



RESEARCH ARTICLE

Residue dissipation kinetics, safety evaluation and consumer risk assessment of fipronil in cowpea and the soil

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Abstract

Fipronil is extensively used for insect pest management in cowpea; however, its residue dynamics under field conditions require a reliable analytical assessment to ensure food safety. In the present study, a sensitive and validated gas chromatography–micro electron capture detector (GC- μ ECD) method was developed for the determination of fipronil residues in cowpea pods, leaves and soil and its dissipation behaviour was evaluated under field conditions. Field experiments were conducted during the kharif season (2023–24) at ICAR-Indian Institute of Vegetable Research, Varanasi, India, using cowpea (*Vigna unguiculata* L.) variety Kashi Kanchan in a randomised block design with three replications. Fipronil 5% SC was applied at the recommended dose (50 g a.i. ha⁻¹) and double the recommended dose using a knapsack sprayer. Samples of pods, leaves and soil were collected at different intervals (0, 1, 2, 5, 7 and 10 days) after the final spray. Residues were extracted and cleaned using a modified QuEChERS method and quantified by GC- μ ECD. The method showed good linearity over the concentration range of 0.01–0.1 μ g mL⁻¹, satisfactory recoveries and a limit of quantification (LOQ) of 0.01 mg kg⁻¹ for all matrices. Fipronil residues dissipated progressively with time, following first-order kinetics and declined below detectable levels within the recommended pre-harvest interval. Therefore, when used at recommended doses, fipronil presents a low risk profile in cowpea cultivation.

Keywords: cowpea pod; dissipation kinetics; fipronil residue; GC- μ ECD; half-life; leaf and soil; risk assessment

Introduction

Cowpea (*Vigna unguiculata* L.) is a nutrient-dense legume vegetable that remains under utilised, yet it may help prevent protein-calorie malnutrition (1). In India, this crop is cultivated over 58000 hectares, yielding 4.8×10^6 t yr⁻¹, with an average productivity of approximately 8.44 t ha⁻¹ (2). It not only offers high-quality livestock feed but also contributes to income generation. However, its production is threatened by various biotic and abiotic challenges. This decrease can be attributed to a complex of insect pests, the most notable of which is the *Maruca vitrata* or cowpea (legume) pod-borer, along with several other pests (3).

There could be a more than 30% reduction in yields if there is a serious borer infestation. (4). Therefore, managing this pest is crucial for the sustainable production of cowpeas. Even with the availability of sustainable and environmentally friendly pest management techniques like host plant resistance (HPR), plant secondary metabolites, bio-control agents, botanicals and defence proteins, farmers still choose synthetic pesticides (5–10). Farmers indiscriminately apply a range of insecticides in an effort to increase yield and lessen the spotted pod borers' negative effects on different pulse crops. There is an ongoing initiative to adopt microbial pesticides, products derived from plants and insecticides, as they are typically considered safer, more eco-friendly and less prone to causing resistance (11, 12).

In these situations, it is imperative to employ novel insecticides because they are highly selective and pose less of a risk to the environment and are safer to pollinators (13–16). Recent research has shown that next-generation insecticides, like fipronil is highly effective at controlling the pod borer *M. vitrata* in soybean and pigeon pea (17). Farmers use insecticides extensively in cowpea crops even though the pesticide regulating body, the Central Insecticide Board and Registration Committee (CIBRC), advises against spraying them on these crops. As such, evaluating the fipronil dissipation pattern in cowpea becomes essential. Fipronil {5-amino-1-(2, 6 dichloro-a, a, a-trifluoro-tolyl)-4-trifluoro-methyl sulfinyl pyraorezole Carbonitrile tetra-zine} for the control of insect pests of vegetables (18). Chemical structure of fipronil is given in Fig. 1. Due to frequent use, residues may build up and interfere with the soils' biological processes, endangering the microorganisms that live there (19). The negligent and excessive use of pesticides also contaminates farmed soil and groundwater (20, 21). There has been research on the dynamics of fipronil residue degradation in the vegetable ecosystem, but there hasn't been any published research on what happens to these substances in the cowpea field environment (22). The present investigation was conducted to evaluate the persistence and dissipation behaviour of fipronil in cowpea under field conditions. It is hypothesised that fipronil residues applied at the recommended agricultural dose dissipate rapidly, follow first-order kinetics and decline to levels below the

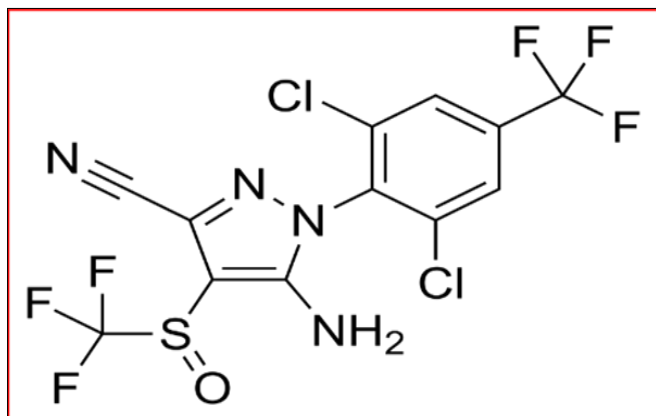


Fig. 1. Chemical structure of fipronil.

maximum residue limit within the pre-harvest interval, thereby posing minimal dietary and environmental risk. Furthermore, it is hypothesised that application at a higher (double) dose influences residue persistence differently across matrices (pods, leaves and soil) due to variations in plant metabolic capacity and soil-mediated degradation processes.

