

DISSIPATION OF SPIROTETRAMAT AND IMIDACLOPRID IN GRAPES AND SOIL

Shashi Vemuri, Ch.Sreenivasa Rao and S.Swarupa

AINP on Pesticide Residues, Prof.Jayashankar Telangana State Agricultural University,
EEI Premises, Rajendranagar, Hyderabad- 500 03, India
Sash_3156@yahoo.co.in

Abstract—A field trial was conducted after October pruning in 2012-13 to determine the residues of spirotetramat 12% (an insecticide having mobility in xylem and phloem) and imidacloprid 12% (240SC) (a neonicotinoid used world wide) a combination product very effective in Grapes. Residue dynamics of spirotetramat and imidacloprid in grapes and soil was studied after spraying the combination formulation in 2 doses. Three sprays were given at initiation of berry formation followed by remaining two sprays at 10 days interval. The samples of immature grape berries were collected at regular intervals 0, 1, 3, 5, 7, 10, 15 and 20 days after last spray and mature berries and soil samples were collected at Harvest and analyzed for spirotetramat and imidacloprid residues. The initial deposits of 1.360 mg/kg of spirotetramat detected in Grape berry samples collected from plots sprayed at double dose (2x-dose) dissipated to 0.994 mg/kg by 1st day, 0.681 mg/kg, 0.223 mg/kg, 0.085 mg/kg by 3, 5 and 7th day and Below Determination Level by 10th day. The initial deposits of 0.820 mg/kg of spirotetramat detected in Grape berry samples collected from plots sprayed Single dose (x-dose), dissipated to 0.524 mg/kg, 0.390 mg/kg, 0.102 mg/kg, and Below Determination Level by 1, 3, 5 and 7th day, respectively. The initial deposits of 1.714 mg/kg of imidacloprid detected in Grape berry samples collected from plots sprayed double dose (2x-dose) dissipated to 1.266 mg/kg by 1st day, 0.904 mg/kg, 0.377 mg/kg, 0.153 mg/kg by 3rd, 5th and 7th day and Below Determination Level by 10th day. The initial deposits of 0.886 mg/kg of imidacloprid detected in Grape berry samples collected from plots sprayed Single dose (x-dose), dissipated to 0.683 mg/kg, 0.519 mg/kg, 0.170 mg/kg and below Determination Level by 1, 3, 5, and 7th day. The residues of spirotetramat and imidacloprid and enol metabolite in mature berries and soil samples collected at harvest from x and 2x dose sprayed plots were Below Determination Level. The initial deposits of 0.886 mg/kg of imidacloprid was detected in Grape berry samples collected from plots sprayed Single dose (x-dose), which dissipated to 0.683 mg/kg by 1st day, 0.519 mg/kg by 3rd day, 0.170 mg/kg by 5th day Determination Level by 7th day. The residues of spirotetramat and imidacloprid and enol metabolite in mature berries and soil samples

collected at harvest from x and 2x dose sprayed plots were Below Determination Level.

Keywords: *spirotetramat, imidacloprid, residues, below detectable level, grape berries*

Introduction

Spirotetramat is a broad spectrum pesticide against sucking insects and is mobile within xylem and phloem and has no cross resistance to the other pesticides. Imidacloprid is a widely used pesticide and used as seed treatment, foliar spray, soil drench to number of crops. The combination product has excellent record against sucking insects [1] and has increased efficacy at different stages of insect. Since Grape has export value and the pesticide residues may affect the export market, experiments were conducted to study the dissipation pattern of Spirotetramat and Imidacloprid on grape as a combination insecticide.

Materials and Methods:

The experiments were carried out at Grape Research Station, YSR Horticultural University, Rajendranagar, Hyderabad and the sample processing analysis has been carried out at AINP on Pesticide Residues during 2013-14 on Thompson seed less variety. The treatments were untreated control, recommended dose of 90 g a.i./ha and the double dose of 180 g a.i./ha of the combination product spirotetramat 12% and imidacloprid 12% (240SC) and the treatments were replicated thrice utilizing a spray volume of 1000 L/ha. For each treatment 10 trees were selected. First spray was given at fruit growth stage and the subsequent 2 applications at 10 days interval. After the third spray samples were collected at 0, 1, 3, 5, 7, 10, 15 and 20 days and at harvest the fruits and soil samples. 500 gm of berries & soil samples collected from each plant were immediately brought to the laboratory, extracted and analyzed on Ultra Performance Liquid Chromatography for Spirotetramat and Imidacloprid and HPLC for Spirotetramat Metabolite Enol. As per the procedure of [2] From each treatment 15 gm of fruits and 10 gm of soil samples were taken for Analysis

