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RESEARCH ARTICLE

Interactive effects of soil moisture, organic amendment and herbicide application on fipronil degradation and structure functional potential of soil microbial community in tropical paddy soil

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Abstract

Fipronil degradation followed first-order kinetics. In sterile soil, residues declined slowly from 2.43–1.05 µg g^{-1} over 30 days, with 30 % dissipation and a half-life of 54.15 days. In non-sterile soil, degradation was faster, decreasing from 1.11–0.02 µg g^{-1}, corresponding to 79.28 % dissipation and a half-life of 11.53 days. Microbial activity accelerated degradation nearly fivefold, contributing 49 % to total dissipation. Fipronil application initially suppressed bacterial and fungal populations, which recovered within 10–30 days, while actinomycetes were largely unaffected. Organic matter (OM) under flooded conditions enhanced bacterial populations (6.61×10^6 CFU g^{-1} soil), whereas fungal populations were higher under OM at field-capacity moisture (4.04×10^4 CFU g^{-1} soil) at 30 days after application (DAA). Enzymatic activities showed transient inhibition followed by recovery, with higher dehydrogenase activity in flooded + OM soils (105 µg triphenyl formazan (TPF) g^{-1} soil 24 hr^{-1}) and increased urease (39 mg NH_4-N g^{-1} soil hr^{-1}) and phosphatase activities (61 µg PNP g^{-1} soil hr^{-1}) under field capacity + OM. Herbicide application consistently suppressed microbial and enzymatic activities across all moisture regimes. Recovery of non-target microbial populations beyond control levels suggests that fipronil and its metabolites may serve as nutrient or energy sources for specific soil microorganisms.

Keywords: fipronil; microbiota; moisture regime; organic matter amendment; soil enzymes

Introduction

Pesticides play a critical role in enhancing agricultural productivity; however, their persistence in the soil environment poses serious ecological risks. The fate of pesticides in soil is governed by several physico-chemical and biological factors, including their solubility, adsorption capacity, persistence and interactions with soil components such as clay, organic matter, moisture and microbial communities. In particular, soils rich in clay and organic matter tend to retain pesticides longer due to their greater surface area and the availability of adsorption sites, which limit pesticide mobility but may prolong their environmental presence (1).

Soil moisture is another key determinant of pesticide behaviour. Increased water availability can promote desorption of pesticides from soil particles, thereby enhancing their mobility and potential leaching. Flooded conditions pose the highest leaching/translocation risk for fipronil in paddy systems. Conversely, field capacity soils may retain pesticides longer, affecting both their efficacy and degradation. The persistence of pesticides is commonly assessed through their half-life, the time required for 50 % of the active ingredient to degrade. Longer half-lives increase the likelihood of environmental transport and accumulation (1).

Once in the soil, pesticides undergo a range of degradation processes including hydrolysis, photodegradation, redox reactions and importantly, microbial degradation. Microorganisms play a vital role in breaking down pesticides; however, their communities and functional activities can be significantly altered by pesticide exposure (2). Pesticide residues have been shown to suppress beneficial soil microbes, particularly nitrogen-fixing and phosphorus-solubilizing bacteria and to inhibit critical enzymatic activities, both of which are essential for maintaining soil health and fertility (3). There is limited information on how different pesticide formulations, residue levels and soil environmental conditions interact to regulate microbially mediated degradation pathways and enzyme dynamics over time, especially under field-relevant moisture and organic matter regimes.

Fipronil [5- amino-3-cyano-1- (2, 6-dichloro-4 trifluoromethylphenyl)-4-trifluoromethylsulfinyl pyrazole] is a phenylpyrazole insecticide and is one of the most persistent, lipophilic and toxic insecticides licensed for use since dieldrin, lindane and DDT (2). It controls a broad spectrum of insects such as rice stem borer, leaf folder, cockroaches, mosquitoes, locust, ticks and fleas at both their larval and adult stages (3). Fipronil is considered a "new generation" insecticide because its mode of action does not follow the common biochemical pathways of classical insecticides such as pyrethroids (sodium channel blockers), organophosphates and carbamates (cholinesterase inhibitors), to which some insects have developed resistance (4). Fipronil exerts its toxicity by blocking the GABA-gated chloride channel in the nervous system, resulting in disruption of neuronal signalling and eventual shutdown of the central nervous system. Fipronil degradation results in the formation of metabolites viz., sulfide, sulfone, amide and desulfinyl (3). These metabolites, except fipronil amide, are more or less toxic and persistent than fipronil and have been reported in diverse environmental samples (4). The half-life of fipronil in soil varies greatly, ranging from 3 days to 7 months. Fipronil, is of particular concern due to its persistence and toxicological effects on non-target soil biota. Its degradation in soil is influenced by multiple factors, including moisture regime, organic amendments and co-application with herbicides. Organic amendments, in turn, may enhance microbial activity and promote biodegradation, while herbicides can have synergistic or antagonistic interactions with insecticides, further affecting soil microbial dynamics. Few studies have demonstrated that repeated exposure to pesticides can promote the establishment of pesticide-degrading microorganisms that use pesticides as nutrient sources (5–7). However, the implications of such microbial adaptation for nutrient cycling efficiency and soil enzyme functionality are not yet well defined. This gap in understanding is especially critical in paddy soil ecosystems, where aerobic and anaerobic conditions driven by water management practices strongly influence microbial metabolic processes, redox-sensitive enzymatic activities and pesticide transformation pathways, thereby potentially intensifying or attenuating the ecological effects of fipronil.

Despite its widespread use, limited information is available on how interacting soil moisture conditions, organic amendments and herbicide co-application collectively influence fipronil degradation and the structure and functional potential of soil microbial communities in tropical paddy soils. Therefore, this study hypothesized that soil moisture regimes, organic amendments and herbicide co-application interactively affect fipronil degradation dynamics and modulate soil microbial community structure and enzymatic activities. To test this hypothesis, soil microcosm experiments were conducted to (i) investigate the biological degradation dynamics of fipronil and (ii) assess its effects on microbial populations and key enzyme activities in tropical paddy soil.

Materials and Methods

Study location

The laboratory study was conducted at Vishweshwaraiah Channel Farm (V.C. Farm), Mandya, Karnataka and biological analyses were carried out at the Department of Pathology, College of Agriculture, V.C. Farm, Mandya. Pesticide residue analysis to study the degradation of fipronil was performed at the Pesticide Residue Analysis Laboratory, Karnataka State Department of Agriculture, Banashankari, Bangalore.

Soil sampling

Soil for the study was collected randomly from the top layer (0–15 cm) of a paddy field at the College of Agriculture, V.C. Farm, Mandya, on 30 August 2024. The collected soil sample was homogenized and air-dried at room temperature in the shade. After drying, the soil was gently crushed using a mortar and pestle and sieved through a 2 mm sieve. The processed soil samples were stored at room temperature in airtight polyethylene containers and analyzed for physico-chemical properties. The methods used to assess these properties are outlined in Table 1.

Table 1. Methods to assess physico-chemical properties

Sl. No.	Properties	Methods	References
	A. Physical properties		
1	Particle size analysis	International pipette method	(8)
	B. Chemical properties		
1	pH (1: 2.5 soil water suspension)	Potentiometric method	(9)
2	Electrical conductivity (1:2.5 soil water extract)	Conductometric method	(9)
3	Organic carbon	Walkley and Black's wet oxidation method	(10)
4	Soil texture	Bouyoucos hydrometer	(11)
5	Cation exchange capacity (CEC)	Ammonium acetate leaching method	(9)
6	Bulk density	Keen's cup	(8)
7	MWHC	Keen's cup	(8)
8	Available nitrogen	Alkaline potassium permanganate method	(12)
9	Available phosphorus	Olsen's method using spectrophotometer	(9)
10	Available potassium and sodium	Ammonium acetate method	(8)
11	Exchangeable calcium and magnesium	Complexometric titration	

Chemical composition of pressmud used as soil amendment

Pressmud used as a soil amendment was obtained from Shri Chamundeswari Sugar Limited and analyzed for various physical and chemical properties viz. moisture content, maximum water holding capacity, pH, EC, organic carbon, N, P, K, Ca and Mg, following standard procedures. The methods adopted for the chemical analysis of pressmud compost used as an organic amendment are given in Table 2.