Materials and Methods

Chemicals and reagents

Fipronil reference standard (95 % purity) was obtained from Sigma-Aldrich (Bangalore, India). Analytical-grade acetone, n-hexane, sodium sulfate (Na_2SO_4), magnesium sulfate (MgSO_4) and sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$) were procured from Thomas Baker (Mumbai, India). Primary secondary amine (PSA, 40 μm) was sourced from Agilent Technologies (Bangalore, India). Activated MgSO_4 was dried at 600 °C for 5 hr before use. Deionised water was produced using a Millipore purification system and filtered through 0.22 μm membrane filters. PTFE syringe filters (0.2 μm) were obtained from Phenomenex (India).

Apparatus

In the experimental setup, the equipment employed included a centrifuge from Kubota, Germany; a Microfuge Pico microcentrifuge by Kendro, D-37,520, Osterode, Germany; a mixer and grinder from Bajaj India Private Limited, Mumbai; a precision balance by Vibra, Adair Dutt, Mumbai, India; a Geni 2 T vortex mixer from Imperials Biomedicals, Mumbai, India; and an ultrasonic bath by Oscar electronics, Mumbai, India.

Reference standard

Certified fipronil reference standards (95 % purity) were obtained from Sigma-Aldrich Pvt. Ltd. (Bangalore, India). A primary stock solution (1000 $\mu\text{g mL}^{-1}$) was prepared by accurately weighing 10 ± 0.1 mg of the reference standard and dissolving it in 10 mL of ethyl acetate. This stock solution was thoroughly mixed and further diluted to obtain a working standard solution of 10 $\mu\text{g mL}^{-1}$. Calibration standards of 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 $\mu\text{g mL}^{-1}$ were prepared by serial dilution. Matrix-matched standards were prepared by spiking blank cowpea extracts obtained using the same extraction protocol (22).

Field experiment

Field experiments were conducted during the kharif season (August 2023–24) at the experimental farm of the ICAR–Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India (25.10° N, 82.52° E), following FAO guidelines. The soil of the experimental field

was sandy loam. The experiment was laid out in a randomised block design (RBD) with eight treatments and three replications. Cowpea (*Vigna unguiculata* L.) variety Kashi Kanchan was grown on raised beds of 4 m \times 5 m following recommended agronomic practices. Fipronil 5% SC was applied at the recommended dose (50 g a.i. ha^{-1}) and double the recommended dose using a hollow cone-nozzled knapsack sprayer with a spray volume of 500 L ha^{-1} . Three sprays were applied at 15-day intervals, with the first spray initiated at flowering upon reaching the economic threshold level. The average minimum and maximum temperatures during the study period were 20 °C and 30 °C, respectively.

Standard solution preparation

The standard solution was prepared by accurately weighing 10 ± 0.1 mg of certified reference material and dissolving it in 10-L of ethyl acetate to obtain a concentration of 1000 $\mu\text{g mL}^{-1}$. A working standard solution (10 $\mu\text{g mL}^{-1}$) was prepared by serial dilution with ethyl acetate. Calibration standards of 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 $\mu\text{g mL}^{-1}$ were prepared and matrix-matched standards were generated using blank cowpea matrix extracts.

Sampling

Samples of cowpea pods were collected from each replication at 0 (2 h after spraying), 1, 2, 5, 7 and 10 days after the final application using a zigzag sampling pattern. The collected samples were placed in labelled sampling bags and stored at -20 °C before analysis to prevent pesticide degradation. Leaf samples were collected following the same protocol over a period of up to 10 days. Field soil samples were collected randomly from each replication at a depth of 0–15 cm at the same time intervals (0, 1, 2, 5, 7 and 10 days) after the last spray. Soil sampling was conducted to evaluate the dissipation pattern and residual levels of fipronil in the soil (23).

Sample extraction and clean-up

Sample extraction was performed using a modified QuEChERS method following established protocols with minor modifications to suit the crop matrix and target pesticide (22). The collected samples were finely chopped and homogenised using a mixer grinder. For extraction, 10 g of cowpea pod or leaf sample was mixed with 10 mL of ethyl acetate containing 1 % acetic acid, vortexed for 2 min and centrifuged at 6000 rpm for 5 min. An aliquot (1.5 mL) of the supernatant was subjected to dispersive solid-phase extraction (d-SPE) cleanup using 225 mg MgSO_4 , 75 mg PSA and 15 mg GCB. The cleaned extract was centrifuged at 10,000 rpm for 5 min and filtered through a 0.22 μm Nylon-6 membrane filter before GC- μECD analysis (Fig. 2). Soil samples (10 g) were pre-hydrated with 10 mL of water for 20 min and extracted following the same procedure using ethyl acetate as the extraction solvent (24, 25).

