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Article

ASSESSMENT OF SOIL MICROBIAL POPULATIONS AND THE DISSIPATION KINETICS OF PENOXSULAM PESTICIDE RESIDUES IN SOIL USING A VALIDATED HPLC-PDA METHOD

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Abstract: We assessed the microbial population in soil samples utilizing a serial dilution plate method. The soil samples were diluted to a ratio of 10^8, and we meticulously distributed specific volumes onto PDA and nutrient agar plates to promote the growth of fungi and bacteria, respectively. Following the preparation and staining of the bacterial cultures, we examined them under a microscope, which revealed the presence of both Gram-negative and Gram-positive cells. Notably, Pseudomonas sp. cultivated in King's B medium exhibited a yellowish-green fluorescent pigment under UV light, indicating a positive result. Understanding the dissipation of Penoxsulam is crucial for assessing its environmental impact and persistence in soil ecosystems. To investigate this, we conducted an experiment applying consistent concentrations: T_0 – Untreated Control, T_1 – Penoxsulam 21.7% SC (Suspension Concentrate) at 1.0 µg/g, and T_2 – Penoxsulam 21.7% SC (Suspension Concentrate) at 2.0 µg/g. The spiked

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samples were subsequently exposed to sunlight, and we collected soil samples at various intervals (0.1, 1, 5, 10, 15, 20, 30, 40, 50 and 60 days). All samples were analyzed until the residues fell below detectable levels. For the quantification of Penoxsulam residues, a validated High-Performance Liquid Chromatography with a photodiode array detector (HPLC-PDA) was employed. Key parameters included a Waters column (150 mm × 4.6 mm ID, 3.5 µm particle size), a sample temperature of 15° C, a detector wavelength of 240 nm, and a column oven temperature of 30° C. The analysis involved a 10.0 µL injection volume with a flow rate of 0.6 mL/min, and the total run time was 8.0 minutes, with Penoxsulam demonstrating a retention time of approximately 3.3 minutes. The detection limit for our method was found to be 0.015 µg/g, with a limit of quantification (LOQ) of 0.05 µg/g based on recovery. We observed that Penoxsulam residues in the aqueous solution decreased to undetectable levels by the 50^{th} day. Using regression analysis on the dissipation data, we calculated the DT50 (Half-Life) of Penoxsulam, which was 21.11 days for the T_1 dose and 21.17 days for the T_2 dose. Investigation findings demonstrate that penoxsulam exerts influence on microbial community composition, which subsequently modulates the rate at which this pesticide undergoes degradation within soil matrices. The established HPLC-PDA analytical method provides dependable quantification of pesticide residues, facilitating comprehensive evaluation of dissipation kinetics. The results indicate that judicious management of penoxsulam application strategies can substantially reduce environmental consequences while preserving soil microbial community integrity. In conclusion, this research emphasizes the critical importance of incorporating microbial evaluation protocols into pesticide residue investigations to advance environmentally responsible agricultural methodologies.

Keywords: Penoxsulam, Dissipation, DT_{50}, Residues, Soil Microbial Populations, HPLC-PDA.

Introduction

Soil microbial populations are a crucial component of soil ecosystems, serving a fundamental function in maintaining fertility and health. Soil is not just a physical entity; it is a dynamic system made up of minerals, soil, water, organic matter, and a rich diversity of microorganisms. Although they constitute less than 0.5 % of the soil's mass, these microorganisms are responsible for 60 – 80 % of the metabolic activity within the soil.[1] The soil microbiome includes various microbial groups: bacteria that decompose organic matter and fix nitrogen, fungi that break down complex compounds and form symbiotic relationships with plants,

archaea that aid in nutrient cycling under extreme conditions, and protozoa and algae that manage bacterial populations and contribute organic matter through photosynthesis.[2] These microbes are essential for critical processes such as nutrient cycling, improving soil structure by binding soil particles, and facilitating both beneficial and harmful interactions with plants.[3] Furthermore, they play a significant environmental role by regulating carbon storage, affecting greenhouse gas emissions, and helping ecosystems adapt to climate change and degradation.[4] Therefore, soil microbial populations are indispensable and frequently underestimated agents in promoting soil fertility, enhancing plant productivity, and ensuring global ecological stability.[5]

Dissipation kinetics refers to the reduction of pesticide residues in environments such as soil, water, and plant tissues over time. This reduction is driven by processes including degradation, volatilization, leaching, and plant metabolism. Understanding dissipation is crucial for estimating residue levels at harvest, establishing preharvest intervals, and evaluating risks to human and ecological health.[6] The core concept of dissipation involves the net loss of pesticides through various transformation processes.[7] Kinetic models that represent dissipation include first-order models, characterized by exponential decline, and multi-compartment or biphasic models that depict more intricate decay patterns.[8] The half-life, or ($t_{1/2}$)[9], signifies the duration needed for the concentration of a pesticide to reduce by half and serves as an important metric derived from concentration versus time data.[10] Numerous factors influence the dissipation of pesticide residues. Chemical characteristics such as solubility, vapor pressure, and inherent degradability affect a pesticide's propensity to undergo various degradation pathways. Furthermore, environmental and soil conditions, including temperature, moisture, pH, organic matter, and sunlight exposure,

impact degradation rates.[11-13] Agronomic practices, including application rate and timing, also significantly affect initial deposition and subsequent residue loss. Data collection techniques for dissipation kinetics involve field studies where crops or soils are sampled over time to quantify residues using validated extraction and chromatographic methods.[14]

