ISSN : 0974-7419 Volume 16 Issue 10

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 16(10) 2016 [433-440]

Dissipation kinetics of spirotetramat and its metabolite in four different soils

Jakka Mastan[1]*, B.N.Srinivas[2], Tentu.Nageswara Rao[1], N.Krishna Rao[1]

[1]Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, (INDIA)

[2] Usharama college of engineering & technology, Telaprolu, Vijayawada, Andhra Pradesh, (INDIA)

E-mail: jmrao2006@gmail.com

ABSTRACT

The laboratory study was conducted to evaluate the persistence of Spirotetramat 240 SC (w/v) in sandy loam, loamy sand, sandy clay and clay soils. The applied dosages are T0 – Untreated Control, T1 – Spirotetramat 240 SC (w/v) @ 0.05 mg/L and T2 – Spirotetramat 240 SC (w/v) @ 1.0 mg/L. Collected the composite soil sample from 0-15 cm depth 2 hours (0 day) after the application. Continued the sampling on 1 3, 5 and 7 days for Spirotetramat and its BYI 08330 enol metabolite. All the samples were analysed for spirotetramat content by a validated HPLC-UV method with an acceptable mean recovery of spirotetramat; 92% for Sandy loam, 93% for Loamy sand, 91% for Sandy clay and 90% for Clay soil. The limit of determination (LOQ) is 0.03 ppm for Spirotetramat and its BYI 08330 enol metabolite. The DT_{50} (Half Life) of Spirotetramat and 08330 enol metabolite calculated by regression analysis from the dissipation data.

KEYWORDS

Spirotetramat;
BYI 08330 enol;
HPLC;
DT50 and Indian soils.

INTRODUCTION

Spirotetramat is a tetramic acid derivative with IUPAC name cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4.5] dec-3-en-2-one. It belongs to ketoenol group. Spirotetramat is sprayed on the leaves of the plant. It reaches all the parts of the plant including the roots and buds, through xylem and phloem present in the plant. Traditional insecticides have one-way transport system, through xylem, and hence are applied on growing plant parts.[1]. However, phloem transports from leaves to roots hence can be applied at any stage of the plant growth. Spirotetramat is widely used for citrus, pome fruit, grape, strawberries, lettuce, mangoes and cotton, etc. This insecticide is effective against aphids, psyllids, scales, whiteflies, mealybugs and selected thrips. In soil laboratory incubations under aerobic conditions in the dark, spirotetramat exhibited very low persistence, forming the major metabolites (>10 % applied radioactivity (AR)) spirotetramat-enol (max. 100 % AR) and spirotetramat-ketohydroxy (24 % AR), which exhibited very low to moderate and low to moderate persistence, respectively[2]. The metabolite spirotetramat-MA-amide was formed at levels triggering consideration for groundwater exposure (5.2 % AR) and exhibited low persistence. Mineralisation of the azaspirodecenyl-3- 14C radiolabel to carbon dioxide accounted for 10 - 19 % AR

Full Paper

after 50 days (range from 4 soils), and reached 12.1 % after 126 days in the single soil where the incubation continued beyond 50 days. The formation of unextractable residues (not extracted by formic acid acidified acetonitrile / water followed by acetonitrile / 1N hydrochloric acid then acetonitrile) for this radiolabel accounted for 21 – 31 % AR after 50 days, and 28 % after 126 days in the single soil where the incubation continued beyond 50 days[3]. In anaerobic soil incubation spirotetramat also exhibited very low persistence forming the same metabolites as under aerobic conditions, with spirotetramat-MA-amide being formed at a slightly higher level than in aerobic incubations at up to 7.2 % AR. In a laboratory soil photolysis study, a novel transformation product 4-methoxy-cyclohexanone was formed at up to 10 % AR. Spirotetramat exhibited medium mobility in soil[4,5]. The metabolites spirotetramat-enol and spirotetramat-ketohydroxy exhibited very high to high soil mobility and spirotetramat-MA-amide exhibited very high soil mobility. Soil adsorption measurements were not available for 4-methoxy-cyclohexanone, therefore the groundwater exposure assessment was completed using the worst case assumption that this metabolite has no soil adsorption potential. The available data indicate that soil mobility is not pH dependent for these compounds[6, 7, 8]. In satisfactory field dissipation studies carried out at 4 sites in the USA (New York, Florida, California and Washington, spray application to the soil surface on bare soil plots in late spring and at three sites to bare soil where previously seeded crops (bush beans, tomatoes and onions) subsequently emerged) spirotetramat exhibited very low persistence[9, 10]. Sample analyses were carried out for spirotetramat, spirotetramat-enol, spirotetramat-ketohydroxy and spirotetramat-MA-amide. During sample handling and extraction spirotetramat-enol was not stable, it converted to spirotetramat-ketohydroxy. When residues were expressed as the sum of these 4 compounds, the total residue exhibited low to moderate persistence (the DT90 were 19 to 78 days).