Instrumental conditions

Fipronil and its metabolite were analysed using an Agilent gas chromatography system (Model 7890B) equipped with an autosampler and a micro-electron capture detector (μECD , ^{63}Ni). The injector temperature was maintained at 275 °C and samples were introduced in split mode at a split ratio of 10:1 with an injection volume of 1 μL . Chromatographic separation was achieved on an HP-5 capillary column (5 % phenyl-methyl siloxane; 30 m \times 0.32 mm i.d., 0.25 μm film thickness). Nitrogen was used as the carrier gas at a flow rate of 1 mL min^{-1} , while nitrogen makeup gas was supplied at 10 mL min^{-1} . The detector temperature was maintained at 300 °C. The oven temperature program was set at 180 °C for 1 min, ramped to 250 °C at 10 °C min^{-1} and held for 5 min. Under these conditions,

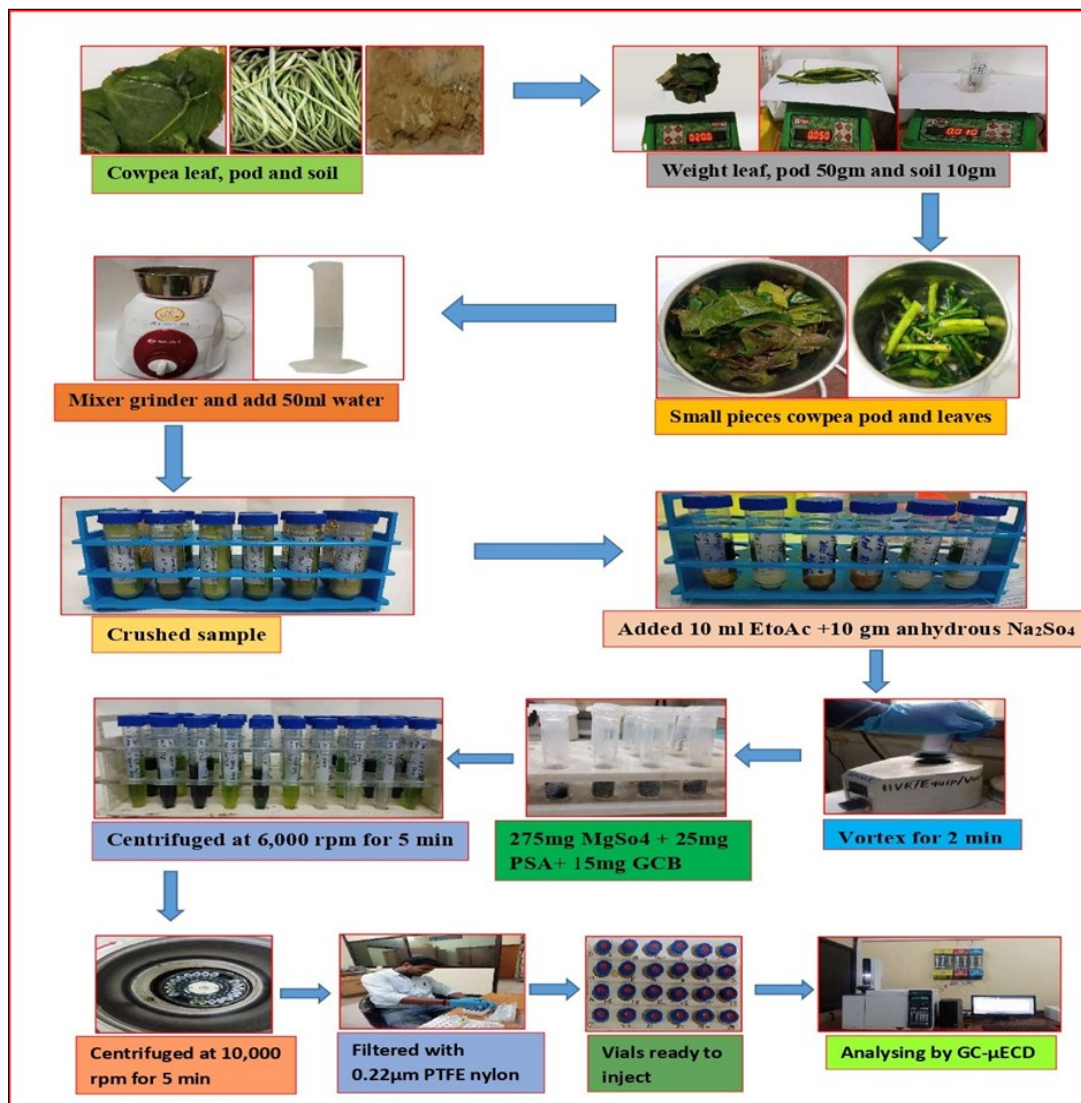


Fig. 2. Extraction and cleanup process of fipronil from cowpea pods, leaf and soil.

fipronil was detected at retention times of approximately 8.70 min in cowpea pod samples and 8.83 min in soil samples (Fig. 3-4). Residue quantification was performed by comparing the peak areas of unknown or spiked samples with those of matrix-matched calibration standards analysed under identical chromatographic conditions.

The peak areas of the matrix-matched standards and the unknown or spiked sample were compared for residue estimate under the same experimental conditions. Pesticide residue in mg kg^{-1} was calculated as per Equation 1.

$$\text{Residue (mg kg}^{-1}\text{)} = (X_1 \times Y_1 \times C) / (X_2 \times Y_2 \times W) \quad (\text{Eqn. 1})$$

Where X_1 represents the area corresponding to the field sample in the chromatogram, while X_2 denotes the peak area associated with the analytical standard. Y_1 is the total volume of the sample measured in mL and Y_2 is the injection volume expressed in microliters. The concentration of the analytical standard is given in mg kg^{-1} and W indicates the weight of the sample in grams.