Table 2. Methods used for chemical analysis

Parameters	Methods	References
pH (1:10)	Potentiometric method	(9)
EC (1:10) (dS m^{-1})	Conductometric method	(9)
Organic carbon (%)	Dry combustion method	(10)
Total nitrogen (%)	Micro kjeldahl method	(8)
Total phosphorous (%)	Spectrophotometry	(8)
Total potassium (%)	Flame photometry	(8)
Calcium and magnesium (%)	Complexometry	(8)
Sulphur (%)	Turbidity using barium chloride	(13)
Fe, Mn, Zn, Cu (mg kg^{-1})	Atomic absorption spectrophotometer	(9)

Laboratory incubation study

Experimental setup

Paddy soil from Mandya (20 g) was accurately weighed into 50 mL Teflon centrifuge tubes, loosely capped, for 9 treatments comprising organic unamended, amended and herbicide-applied

soils under 3 moisture regimes: flooded, saturated and field capacity conditions. These soils were used for microbial population and enzymatic activity assessment. Treatment details are given in Table 3. T_1 to T_3 treatments were without organic amendment and herbicide. Pressmud compost was used as organic amendment for the T_4 to T_6 treatments and was thoroughly mixed with the soil at 0.5 % levels, corresponding to the recommended dose of farm yard manure for paddy fields. A combination of two active ingredients, bensulfuron-methyl (0.6 %) and pretilachlor (6 %) - a pre-emergence herbicide widely used in rice cultivation to control a broad spectrum of weeds was applied to T_7 to T_9 treatments to study the effect of herbicide interaction on fipronil degradation at 10 µg g^{-1} soil, corresponding to the recommended dose for paddy fields. These 9 treatment combinations were replicated thrice, with separate sets maintained for different intervals of fipronil application.

Table 3. Treatment details

Without organic matter	
T_1	Flooded condition
T_2	Saturated condition
T_3	Field capacity condition
With organic matter (Pressmud compost)	
T_4	Flooded condition
T_5	Saturated condition
T_6	Field capacity condition
Herbicide-bensulfuron methyl + Pretilachlor	
T_7	Flooded condition
T_8	Saturated condition
T_9	Field capacity condition

Prior to pesticide application, the soil samples were kept for 10 days at room temperature to allow mixture stabilization. 1 mL of aqueous fipronil suspension was added to each tube, resulting in a final concentration of 12.5 µg a.i. g^{-1} soil, corresponding to the recommended dose for paddy fields. The soil moisture content was maintained by adding deionized water to achieve saturated and field capacity moisture levels (measured by the Keen's cup method) on a weight basis, while a consistent water level was maintained for the flooded treatments by adding deionised water to a specified height. The tubes were stored in a dark, confined space at 30 ± 1 °C. Soil samples were collected for determination of microbial populations and enzyme activities at 1, 5, 15 and 30 DAA.

Redox potential

The redox potential (Eh) of the paddy soil was measured by inserting a combined waterproof ORP/redox meter (Eutech Instruments, USA) into the pot under different moisture regimes and recording the potential difference in mV. The redox potential was significantly negative under flooded conditions (-95 to -122 mV), indicating highly reduced conditions. The redox potential under saturated conditions was around 100–150 mV, suggesting mildly reducing conditions. The redox potential under field capacity moisture was positive (200–300 mV), indicating oxidizing conditions.

Microbial and enzyme activities in paddy soil

For bacteria, fungi and actinomycetes, microbial counts were determined using the standard serial dilution plate count method on nutrient agar, rose bengal agar and starch casein agar respectively (8). 10 g of soil in centrifuge tube kept at different interval was mixed with 90 mL sterilized water blank to give 10 dilutions. Subsequently, serial dilutions were prepared using 9 mL of sterile water blanks. Serial dilutions of the bacterial, fungal and actinomycete suspensions were made by aseptically transferring exactly 1 mL of the sample into sterile test tubes containing 9 mL of sterile saline solution. Each test tube was thoroughly mixed to ensure even distribution of microorganisms. Using a sterile 1 mL pipette, 1 mL of dilution (dilution like 10^2, 10^3, 10^4 and 10^5) transferred aseptically to sterile Petri dishes, cooled and melted agar media were added to their respective dilution. The plates (triplicate) were incubated at 30 ± 1 °C for 2–3 days for bacteria and actinomycetes and for 3–5 days for fungi. The number of microbial colonies found on the plates was counted and their population was expressed as CFU/g of soil for bacteria, fungi and actinomycetes. Media used for culturing microorganisms from soil samples are presented in Table 4.

Table 4. Media used for culturing microorganisms from soil samples

Microorganism	Media
Bacteria	Nutrient agar
Fungus	Martin's Rose Bengal Agar
Actinomycetes	Starch Casein Agar (SCA)

Soil enzyme activities were determined using standard colorimetric methods. Dehydrogenase activity was measured by incubating soil with triphenyl tetrazolium chloride (TTC) and glucose, followed by extraction of the formed triphenyl formazan (TPF) with methanol and recording absorbance at 485 nm (9). Phosphatase activity was estimated by incubating 1 g of soil with buffered sodium *p*-nitrophenyl phosphate and toluene and quantifying the *p*-nitrophenol released spectrophotometrically at 420 nm using a standard curve (10). Urease activity was assayed by incubating pre-treated soil with urea substrate in THAM buffer, terminating the reaction with KCl-Ag_2SO_4 solution and estimating unhydrolyzed urea colorimetrically using Nessler's reagent at 510 nm (11). Enzyme activities were expressed as mg TPF g^{-1} soil hr^{-1}, µmol *p*-nitrophenol g^{-1} soil hr^{-1} and µg urea hydrolyzed g^{-1} soil hr^{-1} respectively.

Fipronil degradation studies in sterile and non-sterile soils

To study the degradation rate of pesticides in sterile and non-sterile soils, two sets of duplicate 20 g air-dried paddy soil samples from Mandya were placed in 100 mL Erlenmeyer flasks and moistened to 80 % of field capacity. One set of Erlenmeyer flasks was tightly plugged with cotton and sterilized by autoclaving at 110 °C and 15 psi pressure for 30 min in an autoclave. The flasks were removed and sterilized again after 72 hr by autoclaving and the process was repeated once more. The contents were cooled in a laminar airflow chamber and the soil in each flask was treated with 1 mL of aqueous fipronil suspension, resulting in a final concentration of 12.5 µg a.i. g^{-1} soil, corresponding to the recommended dose of fipronil for paddy fields. The samples were incubated at 28 ± 1 °C in an incubator. The soil in each flask of the second set (non-sterile) was also treated with 1 mL of aqueous fipronil suspension (12.5 µg mL^{-1}) and incubated in a BOD incubator at 28 ± 1 °C. Soil samples were drawn at 0, 5, 10, 15, 20 and 30 days for the analysis of pesticide residues using liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) following the QuEChERS method (12). Blank sterile and non-sterile soil samples were also incubated under identical conditions.

Analysis of pesticide residue of fipronil and its metabolites

Extraction, purification and analysis of paddy soil for fipronil residue

Soil samples were extracted using a modified QuEChERS (Quick, easy, cheap, effective, rugged and safe) method for pesticide residue analysis. 10 g of soil (in triplicate) were placed in Teflon centrifuge tubes and deionized water was added to adjust the moisture to saturated and field capacity levels on a weight basis. Each sample received 10 mL of acetonitrile, 6 g of anhydrous sodium sulfate and 1.5 g of sodium acetate, followed by vortexing and shaking on a rotospin at 50 rpm for 15 min. Sodium acetate was used instead of citrate salts to maintain a mildly acidic pH (~5.0 –5.5), which enhances the stability of fipronil and minimizes hydrolytic degradation during extraction. In addition, acetate buffering provides effective pH control with lower salt complexity, thereby reducing matrix interferences and yielding cleaner extracts.

Samples were then centrifuged at 5000 rpm for 5 min and 2 mL of the supernatant was transferred into 5 mL Eppendorf tubes containing dispersive solid-phase extraction (d-SPE) sorbents (150 mg magnesium sulfate and 100 mg primary secondary amine (PSA)). The tubes were vortexed and centrifuged again at 10000 rpm for 5 min. The upper solvent layer (1.5 mL) was collected, filtered through a 25 mm, 0.2 µm nylon syringe filter and transferred into LC-MS/MS vials for analysis. For recovery studies, blank soil samples were fortified with fipronil standard solutions at concentrations of 5, 10, 50 and 100 µg g^{-1} to carry out recovery tests.