GEO GRAPHICAL INFORMATION

Place - Nuzvid, Andhrapradesh State
Latitude - 16.7849906
Longitude - 80.8488498
Nature of the study - Dissipation Kinetics
Test matrix - 1. Sandy loam
2. Loamy sand
3. Sandy clay
4. Clay soil

MATERIALS AND METHODS

Reference analytical standards of Spirotetramat (Purity 99.0 %) and BYI 08330 enol metabolite (Purity 99.0 %) were obtained from Sigma Aldrich. The test item Spirotetramat 240 SC (w/v) was purchased from local market. Acetonitrile, Water HPLC grade, Formic acid AR grade, and Celite 545 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 ATvp pump and SPD-20A UV/VIS CTO-20A Column oven using LC soluition software, Hamilton syringe (50 µl) – 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 Mettler AG-245 analytical balance, capable of weighing 0.01 mg supplied by M/s. Mettler Toledo, Switzerland.

Chromatographic separation parameters

Instrument - Shimadzu High Performance Liquid Chromatograph system equipped with LC-20 ATvp pump and SPD-20A UV/VIS CTO-20A Column oven using LC soluition software.
Column used - Phenomenex C_{18} (25cm length x 4.6mm i.d)
Wave length - 235 nm
Mobile phase - Acetonitrile : Water : 0.04 % formic acid (40:60)
Flow rate - 1.5 ml/min for 5 min 2 ml/min from 6 min up to 22 min Stop time 22.0 min
Column oven - 30°C
Injected volume - 10 µl

Retention time
(Approximately)
Metabolite Enol- 5.3 minutes
Spirotetramat - 20.2 minutes

METHOD VALIDATION

Calibration details

Preparation of stock solution of reference analytical standard

Spirotetramat and its BYI 08330 enol metabolite

Accurately weighed about 10 mg of reference analytical standard of Spirotetramat and its metabolite in 10 ml volumetric flask and dissolved the content of the flask and the volume was made upto the mark using acetonitrile.

Preparation of calibration solutions

Different known concentrations of Spirotetramat and its metabolite (1.0 – 0.01ppm) were prepared in mobile phase by diluting the stock solution. Injected the standard solutions and measured the peak area. Resulting from the elution of the compound. The details were given in the TABLE 1 A calibration curve has been plotted for the concentration of the standards injected versus area observed and the linearity the method was determined form the correlation coefficient. Calibration curve is shown in Figure 1.

TABLE 1

Injected concentration (ppm)	Response in Area (µV-sec)	
	BYI 08330 enol metabolite	Spirotetramat
1	111031	89191
0.5	54711	43675
0.2	21162	17800
0.1	11936	8859
0.05	5564	4582
0.01	1130	932

Recovery-limit of determination

Recovery studies in sandy loam, loamy sand, sandy clay and clay soil was conducted by fortifying different concentrations of Spirotetramat and its BYI 08330 enol metabolite standard in the range (0.3 – 0.03 ppm). The samples were homogenized, extracted and analysed for Spirotetramat and its BYI 08330 enol metabolite content, as described in the method of analysis. The mean recovery percentage of spirotetramat ; 92% for Sandy loam, 93% for Loamy sand, 91% for Sandy clay and 90% for Clay soil.

