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DETERMINATION OF RESIDUES AND DISSIPATION KINETICS OF THIDIAZURON AND ITS METABOLITE IN DIFFERENT TROPICAL SOILS

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ABSTRACT

Thidiazuron play an important role in the production of crops, but their residues may cause numerous human/environmental problems. A study was conducted to evaluate the residues and dissipation kinetics of thidiazuron 50% WP in four different soils. The applied dosages are T0 – Untreated Control, T1 – thidiazuron 50% WP at 1 mg/kg of water and T2 – thidiazuron 50% WP at 2 mg/kg. Representative soil samples were collected on 0, 1, 3, 5, 10 and 20 days after spraying the formulation for the determination of residues of thidiazuron and its metabolite 1,2,3-Thiadiazol-5-ylurea by using a validated HPLC-PDA method. The method was used for the excellent separation of thidiazuron and its metabolite 1,2,3-Thiadiazol-5-ylurea in two different type soils is very short (around 15 min for a chromatographic run). he mean percent recovery of thidiazuron in Sandy loam, Loamy sand, Sandy clay and Clay soils was 85 ± 1.19; 87 ± 1.56; 89 ± 1.41 and 86 ± 1.78 at 0.03 mg/kg fortification level, 92 ± 2.15; 94 ± 2.56; 90 ± 2.69 and 95 ± 1.89 at 0.3 mg/kg fortification level. The mean percent recovery of metabolite 1, 2, 3-Thiadiazol-5-ylurea in Sandy loam, Loamy sand, Sandy clay and Clay soils was 89 ± 1.36; 86 ± 1.11; 87 ± 1.32 and 88 ± 1.77 at 0.03 mg/kg fortification level, 90 ± 2.41; 93 ± 2.82; 90 ± 2.71 and 92 ± 2.32 at 0.3 mg/kg fortification level. The LOQ was 0.03 mg/kg for thidiazuron and its metabolite 1, 2,3-Thiadiazol-5-ylurea. The half-life of thidiazuron was 4.73 and 4.90 days for T1 and T2 recommended doses in sandy loam, 4.61 (T1) and 4.84 (T2) days in loamy sand, 4.66 (T1) and 4.79 (T2) days in sandy clay and 4.71 (T1) and 4.78 (T2) days in clay soils respectively. Dissipation of thidiazuron in different tropical soils followed first order kinetics and this review describes a fast, simple sensitive analytical HPLC method.

KEY WORDS: ***Thidiazuron, 1, 2, 3-Thiadiazol-5-ylurea, HPLC, DT50, Tropical soils***

INTRODUCTION

Pesticides are valuable for elevated agricultural production. The secure use of the pesticide relies upon its toxicological properties and its distribution and persistence within the environment, along with consideration of any unusual photoproducts and metabolites that are probably formed. Fallacious utilization of pesticides ends in environmental contamination. There has been increased research interest aimed at understanding the process that controls the penetration of pesticides into the soil and their subsequent contamination of groundwater and surface water. Accumulation of these poisonous pesticides in groundwater is mainly caused by the physical, chemical, and biological processes. Thermal decomposition, changes in pH of water and hydrolysis cause the breakdown and degradation of pesticides.Thidiazuron [1-phenyl-3-(1, 2, 3-thidiazol-5-yl) urea] is a plant growth regulator developed by Schering AG (now Aventis Crop Science). It has been used to stimulate high rate of axillary shoot proliferation on many woody plant species[1]. Thidiazuron directly promotes growth due to its own biological activities in a fashion similar to that of an N-substituted cytokinin or it may induce the synthesis and accumulation of an endogenous cytokinin[2]. In China, thidiazuron is being investigated for promoting growth of apple[3]. To the best of our knowledge, no analytical methods are published in literatures to determine thidiazuron residues and dissipation kinetics in different tropical soils. Although thidiazuron residues have been reported in some environmental

materials, such as water[4], cotton seed[5- 6.] Therefore, the development of a readily applicable method for residual analysis of thidiazuron in tropical soils is necessary. In this paper, a simple HPLC–PDA method combined with solid phase extraction was established to detect the residues of thidiazuron and its metabolite in tropical soils.