Analytical procedure by LC-MS/MS

All samples were analysed using a Shimadzu LCMS-8045 equipped with a triple quadrupole MS/MS system. Chromatographic separation was performed using a Shim-pack GISS C18 column (100 mm × 2.1 mm × 3 µm particle size), containing an octadecyl stationary phase with high-purity porous spherical silica support. The mobile phase was isocratic, consisting of methanol/water (50:50, v/v), with a flow rate of 0.4 mL/min. Nitrogen was used as the MS/MS system gas at a constant flow rate of 3.0 L/min and the source temperature was maintained 375 °C. The nebulizer pressure was 35.0 psi and the injection volume was 1 µL. Spectral acquisition was performed using negative electrospray ionization (ESI) mode, with multiple reaction monitoring (MRM). A standard solution of 1 µg g^{-1} of the parent compound was initially scanned in SCAN mode over an m/z range of 330–451. Based on the m/z ratios obtained, the parent compound (fipronil) was subsequently analysed in MRM mode using selected precursor and product ion transitions.

Instrumentation-method validation

Calibration linearity (R^2) and analytical sensitivity were evaluated by plotting a calibration curve (0.001–0.100 µg g^{-1}) using standards. The limit of detection (LOD) and limit of quantification (LOQ) were calculated by dividing three and ten times respectively, the average standard deviation of the peak area across all calibration levels by the slope of the calibration curve (13). The trueness and precision of the method were assessed through a recovery experiment conducted by spiking the control matrix with the respective compounds at different concentration levels of 0.005, 0.010 and 0.100 µg g^{-1}, followed by processing using the aforementioned method. Precision was evaluated by calculating the relative standard deviation (RSD) in terms of intra-day repeatability check.

Statistical analysis

Individual datasets on fipronil residues and other soil parameters were statistically analyzed using IBM SPSS Statistics version 27.0.1 software package. Treatment effects were assessed using analysis of variance (ANOVA) under a factorial completely randomized design (CRD). Differences among treatment means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. The dissipation curves of fipronil were analyzed using non-linear regression in Microsoft Excel. Degradation followed first-order kinetics, described by the equation $C = C_0 e^{-kt}$, where C is the chemical concentration (mg kg^{-1}) at time t (days), C_0 is the initial concentration (mg kg^{-1}) and k is the first-order rate constant (d^{-1}), independent of C and C_0. The correlation coefficient (R^2) was used to evaluate the congruence between the data and the first-order kinetic model. The dissipation of these compounds was expressed as DT_{50} ($t_{1/2}$), the time required for 50 % of the pesticide to degrade, calculated using Hoskins' formula: $DT_{50} = \ln(2)/k$.

Results and Discussion

Soil characteristics

Soil under study is sandy clay loam in texture, with sand 50.3 %, silt 19.5 % and clay 30.2 %. It belongs to Alfisol (Typic Rhodustalfs). The soil had low bulk density (1.32 g cm^{-3}), 23.66 % water holding capacity (WHC). Soil was alkaline in pH (8.38), medium in organic carbon content (0.60 %), had normal soluble salt content (EC = 0.28 dS m^{-1}) and medium cation exchange capacity (CEC = 17.7 cmol (p^+) kg^{-1} soil), with medium available nitrogen, available phosphorus, exchangeable calcium, exchangeable magnesium, exchangeable sodium and low available potassium levels (Table 5).

Chemical composition of pressmud used as soil amendment

The pressmud was nearly neutral (pH = 7.74), with 35.65 % total organic carbon (OC). 1.28 % total nitrogen (C:N ratio 27.85), 3.3 % total phosphorus, 1.82 % total potassium, 2.5 % total calcium, 1.0 % total magnesium, 1.20 % total sulfur, 115 ppm total Zn, 80 ppm total Cu, 400 ppm total Fe and 450 ppm total Mn (Table 5).

Table 5. Physico-chemical characteristics of paddy soil and chemical composition of press mud compost used in the study

Particulars	Mandya soil	Particulars	Press mud compost
pH	8.38	pH	7.74
EC	0.28	Total OC (%)	35.65
Total organic carbon (%)	0.6	Moisture content(%)	55.25
Sand (%)	50.3	MWHC (%)	62.73
Silt (%)	19.5	Total Nitrogen (%)	1.28
Clay (%)	30.2	C:N ratio	27.85
Soil texture	Sandy Clay Loam	Total Phosphorus (%)	3.3
Bulk density(gcc^{-1})	1.32	Total Potassium (%)	1.82
CEC (cmol(p^+)/kg)	17.7	Total Ca (%)	2.5
MWHC (%)	23.66	Total Mg (%)	1.0
Available N (kg ha^{-1})	401.4	Total S(%)	1.20
Available P_2O_5 (kg ha^{-1})	53.60	Total Zn (ppm)	115
Available K_2O (kg ha^{-1})	75.66	Total Cu (ppm)	80
Exchangeable Ca (me/100g)	5.8	Total Fe (ppm)	400
Exchangeable Mg (me/100g)	2.6	Total Mn (ppm)	450
Exchangeable Na (me/100g)	3.8		

Note: MWHC-Maximum water holding capacity

Validation of LC-MS/MS

Calibration curve for fipronil

The 100 µg g^{-1} stock solutions of fipronil were prepared. This solution was diluted to 0.001, 0.005 0.01, 0.025, 0.05, 0.75 and 0.1 µg g^{-1} by taking desired amount of stock solution. One microliter sample of each solution was injected in LC-MS/MS. The detector response was calculated in terms of peak area. A calibration curve was plotted against the average detector response in terms of peak area and concentration (Table 6, Fig. 1, 2).

Table 6. Linearity (R^2) and sensitivity of (0.005–0.100 µg g^{-1}) of fipronil standard

Actual Concentration (µg g^{-1})	Area	Observed Concentration (µg kg^{-1})
BLANK	-	-
0.005	585622	5.6609
0.010	1029554	9.257
0.025	2672967	22.5694
0.050	6379376	52.5931
0.075	8711682	71.4859
0.100	12655618	103.4337

Recovery experiment of fipronil in paddy soil

Recovery experiments were carried out to ensure that the methods used in the analysis of paddy soil samples were accurate and reliable. Untreated paddy soil samples were fortified (spiked) with standard solutions of fipronil at various fortification levels. Fipronil recovery studies in paddy soil were also carried out to ensure the accuracy of the findings. The paddy soil sample was spiked with fipronil at 0.005, 0.01 and 0.1 µg g^{-1} fortification levels and analyzed. The mean percent recovery (Table 7) of the fipronil in paddy soil was found to be consistent (86.4 % to 95.47 %). The matrix effect was calculated using the following equation: ME % = (Peak area of matrix standard - Peak area of solvent standard)/Peak area of solvent standard *100. The Table 7 highlighted that ME fell below 20 %. If the % ME fell less than 20 %, the matrix effect was considered negligible (13).

Rate kinetics of fipronil degradation in sterile and non-sterile soil

The role of microbial activity in the degradation of fipronil was evaluated by comparing dissipation patterns in sterile and non-sterile paddy sandy clay loam soils. Sterilization was achieved by autoclaving to eliminate biological activity, thereby allowing assessment of non-biological degradation processes alone.

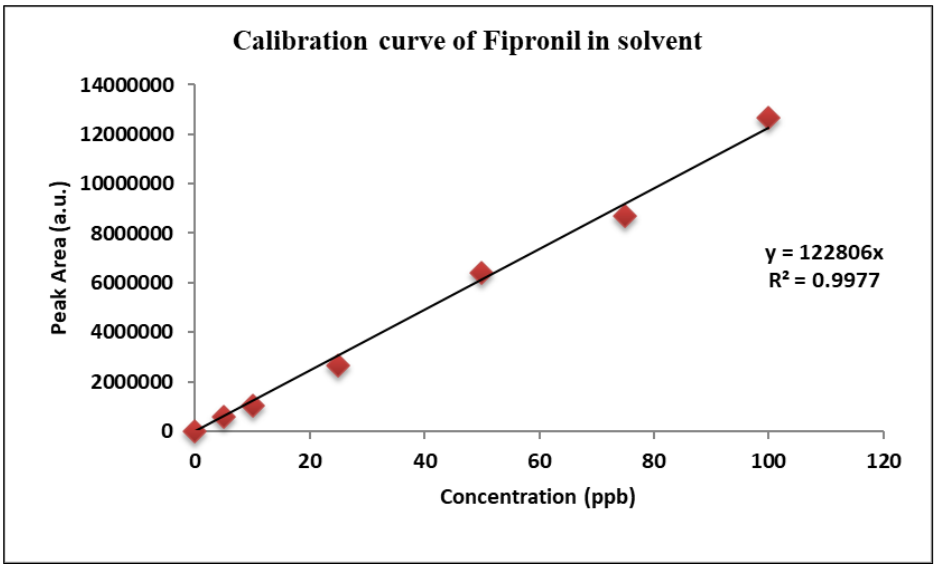

Fig. 1. A standard curve of fipronil in solvent.