Method of analysis

Extraction

Weighed 50 g of soil sample in to a extraction bottle. Added 75 ml of acetonitrile/water containing 0.22 ml of formic acid / L (4:1 - extracting solvent). Extracted the residues using end over end shaker for 30 minutes. The same extraction procedure repeated once again with 50 ml of extracting

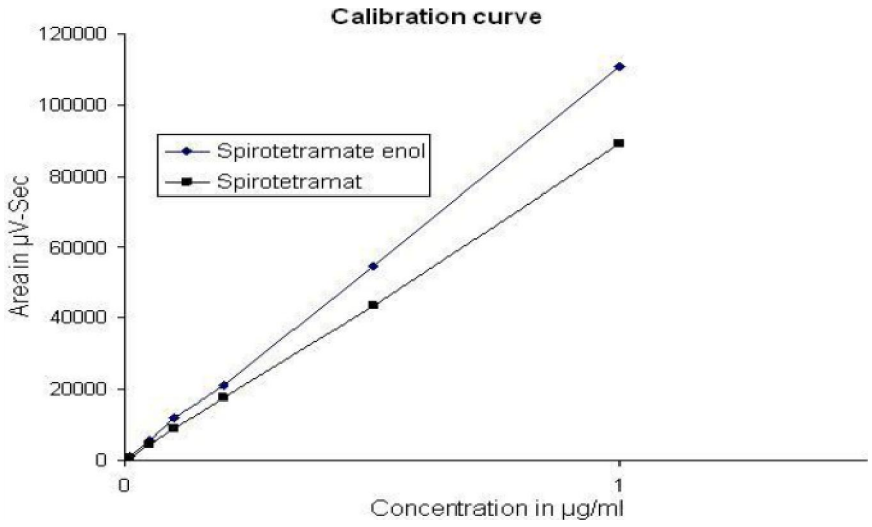

Figure 1

solvent. Added 2 g of celite 545 to the extract and passed through strata C18 E tube after equilibration with extraction solvent. After elution of extract, residues again eluted with 50 ml of extracting solvent. Combined the extracts and reduced the volume of extract under vacuum at 40°C using buchi rota vapour. Residues were reconstituted with 5 ml of acetonitrile, injected in HPLC.

Method of calculation

Spirotetramat and its metabolite content (ppm)

$$= \frac{A \times B \times C}{D \times E} \times F$$

Where; A - Peak area in sample (µV-sec); B - Volume of the sample (ml); C - Concentration of the standard (ppm); D - Peak area in standard (µV-sec); E - Weight of the sample (g); F - Dilution Factor

RESULTS AND DISCUSSION

Method validation

Linearity

Spirotetramat and its BYI 08330 enol metabolite

The method was found to be linear with a correlation coefficient of 1.000 when tested in the range 1.0 to 0.01 ppm.

Recovery

Spirotetramat and its BYI 08330 enol metabolite

The mean recovery percentage of Spirotetramat is 92% for sandy loam, 93% for loamy sand, 91% for sandy clay and 90% for clay soil. The mean recovery percentage of BYI 08330 enol metabolite is 93% for sandy loam, 94% for loamy sand, 95% for sandy clay and 90% for clay soil. From the data limit of determination (LOQ) can be established as 0.01 ppm for Spirotetramat and its BYI 08330 enol metabolite.

Persistence in sandy loam soil

Spirotetramat

The initial concentration of Spirotetramat in sandy loam soil was 0.046 ppm and 0.097 ppm in T1 and T2 dosages respectively, which on 1st day had dissipated to 0.038 ppm and 0.066 ppm in T1 and T2 dosages respectively which on 3rd day residues went below the limit of determination in T1 and 0.056 ppm in T2 dosages which on 5th day residues went below the limit of determination in T1 and 0.037 ppm in T2 dosages. On Analysis of 7th day samples showed that residues were below the limit of determination in both the tested dosages.

BYI 08330 enol metabolite

Analysis of samples collected on all the sampling occasions showed that the residues of BYI 08330 enol metabolite were below the limit of determination in both the tested dosages.

The half life values calculated for Spirotetramat in sandy loam soil are 3.63 days and 3.96 days at the tested dosages T1 and T2, respectively.

Persistence in loamy sand soil

Spirotetramat

The initial concentration of Spirotetramat in loamy sand soil was 0.044 ppm and 0.081 ppm in T1 and T2 dosages respectively, which on 1st day had dissipated to 0.036 ppm and 0.062 ppm in T1 and T2 dosages respectively which on 3rd day residues were below the limit of determination in T1 and 0.047 ppm in T2 dosages which on 5th day residues went below the limit of determination in T1 and 0.031 ppm in T2 dosages. On Analysis of 7th day samples showed that residues were below the limit of determination in both the tested dosages.

BYI 08330 enol metabolite

Analysis of samples collected on all the sampling occasions showed that the residues of BYI 08330 enol metabolite were below the limit of determination in both the tested dosages.