Dissipation kinetics

Fipronil dissipation in samples was studied by subjecting the data to

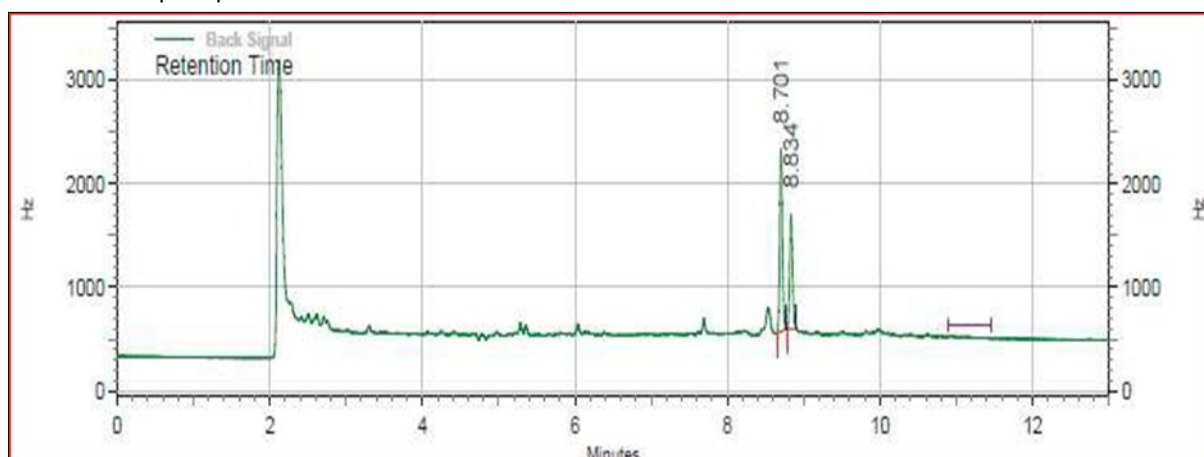


Fig. 3. Chromatogram of fipronil in cowpea pod.

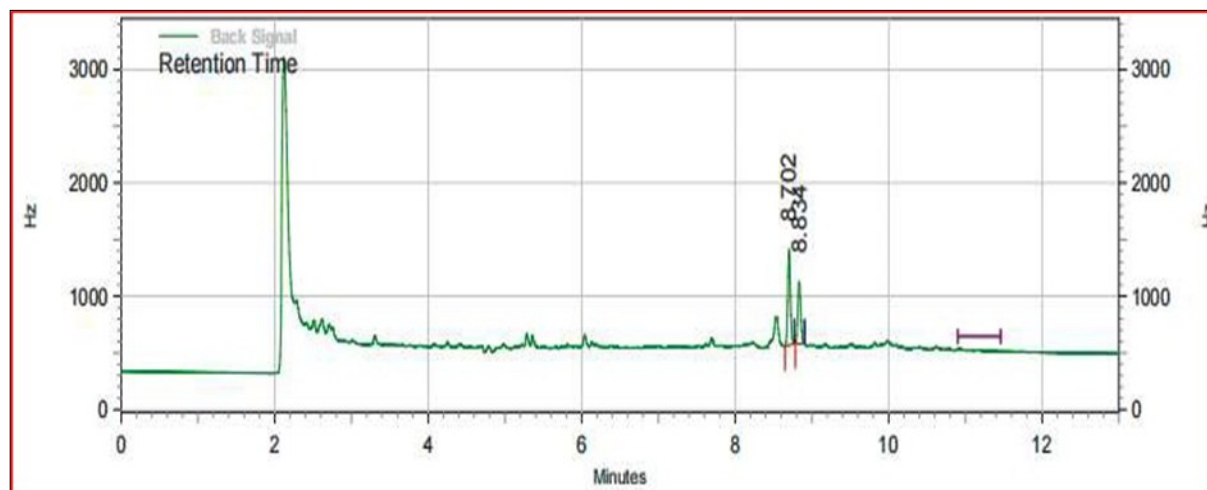


Fig. 4. Chromatogram of fipronil in the soil.

the first-order kinetics as given in Equation 2 (25).

$$A_t = A_0 e^{-kt} \quad (\text{Eqn. 2})$$

Where, A_t is a representation of the concentration at a specific time, t , where t is the duration, k is the rate constant for the pesticide or insecticides' dissipation and A_0 represents its initial concentration. In order to estimate the half-life ($t_{1/2}$) of the parent pesticide, the residue data underwent mathematical analysis according to equation 3 (25).

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

Results and Discussion

Method validation

The validation of the analytical method for assessing fipronil residues in cowpea pods, leaves and soil adhered to the SANTE guideline (24). At five distinct levels, the recovery percentage was assessed for both the entire cowpea pod and leaf samples, as well as for the soil matrices considered separately. The percentage recoveries at concentrations of 0.01, 0.02, 0.04, 0.08 and 0.1 mg kg^{-1} fell within the range of 80.00–90 % for all matrices (Table 1). The relative standard deviation (RSD) remained under 20 %, thus complying with the SANTE guidelines (24). Fipronil was identified during the entire runtime, which lasted approximately 13 min. In these conditions, the fipronil peak at 8.834 min, representing the retention time (RT), adhered to standard chromatographic conditions. The calibration curve, accompanied by the coefficient of determination (R^2), demonstrated high linearity for the method. Specifically, R^2 values were 0.9989 for the solvent standard, 0.9983 for the matrix-matched standard (cowpea pods), 0.9998 for the matrix-matched standard (leaf) and 0.9998 for the matrix-matched standard (soil).