Penoxsulam is a synthetic herbicide belonging to the triazolopyrimidine class (CAS number 219714-96-2, molar mass approximately 483.37 $g \cdot mol^{-1}$) that is primarily utilized for managing aquatic plants, as well as certain emergent and floating vegetation.[15] Its mode of action involves the inhibition of acetolactate synthase (ALS), a crucial enzyme for the production of branched-chain amino acids in plants, leading to stunted growth and eventual die-off of the plants. It is classified under WSSA Group 2. Penoxsulam effectively targets submerged, emergent, and floating-leaf aquatic plants, with application methods that may include foliar, surface, or subsurface treatments, depending on the specific product labeling.[16] Since its approval for aquatic use in the India, it has been used to manage aquatic weeds in ponds and lakes, adhering to specific rates and restrictions as outlined on product labels.[17] Environmental assessments consider factors such as persistence, mobility, and non-target effects during the regulatory review process. Safety protocols emphasize the necessity of following labeling instructions to mitigate risks to humans and non-target species, including guidelines for buffer zones and other precautionary measures specified in the labeling documents.[18-20] Practical advice suggests confirming the product's suitability for specific water bodies, strictly adhering to the labeled application rates and safety protocols, and consulting state pesticide regulators or the EPA for inquiries regarding localized risks, particularly concerning drinking water and endangered species.[21]

Residues of Penoxsulam in agricultural soils can be notably substantial, with their persistence being affected by several factors such as soil type, application rate, and environmental conditions. Research indicates that detectable residues may persist throughout the growing season, highlighting the necessity for specific extraction and analysis techniques like HPLC.[22] Important factors influencing dissipation include organic matter, soil texture, pH, and moisture content, where a higher organic carbon level typically leads to slower dissipation rates.[23] Analytical techniques such as matrix solid-phase dispersion and HPLC are essential for accurately quantifying residues. The practical implications underscore the importance of monitoring residual phytotoxicity prior to planting subsequent crops and evaluating the risks to aquatic organisms.[24] A monitoring checklist should include sampling soil at different depths, employing validated extraction and analysis techniques, and adopting mitigation strategies to reduce off-site movement.[25]

Materials and methods

Materials

The reference analytical standard for Penoxsulam, with a purity of 99.61 % (g/g), was obtained from Ehrenstorfer. The test item, Penoxsulam 21.7 % SC (Suspension Concentrate), was purchased from the local market in Hyderabad. Acetonitrile of HPLC grade was sourced from Merck India Limited. Soil samples were collected from the Keesaragutta forest area in Telangana.

Estimation of soil microbial population (Fungi and bacteria)

Culture medium for fungi

Potato Dextrose Agar (PDA) medium is formulated using the following ingredients: 250.0 g of peeled potato, 5.0 g of glucose, 15.0 g of

agar, and 1000 mL of distilled water, with a target pH of 6.3. The preparation method includes slicing the peeled potato into thin slices, boiling them in 500 mL of distilled water, and filtering the mixture. After obtaining the potato extract, glucose is incorporated into the solution. The total volume is then adjusted to 1000 mL, and agar is added prior to sterilization.

Culture medium for bacteria

Nutrient Agar (NA) is a microbiological culture medium that includes the following components: 5.0 g of Glucose, 5.0 g of Bacto peptone, 5.0 g of Sodium chloride, 15.0 g of Agar, and 1000 mL of Distilled water. The pH level of this medium was adjusted to remain within the range of 6.8 to 7.2.

Method of inoculation

The enumeration of the microbial population, including fungi and bacteria, in soil samples was conducted through the serial dilution plate method. One gram of soil was introduced into a test tube containing 9 mL of sterile distilled water (10^{-1} dilution) and mixed thoroughly using a cyclo mixer. A further dilution was performed by transferring 1 mL of mother suspension into another test tube with 9 mL of sterile distilled water (10^{-2} dilution), followed by serial dilutions up to 10^{-8}. From the dilutions ranging from 10^{-4} to 10^{-6} for fungi and from 10^{-6} to 10^{-8} for bacteria, 0.1 mL of the suspension was extracted with a micropipette and placed onto PDA and nutrient agar plates, respectively, under aseptic conditions in a laminar air flow environment. The plates were labeled with the inoculation date and time. After inoculation, all Petri dishes were incubated in a BOD incubator at 28 ± 2°C for seven days for fungi and at 35 ± 2 °C for two days for bacteria.