MATERIALS AND METHODS

Reference analytical standards of thidiazuron (Purity 98.1%) and 1, 2, 3-Thiadiazol-5-ylurea (Purity 91.2%) were obtained from Sigma Aldrich. The test item thidiazuron 50%WP was purchased from hyderabad local market. Acetonitrile HPLC grade, Water HPLC grade, Acetone AR grade, Toluene AR grade, Methanol AR grade, dieethyl ether AR grade and silica gel were obtained from the Merck India limited. Distilled water was purified by using the Milli-Q Plus apparatus (Millipore, Bedford, MA, USA). Shimadzu High Performance Liquid Chromatograph system equipped with LC-20 AT_{VP} pump and SPD-20A UV/VIS CTO-20A Column oven using LC solution software, Kyoto, Japan, Hamilton syringe (25µl) – M/s. Hamilton Inc., New York, USA, Volumetric flasks, pipettes, measuring cylinder and glass columns - All 'A' grade glassware supplied by M/s.Borosil Glass and Glassware Mumbai, India and LCGC AS 60/220.R2 analytical balance, capable of weighing 0.01 mg supplied by LCGC rad wag, India.

Chromatographic Separation Parameters

Instrument : Shimadzu High Performance Liquid Chromatograph system equipped with LC-20 AT_{VP} pump and SPD-20A UV/VIS CTO-20A Column oven using LC soluition software.

Column used	:	Phenomenex C18 (25cm length x 4.6mm i.d)
Wave length	:	250 nm
Mobile phase	:	Acetonitrile : Water (55:45 v/v)
Flow rate	:	0.8 ml/min
Column Temperature:		35°C
Injected volume	:	20 µl
Run time	:	15 min
Retention time	:	Thidiazuron – 5.3 minutes,
Metabolite (1,2,3-Thiadiazol-5-ylurea) – 4.0 minutes		

Method of Analysis

Accurately weighed 20 g of soil sample into a 250 ml round bottom flask and extracted with 100 mL of methanol in an extractor for 1 hour. Filtered and the filtrate was evaporated to near dryness on a rotary vacuum evaporator. Added 50 ml of acetonitrile into a same flask and evaporated the mixture to dryness. The residue was taken in 5 mL methanol and sonicated in an ultrasonic bath. Further added 10 ml of toluene and evaporated to about 5 mL for column clean up.

Clean up

5 g of Silica gel was packed using acetonitrile in a 30 cm chromatographic column which has 3 cm glass wool plug at lower end and covered the top with 0.5 g Na2SO4. The residue obtained from partition step was transferred quantitatively into the pre washed column and eluted with 50 ml of elution solvent (diethyl ether). Discarded the first 15 ml and collected the eluate. The residue was concentrated to near dryness and taken in mobile phase for HPLC analysis.

Method Validation

The method for the determination of thidiazuron and its metabolite 1, 2, 3-Thiadiazol-5-ylurea was validated in terms of method specificity, linearity, assay accuracy and Limit of quantification[7].

Specificity

The standard, sample solutions, sandy loam, loamy sand, sandy clay and clay soil control samples, Spiked soil samples, mobile phase solvents i.e., acetonitrile and HPLC water were injected into HPLC and checked for specificity and it was observed that there was no interference observed with the main peak.

Linearity

Preparation of Stock Solution of Reference Standard of thidiazuron

Accurately weighed 0.00536 g of reference standard of thidiazuron in 10 mL volumetric flask and the volume was brought upto the mark using acetonitrile.

Preparation of Stock Solution of Reference Standard of 1,2,3-Thiadiazol-5-ylurea

Accurately weighed 0.00521 g of reference standard of 1,2,3-Thiadiazol-5-ylurea in 10 mL volumetric flask and the volume was made upto the mark using acetonitrile.

Preparation of Calibration Solutions of Thidiazuron and 1,2,3-Thiadiazol-5-ylurea

Different known concentrations of thidiazuron and 1,2,3-Thiadiazol-5-ylurea (0.03-5 mg/L) were prepared in mobile phase by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient. The details were presented in Table 1. Calibration curve was shown in Figure 1.