A. *Extraction and Clean up Procedure for Spirotetramat, its Metabolite and Imidacloprid.*

QuEChERS Extraction and clean up for Grapes (immature raw berries and mature berries) samples

The Grape berry samples were analyzed for Spirotetramat and Imidacloprid pesticide residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method at the laboratory. [3]. The collected Grape berry samples were homogenized with robot coupe blixer, and homogenized 15±0.1g sample was taken in 50ml centrifuge tube. The sample tube was then added with 30±0.1 ml acetonitrile. The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. The samples were then added with 3±0.1g sodium chloride and mixed by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube and added with 9±0.1g anhydrous sodium sulphate to remove the moisture content. 8 ml of extract was taken into 15 ml tube, containing 0.4±0.01g PSA sorbent (for dispersive solid phase d-SPE cleanup) and 1.2±0.01g anhydrous magnesium sulphate. The sample tube was vortexed for 30sec then followed by centrifugation for 5min at 2500-3000rpm. The extract of about 2ml was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1ml Acetonitrile for UPLC and HPLC analysis (Spirotetramat , enol and Imidacloprid) under standard operational conditions. The extract of about 4ml was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1ml Acetonitrile for HPLC analysis (enol 0.05 fortification only) under standard operational conditions.

Extraction and clean up for soil samples:

The soil samples were analyzed for Spirotetramat and Imidacloprid pesticide residues following the QuEChERS method after validation of the method at the laboratory. 500 grms of soil collected from the field in Polythene bags was sieved, homogenized and dried at room temperature. 10±0.1 g sample was taken into 50 ml centrifuge tube. The sample tube was then added with 20±0.1 ml acetonitrile. The samples were then added with 1±0.1 g sodium chloride and 4±0.1 g Magnesium sulphate mixed by shaking gently followed by centrifugation for 3 min at 3300 rpm to separate the organic layer. The top organic layer of about 10 ml was taken into the 15 ml centrifuge tube containing 1.5±0.1g Magnesium sulphate and 0.25 grms PSA and sonicated for 1min to remove air bubbles and centrifuge for 10 min at 3000rpm. The extract of about 2ml(1gm sample) was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted 1ml Acetonitrile for UPLC and HPLC analysis (enol and Imidacloprid) under standard operational conditions. The extract of about 4ml(2gm sample) was transferred into test

tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted 1ml Acetonitrile for UPLC analysis (Spirotetramat) under standard operational conditions.

HPLC parameters for Enol metabolite analysis

HPLC	SHIMADZU LC20
Detector	HPLC Photo Diode Array Detector (PDA)
Column	UPLC Column Kinetex 5U XB – C18 column, 100X 4.6mm ID.
Wave Length	270 nm
Solvents in Pump A Solvents in Pump B	Water Acetonitrile
Solvents Gradient Program	Water + Acetonitrile (60+40) Mixture run for 15min.
Solvent Gradient rate	0.5 ml/min
Quantity of sample injected	20µl
Run time	15 min
Retention Time	Spirotetramat Enol metabolite – 2.21min
LC Program	Time Acetonitrile Water 0.00 40 60 15.0 60 40

UPLC parameters for Spirotetramat

UPLC	WATERS UPLC 'H' CLASS SYSTEM
Detector	UPLC Photo Diode Array Detector (PDA)
Column	UPLC Column Kinetex 5U–C18 column, 100X 4.6mm ID.
Wave Length	210 nm
Solvents in Pump A Solvents in Pump B	Water Acetonitrile
Solvents Gradient Program	Water + Acetonitrile (45+55)
Solvent Gradient rate	0.3 ml/min
Quantity of sample injected	10 µl
Run time	15 min
Retention Time	Spirotetramat – 6.8min
LC Program	Time Acetonitrile Water 0.00 55 45 10.0 65 35 15.00 55 45

UPLC parameters for imidacloprid

UPLC	WATERS UPLC 'H' CLASS SYSTEM
Detector	UPLC Photo Diode Array Detector(PDA)
Column	UPLC Column Kinetex 5UXB-C18 column,100X 4.6mm ID.
Wave Length	270 nm
Solvents in Pump A Solvents in Pump B	Water Acetonrile
Solvents Gradient Program	Water + Acetonrile (10+90)
Solvent Gradient rate	0.3 ml/min
Quantity of sample injected	10 µl
Run time	10 min
Retention Time	imidacloprid – 3.2min
LC Program	Time Acetonitrile Water 0.00 90 10 3.0 0 10 90 3.01 90 10 10.00 90 10

Results of Fortification and Recovery Studies**Spirotetramat Fortification and Recovery studies**

The control Grapes and soil samples were fortified with required quantity of spirotetramat and its metabolite and imidacloprid so as to obtain 0.05 mg/kg, 0.25 mg/kg, 0.5 mg/kg fortification levels and the samples were extracted and cleaned up as per QuEChERS method to validate the suitability of method.