Identification of fungal and bacterial spp

Identification of *Trichoderma* spp

The process of isolating Trichoderma species was carried out using a selective culture medium, which resulted in the emergence of colonies that exhibited a color spectrum from white to yellowish and ultimately green. The conidiophores of these fungi are notable for having phialides that are produced individually. The selective culture medium, as detailed by Elad and Chet (1983), includes the following ingredients: 3.0 g of glucose, 1.0 g of ammonium nitrate, 0.90 g of dipotassium hydrogen phosphate, 0.20 g of magnesium sulfate, 0.15 g of potassium chloride, 0.15 g of rose bengal, 0.30 g of Dexon (60 % WP), 0.25 g of chloramphenicol, 0.20 g of PCNB (75 % WP), and 15 g of agar, all dissolved in 1000 mL of distilled water.

Identification of *Pseudomonas* spp

The medium specifically designed for isolating Pseudomonas species is called King's B medium, which acts as a selective medium for Pseudomonas. The formulation includes 20.0 g of peptone, 10 mL of glycerol, 1.5 g of dipotassium hydrogen phosphate, 1.5 g of magnesium sulfate, 100 µg/g of cycloheximide, 50 µg/g of ampicillin, 12.5 µg/g of chloramphenicol, 15.0 g of agar, and 1000 mL of distilled water. It is prepared to achieve a pH range of 6.5 – 7.0. Notably, cycloheximide, ampicillin, and chloramphenicol are added after autoclaving to ensure their efficacy.

Gram staining method

A loop containing a bacterial culture was placed on a clean glass slide, where a smear was prepared, air-dried, and heat-fixed. The smear was then treated with ammonium oxalate crystal violet for one minute. The excess stain was subsequently removed, and the slide was rinsed

with a gentle stream of water. Following this, Lugol's iodine solution was applied and left for one minute before the smear was decolorized using 95 percent ethyl alcohol. The smear was rinsed again with a gentle stream of water and counterstained with safranin for 30 seconds. The Gram-negative cells were noted to be red, while the Gram-positive cells appeared violet.

Fluorescens pigment production

The test tubes containing sterilized King's B medium were inoculated with the culture of *Pseudomonas* sp. incubated for two days. The microorganisms produced pigments were followed after incubation.

Standard stock solution

A precise amount of 10.04 mg of Penoxsulam reference standard, exhibiting a purity of 99.61 %, was accurately weighed directly into a 10 mL volumetric flask. The compound was dissolved in 5 mL of acetone, subjected to sonication, and subsequently diluted to the mark with the same solvent. This procedure yielded a solution concentration of 1 mg/mL, which was then stored in a freezer at -18 °C. The stock standard solutions were deemed stable for use for a duration of up to 3 months. Working standards of appropriate concentrations were prepared from the stock solutions by diluting with acetone immediately prior to sample preparation.

Sample stock solution

An exact amount of 45.98 mg of Penoxsulam, which has a purity of 21.7 %, was placed into a 10 mL volumetric flask. It was dissolved in 5 mL of acetone, subjected to sonication, and then adjusted to the mark with additional acetone. This process produced a solution concentration of

997.77 µg/mL. The stock sample solution was subsequently used for the preparation of dose samples (T_1 and T_2) in soil.

Sample application in soil

In the study, three distinct replication doses of Penoxsulam were established in soil to evaluate its effects. The untreated control, designated as T_0, serves as the baseline for comparison. The second dose, T_1, consists of a concentration of 1 µg/g, which was achieved by incorporating 1.0 mL of the Penoxsulam sample stock solution into 1000 g of soil. The third dose, T_2, features a higher concentration of 2 µg/g, accomplished by adding 2.0 mL of the test item stock solution to the same amount of soil. This structured approach allows for a systematic assessment of the compound's impact at varying concentrations.

Sampling data

The study involved exposing samples to direct sunlight while taking aliquots at predetermined intervals. Concurrently, water samples were collected, with temperatures ranging from 23 °C to 43 °C. This approach aimed to assess the effects of sunlight exposure on the samples under varying temperature conditions and for the purpose of extraction and analysis, 50 g of samples were uniformLy collected from various depths, covering the entire soil within the container. Soil samples were gathered on designated days: 0.1, 1, 5, 10, 15, 20, 30, 40, 50 and 60 days. The laboratory conditions were maintained at a temperature between 20 °C and 25 °C.

Selected parameters for HPLC separation

The procedure involved High-Performance Liquid Chromatography equipped with a photodiode array detector (HPLC-PDA), characterized by key parameters that included a Waters column (C18) of dimensions 150 mm × 4.6 mm ID and a particle size of 3.5 µm, a sampler temperature of 15° C,

a detector wavelength maintained at 240 nm, and a column oven temperature of 30° C. A 10.0 µL injection volume was utilized, with separation was performed at a flow rate of 0.6 mL/min. The mobile phase ratio utilized in the analysis was composed of acetonitrile and 0.1% orthophosphoric acid in a proportion of 75:25. The total duration of the analysis was 8.0 minutes, and the retention time for Penoxsulam was found to be around 3.2 minutes.