Linear calibration was achieved in the range of 0.01–0.5 mg kg^{-1} (Fig. 5). For every matrix, the limits of detection (LOD) and quantitation (LOQ) were found to be 0.005 mg kg^{-1} and 0.01 mg kg^{-1} , respectively. On average, percentages of matrix effect (ME) were less than 20 %. The methodology is considered appropriate for determining fipronil residues in cowpea pods, leaves and soil matrices. The optimised data from the present investigation confirm the compliance with internationally accepted standards for estimating pesticide residues (27). Comparable findings were achieved through the application of a standardised liquid chromatographic technique (24). This method exhibited a recovery

Table 1. Percentage recovery of fipronil in cowpea pod, leaf and soil

Level of fortification (mg kg^{-1})	% Recovery	% RSD
Pod		
0.01	82.87	1.848
0.02	83.33	9.165
0.04	85.33	7.160
0.08	91.00	1.099
0.1	83.33	3.666
Leaf		
0.01	81.67	2.549
0.02	80.00	6.250
0.04	88.67	5.677
0.08	90.33	1.691
0.1	85.33	4.879
Soil		
0.01	81.67	1.870
0.02	84.00	9.091
0.04	84.00	2.381
0.08	92.00	2.876
0.1	90.67	6.739

RSD: Relative standard deviation

rate of up to 93.67 % for quantifying residues of acetamiprid and buprofezin pesticides in the paddy matrix, aligning with international standards. The National Sample Survey office effectively illustrated how the matrix-matched calibration technique plays a crucial role in minimising the effects of matrix-induced false pretension (28).

Kinetics of residue dissipation in cowpea pods, leaves and soil

Pesticide residues have the potential to degrade rapidly, influenced by processes such as microbial breakdown, volatilisation and microbial decay and environmental factors like weathering, heat and sunlight. Moreover, the metabolic processes in plants, especially during different growth phases such as when fruits reach maturity, can play a role in decreasing pesticide residues (29). The dissipation behaviour of fipronil initially exhibited a faster rate, which gradually decelerated over time, following an exponential degradation pattern consistent with first-order kinetics. The coefficient of determination (R^2) values were 0.9259 and 0.8729 for the recommended dose (RD) and double dose (DD), respectively (Fig. 6). The half-life of fipronil residues in cowpea pods was calculated as 1.74 days for RD and 1.55 days for DD (Table 2). Two hours after the final application, the initial residue levels in cowpea pods were 0.67 mg kg^{-1} for RD and 0.96 mg kg^{-1} for DD, representing the initial deposition of fipronil at the respective doses (Table 3). In leaves, fipronil residues also followed a well-defined exponential first-order dissipation pattern, with R^2 values of 0.9387 and 0.9922 for RD and DD, respectively (Fig. 7). The initial residue levels in leaves were 0.85 mg kg^{-1} for RD and 0.99 mg kg^{-1} for DD (Table 4). Notably, the half-life of fipronil in

