033	BQL	BQL	BQL	0.044	77.44
45	0.010	0.009	BQL	BQL	BQL	0.019	88.69	0.005	0.022	BQL	BQL	BQL	0.027	86.15
60	0.003	BQL	BQL	BQL	BQL	0.003	98.21	0.003	0.004	BQL	BQL	BQL	0.007	96.41
90	BQL	BQL	BQL	BQL	BQL	BQL	-	BQL	BDL	BQL	BQL	BQL	BQL	-
120	BQL	BQL	BQL	BQL	BQL	BQL	-	BQL	BDL	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^2)	y = -0.0292x + 2.3484 R^2 = 0.92							y = -0.0211x + 2.1714 R^2 = 0.72						
DT50 (days)	23.7							32.9						
DT90 (days)	78.8							109.2						

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