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Residue Dynamics of Spirotetramat and Imidacloprid in/on Mango and Soil

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Abstract Spirotetramat is a unique insecticide having both phloem and xylem mobility and imidacloprid, a neonicotinoid insecticide, is one of the most widely used in the world. The combination formulation is very effective against sucking pests of mango. Residue dynamics of spirotetramat and imidacloprid in/on mango and soil was studied following application of the combination formulation, spirotetramat 12 % + imidacloprid 12 % (240 SC) at 90 and 180 g a.i. ha^{-1}. Spirotetramat residues in/on mango fruits were 0.327 and 0.483 mg kg^{-1} after giving 3 applications at 90 and 180 g a.i. ha^{-1}, respectively. The residues remained on mango fruits for 7 days and dissipated with the half-life of 3.3 and 5.2 days, respectively. Residues of spirotetramat-enol, the major metabolite of spirotetramat in plant, were not detected in mango fruits. Initial residues of imidacloprid on mango fruits from the two treatments were 0.329 and 0.536 mg kg^{-1}, respectively. Imidacloprid residues remained on mango fruits beyond 15 days and dissipated with the half-life of 5.2 and 8.2 days. The residues of spirotetramat, spirotetramat-enol and imidacloprid were found below quantifiable limit of 0.05 mg kg^{-1} in mature mango fruits and field soil at harvest.

Keywords Imidacloprid · Mango · Persistence · Spirotetramat · Spirotetramat-enol

Spirotetramat [*cis*-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4.5]dec-3-en-2-one] is used for control of a broad spectrum of sucking insects such as aphids, whiteflies, scales, mealy bugs etc. Spirotetramat exhibits an excellent systemic and translaminar efficacy. Once sprayed on the crop it can even protect the roots from aphid attack and newly-grown leaves that develop after the spray are also protected. This compound is mobile within the phloem of the plants and can control hidden pests. These outstanding properties of spirotetramat are considered unique among recently developed insecticides (Anonymous 2008a). Spirotetramat reportedly showed no cross resistance to any other commercially available insecticide (van Waetermeulen et al. 2007). Spirotetramat-enol [*cis*-3-(2,5-dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4,5]dec-3-en-2-one] is the major metabolite of spirotetramat in plants. Due to its physicochemical properties the primary metabolite spirotetramat-enol fulfills the requirements for a phloem-systemic insecticide.

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine], a neonicotinoid insecticide, is widely used all over the world. It is applied as seed treatment, soil drench, foliar spray, and as tree injection to a large variety of crop and non-crop plants. Imidacloprid is very effective to control mango hoppers at very low doses (Singh et al. 2010; Verghese 2000). Imidacloprid could control hoppers effectively and increased the yield significantly. The combination formulation of spirotetramat and imidacloprid, Movento® Plus (spirotetramat 12 % + imidacloprid 36 % − 480 SC) has shown outstanding property against sucking pests (Lozano et al. 2008). As reported by Salles (2002) a combination of both active ingredients resulted in an increased efficacy in different phases of insect development. Besides, it gave a good control of plant viruses, a major problem of sucking insects besides the direct damage. The combination formulation is also a suitable tool for resistance management and has shown outstanding performance against sucking pests. The

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combination formulation spirotetramat 12 % + imidacloprid 12 % − 240 SC is marketed by Bayer Crop Science, India and is under consideration for its registered used against mango hoppers and aphids. This study was therefore carried out to evaluate the residue dynamics of spirotetramat, its metabolite spirotetramat-enol and imidacloprid on mango following application of the combination formulation spirotetramat 12 % + imidacloprid 12 % − 240 SC in/on mango and soil.

Materials and Methods

Standard reference materials of spirotetramat (99.2 % purity), its metabolite spirotetramat-enol (99.1 % purity), imidacloprid (99.2 % purity) and the combination formulation (spirotetramat 12 % + imidacloprid 12 % − 240 SC) were obtained from Bayer Crop Science Limited, India. Stock solutions of spirotetramat (1,000 μg mL^{-1}), its metabolite spirotetramat-enol (1,000 μg mL^{-1}) and imidacloprid (1,000 μg mL^{-1}) were prepared with gradient high pressure liquid chromatography (HPLC) grade acetonitrile. Further dilutions were made with gradient HPLC grade acetonitrile to obtain the working standards.