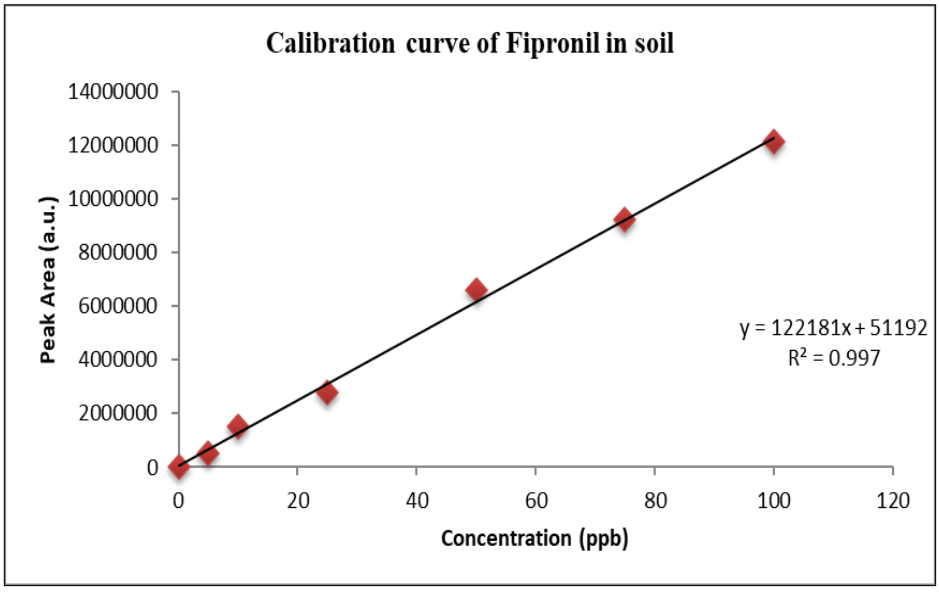

Fig. 2. A standard curve of fipronil in paddy soil.

Table 7. Recovery results and Matrix effect of fipronil in paddy soil

Spiking level µg g^{-1}	R (%)	RSD (%)	ME (%)
0.05	86.40	7.23	-18.66
0.10	90.50	5.99	-16.45
1.0	95.47	4.99	-4.56

Under sterile conditions, the initial residue of fipronil was 2.43 µg g^{-1} which dissipated to 2.18, 1.98, 1.50 and 1.05 µg g^{-1} at 5, 10, 15 and 30 days respectively. In non-sterile soil, the initial deposit was 1.11 µg g^{-1}, which dissipated to 0.79, 0.40, 0.11 and 0.02 µg g^{-1} over the same time intervals. The dissipation curves of fipronil showed a gradual loss under sterile conditions and a rapid loss under non-sterile conditions, with 30 % and 79.28 % dissipation respectively, after 30 days of incubation (Fig. 3). The dissipation of fipronil in both sterile and non-sterile soils followed first-order kinetics, exhibiting a linear relationship between incubation time and the logarithm of pesticide concentration remaining in soil (Table 8, Fig. 4). The half-lives of fipronil in sterile and non-sterile soils, calculated from the slopes of the dissipation curves, were 54.15 and 11.53 days respectively. This indicates a nearly five-fold increase in the degradation rate of fipronil in non-sterile soil compared with sterile soil, highlighting the significant contribution of soil microorganisms to fipronil degradation.

For all the pesticides, decomposition was relatively slow under sterile conditions and quite rapid under non-sterile conditions. In the absence of microbial activity, sterile soil is presumed to behave like normal soil in terms of its adsorption characteristics. The persistence of pesticides was greater in sterile than in non-sterile sandy clay loam soil, indicating a positive role of soil microbes in pesticide degradation. Microbial activity contributed approximately 49.3 % to the total degradation of fipronil (Fig. 3). These results indicated that, in non-sterile soil, both biological and non-biological agencies were responsible for degradation of pesticides, whereas in sterile soil, only non-biological agencies were involved. Therefore, it can be concluded that the higher degradation observed in non-sterile soil was due to soil microorganisms.

In the present study, the sandy clay loam soil also favoured faster dissipation under non-sterile conditions, consistent with the premise that microbial viability and adaptability strongly influence degradation dynamics. Several studies on the microbial degradation of fipronil have reported the formation of the fipronil sulfide metabolite, confirming the role of microbial enzymes in this process (14–18).

The higher persistence of fipronil in sterile soil can be attributed to the absence of active microbial populations, limiting degradation processes to abiotic mechanisms such as hydrolysis, oxidation and reduction. The gradual decrease in residue under sterile conditions suggests that physico-chemical interactions such as sorption, coupled with limited abiotic degradation, primarily governed fipronil dissipation. In contrast, dissipation in non-sterile soil was much more rapid, with 79.28 % degradation observed by 30 DAA, compared to 30 % under sterile conditions. This accelerated degradation can be linked to microbial enzymatic activity that metabolizes fipronil into fipronil sulfide, sulfone and amide, as reported in earlier studies (14).

The contribution of microbial activity to fipronil degradation in the present study was approximately 49.3 %, confirming that biological processes play a dominant role compared to abiotic mechanisms. This observation is further supported by isolation studies of *Paracoccus* sp., *Bacillus* strains and *Streptomyces rochei*, all of which have demonstrated the ability of soil microorganisms to utilize fipronil as a carbon and energy source (15–17). These microbial degraders not only accelerate fipronil dissipation but also transform it into metabolites, some of which can undergo complete mineralization (14).

The persistence of fipronil under sterile conditions suggests that soil adsorption and abiotic degradation may delay its mobility but do not eliminate it rapidly. This observation aligns with other studies, which have emphasized the role of physico-chemical properties such as solubility, hydrophobicity and ionization potential, in determining pesticide behaviour in soil. The sandy clay loam texture with moderate organic matter likely facilitated sorption, thereby limiting bioavailability and slowing abiotic degradation (18).

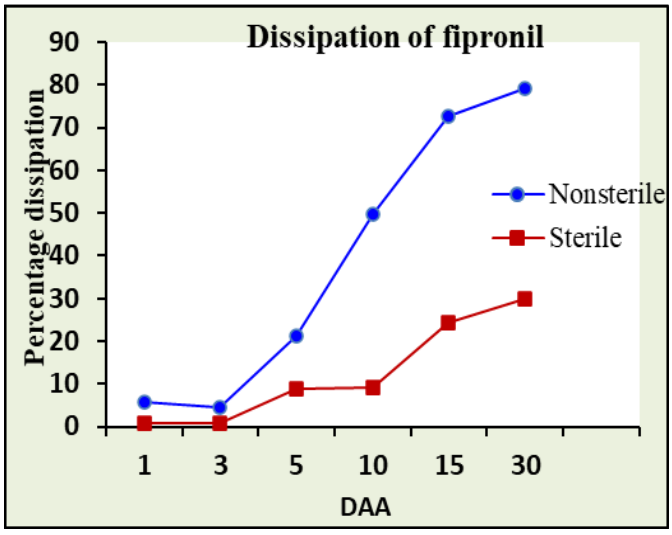

Fig. 3. Percent dissipation of fipronil in sterile and non-sterile paddy soil.

Table 8. Residues and percent dissipation of fipronil (µg g^{-1}) in sterile and nonsterile paddy soil

DAA	Nonsterile		Sterile	
	Average Residue level (µg g^{-1})	Dissipation (%)	Average Residue level (µg g^{-1})	Dissipation (%)
0	1.11 ± 0.08	-	2.43 ± 0.32	-
1	1.05 ± 0.14	5.86	2.41 ± 0.21	0.82
3	1.00 ± 0.05	4.60	2.39 ± 0.15	0.83
5	0.79 ± 0.05	21.31	2.18 ± 0.10	8.79
10	0.40 ± 0.05	49.65	1.98 ± 0.26	9.17
15	0.11 ± 0.01	72.73	1.50 ± 0.08	24.24
30	0.02 ± 0.002	79.28	1.05 ± 0.07	30.00
$t_{1/2}$= ln (2)/k	11.53 days		54.15 days	
Equation	y = -0.0601x + 0.1124		y = -0.0128x + 0.3996	
R^2	0.98		0.98	