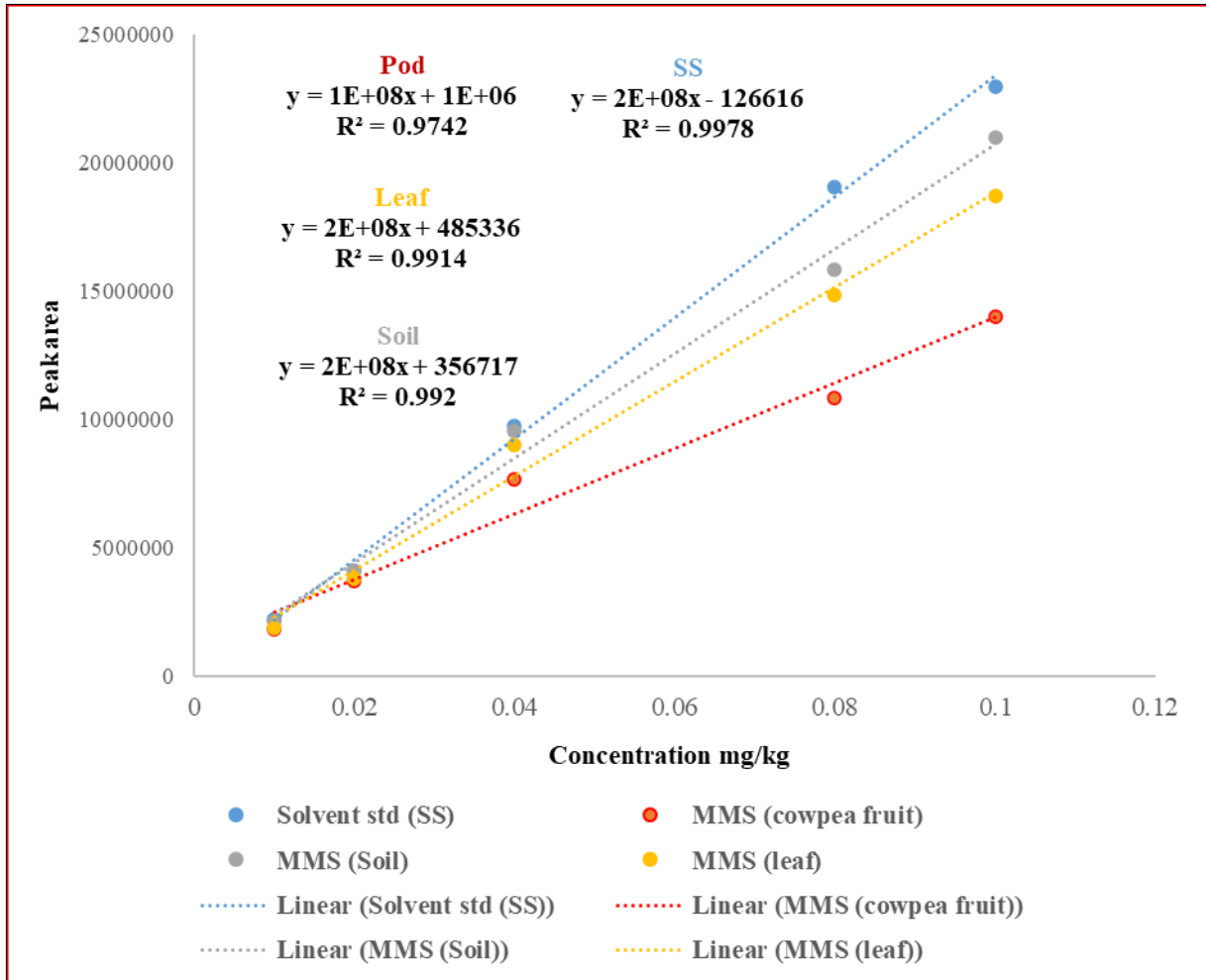


Fig. 5. Linearity graph for solvent standard and matrix-matched standard (MMS) for all the matrices.

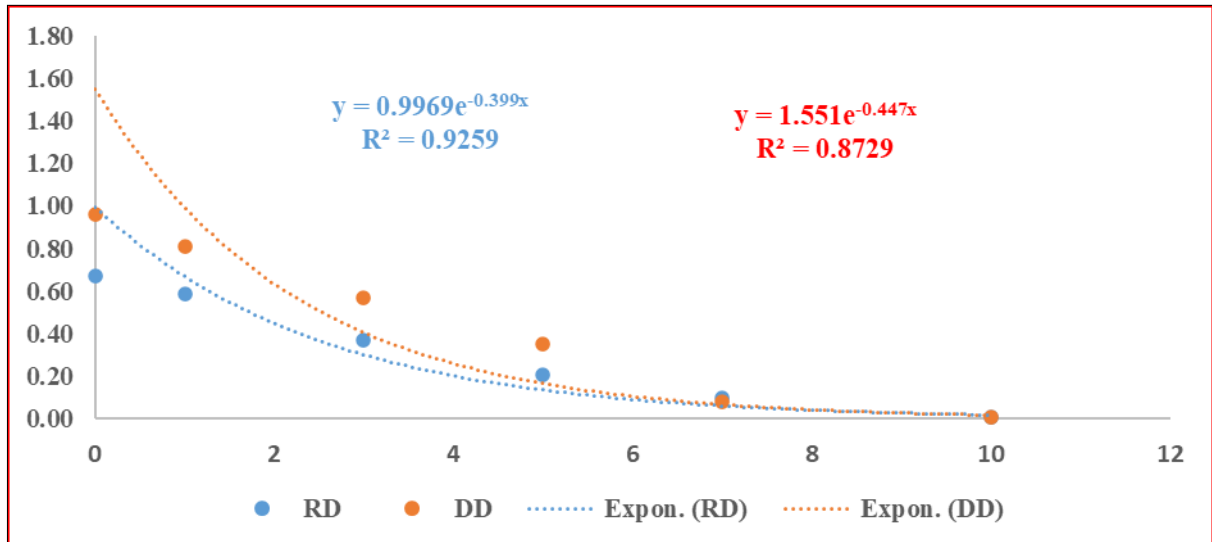


Fig. 6. Dissipation pattern of fipronil in cowpea pods under field conditions.

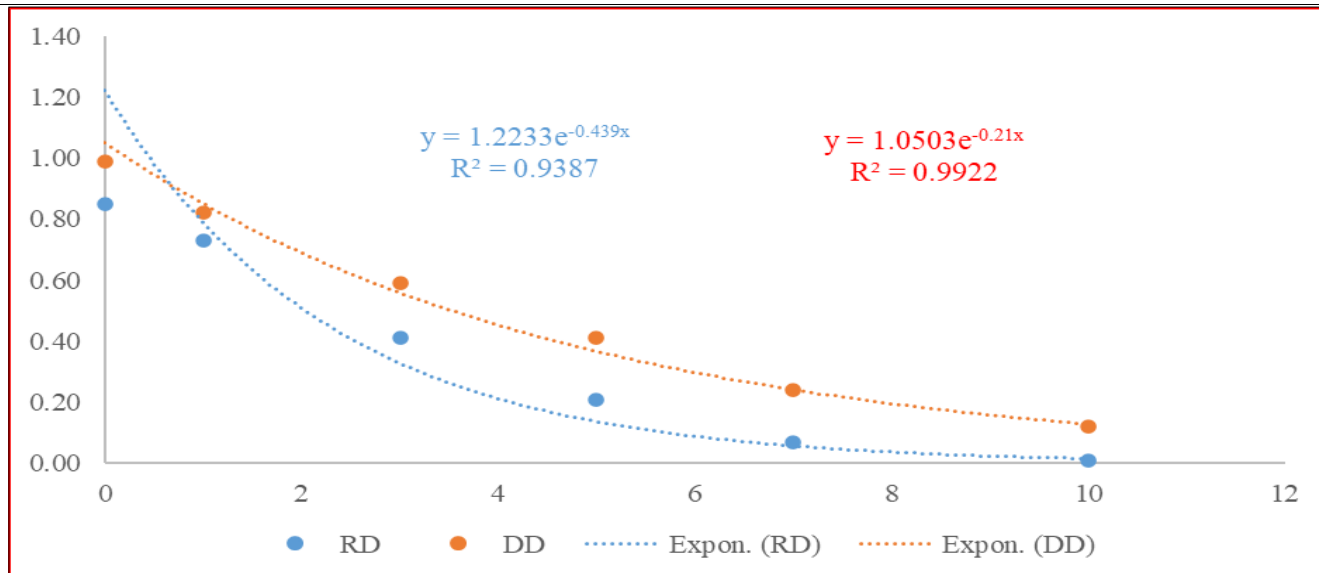
Table 2. Coefficient of determination and half-life of fipronil in different matrices of the cowpea