The half life values calculated for Spirotetramat in loamy sand soil are 3.45 days and 3.77 days at the tested dosages T1 and T2, respectively.

Persistence in sandy clay soil

Spirotetramat

The initial concentration of Spirotetramat in sandy clay soil was 0.046 ppm and 0.084 ppm in T1 and T2 dosages respectively, which on 1st day had

dissipated to 0.037 ppm and 0.063 ppm in T1 and T2 dosages respectively. On 3rd day residues were below the limit of determination in T1 and 0.045 ppm in T2 dosages which on 5th day residues went below the limit of determination in T1 and 0.033 ppm in T2 dosages. On Analysis of 7th day samples showed that residues were below the limit of determination in both the tested dosages.

BYI 08330 enol metabolite

Analysis of samples collected on all the sampling occasions showed that the residues of BYI 08330 enol metabolite were below the limit of determination in both the tested dosages.

The half life values calculated for Spirotetramat in sandy clay soil are 3.18 days and 3.82 days at the tested dosages T1 and T2, respectively.

Persistence in clay soil

Spirotetramat

The initial concentration of Spirotetramat in clay soil was 0.045 ppm and 0.089 ppm in T1 and T2 dosages respectively, which on 1st day had dissipated to 0.035 ppm and 0.057 ppm in T1 and T2 dosages respectively. On 3rd day residues were below the limit of determination in T1 and 0.045 ppm in T2 dosages. On Analysis of 5th day samples showed that residues were below the limit of determination in both the tested dosages.