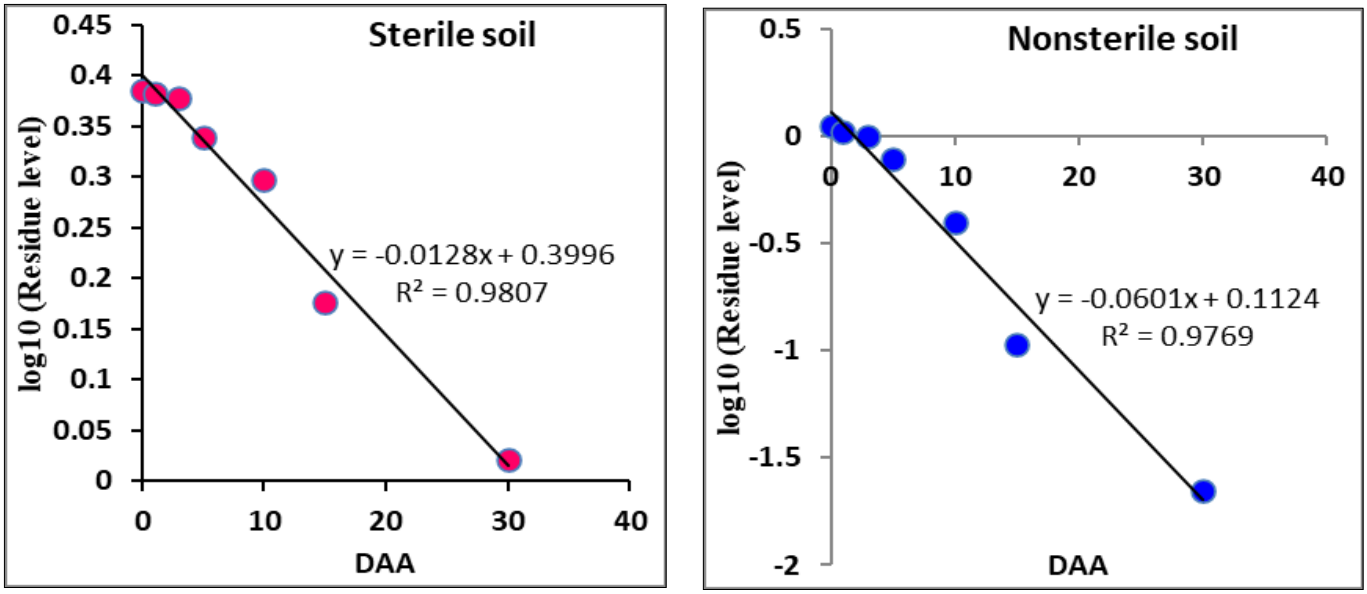

Fig. 4. First order dissipation of fipronil in sterile and nonsterile paddy soil.

Overall, the study demonstrates that microbial activity substantially accelerates fipronil degradation in paddy soils, thereby reducing its persistence and potential carryover risk. The results reinforce the role of microorganisms as key regulators of pesticide fate, consistent with literature on other pesticides showing faster degradation in biologically active soils (19). The higher dissipation rate in non-sterile soil underscores the ecological importance of maintaining microbial diversity and activity in agricultural soils to ensure faster breakdown of pesticides and to minimize environmental contamination risks.

Microbial activities in paddy soil

Effect of fipronil on bacterial population in paddy soil

The initial bacterial population in paddy soil was 5.34 × 10^6 CFU g^{-1} soil. Following fipronil application, a significant decline in bacterial numbers was observed on the first day, with populations decreasing to 0.94-2.32 × 10^6 CFU g^{-1} soil across treatments (Table 9, Fig. 5A). This initial suppression was transient and by the 5th DAA, bacterial populations showed a significant recovery, increasing to 3.23-6.73 × 10^6 CFU g^{-1} soil. At the end of the incubation period (30 DAA), bacterial counts ranged from 3.11-6.61 × 10^6 CFU g^{-1} soil, approaching levels comparable to the untreated control. In the control soil, bacterial populations remained relatively stable throughout the study, varying between 4.95 and 5.34 × 10^6 CFU g^{-1} soil. Combined application of herbicide with fipronil resulted in a marked reduction in bacterial population compared to fipronil alone. In this treatment, bacterial counts declined sharply to 0.94-0.98 × 10^6 CFU g^{-1} soil at 1 DAA and increased only to 3.23-4.21 × 10^6 CFU g^{-1} soil by 5 DAA, indicating prolonged suppression. Among soil amendments, organically amended soil supported significantly higher bacterial populations (5.66-6.61 × 10^6 CFU g^{-1} soil at 30 DAA) compared to organic unamended soil (4.42-5.11 × 10^6 CFU g^{-1} soil at 30 DAA). Under different moisture regimes, flooded conditions consistently recorded higher bacterial populations, with values of 5.11, 6.61 and 4.09 × 10^6 CFU g^{-1} soil in organic unamended (T_1), organic amended (T_4) and herbicide-treated (T_7) soils respectively, compared to the control.

The initial decline in bacterial population following fipronil application indicates a short-term toxic or inhibitory effect on soil microorganisms. Such early suppression is commonly observed after pesticide application and is attributed to physiological stress imposed on sensitive microbial groups. Similar transient inhibitory

Table 9. Changes in microbial population in soil under different moisture regimes, organic amendment and herbicide

DAA	Bacteria(× 10^6 cfu g^{-1} soil)				Fungi(× 10^4 cfu g^{-1} soil)				Actinomycetes (× 10^2 cfu g^{-1} soil)			
	1	5	15	30	1	5	15	30	1	5	15	30
T_1	2.32 ^{c}	6.12 ^{g}	5.15 ^{ef}	5.11 ^{cd}	0.80 ^{a}	3.76 ^{bc}	3.30 ^{bcd}	3.34 ^{bcd}	0.83 ^{bc}	0.86 ^{ab}	0.87 a	0.88 a
T_2	1.38 ^{b}	5.23 ^{ef}	5.03 ^{de}	4.99 ^{cd}	0.82 ^{a}	3.98 ^{c}	3.34 ^{bcd}	3.36 ^{bcd}	0.83 ^{bc}	0.87 ^{an}	0.88 a	0.88 a
T_3	1.34 ^{b}	4.54 ^{cd}	4.46 ^{cd}	4.42 ^{bc}	0.82 ^{a}	4.02 ^{c}	3.68 ^{cde}	3.62 ^{cde}	0.84 ^{bc}	0.88 ^{ab}	0.89 a	0.89 a
T_4	2.42 ^{c}	6.73 ^{h}	6.65 ^{h}	6.61 ^{f}	1.00 ^{b}	4.56 ^{d}	3.88 ^{def}	3.80 ^{de}	0.93 ^{c}	0.96 ^{b}	1.12 b	1.10 b
T_5	2.22 ^{c}	5.78 ^{fg}	6.04 ^{gh}	6.00 ^{ef}	1.01 ^{b}	4.89 ^{d}	4.21 ^{ef}	4.02 ^{e}	0.93 ^{c}	0.96 ^{b}	1.13 ^{b}	1.10 ^{b}
T_6	1.36 ^{b}	5.11 ^{de}	5.70 ^{fg}	5.66 ^{de}	1.02 ^{b}	5.02 ^{d}	4.34 ^{f}	4.04 ^{e}	0.95 ^{c}	0.98 ^{b}	1.14 ^{b}	1.12 ^{b}
T_7	0.98 ^{a}	4.21 ^{bc}	4.13 ^{bc}	4.09 ^{b}	0.72 ^{a}	3.34 ^{ab}	2.66 ^{a}	2.71 ^{a}	0.17 ^{a}	0.78 ^{a}	0.88 ^{a}	0.84 ^{a}
T_8	0.96 ^{a}	3.87 ^{b}	3.79 ^{b}	3.75 ^{ab}	0.73 ^{a}	3.43 ^{ab}	2.97 ^{ab}	3.05 ^{ab}	0.17 ^{a}	0.80 ^{a}	0.89 ^{a}	0.85 ^{a}
T_9	0.94 ^{a}	3.23 ^{a}	3.15 ^{a}	3.11 ^{a}	0.74 ^{a}	3.65 ^{bc}	3.25 ^{abc}	3.20 ^{abc}	0.17 ^{a}	0.81 ^{a}	0.90 ^{a}	0.86 ^{a}
T_{10}	5.34 ^{d}	5.08 ^{de}	5.00 ^{de}	4.95 ^{cd}	3.08 ^{c}	3.09 ^{a}	3.19 ^{abc}	3.25 ^{bc}	0.79 ^{b}	0.81 ^{a}	0.85 ^{a}	0.86 ^{a}
R^2	**0.98**	**0.89**	**0.88**	**0.83**	**0.98**	**0.78**	**0.82**	**0.74**	**0.96**	**0.44**	**0.7**	**0.67**

Values marked by different letter differ significantly according to Duncan's multiple range test ($p \leq 0.05$)

T_1:	FL + WOM	T_5:	ST + OM	T_9:	FC + WD
T_2:	ST + WOM	T_6:	FC + OM	T_{10}:	Control
T_3:	FC + WOM	T_7:	FL + WD		
T_4:	FL + OM	T_8:	ST + WD		

DAA- Days after application of fipronil; FL- flooded; ST- saturated; FC- field Capacity; WOM-without organic amendment; OM- organic amendment (press mud); WD- herbicide (Bensulfuron methyl 0.6 % + Pretilachlor 6 % GR); Control-without crop + FC + WOM.

effects have been reported for fipronil and other pesticides, where microbial populations decrease shortly after exposure but recover as pesticide degradation proceeds and microbial adaptation occurs (6).