Dose	Regression equation	Coefficient of determination (R^2)		Half - lives ($t_{1/2}$)
		Pod		
RD	$y = 0.9969e^{-0.399x}$	0.9259		1.74
DD	$y = 1.551e^{-0.447x}$	0.8729		1.55
		Leaf		
RD	$y = 1.2233e^{-0.439x}$	0.9387		1.58
DD	$y = 1.0503e^{-0.21x}$	0.9922		3.30
		Soil		
RD	$y = 0.531e^{-0.398x}$	0.9982		1.74
DD	$y = 1.3294e^{-0.404x}$	0.9406		1.72

RD: Recommended dose; DD: Double dose

Table 3. Percentage reduction of fipronil residue on different days of sampling in cowpea pods

Days after spray	Recommended dose (RD)		Double dose (DD)	
	Residues (mg kg ⁻¹)	% decrease residue	Residues (mg kg ⁻¹)	% decrease residue
0	0.67	0.00	0.96	0.00
1	0.59	11.94	0.81	15.63
3	0.37	44.78	0.57	40.63
5	0.21	68.66	0.35	63.54
7	0.10	85.07	0.08	91.67
10	0.01	98.51	0.01	98.96

**Fig. 7.** Dissipation pattern of fipronil in leaf under field conditions.

leaves increased from 1.58 days at RD to 3.30 days at DD, indicating slower degradation at higher concentrations. In soil, the initial residue levels were 0.52 mg kg⁻¹ for RD and 0.89 mg kg⁻¹ for DD (Table 5). The dissipation of fipronil in soil also conformed to exponential first-order kinetics, with R² values of 0.9982 and 0.9406 for RD and DD, respectively (Fig. 8). The calculated half-lives in soil were 1.74 days for RD and 1.72 days for DD, showing slightly faster degradation compared to leaves at the higher dose (Table 2). These results collectively indicate that fipronil dissipates more rapidly in soil than in leaves at higher doses, likely due to microbial activity and soil physicochemical properties, whereas plant metabolism may be saturated at elevated residue levels. Our findings contribute to a comprehensive understanding of fipronil residue dynamics in cowpea and can aid in mitigating pesticide residue load in the ecosystem.

Consumer risk assessment

In all dosage scenarios, the residues underwent degradation until they fell below the Maximum residue limit (MRL). The daily limit for fipronil intake (ADI-acceptable daily intake) is established at 0.0002 mg kg⁻¹ of body weight. The multiplier is computed by dividing the ADI by a child's average body weight, which is 16 kg. This results in an estimated maximum permissible intake (MPI) for fipronil of 0.0032 mg per person per day, as shown in Table 6. As reported by the NSSO in 2012, the average daily consumption of cowpea per

person is 0.005 kg (28), which is below the MPI value of 0.0032 mg per person per day noted across all sampling days. This indicates that the levels of residues in the diet are within acceptable limits (26). Consequently, research has indicated that using fipronil for pest control in cowpea, when administered at a prescribed dose consistent with recognised agricultural practices, results in minimal risk.

Soil ecological risk assessment

The risk quotient (RQ) calculation enabled the evaluation of environmental risks for earthworms and other arthropods. The RQ values for earthworms (*E. foetida*) ranged from 0.52 to 0.01 for RD and 0.89 to 0.01 for DD, spanning the period from 0 (2 hr) to 10 days after the application of fipronil in the field soil. For the parasitic wasp (*Ahidius rhopalosiphii*) that preys on aphids, the RQ values ranged from 0.69 to 0.01 for RD and 1.19 to 0.01 for DD, as shown in Table 7. The RQ values reveal that fipronil residues present a negligible risk (RQ < 0.01) to a minor risk (0.01 ≤ RQ < 0.1) for earthworms. For arthropods, the RQ can range from almost negligible to low risk, except for the day right after the final DD application. Therefore, it can be inferred that using fipronil application on cowpea is environmentally safe for the soil when applied at the recommended dosages.

Table 5. Percentage reduction of fipronil residue on different days of sampling in soil.

Days after the spray	Recommended dose (RD)		Double dose (DD)	
	Residues (mg kg ⁻¹)	% Decrease residue	Residues (mg kg ⁻¹)	% Decrease residue
0	0.52	0.00	0.89	0.00
1	0.37	35.76	0.71	20.22
3	0.17	69.98	0.46	48.31
5	0.07	88.55	0.29	67.42
7	0.03	94.01	0.18	79.78
10	0.01	98.24	0.01	98.88