Table 1
Calibration details – Copper Oxychloride

Concentration in mg/L	Peak area (mV-sec)	
	Thidiazuron	Metabolite 1,2,3-Thiadiazol-5-ylurea
0.03	354	295
0.1	1145	987
0.5	5265	4887
1	10254	9674
2	20587	18594
5	51852	47695

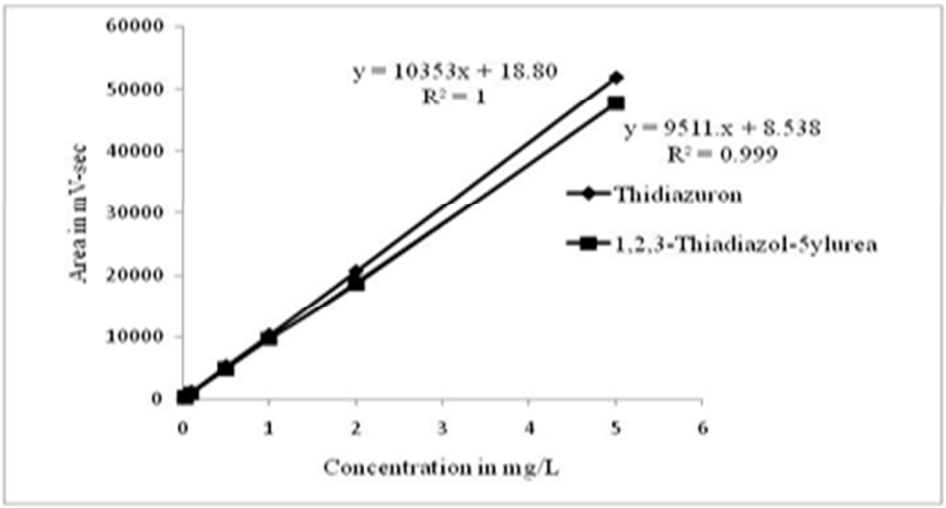

Figure 1
Representative Calibration curve of thidiazuron and 1,2,3-Thiadiazol-5-ylurea

Recovery–Limit of Determination

Recovery studies in sandy loam, loamy sand, sandy clay and clay soil were conducted by fortifying three different concentrations (0.03 and 0.30 mg/kg) of standard solutions of thidiazuron and Metabolite 1,2,3-thiadiazol-5-ylurea. The samples were homogenized, extracted and analyzed for thidiazuron and metabolite 1,2,3-thiadiazol-5-ylurea content, as described in the method of analysis.

Limit of Quantification (LOQ)

Based on the recovery percentage[8- 10], the limit of Quantification (LOQ) was 0.03 mg/kg for thidiazuron and its metabolite 1, 2,3-Thiadiazol-5-ylurea.

Method of Calculation

$$\text{Thidiazuron, its Metabolite 1,2,3-Thiadiazol-5-ylurea in mg/kg} = \frac{A \times B \times C}{D \times E} \times F$$

Where,

A – Peak area in sample (mV-sec)
B – Volume of the sample (ml)
C – Concentration of the standard (mg/L)
D – Peak area in standard (mV-sec)
E – Weight of the sample (g)
F – Dilution Factor

Experimental Design for Dissipation study

Common Name : Thidiazuron
IUPAC Name : 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea
Type of formulation : Wettable powders (WP)
Type of Soils : Sandy loam, Loamy sand, Sandy clay and Clay
Location : GITAM University, Rudraram (Latitude - 17.5620838 and Longitude - 78.16678539999998)
Dose rate : T_0– Untreated Control
T_1 – Thidiazuron 50% WP@ 1.0 mg/kg
T_2 – Thidiazuron 50% WP @ 2.0 mg/kg
Method of application : T0- Dosage
0.8 mL of acetonitrile was added to the 100 ml of flask and made up to the mark with distilled water.