The subsequent recovery of bacterial populations after five days and their return to near-normal levels by 30 DAA suggests that soil microorganisms adapted to the presence of fipronil. This adaptation may involve the selection of tolerant or pesticide-degrading microbial communities capable of utilizing fipronil or its metabolites as carbon or energy sources. The observed recovery supports earlier findings that microbial communities in agricultural soils can regain functional stability following initial pesticide-induced stress.

Organic amendment played a crucial role in enhancing bacterial populations, particularly at later stages of incubation. The higher bacterial counts observed in organically amended soils indicate that organic matter improves nutrient availability, enhances microbial habitat and buffers the toxic effects of pesticides, thereby supporting microbial resilience and regrowth (7). In contrast, the combined application of herbicide and fipronil resulted in prolonged suppression of bacterial populations, suggesting additive or synergistic toxicity under multiple chemical stresses. This highlights the potential ecological risk of pesticide mixtures, especially when applied simultaneously at field-relevant doses (20).

Soil moisture regime also significantly influenced bacterial dynamics. Flooded conditions supported higher bacterial populations across treatments, likely due to improved substrate availability, reduced pesticide toxicity under anaerobic conditions and enhanced microbial metabolic activity. These findings are consistent with earlier reports indicating that recommended doses of fipronil do not adversely affect microbial biomass and enzyme activity under favourable moisture conditions (21).

Overall, the results demonstrate that while fipronil initially suppresses soil bacterial populations, this effect is temporary and strongly modulated by soil amendments and moisture regime. The addition of organic matter and flooded conditions promote microbial recovery, whereas combined pesticide applications exert sustained negative effects. These observations emphasize the importance of soil management practices in mitigating pesticide-induced microbial stress and maintaining soil biological health in paddy ecosystems.

Effect on fungal population in paddy soil

The initial fungal population in paddy soil was 3.08×10^4 CFU g^{-1} soil. Following fipronil application, a significant decline in fungal numbers was recorded at 1 DAA, with populations decreasing to $0.72\text{-}1.02 \times 10^4$ CFU g^{-1} soil across treatments (Table 9 and Fig. 5B). This initial reduction was temporary and a significant increase in fungal population was observed by the fifth day. By 15 DAA, fungal counts increased to $2.66\text{-}4.34 \times 10^4$ CFU g^{-1} soil and at the end of the experiment (30 DAA), populations ranged from $2.71\text{-}4.04 \times 10^4$ CFU g^{-1} soil, approaching pre-application levels. In the untreated control (T_{10}), fungal populations remained stable throughout the study, varying narrowly between 3.09 and 3.25×10^4 CFU g^{-1} soil. The combined application of herbicide with fipronil caused the greatest suppression of fungal populations, with counts declining to $0.72\text{-}0.74 \times 10^4$ CFU g^{-1} soil at 1 DAA. However, even under these treatments, fungal populations showed significant recovery after five days. Among soil amendments, organically amended soils consistently supported higher fungal populations compared to unamended soils. The moisture regime significantly influenced fungal abundance, with field capacity conditions recording the highest fungal populations, followed by saturated and flooded soils.

The observed reduction in fungal population immediately following fipronil application indicates a short-term inhibitory effect on soil fungi. Fungal communities are often sensitive to pesticide exposure due to disruption of membrane integrity, enzymatic activity and nutrient uptake. The transient nature of this suppression suggests that fipronil initially affected susceptible fungal groups, while more tolerant or adaptive species gradually re-established dominance as the pesticide dissipated. The pronounced suppression observed under combined herbicide and fipronil treatments highlights the cumulative stress imposed by multiple agrochemicals. Such combined applications can intensify toxicity by affecting multiple metabolic pathways simultaneously, thereby delaying fungal recovery. Nevertheless, the gradual increase in fungal populations after five days indicates the adaptive resilience of soil fungi, even under elevated chemical stress (7).

Organic amendment markedly enhanced fungal recovery and population size, likely by improving carbon availability and providing protective microhabitats that buffer toxic effects. Fungi are particularly responsive to organic substrates and the availability of decomposable organic matter may have supported rapid recolonization and growth following the initial stress. These findings reinforce the role of organic amendments in sustaining microbial stability in pesticide-treated soils (20).

The moisture regime played a decisive role in regulating fungal dynamics. The highest fungal populations observed under field capacity conditions suggest that optimal aeration and moisture balance favour fungal activity and survival. In contrast, flooded conditions may limit fungal proliferation due to reduced oxygen availability, while saturated soils may impose diffusion constraints, collectively explaining the lower fungal counts under these regimes. Overall, the results demonstrate that fipronil exerts a temporary inhibitory effect on soil fungal populations, with recovery strongly influenced by soil management practices. Organic amendments and optimal moisture conditions promote fungal resilience, whereas combined pesticide applications prolong suppression. These findings highlight the importance of integrated soil and pesticide management strategies to maintain fungal diversity and functional stability in paddy ecosystems (7).

Effect on actinomycetes population

The actinomycetes population was unaffected by the application of fipronil to paddy soil (Table 9, Fig. 5C). The initial actinomycetes population ranged from $0.83\text{-}0.95 \times 10^2$ CFU g^{-1} soil, except in herbicide-applied soil, where populations remained suppressed until the end of the experiment (30 DAA). However, herbicide application along with fipronil indicated a decline in actinomycetes population, with counts decreasing to 0.17×10^2 CFU g^{-1} soil at 1 DAA. Significantly higher actinomycetes populations were observed in organically amended soils ($1.10\text{-}1.12 \times 10^2$ CFU g^{-1} soil at 30 DAA) compared to unamended soils ($0.88\text{-}0.89 \times 10^2$ CFU g^{-1} soil at 30 DAA). Among the different moisture regimes and field capacity conditions ($0.89\text{-}1.12 \times 10^2$ CFU g^{-1} soil at 30 DAA) recorded significantly higher populations, followed by saturated and flooded soils ($0.88\text{-}1.10 \times 10^2$ CFU g^{-1} soil at 30 DAA).