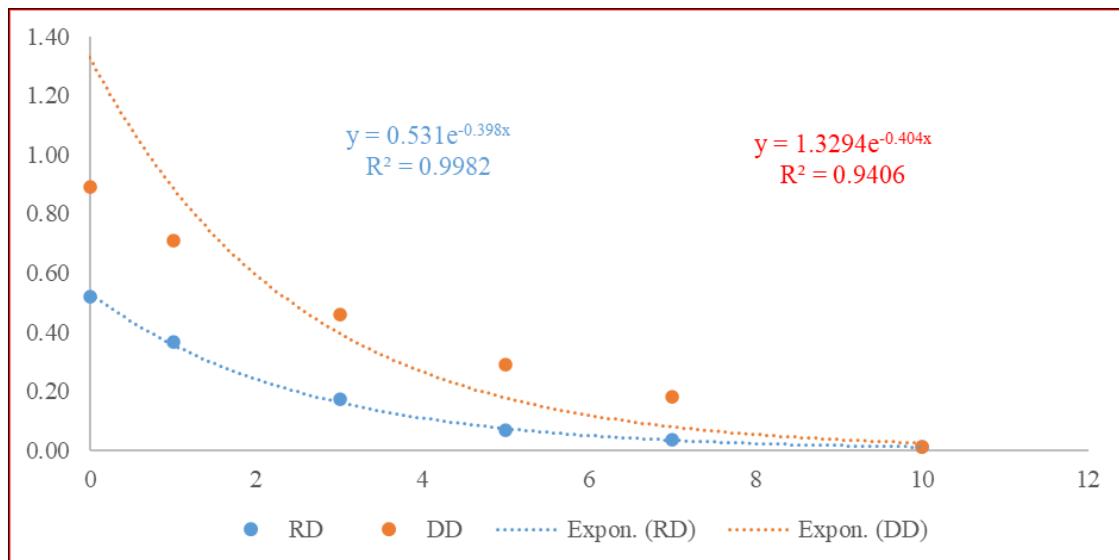


Fig. 8. Dissipation pattern of fipronil in the soil under field conditions.

Table 6. Safety evaluation of day-wise residues of fipronil in cowpea pods

Days after the spray	Recommended dose (RD)				Double the recommended dose (DD)			
	Residues (mg kg ⁻¹)		Dietary exposure (mg person ⁻¹ day ⁻¹)		Residues (mg kg ⁻¹)		Dietary exposure (mg person ⁻¹ day ⁻¹)	
	Rural	Urban	Rural	Urban	Rural	Urban	Rural	Urban
0	0.67	0.0020	0.00335	0.96	0.0029	0.0048		
1	0.59	0.0018	0.00295	0.81	0.0024	0.00405		
3	0.37	0.0011	0.00185	0.57	0.0017	0.00285		
5	0.21	0.0006	0.00105	0.35	0.0011	0.00175		
7	0.10	0.0003	0.0005	0.08	0.0002	0.0004		
10	0.01	0.0000	0.00005	0.01	0.0000	0.00005		

Table 7. Soil ecological risk assessment.

Days	Earthworms							
	RD	LC ₅₀	PNCE (mg/kg)	RQs	DD	LC ₅₀	PNCE (mg/kg)	RQs
0	0.52	>1000	1.00	0.52	0.89	>1000	1.00	0.89
1	0.37	>1000	1.00	0.37	0.71	>1000	1.00	0.71
3	0.17	>1000	1.00	0.17	0.46	>1000	1.00	0.46
5	0.07	>1000	1.00	0.07	0.29	>1000	1.00	0.29
7	0.03	>1000	1.00	0.03	0.18	>1000	1.00	0.18
10	0.01	>1000	1.00	0.01	0.01	>1000	1.00	0.01

Days	Arthropods							
	RD	LC ₅₀	PNCE (mg/kg)	RQs	DD	LC ₅₀	PNCE (mg/kg)	RQs
0	0.52	>750	0.75	0.69	0.89	>750	0.75	1.19
1	0.37	>750	0.75	0.49	0.71	>750	0.75	0.95
3	0.17	>750	0.75	0.23	0.46	>750	0.75	0.61
5	0.07	>750	0.75	0.09	0.29	>750	0.75	0.39
7	0.03	>750	0.75	0.05	0.18	>750	0.75	0.24
10	0.01	>750	0.75	0.01	0.01	>750	0.75	0.01

LC₅₀: Lethal concentration 50; RD: Recommended dose; DD: Double dose; RQ: Risk quotient; PNCE: Predicted no-effect concentration.

Conclusion

The validated GC- μ ECD method was reliable and sensitive for quantifying fipronil residues in cowpea pods, leaves and soil, with a low LOQ of 0.01 mg kg⁻¹, satisfactory recoveries and acceptable precision across all matrices. Fipronil dissipated rapidly under field conditions, exhibiting short half-lives in plant matrices (\approx 1.7 days) and even faster degradation in soil, irrespective of application rate. Dietary exposure assessment confirmed that residue levels were well below the maximum permissible intake, indicating minimal risk to consumers. Ecological risk assessment revealed negligible to low risk to earthworms and arthropods and negligible to medium risk to birds, with higher risk observed only immediately after application at the double dose. Among household processing methods, blanching was the most effective in reducing fipronil residues in cowpea pods. Future research should focus on multi-location and multi-season

field studies to assess the influence of varying agro-climatic conditions on fipronil dissipation. Detailed investigations into the role of plant metabolism, photodegradation, soil physicochemical properties, microbial activity and the behaviour of fipronil metabolites are also recommended to further refine environmental risk assessment and support safer and sustainable pest management practices in cowpea cultivation.

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Authors' contributions

SM contributed to methodology, investigation, supervision, data curation, writing original draft, writing. RK contributed to methodology and investigation. A contributed methodology and investigation. KS contributed the review and editing, formal analysis and data curation of the manuscript. PAD contributed to conceptualisation, formal analysis, writing-review & editing. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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