T1- Dosage

Stock solution was prepared by dissolving 0.01002 g of test item into a 100 ml volumetric flask. 0.8 ml of acetonitrile was added to the flask and sonicated to dissolve the test item and flask was made up to the mark with distilled water and shaken well to homogenise the content.20 mL of above stock solution was pipetted out and thoroughly mixed with 1 kg of soil.

T2 - Dosage

Stock solution was prepared by dissolving 0.02001 mg of test item into a 100 ml volumetric flask. 0.8 ml of acetonitrile was added to the flask and sonicated to dissolve the test item and flask was made up to the mark with distilled water and shaken well to homogenise the content. 20 mL of above stock solution was pipetted out and thoroughly mixed with 1 kg of soil.

Note: The soil was wetted with 20 mL of water before adding the test solution.
No. of samples taken per test/treatment : Three
Sample Collection : At each sampling intervals, soil samples were collected randomly at different predetermined places in the soil container.
Sampling intervals (Days) : 0, 1, 3, 5, 10 and 20

RESULTS AND DISCUSSION

Linearity

The method was found to be linear with a correlation co-efficient (CC) of 1.000 for thidiazuron and 0.999 for its metabolite 1,2,3-Thiadiazol-5-ylurea when tested in the range 0.03 – 5.0 mg/L.

Recovery

The mean percent recovery of thidiazuron in Sandy loam, Loamy sand, Sandy clay and Clay soils was 85 ± 1.19; 87 ± 1.56; 89 ± 1.41 and 86 ± 1.78 at 0.03 mg/kg fortification level, 92 ± 2.15; 94 ± 2.56; 90 ± 2.69 and 95 ± 1.89 at 0.3 mg/kg fortification level. The mean percent recovery of metabolite 1, 2, 3-Thiadiazol-5-ylurea in Sandy loam, Loamy sand, Sandy clay and Clay soils was 89 ± 1.36; 86 ±

1.11; 87 ± 1.32 and 88 ± 1.77 at 0.03 mg/kg fortification level, 90 ± 2.41; 93 ± 2.82; 90 ± 2.71 and 92 ± 2.32 at 0.3 mg/kg fortification level.

Dissipation in Sandy Loam Soil

Thidiazuron

The residues of thidiazuron in sandy loam soil samples collected on '0' day after the application showed the initial concentrations 0.982 mg/kg and 1.995 mg/kg in T1 and T2 dosages, respectively. Analysis of 1st day samples showed the dissipation of residues to 0.669 mg/kg and 1.339 mg/kg and by 3rd day, residues dissipated to 0.521 mg/kg and 1.021 mg/kg in T1 & T2 dosages, respectively. Analysis of 5th day samples showed the residues 0.425 mg/kg and 0.809 mg/g and 0.087 mg/kg and 0.186 mg/kg in T1 and T2 tested dosages on 10th day. Complete dissipation of residues of thidiazuron to below the determination limit at both the tested dosages (T1 and T2) was observed by 20th day.

Metabolite – 1,2,3-Thiadiazol-5-ylurea

The residues of metabolite 1,2,3-Thiadiazol-5-ylurea in sandy loam soil samples collected on '0' day, 1st day, 3rd day and 5th day showed below the determination limit at both the tested dosages (T1 and T2). Analysis of 7th day samples showed the residues 0.069 mg/kg and 0.139 mg/kg and by 10th day, residues dissipated to 0.036 mg/kg and 0.076 mg/kg in T1 & T2 dosages, respectively. Complete dissipation of residues of metabolite 1,2,3-thiadiazol-5-ylurea to below the detectable limit at both the tested dosages (T1 and T2) was observed by 20th day.

Dissipation in Loamy Sand Soil

Thidiazuron

The residues of thidiazuron in sandy loam soil samples collected on '0' day after the application showed the initial concentrations 0.955 mg/kg and 1.971 mg/kg in T1 and T2 dosages, respectively. Analysis of 1st day samples showed the dissipation of residues to 0.603 mg/kg and 1.128 mg/kg and by 3rd day, residues dissipated to 0.502 mg/kg and 1.012 mg/kg in T1 & T2 dosages, respectively. Analysis of 5th day samples showed the residues 0.489 mg/kg and 0.929 mg/k and 0.079 mg/kg and 0.163 mg/kg in T1 and T2 tested dosages on 10th day. Complete dissipation of residues of thidiazuron to below the determination limit at both the tested dosages (T1 and T2) was observed by 20th day.