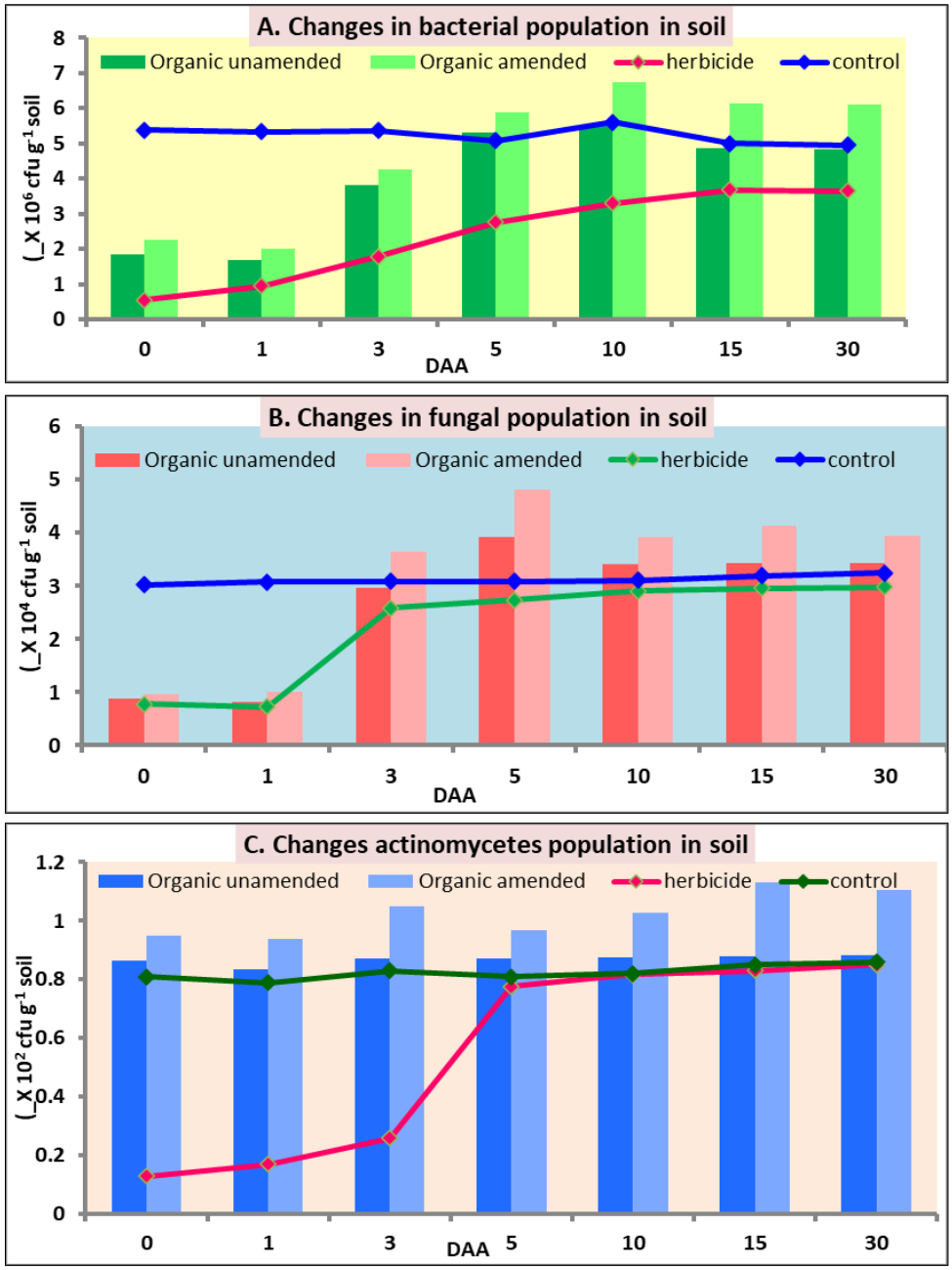

Fig. 5. Changes in microbial activity in soil under different moisture regimes, organic amendment and herbicide application. (A- bacteria; B- fungi; C- actinomycetes)

Similar findings have been reported in other studies, where soils treated with various pesticides showed no significant inhibition of total microbial populations (20). In fact, insecticides such as HCH, phorate, carbofuran and fenvalerate have been shown to significantly enhance bacterial, fungal and actinomycete populations, indicating that soil microbes can utilize pesticide residues and their breakdown products as energy and nutrient sources (22). This sustained microbial activity may contribute to the faster biodegradation of fipronil in the soil.

In contrast to fungi, actinomycete populations were largely unaffected by fipronil application, maintaining relatively stable levels throughout the experimental period. This resilience suggests that actinomycetes are less sensitive to fipronil-induced stress, a finding consistent with earlier studies reporting minimal inhibition of total microbial populations under pesticide treatments (20). Interestingly, a decline in actinomycetes was observed when fipronil was applied in combination with herbicide, again emphasizing the adverse impact of multiple pesticide inputs. Conversely, organic amendments consistently enhanced actinomycete abundance, while field capacity moisture conditions supported higher counts than saturated or flooded regimes. These findings corroborate earlier observations demonstrating that several insecticides, including HCH, phorate and carbofuran, can stimulate microbial growth by serving as carbon or nutrient sources. Such microbial persistence and in some cases enhancement under pesticide stress, not only highlights the adaptive potential of soil microbiota but also suggests their role in facilitating pesticide degradation and turnover in soil ecosystems (22).

Overall, our results confirm that while fipronil causes an initial inhibitory effect on soil microbial populations, this impact is transient and recoverable. The degree of recovery, however, depends on soil management practices such as organic amendment and moisture regimes, whereas combined pesticide inputs such as herbicides, exacerbate the negative effects. This aligns with broader evidence that pesticide effects on soil microbes are dose- and environment-dependent (23).

Effect of fipronil on enzyme activities in paddy soil

Dehydrogenase dynamics in paddy soil

The data presented in Table 10 and Fig. 6A reveal that significant inhibition of dehydrogenase activity occurred following fipronil application up to 5 DAA, with values ranging from 17–49 µg TPF g^{-1} soil 24 hr^{-1}. After 5 DAA, dehydrogenase activity increased significantly across all treatments and eventually stabilized at levels comparable to the control (93 µg TPF g^{-1} soil 24 hr^{-1}). The results of the present study further indicate that dehydrogenase activity under flooded conditions (34–105 µg TPF g^{-1} soil 24 hr^{-1}) was significantly higher than under field capacity conditions (32–48 µg TPF g^{-1} soil 24 hr^{-1}). Application of herbicide exerted a negative effect on dehydrogenase activity, with values ranging from 11–35 µg TPF g^{-1} soil 24 hr^{-1} from 0–30 DAA. Dehydrogenase activity was significantly more pronounced in flooded soil, as most dehydrogenases are anaerobic in origin. Similar to the present findings, previous studies reported that dehydrogenase activity varied significantly with different doses of chlorantraniliprole (40 and 80 g a.i. ha^{-1}), showing an initial decline followed by a subsequent increase at 45 DAA (23).

Urease dynamics in paddy soil

Urease is a key enzyme in the soil nitrogen cycle, catalyzing the hydrolysis of urea into CO_2 and NH_3. Urease activity in soil declined significantly during the initial days up to 5 DAA, with values ranging from 2–13 mg NH_4-N g^{-1} soil hr^{-1} (Table 10 and Fig. 6B). After 5 DAA, urease activity increased significantly across treatments compared to 10 DAA and further the activity remained constant as that of control (32–36 mg NH_4-N g^{-1} soil hr^{-1}). Urease activity did not vary significantly among different moisture regimes or between organically amended and unamended soils. However, significant variation was observed in herbicide-applied soils where activity ranged from 2–5 mg NH_4-N g^{-1} soil hr^{-1} at 1 DAA. These results indicate that application of fipronil 0.3 GR affected urease activity at the initial stage of the experiment and the subsequent recovery of urease activity in soil indicates that the ecosystem was able to overcome the disturbance caused by fipronil application by harvest time. The highest urease activity at the recommended dose of chlorantraniliprole (40 g a.i. ha^{-1}) was found compared to the control and its activity increased over the time (23).

Phosphatase in paddy soil

The result showed that acid phosphatase activity (53–61 µg PNP g^{-1} soil hr^{-1}) in paddy soil was higher than alkaline phosphatase activity (11–19 µg PNP g^{-1} soil hr^{-1}). Both acid and alkaline phosphatase activities were significantly lower in fipronil-treated soils compared to the control (Table 10 and Fig. 6C & D). An inhibitory effect was observed during the initial days; however, enzyme activity increased significantly from 5 DAA and remained relatively constant until the final observation at 30 DAA. Phosphatase activity was significantly higher under field capacity conditions than under flooded conditions, highlighting the role of soil aeration in regulating enzyme-mediated phosphorus cycling. Aerobic conditions at field capacity favour microbial proliferation and enzyme synthesis, whereas prolonged flooding induces anaerobic conditions that suppress aerobic microbial activity and enzyme production. These observations align with earlier studies showing reduced phosphatase activity under anaerobic or continuously flooded soils (22).

Herbicide application exerted a negative effect on phosphatase activity, likely due to additional stress on soil microorganisms and interference with microbial metabolic processes involved in phosphorus mineralization (23). In contrast, organic amendment significantly enhanced phosphatase activity compared to unamended soil (23). The stimulatory effect of organic amendments can be attributed to improved substrate availability, enhanced microbial biomass and protection of enzymes through organo-mineral complexes, which together promote higher enzymatic activity.