Method validation

Method validation is essential for ensuring the reliability of analytical results. This study focused on key parameters such as linearity, recovery, repeatability, and the limits of detection (LOD) and quantification (LOQ). The accuracy of the method was assessed through recovery tests involving samples spiked at concentrations of 0.05, and 5.0 µg/mL. Linearity was evaluated by preparing a series of known concentrations (0.015, 0.05, 0.5, 1.0, 5.0, and 10.0 µg/mL) through dilution of a stock solution. The LOD was defined as the lowest concentration that produced a response 3.3 times greater than the baseline noise, determined from the analysis of a control sample. The LOQ was established based on recovery metrics, ensuring that the method could reliably quantify the analyte at specified levels.

Method of analysis

Prior to injecting the sample, an extraction procedure of pesticide residues from the soil matrix has been used. The resulted sample extracts were subsequently injected into HPLC for residue quantification.

Dissipation kinetics studies

The study aimed to investigate the dissipation kinetics of Penoxsulam residues in soil at two concentrations, 1.0 µg/g (T_1) and

2.0 µg/g (T_2), with triplicate samples and control groups for comparison. The samples were exposed to direct sunlight, and aliquots were collected at specified intervals to monitor changes. Soil temperatures during the experiment varied between 26 to 38°C, and all samples were stored at 5°C in darkness prior to High-Performance Liquid Chromatography (HPLC) analysis. The collection of samples continued until the concentrations of both T_1 and T_2 fell below the limit of quantification (LOQ). Following the analysis, the half-life (DT50) of Penoxsulam was calculated using a designated formula, providing insights into the degradation rate of the compound in soil under the given conditions.

The first-order kinetics form is:

$$\ln \frac{C_0}{C} = k \cdot t$$

$$\text{Rate constant} = \ln \frac{C_0}{C} = f(t)$$

$$t_{1/2} = \text{DT50} = \frac{ln2}{k} = \frac{0.6931}{k}$$

where,

C_0 – Pesticide concentration at time zero

C_t – Pesticide concentration at time t,

k – The rate constant

Extraction procedure

A 10 g soil sample was subjected to two extraction cycles with 25 mL of acetonitrile, utilizing an orbital shaker for 30 minutes at room temperature (25°C). The resulting mixture will be filtered using a 0.45 µm Whatman filter paper and then the filtrate evaporated to near dryness with the help of an ultrasonic water bath. The remaining material will then be re-dissolved in 10 mL of acetonitrile for HPLC analysis.

Results and discussion

Estimation of soil microbial population (fungi and bacteria)

Test tubes containing sterilized King's B medium were inoculated with a culture of Pseudomonas sp. and incubated for two days. The appearance of a yellowish-green fluorescent pigment, visible under UV light (366 nm), suggested positive results. The culture was identified as Pseudomonas through Gram staining and fluorescence pigmentation tests. The Gram stain results indicated that the culture is Gram-negative. In the slide, the crystal violet was washed away from the cells, resulting in a red coloration of the cells. By utilizing Gram's staining technique, the culture was also identified as Bacillus. The Gram stain results indicated that this culture is Gram-positive. In this case, crystal violet was retained within the cells, leading to a deep violet appearance. The cells were rod-shaped, straight, and formed chains. All microbial colonies were evaluated and counted using a colony counter. The microbial organisms per gram of soil samples were calculated by multiplying the number of colonies observed by the dilution factor.

Specificity

The method's specificity was established based on the injected diluent solvent, reference solution (Linearity standard solution-1), sample solution (P1), and soil control, which were all subjected to HPLC-PDA injection. Data analysis demonstrated that there were no interference peaks detected at the analyte's retention time among these solutions, thus affirming their independence from one another.

Linearity

The study involved preparing various concentrations of fungicides (0.015, 0.05, 0.5, 1.0, 5.0, and 10.0 µg/mL) through serial dilution in 10 mL volumetric flasks. The prepared standard solutions were analyzed using High-Performance Liquid Chromatography (HPLC), with the results documented in a series of chromatograms. A calibration curve was established to demonstrate the correlation between the concentration of the standards and the corresponding peak areas observed. The analysis of six standard concentration solutions confirmed the linearity of the method, resulting in a linear regression equation of Y = 15524.64X + 4.55, accompanied by a perfect correlation coefficient of 1.0000. This data facilitated the construction of a calibration curve, further illustrated in the accompanying figure 2. The relative error calculated using replicate of concentration of linearity solutions vs peak areas. The average relative error obtained 0.0. The results are presented in Table 2.

Table 1. Serial dilutions for linearity standard solutions and their response

Standard Stock (µg/mL)	Volume taken (mL)	Final volume (mL)	Final concentration (µg/mL)	Peak area
0.50	0.300	10	0.015	238
0.50	1.000	10	0.05	789
10.00	0.500	10	0.50	7854
10.00	1.000	10	1.00	15453
1000.08	0.050	10	5.00	77582
1000.08	0.100	10	10.00	155277

Table 2. The relative error results.