Metabolite – 1,2,3-Thiadiazol-5-ylurea

The residues of metabolite 1,2,3-Thiadiazol-5-ylurea in sandy loam soil samples collected on '0' day, 1st day, 3rd day and 5th day showed below the determination limit at both the tested dosages (T1 and T2). Analysis of 7th day samples showed the residues 0.058 mg/kg and 0.111 mg/kg and by 10th day, residues dissipated to 0.032 mg/kg and 0.066 mg/kg in T1 & T2 dosages, respectively. Complete dissipation of residues of metabolite 1,2,3-thiadiazol-5-ylurea to below the detectable limit at both the tested dosages (T1 and T2) was observed by 20th day.

Dissipation in Sandy Clay Soil

Thidiazuron

The residues of thidiazuron in sandy loam soil samples collected on '0' day after the application showed the initial concentrations 0.925 mg/kg and 1.902 mg/kg in T1 and T2 dosages, respectively. Analysis of 1st day samples showed the dissipation of residues to 0.615 mg/kg and 1.333 mg/kg and by 3rd day, residues dissipated to 0.519 mg/kg and 1.011 mg/kg in T1 & T2 dosages, respectively. Analysis of 5th day samples showed the residues 0.468 mg/kg and 0.932 mg/k and 0.079 mg/kg and 0.168 mg/kg in T1 and T2 tested dosages on 10th day. Complete dissipation of residues of thidiazuron to below the determination limit at both the tested dosages (T1 and T2) was observed by 20th day.

Metabolite – 1,2,3-Thiadiazol-5-ylurea

The residues of metabolite 1,2,3-Thiadiazol-5-ylurea in sandy loam soil samples collected on '0' day, 1st day, 3rd day and 5th day showed below the determination limit at both the tested dosages (T1 and T2). Analysis of 7th day samples showed the residues 0.062 mg/kg and 0.119 mg/kg and by 10th day, residues dissipated to 0.034 mg/kg and 0.070 mg/kg in T1 & T2 dosages, respectively. Complete dissipation of residues of metabolite 1, 2,3-thiadiazol-5-ylurea to below the detectable limit at both the tested dosages (T1 and T2) was observed by 20th day.

Dissipation in Clay Soil

Thidiazuron

The residues of thidiazuron in sandy loam soil samples collected on '0' day after the application showed the initial concentrations 0.931 mg/kg and 1.887 mg/kg in T1 and T2 dosages, respectively. Analysis of 1st day samples showed the dissipation of residues to 0.672 mg/kg and 1.349mg/kg and by

3rd day, residues dissipated to 0.429 mg/kg and 1.002 mg/kg in T1 & T2 dosages, respectively. Analysis of 5th day samples showed the residues 0.365 mg/kg and 0.739 mg/k and 0.073 mg/kg and 0.158 mg/kg in T1 and T2 tested dosages on 10th day. Complete dissipation of residues of thidiazuron to below the determination limit at both the tested dosages (T1 and T2) was observed by 20th day.

Metabolite – 1,2,3-Thiadiazol-5-ylurea

The residues of metabolite 1,2,3-Thiadiazol-5-ylurea in sandy loam soil samples collected on '0' day, 1st day, 3rd day and 5th day showed below the determination limit at both the tested dosages (T1 and T2). Analysis of 7th day samples showed the residues 0.053 mg/kg and 0.106 mg/kg and by 10th day, residues dissipated to 0.035 mg/kg and 0.071 mg/kg in T1 & T2 dosages, respectively. Complete dissipation of residues of metabolite 1,2,3-thiadiazol-5-ylurea to below the detectable limit at both the tested dosages (T1 and T2) was observed by 20th day. The dissipation curve plotted between concentration of the analyte and sampling occasions is presented in Figure 2, Figure 3, Figure 4 and Figure 5**.** DT50 value was calculated using the following formula