Table- 1 Recoveries of spirotetramat metabolite i.e enol and Imidaclorid various fortification levels

	In grape samples		In soil samples			
	0.05 mg/kg 0.25mg/kg	0.05 mg/kg 0.25mg/kg	0.05 mg/kg	0.25mg/kg	0.5 mg/kg	0.05 mg/kg
spirotetramat	111.1	107.8	105.3	85.06	83.75	88.57
spirotetramat metabolite i.e enol	95.7	106.8	90.7	102.7	101.5	88.8
Imidacloprid	112.1	93.7	86.3	94	106	109
Limit of Quantitation/determination (LOQ)	Spirotetramat -0.05mg/kg Spirotetramat Metabolite Enol - 0.05mg/kg Imidacloprid -0.05mg/kg					

Results and conclusion

The Grape berry and Soil samples collected at regular intervals were analyzed for spirotetramat and its Metabolite enol and imidacloprid residues and the

residue data is presented in Tables 2and 3.The initial deposits of 1.360 mg/kg of spirotetramat detected in Grape berry samples collected from plots sprayed with double dose180 g.ai./ha (2x-dose)dissipated to 0.994 mg/kg by 1st day, 0.681 mg/kg ,0.223 mg/kg, 0.085 mg/kg by 3,5 and 7 days and Below Determination Level by 10th day. The initial deposits of 0.820 mg/kg of spirotetramat detected in Grape berry samples collected from plots sprayed Single dose90 g.a.i/ha (x-dose) dissipated to 0.524 mg/kg by 1st day, 0.390 mg/kg 0.102 mg/kg by 3 and 5 days and Below Determination Level by 7th day. The initial deposits of 1.714 mg/kg of imidacloprid detected in Grape berry samples collected from plots sprayed at double dose (2x-dose) dissipated to 1.266 mg/kg, 0.904 mg/kg, 0.377 mg/kg, 0.153mg/kg by 1,3,5,and 7 days and Below Determination Level by 10th day. The initial deposits of 0.886 mg/kg of imidacloprid detected in Grape berry samples collected from plots sprayed at Single dose(x-dose) , dissipated to 0.683 mg/kg by 1st day, 0.519 mg/kg by 3rd day, 0.170 mg/kg by 5th day and below Determination Level by 7th day. The residues of spirotetramat and imidacloprid and enol metabolite in mature berries and soil samples collected at harvest from x and 2x dose sprayed plots were Below Determination Level.Information on the residues of spirotetramat on grapes is not available. However information on mango, peach is available [2]and [4].In peaches spirotetramat at 180 g a.i./ha resulted in residues of of 0.14 mg /kg after 7 days. However In the present studies residues of spirotetramat were 0.22 mg/kg after 5 days and dissipated to 0.08 by 7th day at the higher dose. In the present study no enol metabolite was detected as it oxidases to BY 108330-ketohydroxy and subsequently to Y108330- MA- amide [5]. The weather conditions at Hyderabad might have dissipated the enol to other metabolites.In the field soil analysed at harvest no residues of spirotetramat or enol were detected. The residues of imidacloprid in grapes were upto 5 days in normal dose and 7 days in higher dose. These are in conformity of the reports of [6].No residues were detected in the soil at harvest