Code	Replication	Concentration in µg/mL	Peak area	Relative Response factor	Relative error
Linearity solution - 0.015 µg/mL	R1	0.015	238	15866.67	252.97
	R2	0.015	235	15666.67	52.97
	R3	0.015	233	15533.33	-80.36
Linearity solution - 0.05 µg/mL	R1	0.05	789	15780.00	166.31
	R2	0.05	792	15840.00	226.31
	R3	0.05	786	15720.00	106.31
Linearity solution - 0.5 µg/mL	R1	0.5	7854	15708.00	94.31
	R2	0.5	7798	15596.00	-17.69
	R3	0.5	7887	15774.00	160.31
Linearity solution - 1.0 µg/mL	R1	1.0	15453	15453.00	-160.69
	R2	1.0	15365	15365.00	-248.69
	R3	1.0	15396	15396.00	-217.69
Linearity solution - 5.0 µg/mL	R1	5.0	77582	15516.40	-97.29
	R2	5.0	78003	15600.60	-13.09
	R3	5.0	78107	15621.40	7.71
Linearity solution - 10.0 µg/mL	R1	10.0	155277	15527.70	-85.99
	R2	10.0	155309	15530.90	-82.79
	R3	10.0	155508	15550.80	-62.89
		Average		15613.69	
		STD		145.78	
		% RSD		0.93	
		Average Relative error			0.00

Relative Response factor = Peak area / Concentration in µg/mL

Relative error = (At each point Relative Response factor – Average Relative Response factor)

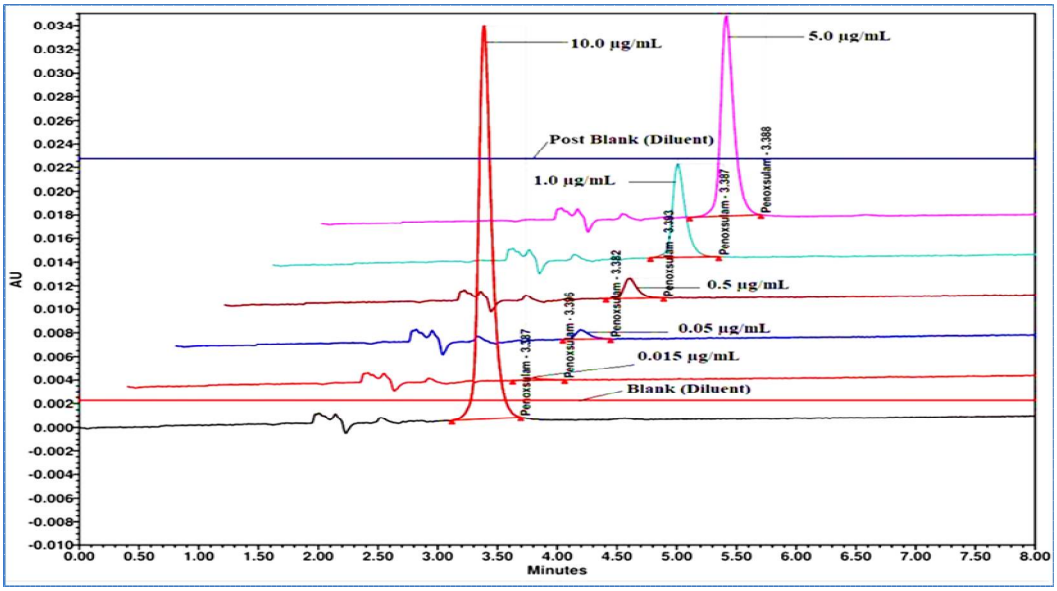


Figure 1. Representative chromatograms at various amounts of Penoxsulam.

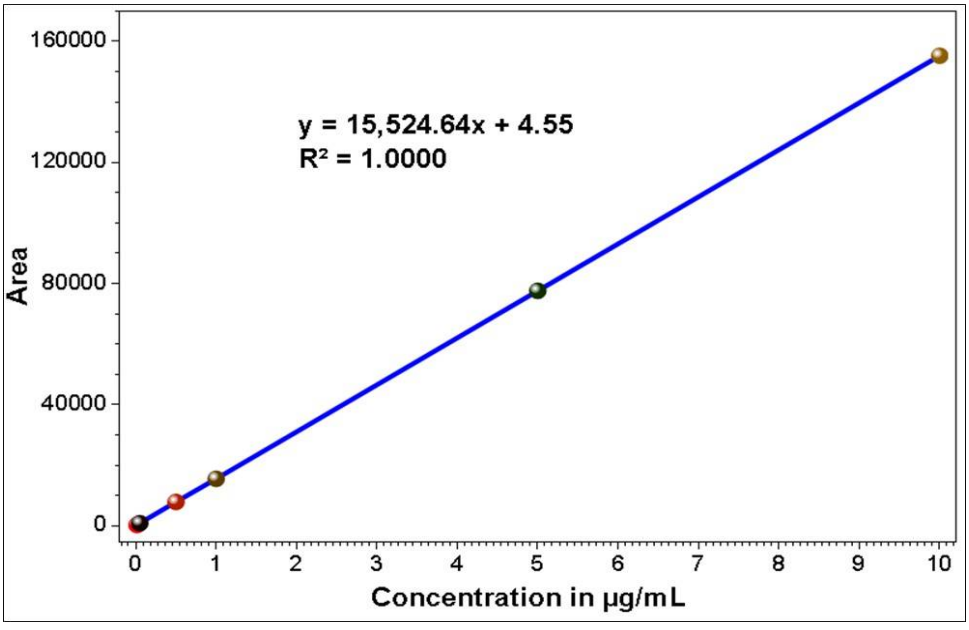


Figure 2. Calibration curve of Penoxsulam.