$$DT50 = \ln 2/ (k)$$

Where,'k' is slope of the curve obtained from the dissipation data. The calculated DT 50 (Time required to degrade 50% of residues) values are presented in Table 2, 3, 4 and 5. The rate constant value was calculated by linear regression equation from the first order rate equation

$$K = \ln a/a–x/dt$$

Where, dt is the time interval between T1 and T2 and a, x are the concentration of pesticides at times T1 and T2 respectively. A plot of concentration of the residues and rate with the R2 indicates first order kinetics in dissipation of both the fungicides. The DT50 (Half Life) of copper oxychloride and Kasugamycin calculated by regression analysis from the dissipation data.

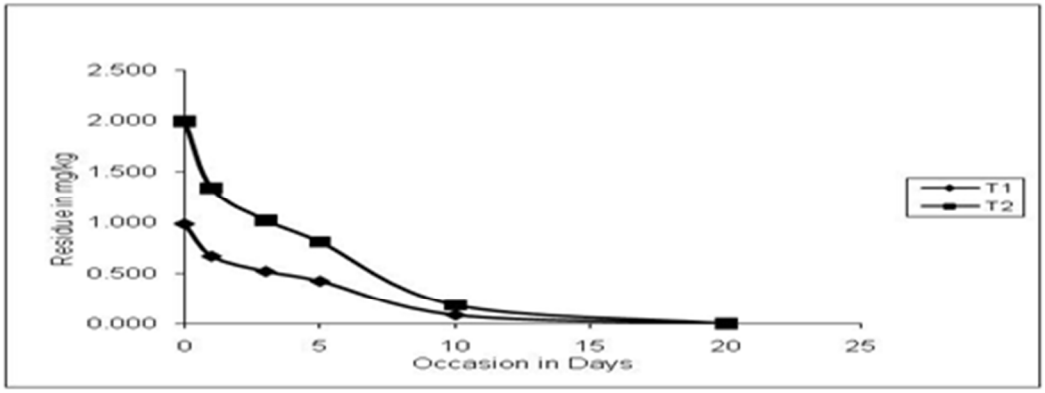

Figure 2
Dissipation curve of thidiazuron in Sandy Loam Soil

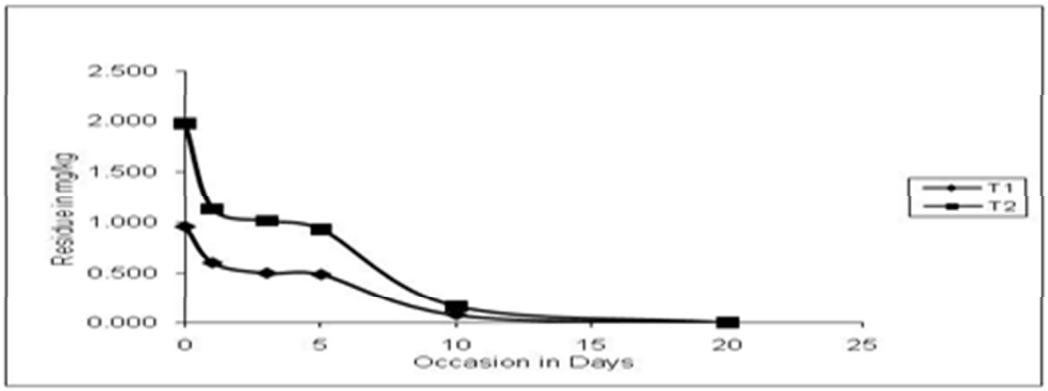

Figure 3
Dissipation curve of thidiazuron in loamy Sand

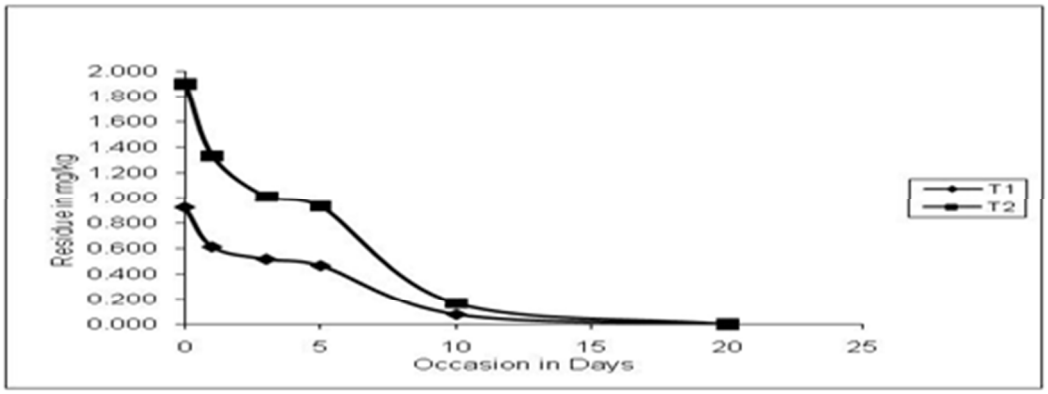

Figure 4
Dissipation curve of thidiazuron in sandy clay

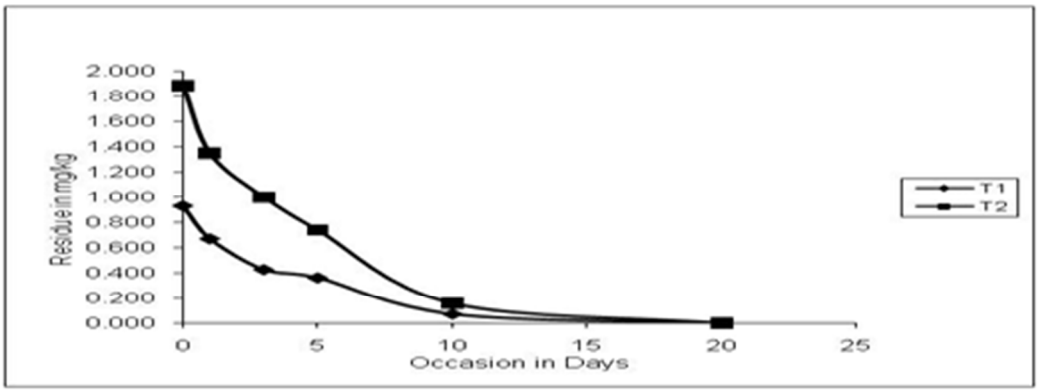

Figure 5
Dissipation curve of thidiazuron in clay

Table 2
Regression Analyses – Sandy Loam Soil for Thidiazuron

Parameters	**Dosages**	
	T1	**T2**
Regression equation	Y = 0.0002– 0.0999 * X	Y = 0.2943 – 0.0978* X
Half-life (Days)	3.01	3.08
Correlation co-efficient	-0.980	-0.986

Table 3
Regression Analysis – Loamy sand for Thidiazuron

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0106 – 0.10000 * X	Y = -2812 – 0.0986* X
Half-life (Hours)	3.01	3.05
Correlation co-efficient	-0.955	-0.957

Table 4
Regression Analysis – Sandy clay for Thidiazuron

Parameters	**Dosages**	
	T1	**T2**
Regression equation	Y = -0.0122- 0.1000* X	Y = 0.2991 – 0.0996 * X
Half-life (Days)	3.01	3.02
Correlation co-efficient	-0.961	-0.969

Table 5
Regression Analysis – Clay for Thidiazuron

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.0251 – 0.1063 * X	Y = 0.2904 – 0.1041* X
Half-life (Hours)	2.83	2.89
Correlation co-efficient	-0.987	-0.988

CONCLUSIONS

A simple and inexpensive HPLC method was developed and validated for the determination of thidiazuron residues in different tropical soils. The developed analytical procedure involves optimization of the extraction process, SPE for purification, and HPLC– PDA analysis. The satisfactory validation parameters such as linearity, recovery and LOQ and DT 50 values were established by following South African National Civic Organization (SANCO) guidelines[11]. As a result of this study the procedure could be useful for regular agencies, residue labs and research scholars to monitoring of thidiazuron residues in different commodities (crop, water, soil and air samples).

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