Table 2: Residues of spirotetramat in Grapes and soil

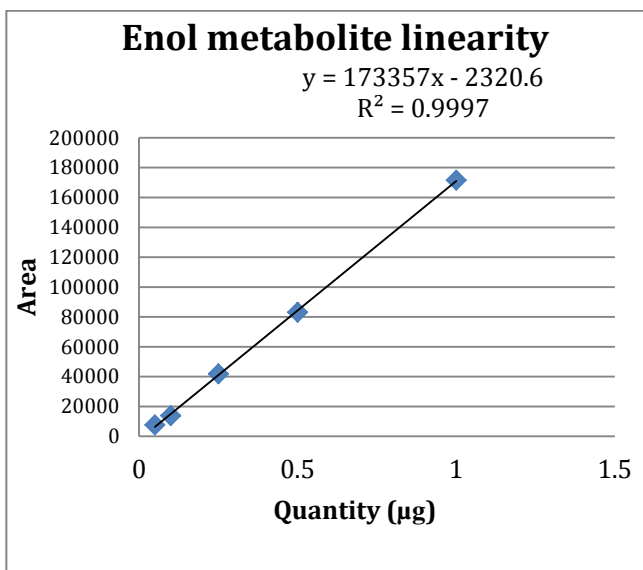
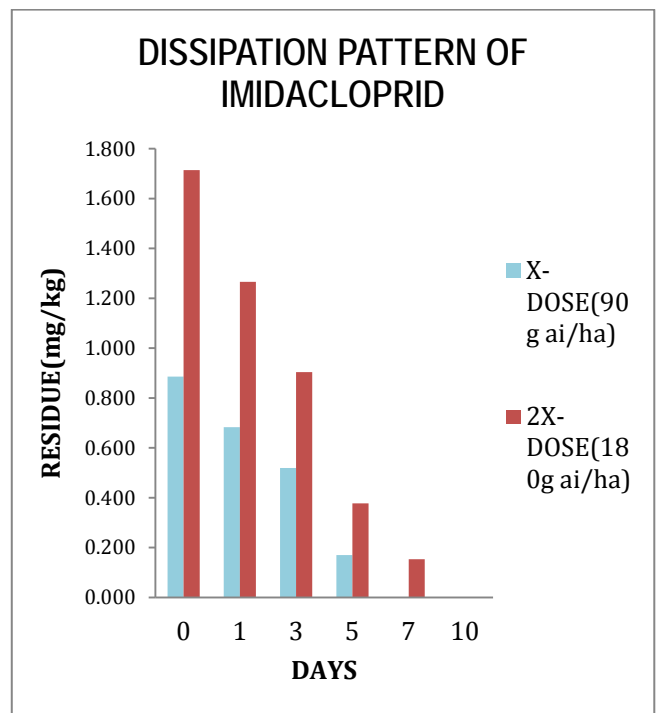
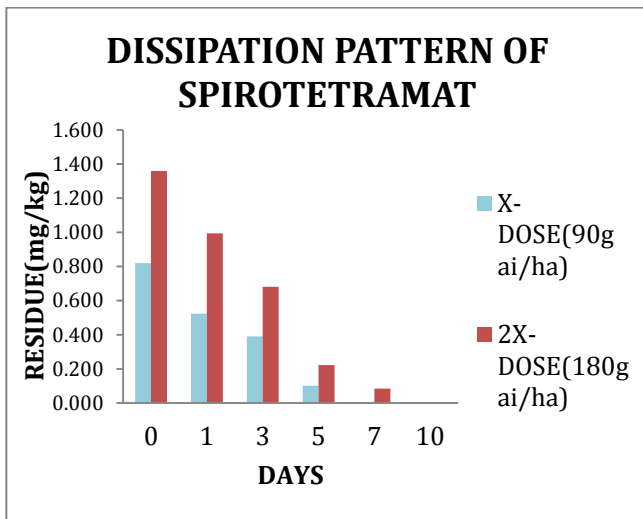
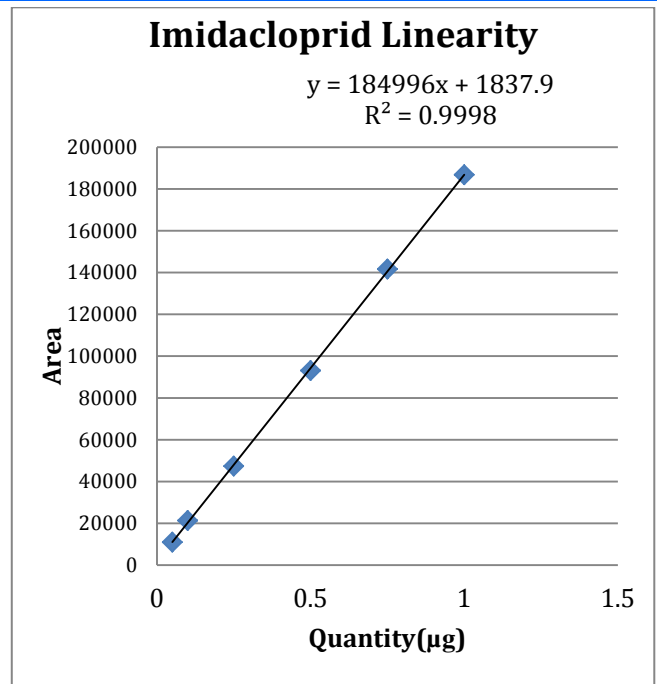
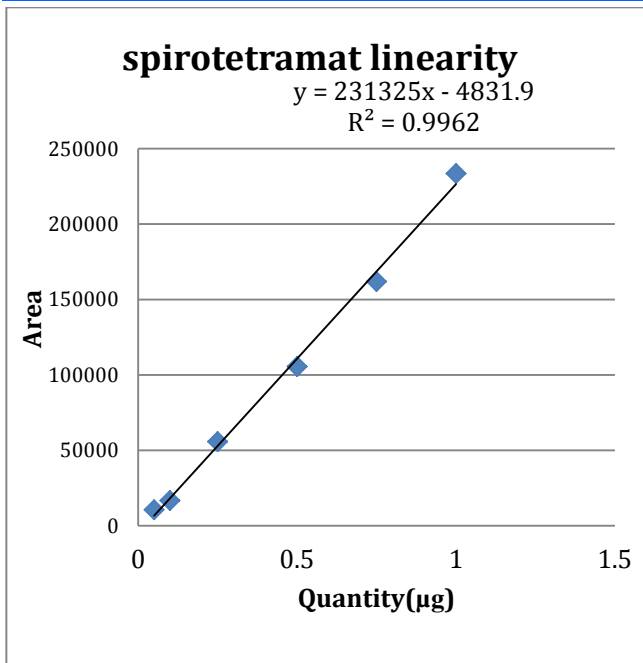
Days after treatment	spirotetramat residues (mg/kg)							
	X Dose- 90 g ai/ha				2X Dose – 180g ai/ha			
	R1	R2	R3	Mean	R1	R2	R3	Mean
0	0.832	0.816	0.803	0.820	1.318	1.376	1.384	1.360
1	0.564	0.539	0.468	0.524	0.980	0.950	1.053	0.994
3	0.395	0.393	0.382	0.390	0.685	0.696	0.664	0.681
5	0.112	0.102	0.093	0.102	0.252	0.215	0.202	0.223
7	BDL	BDL	BDL	BDL	0.091	0.072	0.092	0.085
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mature berries 4at Harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil at Harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Regression equation	Y=0.752-0.130X				Y=1.249-0.181X			
DT 50	5.07				3.64			