Recovery and repeatability

The validation of the analytical method was conducted to evaluate its effectiveness in recovering the test item from soil at two distinct concentration levels, which confirms its dependability and accuracy. The results show that the method efficiently recovers the test item, making it suitable for environmental assessments and providing a detailed understanding of its performance across various conditions.

Preparation of test item stock solution

A measured quantity of 5.02 mg of Penoxsulam, which has a purity of 21.7 %, was placed into a 100 mL volumetric flask. The sample was dissolved in 20 mL of acetonitrile, and sonication was applied to ensure complete dissolution. The solution was then diluted to the mark with acetonitrile, achieving a final concentration of 10.89 µg/mL. This preparation method provides an accurate concentration for later analysis or experimentation.

Preparation of 0.05 µg/g fortification level

Six replications were carried out, in which a 0.115 mL aliquot of a test item stock solution, with a concentration of 10.89 µg/mL, was mixed with 25 grams of soil. This experimental configuration enables the evaluation of the test item's behavior and effects in a soil matrix, laying the groundwork for further analysis and consideration of its environmental consequences. The use of several replications bolsters the reliability and validity of the results obtained from this investigation.

Preparation of 0.5 µg/g fortification level

A total of 1.148 mL of a stock solution of the test item, with a concentration of 10.89 µg/mL, was added to 25 grams of soil. This procedure was carried out in six independent replications to confirm the

reliability and accuracy of the results. The methodology indicates a methodical approach to evaluating the interaction or impact of the test item within the soil matrix.

The evaluation included an assessment of the samples for recovery and repeatability utilizing High-Performance Liquid Chromatography (HPLC). The accuracy was established by computing the percentage recovery, whereas repeatability was measured through the percentage relative standard deviation (% RSD). The outcomes of these evaluations are outlined in Table 3, and the representative chromatograms that depict the results are shown in Figure 3.

Table 3. Recovery and repeatability of Penoxsulam 37.12 % EC in soil.

Replication	**% of Recovery at 0.05 µg/g Fortification Level**	**% of Recovery at 0.5 µg/g Fortification Level**
R1	85.12	94.28
R2	87.55	95.17
R3	84.26	94.95
R4	86.41	96.03
R5	85.32	95.62
R6	84.79	95.34
Mean	85.58	95.23
STDEV	1.20	0.60
% of RSD	1.40	0.63

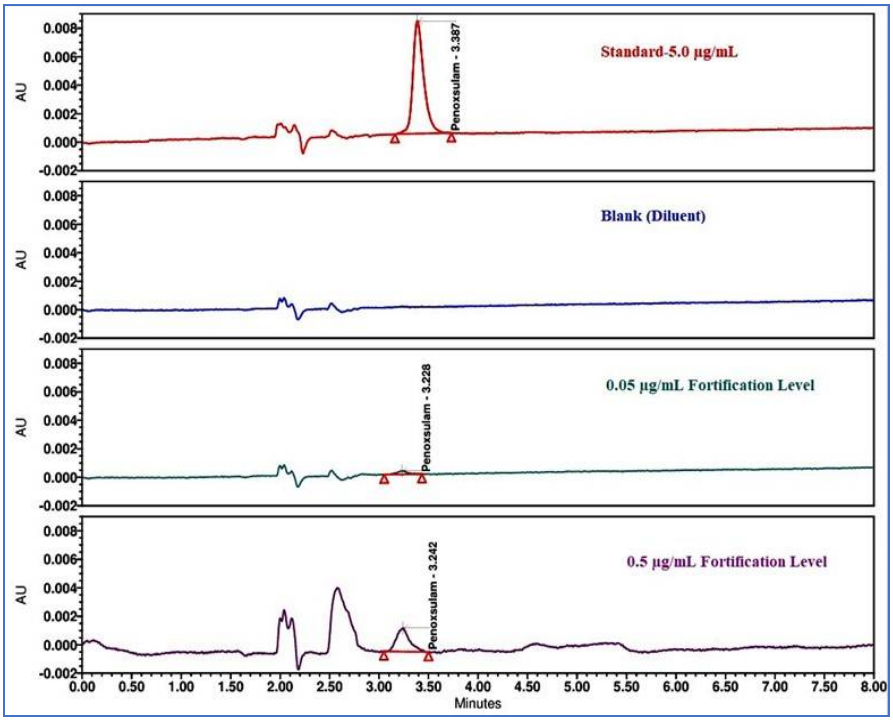


Figure 3. Representative recovery and repeatability chromatograms of Penoxsulam.