BYI 08330 enol metabolite

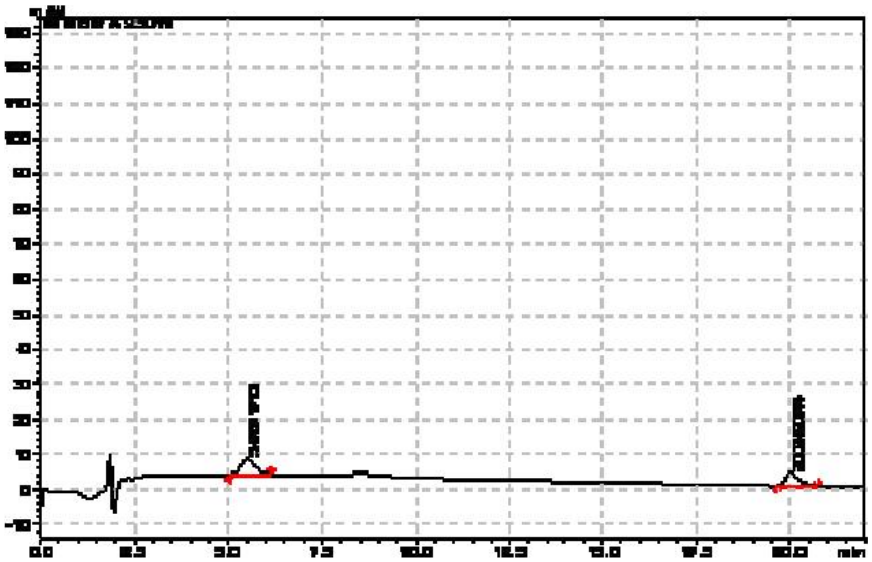

Figure 2 : Representative chromatogram of standard 1.0 ppm

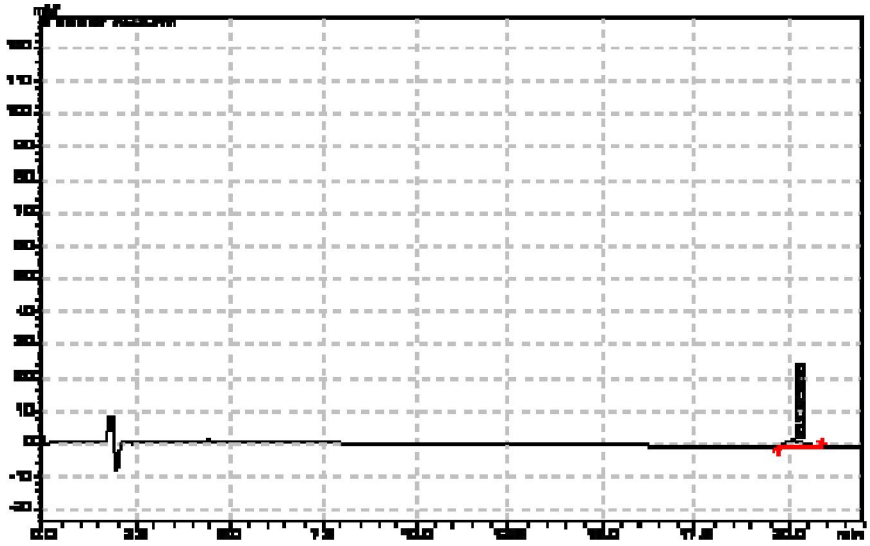

Figure 3 : Representative chromatogram of sandy loam 0 day

Full Paper

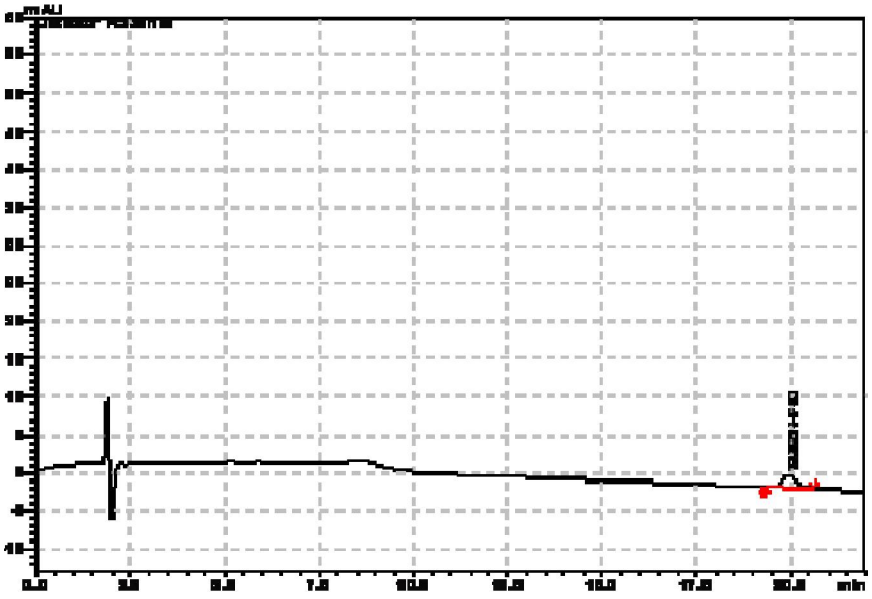

Figure 4 : Representative chromatogram of loamy sand 0 day

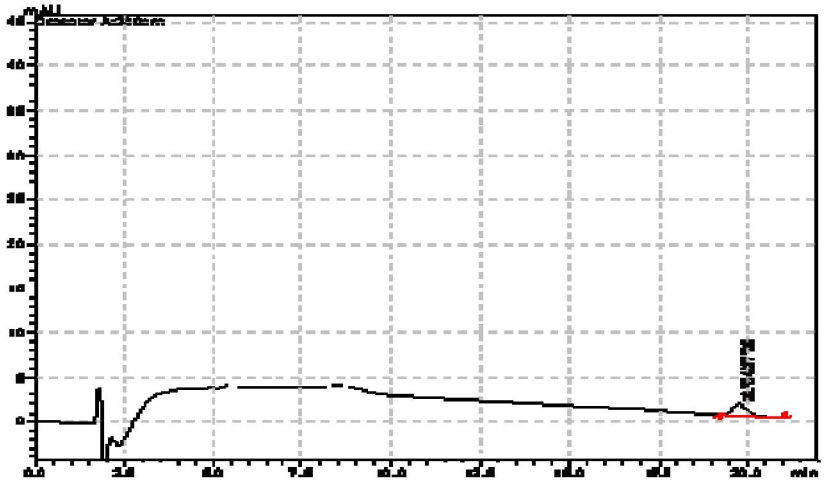

Figure 5 : Representative chromatogram of sandy clay 0 day

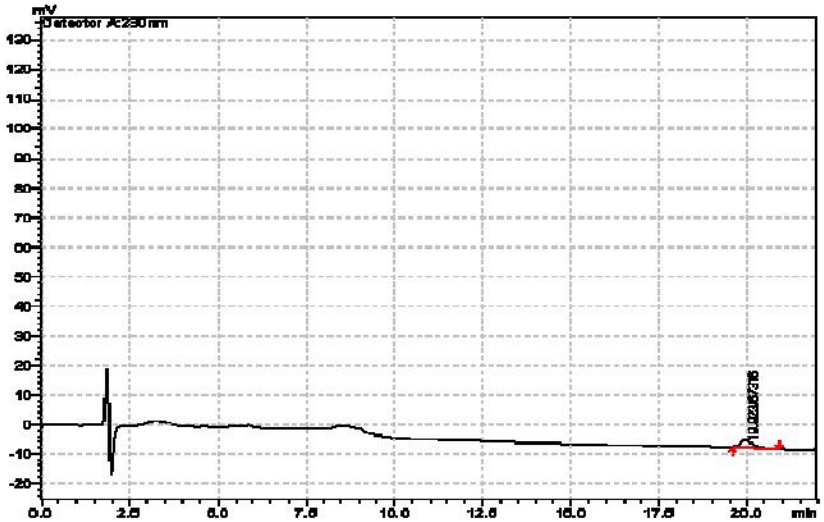

Figure 6 : Representative chromatogram of clay 0 day

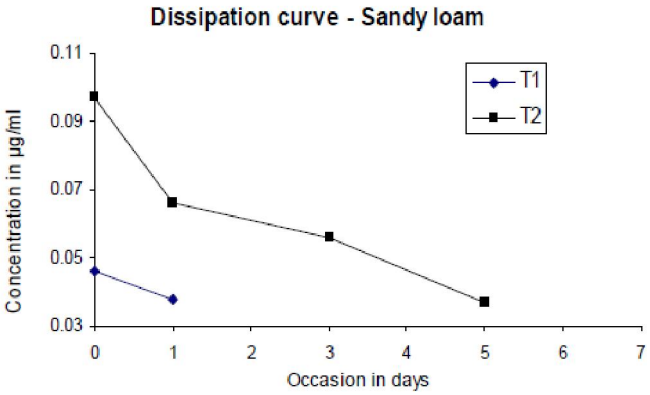