Residue study of spirotetramat and imidacloprid on mango was carried out at the experimental farm of Indian Institute of Horticultural Research, Bangalore, India during April–June 2010 on mango variety, Totapuri. For every treatment 10 trees were selected. The spray volume taken was 1,000 L ha^{-1}. The treatments were untreated control, recommended dose, 90 g a.i. ha^{-1} and double dose, 180 g a.i. ha^{-1} of the combination formulation of spirotetramat 12 % + imidacloprid 12 % − 240 SC. Untreated control trees were sprayed with water. The first spray application was given at the fruit growth stage, further 2 sprays were given at 10 day intervals using triple action/hollow cone nozzle sprayer. After the third spray, residue analysis of spirotetramat, spirotetramat-enol and imidacloprid was carried out on 0 (1 h), 1, 3, 5, 7, 10, 15, 20 days and at harvest (60 days after last spray). Mango fruit samples, 500 g approximately from each tree was harvested, pooled together and brought to the laboratory for processing. From each treatment 5 kg mango samples were collected. The mango fruits were cut into small pieces mixed in a Waring blender and a representative samples in 3 replicates were processed for analysis of spirotetramat and imidacloprid separately. At the time of harvest matured mango fruits (whole fruit) and pulp (after peeling of the fruits) were analyzed. At harvest soil samples were collected from area under the canopy of each mango tree. At each site two soil plugs about 15 cm deep and 3–5 cm diameter were collected using small garden trowel. The soil samples were mixed thoroughly, air dried and passed through 2 mm sieve. A representative 100 g sample in triplicates was processed for residue analysis of spirotetramat and imidacloprid separately.

Residue analysis of spirotetramat and its metabolite spirotetramat-enol in/on mango field samples were carried out as per Mohapatra et al. (2012). The method followed the QuEChERS analytical method for analysis of pesticide residues in produce (Anastassiades et al. 2003). A representative mango samples (10 g) in 3 replicates was placed in 50 mL Teflon tubes. To the tube 10 mL of gradient HPLC grade acetonitrile was added and shaken vigorously for 1 min. To the tube containing the fortified samples 4 g anhydrous magnesium sulphate and 1 g sodium chloride was added and spinned for 2 min. The tubes were centrifuged at 10,000 rpm for 10 min. An aliquot (4 mL) of the upper acetonitrile extract was placed in a centrifuge tube containing 50 mg primary secondary amine (PSA) sorbent and 150 mg anhydrous magnesium sulphate. The tubes were shaken vigorously for 1 min and centrifuged for 10 min at 10,000 rpm. About 2 mL of the supernatant acetonitrile phase was taken in a vial and injected into HPLC after passing through Millipore 0.45 μm filters.

Extraction and clean up of imidacloprid residues in mango fruits was carried out in the following manner. A 50 g portion of mango representative sample was homogenized with 100 mL acetonitrile in a Waring blender and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed with acetonitrile (2 × 50 mL) and the combined extracts were collected in a 500 mL flask. The acetonitrile fraction was concentrated under reduced pressure in a rotary vacuum evaporator. The aqueous extract was transferred into a 1 L separatory funnel and diluted with 80 mL of distilled water. The aqueous phase was partitioned with mixture of hexane + ethyl acetate (5:5, v v^{-1}; 3 × 50 mL) after adding 25 mL saturated sodium chloride solution. The combined solvent fraction was dried over anhydrous sodium sulphate, concentrated to 5 mL and subjected to column chromatography. The column was packed with 5 g of florisil in between 1 inch layer of sodium sulphate. Imidacloprid residues were eluted with 100 mL of gradient HPLC grade acetonitrile. The elute was concentrated to 5 mL and analyzed by HPLC after passing through Millipore 0.45 μm filters.