Detection and quantification limits

The established limit of quantification was 0.05 µg/g, indicating the lowest fortification level that achieves satisfactory average recoveries ranging from 84 % to 87 %, with a relative standard deviation of less than 2 %. This quantification limit implies that the analyte peak can be reliably observed at about ten times the baseline noise in the chromatogram. Moreover, the limit of detection was found to be 0.015 µg/g, which is roughly equivalent to three times the background level recorded in the control injection at the retention time of the target peak.

Dissipation details of Penoxsulam in soil

The investigation monitored the degradation of Penoxsulam in soil over a duration of 60 days, starting with initial concentrations of 0.982 µg/g for treatment 1 (T_1) and 1.975 µg/g for treatment 2 (T_2) on day 0.1. By day

1, concentrations had decreased to 0.859 µg/g for T_1 and 1.701 µg/g for T_2, signifying a slightly initial reduction in residue levels. This trend continued with further decreases observed on day 5, where T_1 showed 0.605 µg/g and T_2 showed 1.236 µg/g. As the days advanced, the concentrations continued to decline, reaching 0.522 µg/g for T_1 and 1.098 µg/g for T_2 by day 10, and further diminishing to 0.469 µg/g for T_1 and 0.902 µg/g for T_2 by day 20. On day 30, the levels were recorded at 0.396 µg/g for T_1 and 0.805 µg/g for T2, followed by a drop to 0.298 µg/g for T_1 and 0.608 µg/g for T_2 on day 40. By day 50, the concentrations were drastically dropped at 0.126 µg/g for T_1 and 0.255 µg/g for T_2. Ultimately, both treatments reached below detectable levels (BDL) by day 60, indicating the complete dissipation of Penoxsulam residues in the soil. The representative chromatograms that support these conclusions are illustrated in the accompanying Figure 4 and Figure 5.

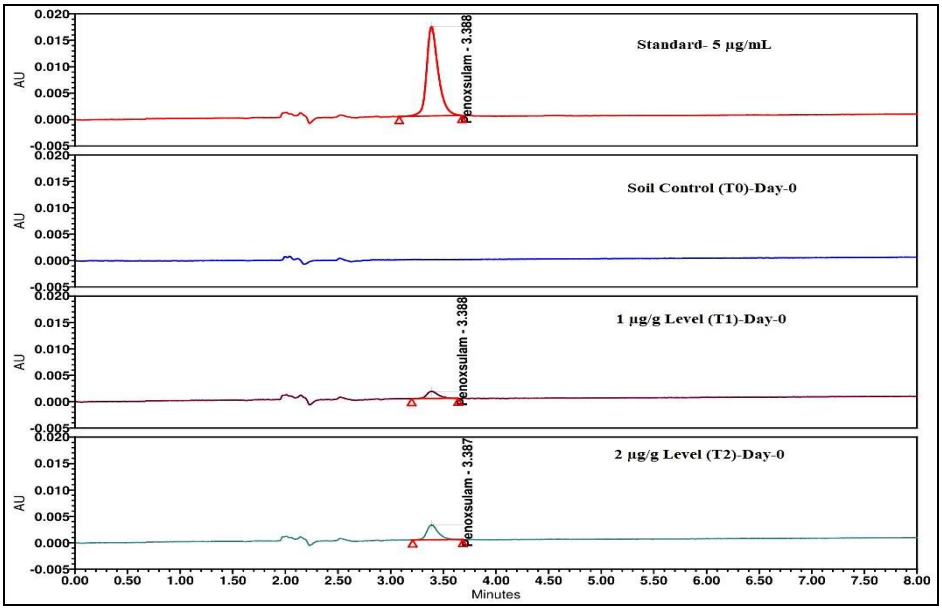


Figure 4. Representative initial chromatograms of Penoxsulam in soil.

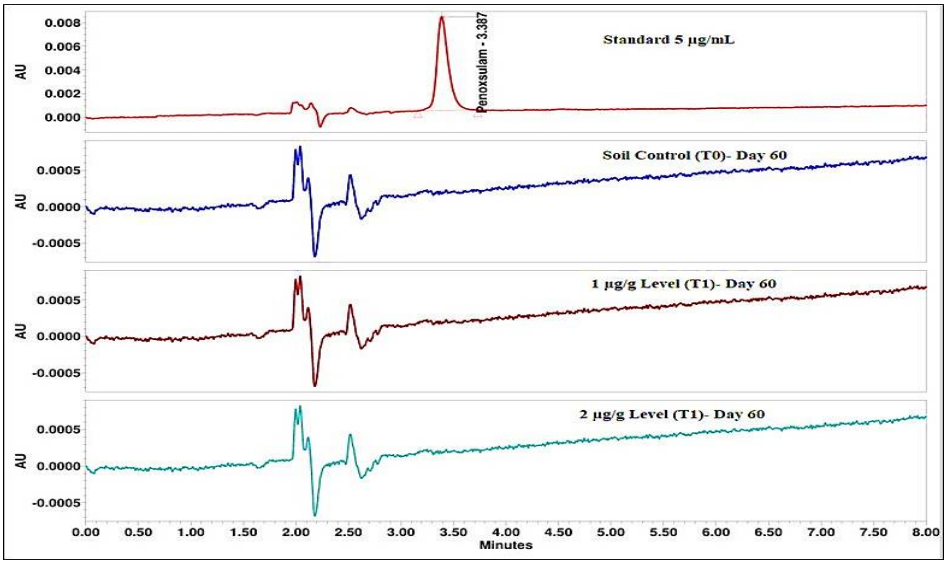


Figure 5. Representative chromatograms of extracted soil samples after 60 days.