Figure 7 : Dissipation curve of spirotetramat in sandy loam

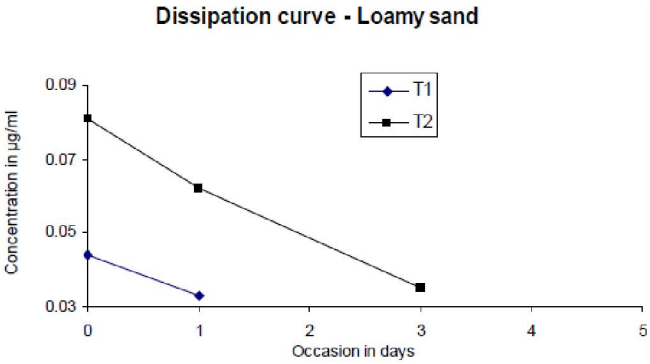

Figure 8 : Dissipation curve of spirotetramat in loamy sand

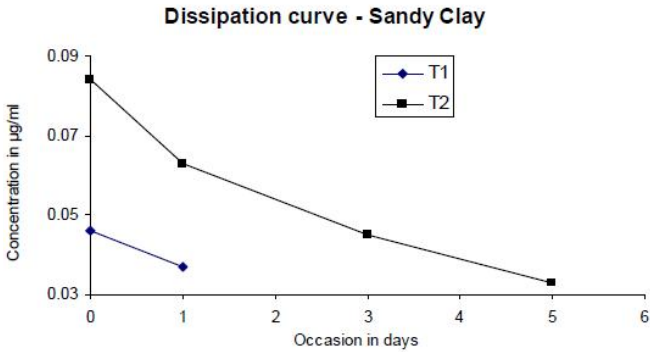

Figure 9 : Dissipation curve of spirotetramat in sandy clay

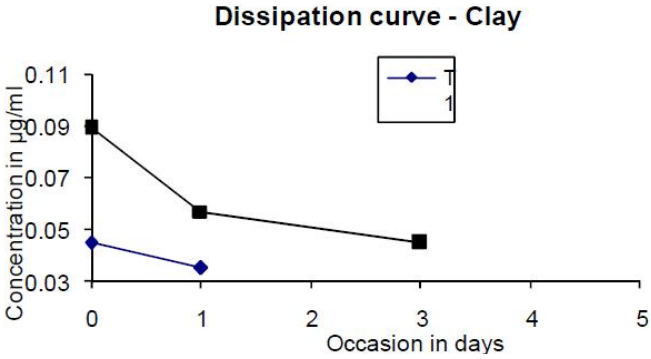

Figure 10 : Dissipation curve of spirotetramat in clay

Analysis of samples collected on all the sampling occasions showed that the residues of BYI 08330 enol metabolite were below the limit of determination in both the tested dosages.