Table 10. Changes in enzyme activity in soil under different moisture regimes, organic amendment and herbicide

DAA	Dehydrogenase activity (µg TPF g^{-1} soil 24 hr^{-1})				Urease activity (mg NH_4-N g^{-1} soil hr^{-1})				Acid phosphatase activity (µg PNP g soil hr^{-1})				Alkali phosphatase activity (µg PNP g soil hr^{-1})			
	1	5	15	30	1	5	15	30	1	5	15	30	1	5	15	30
T_1	34.00^{b}	45.00^{bc}	95.00^{cd}	99.00^{de}	3.00^{ab}	20.00^{d}	31.00^{b}	36.00^{bc}	2.00^{ab}	11.00^{b}	44.00^{cd}	53.00^{bc}	3.00^{b}	6.00^{b}	9.00^{b}	11.00^{c}
T_2	32.00^{b}	43.00^{bc}	93.00^{cd}	97.00^{de}	4.00^{bc}	24.00^{ef}	32.00^{b}	37.00^{bc}	3.00^{abc}	13.00^{bc}	45.00^{cd}	54.00^{bcd}	4.00^{c}	6.00^{b}	14.00^{d}	14.00^{d}
T_3	31.00^{b}	41.00^{b}	45.00^{b}	45.00^{bc}	5.00^{cd}	27.00^{gh}	34.00^{b}	39.00^{c}	4.00^{bc}	13.00^{bc}	46.00^{cd}	57.00^{cd}	5.00^{d}	8.00^{c}	15.00^{de}	16.00^{ef}
T_4	36.00^{b}	49.00^{c}	102.00^{cd}	105.00^{de}	3.00^{ab}	22.00^{de}	31.00^{b}	34.00^{b}	3.00^{abc}	11.00^{b}	42.00^{bc}	55.00^{bcd}	3.00^{b}	6.00^{b}	14.00^{d}	15.00^{de}
T_5	34.00^{b}	47.00^{bc}	105.00^{d}	108.00^{e}	5.00^{cd}	25.00^{fg}	32.00^{b}	37.00^{bc}	4.00^{bc}	12.00^{bc}	48.00^{d}	58.00^{cd}	3.00^{b}	8.00^{c}	16.00^{e}	17.00^{f}
T_6	32.00^{b}	44.00^{bc}	47.00^{b}	48.00^{c}	6.00^{d}	28.00^{h}	35.00^{b}	39.00^{c}	5.00^{c}	14.00^{c}	55.00^{e}	61.00^{d}	5.00^{d}	9.00^{d}	18.00^{f}	19.00^{g}
T_7	12.00^{a}	19.00^{a}	32.00^{a}	35.00^{b}	2.00^{a}	10.00^{a}	20.00^{a}	22.00^{a}	1.00^{a}	9.00^{a}	36.00^{a}	44.00^{a}	0.74^{a}	4.00^{a}	7.00^{a}	7.00^{a}
T_8	11.00^{a}	18.00^{a}	28.00^{a}	31.00^{a}	4.00^{bc}	13.00^{b}	20.00^{a}	21.00^{a}	2.00^{ab}	12.00^{bc}	36.00^{a}	48.00^{ab}	0.89^{a}	4.00^{a}	8.00^{ab}	9.00^{b}
T_9	11.00^{a}	17.00^{a}	24.00^{a}	28.00^{a}	5.00^{cd}	16.00^{c}	22.00^{a}	23.00^{a}	2.00^{ab}	12.00^{bc}	38.00^{ab}	49.00^{ab}	1.30^{a}	6.00^{b}	11.00^{c}	10.00^{bc}
T_{10}	92.00^{c}	93.00^{d}	91.00^{c}	93.00^{d}	35.0^{e}	35.00^{i}	35.00^{b}	34.00^{b}	44.00^{d}	43.00^{d}	44.00^{cd}	43.00^{a}	14.00^{e}	15.00^{e}	12.00^{c}	14.00^{d}
R^2	**0.97**	**0.98**	**0.95**	**0.96**	**0.99**	**0.96**	**0.86**	**0.89**	**0.99**	**0.99**	**0.74**	**0.62**	**0.99**	**0.97**	**0.94**	**0.93**

Values marked by different letter differ significantly according to Duncan's multiple range test ($p \leq 0.05$)

T_1: FL + WOM T_5: ST + OM T_9: FC + WD

T_2: ST + WOM T_6: FC + OM T_{10}: Control

T_3: FC + WOM T_7: FL + WD

T_4: FL + OM T_8: ST + WD

DAA- Days after application of fipronil; FL- flooded; ST-saturated; FC- field capacity; WOM- without organic amendment; OM- organic amendment (press mud); WD- herbicide (Bensulfuron methyl 0.6 % + Pretilachlor 6 % GR); Control- without crop + FC + WOM.

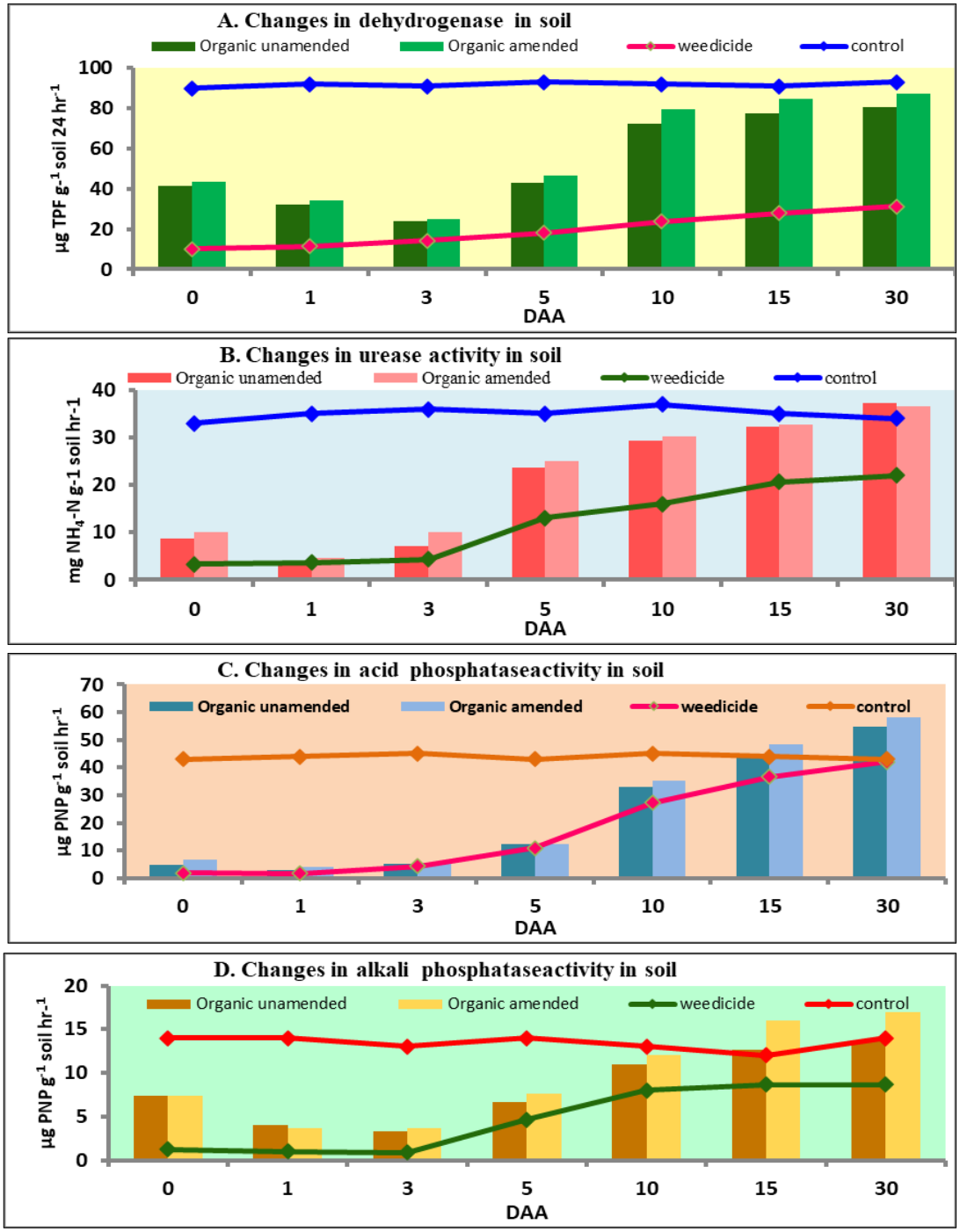

Fig. 6. Changes in enzyme activity in soil under different moisture regimes, organic amendment and herbicide application (A- dehydrogenase; B-urease; C- acid phosphatase; D- alkali phosphatase).

Conclusion

Fipronil degradation in paddy soil was primarily driven by microbial activity, with soil moisture, organic amendment and herbicide application significantly influencing its fate. Faster first-order dissipation in non-sterile soils confirmed the major role of microorganisms. Organic amendment and optimal moisture conditions enhanced microbial recovery and enzymatic activities, whereas herbicide application exerted inhibitory effects. Overall, the combined use of organic amendments and appropriate moisture management emerges as a practical and sustainable strategy to accelerate fipronil dissipation while restoring soil biological health in paddy ecosystems.

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Authors' contributions

AKR, BT, VL, KN and YSB designed and conceived the study. PDC conducted the experiments; analysed and interpreted the data and drafted the manuscript. AKR and BT provided resources and supervised. KN, VL and YSB critically reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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