The dissipation curve, which illustrates the relationship between the concentration of the analyte and the sampling occasions, is shown in Figure 6. The DT50 value was determined using the formula provided below

$$DT50 = \frac{ln2}{k}$$

where,

'k' is the slope of the curve obtained from the dissipation data.

The values for DT 50, indicating the duration required to break down 50% of the residues, can be found in Table 4. The calculation of the rate constant was performed through a linear regression equation based on the first-order rate equation.

$$K = \ln a/(a-x)/dt$$

where, dt is the time interval between t_1 and t_2 and a, x are the concentrations of pesticides at times t_1 and t_2, respectively. A plot of concentration of the residues and rate with the R^2 indicates first-order kinetics in the dissipation of both the fungicides. The DT50 (Half Life) of

Penoxsulam was calculated by regression analysis from the dissipation data. The results were presented in Table 4.

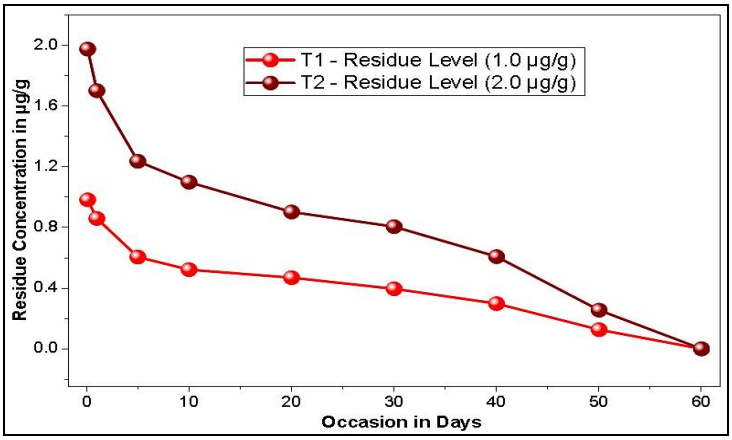


Figure 6. Dissipation curve of Penoxsulam in soil.

Table 4. Regression analysis of Penoxsulam in Soil.

Occasion (Days)	Sample T_1 (µg/g)	SampleT_2 (µg/g)	Log T1	Log T2
0.1	0.982	1.975	-0.0079	0.2956
1	0.859	1.701	-0.0660	0.2307
5	0.605	1.236	-0.2182	0.0920
10	0.522	1.098	-0.2823	0.0406
20	0.469	0.902	-0.3288	-0.0448
30	0.396	0.805	-0.4023	-0.0942
40	0.298	0.608	-0.5258	-0.2161
50	0.126	0.255	-0.8996	-0.5935
60	BDL	BDL	0.0000	0.0000
	Intercept		-0.0917	0.2128
	CC		-0.9527	-0.9527
	slope		**-0.0143**	**-0.0142**
	Rate Constant (k)		**0.0328**	**0.0327**
	t1/2 (DT50) in Days		**21.11**	**21.17**

Conclusions

The investigation outlines a rapid and effective analytical method using HPLC-PDA for the quantification of Penoxsulam residues in soil. By utilizing acetonitrile and 0.1% orthophosphoric acid as mobile phases, the method achieves excellent separation and resolution within a short analysis time of roughly eight minutes per chromatographic run. We rigorously validated the method's accuracy through recovery tests, utilizing samples spiked at precise concentrations of 0.05 and 0.5 µg/mL. Linearity was definitively established by preparing a comprehensive series of known concentrations, ranging from 0.015 to 10.0 µg/mL, achieved through precise dilution of a stock solution. The LOD was precisely determined as the lowest concentration yielding a response 3.3 times above the baseline noise, consistently derived from the analysis of a control sample. The LOQ was confidently established based on robust recovery metrics, unequivocally ensuring the method's reliable quantification of the analyte at the specified levels. This analytical method, along with the dissipation data, is well-equipped to support routine monitoring and assist residue laboratories and researchers in assessing Penoxsulam residues across various commodities, including crops, water, and soil samples. Moreover, the study explores the dissipation kinetics of the Penoxsulam herbicide in soil, focusing on its environmental impact and persistence in soil ecosystems. The results indicate that the half-life (DT_{50}) of Penoxsulam is approximately 21days for both the T_1 and T_2 doses, highlighting its persistence in the environment.

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Author contributions

V. Sridhar: Methodology, Field survey, Data curation, Formal analysis. K. Kiranmai: Visualization, Validation. Y. Prashanthi: Conceptualization, Methodology, Writing-review.

Tentu Nageswara Rao: Writing original draft.

Conflicts of interest

The authors declare no competing interests

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