The half life values calculated for Spirotetramat in sandy clay soil are 2.76 days and 3.27 days at the tested dosages T1 and T2, respectively. The typical standard and 0th day occasion soil sample chromatograms are showed Figure 2, 3, 4, 5 and 6.

The dissipation curve plotted between concentration of the analyte and sampling occasions were presented in Figure 7 to 10. 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

TABLE 2 : Regression analysis of spirotetramat in sandy loam

Parameters	Dosages	
	T1	T2
Regression equation	Y = -1.337 – 0.083 * X	Y = -1.048 – 0.076 * X
Half-life (days)	3.63	3.96
Correlation co-efficient	1.0000	0.9719

TABLE 3 : Regression analysis of spirotetramat in loamy sand

Parameters	Dosages	
	T1	T2
Regression equation	Y = -1.357 – 0.087 * X	Y = -1.104 – 0.080 * X
Half-life (days)	3.45	3.77
Correlation co-efficient	1.0000	0.9948

Full Paper

TABLE 4 : Regression analysis of spirotetramat in sandy clay

Parameters	Dosages	
	T1	T2
Regression equation	Y = -1.337 – 0.095 * X	Y = -1.099 – 0.079 * X
Half-life (days)	3.18	3.82
Correlation co-efficient	1.0000	0.9928

TABLE 5 : Regression analysis of spirotetramat in clay

Parameters	Dosages	
	T1	T2
Regression equation	Y = -1.347 – 0.109 * X	Y = -1.091 – 0.092 * X
Half-life (days)	2.76	3.27
Correlation co-efficient	1.0000	0.9340

to 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 Spirotetramat and its BYI 08330 enol metabolite. The DT50 (Half Life) of Spirotetramat and its BYI 08330 enol metabolite calculated by regression analysis from the dissipation data.

CONCLUSION

Satisfactory validation parameters such as linearity, recovery, precision and LOQ and DT 50 values were established by following South African National Civic Organization (SANCO) and Environmental Protection Agency (EPA) guidelines[11]. Therefore, the proposed analytical procedure and dissipation data could be useful for regular monitoring, residue labs and research scholars to determine the Spirotetramat and its BYI 08330 enol metabolite residues in different commodities (crop, water and soil samples).

ACKNOWLEDGEMENTS

The authors are thankful to the Dr. B. Gowtham Prasad, SVV University, for providing necessary facility to conduct the Laboratory experiment.

REFERENCES

[1] S.Mohapatra, M.Deepa, G.K.Jagadish; Bull environ contam toxicol., **88(2)**, 124-128 **(2012)**.
[2] Singh, Balwinder, Mandal, Kousik, Sahoo, K.Sanjay, Bhardwaj; Journal of Chromatography A., **1299**, 71–77 **(2013)**.
[3] Shashi Vemuri, Ch.Sreenivasa Rao, S.Swarupa; Journal of multidisciplinary engineering science and technology, **1(5)**, 319-324 **(2014)**.
[4] C.K.Hindumathy, V.Gayathri; Bioremed Biodeg., **47(5)**, 178-184 **(2013)**.
[5] Ismail Sahid et al.; Pertanika., **15(2)**, 121-125 **(1992)**.
[6] G.Kiran, Chaudhari; Advances in Applied Science Research., **4(6)**, 246-248 **(2013)**.
[7] R.Lal, D.M.Saxena; Microbiol.Rev., **46**, 95 **(1982)**.
[8] Lipika Patnaik et al.; Asianj.Exp.Biol.Sci., **4(2)**, 219-225 **(2013)**.
[9] Metin Diurak, Ferda Ukazanici; Turk J Biol., **25**, 51-58 **(2001)**.
[10] G.S.Wagh et al.; Universal journal of Enviromental Research and Technology., **3(1)**, 93-99 **(2013)**.
[11] SANCO Guidelines; Document NO., SANCO/10684/2009, **(2009)**.