BDL- Below Determination Level (< 0.05mg/kg)

Table 3: Residues of Imidacloprid Grapes and soil

Days after treatment	Imidacloprid residues (mg/kg)							
	X Dose – 90 g ai/ha				2X Dose – 180 g ai/ha			
	R1	R2	R3	Mean	R1	R2	R3	Mean
0	0.883	0.889	0.887	0.886	1.712	1.714	1.715	1.714
1	0.685	0.681	0.684	0.683	1.264	1.266	1.267	1.266
3	0.518	0.521	0.520	0.519	0.904	0.902	0.904	0.904
5	0.174	0.170	0.165	0.170	0.379	0.375	0.377	0.377
7	BDL	BDL	BDL	BDL	0.157	0.155	0.148	0.153
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mature berries at Harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil at Harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Regression equation	Y=0.868-0.134				Y=1.584-0.219			
DT 50	4.92				3.01			

BDL- Below Determination Level (< 0.05mg/kg)



REFERENCES

- [1] Lozano F, Kemper K, Tundisi H (2008). Field development of Movento plus for sucking pest control in Brazil. Bayer crop Sci. J. 61: 329-341
- [2] Mohapatra S, Deepa M, Jagdish GK (2012) A simple analytical method for analysis of spirotetramat enol by HPLC Bull Environ Contam Toxicol 88: 124-128
- [3] Anatassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning

and dispersive solid phase extraction for the determination of pesticide residues in produce. J.Assoc.of Anal.Chem 86:412-431

[4]Sur R (2008) Metabolism of spirotetramat in plants Bayer crop Sci J 61: 227-244

[5]Babczinski P, Hellpointer E(2008) Environmental fate of Spirotetramat Bayer Crop Sc J. 61:181-202

[6]Mohapatra S,Deepa M, Jagdish GK (2011) Residues of betacyfluthrinand imidacloprid in/on Mango (Mangifera indica L.) Bull Environ Contam Toxicol87:202-207