For extraction of spirotetramat, spirotetramat-enol and imidacloprid from soil a representative 100 g soil in triplicate was extracted with acetonitrile + water (2 + 1 by volume; 100 mL) and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed with acetonitrile + water mixture (2:1 v v^{-1}; 2 × 50 mL) and the combined extracts were collected in a 500 mL flask. The acetonitrile fraction was concentrated under reduced pressure in a rotary vacuum evaporator. For

Table 1 Recovery of spirotetramat, and spirotetramat-enol from mango and soil at various fortification levels

Fortified concentration (mg kg^{-1})	Mean recovery (%) ± SD[a]					
	Spirotetramat			Spirotetramat-enol		
	Mango whole fruit	Pulp	Soil	Mango whole fruit	Pulp	Soil
0.05	70.29 ± 4.89	72.55 ± 5.34	75.76 ± 3.95	80.63 ± 5.86	78.35 ± 5.61	86.42 ± 6.55
0.50	81.58 ± 3.65	80.86 ± 4.25	82.81 ± 5.44	91.26 ± 5.02	87.02 ± 5.02	93.54 ± 5.62
1.00	90.08 ± 5.26	87.18 ± 3.90	91.32 ± 6.16	100.35 ± 4.76	95.36 ± 3.84	101.24 ± 4.16

[a] Average of five replicate analyses ± standard deviation

partitioning of spirotetramat and spirotetramat-enol the aqueous extract was transferred into a 1 L separatory funnel, partitioned with dichloromethane (3 × 50 mL) after adding 25 mL saturated sodium chloride solution. Imidacloprid residues were partitioned into hexane + ethyl acetate (5:5 v v^{-1}; 3 × 50 mL) mixture after adding 25 mL saturated sodium chloride solution. The solvent fractions obtained for all pesticides were concentrated to dryness, redissolved in 5 mL gradient HPLC grade acetonitrile without any clean-up. The residues were analyzed by HPLC after passing through Millipore 0.45 µm filters.

Pesticide free mango fruit samples in 5 replicates were fortified with spirotetramat, spirotetramat-enol and imidacloprid at 0.05, 0.5 and 1.0 mg kg^{-1}. Mango samples without added insecticides served as controls. The fortified mango and soil samples were extracted and analyzed as described above for spirotetramat, spirotetramat-enol and imidacloprid before carrying out field sample analysis.

The residues of spirotetramat, spirotetramat-enol and imidacloprid were estimated by Shimadzu HPLC, Prominence LC 20 AT with a photo diode array (PDA) detector. The column used was Phenomenex C18, Luna, 250 × 4.6 mm i.d. acetonitrile : water at a proportion of 40:60 was used as the mobile phase at a flow rate of 1 mL min^{-1}. The injection volume taken was 20 µL. Spirotetramat and spirotetramat-enol residues were detected at a wavelength of 250 nm and imidacloprid at 270 nm. The retention times were 5.06, 7.5 and 8.8 min for imidacloprid, spirotetramat-enol and spirotetramat, respectively. The residue data was subjected to statistical analysis to compute the residual half-life ($t_{1/2}$) and pre-harvest interval (PHI) (Hoskins 1961).

Results and Discussion

The results of the recovery study of spirotetramat, spirotetramat-enol and imidacloprid carried out at the fortification level of 0.05, 0.5 and 1.0 mg kg^{-1} in mango whole fruit (with peel), pulp (without peel) and soil are presented in Tables 1 and 2. In mango whole fruit recovery of spirotetramat was in the range of 70.29 %–90.08 %, in pulp in the range of 72.55 %–87.18 % and in soil the recovery was 75.76 %–91.32 %. In mango whole fruit recovery of spirotetramat-enol was in the range of 80.63 %–100.35 %, in pulp 78.35 %–95.36 % and in soil the recovery was 86.42 %–101.24 %. In mango whole fruit recovery of imidacloprid is in the range of 85.55 %–88.56 %, in pulp 82.14 %–86.72 % and in soil the recovery was 87.85–89.56 %.

Table 2 Recovery of imidacloprid from mango and soil at various fortification levels

Fortified concentration (mg kg^{-1})	Mean recovery (%) ± SD[a]		
	Mango whole fruit	Pulp	Soil
0.05	85.55 ± 2.65	82.14 ± 5.33	87.85 ± 2.34
0.50	87.68 ± 4.52	85.49 ± 4.78	88.64 ± 3.44
1.00	88.56 ± 3.45	86.72 ± 5.62	89.56 ± 5.12

[a] Average of five replicate analyses ± standard deviation

Spirotetramat residues on mango fruits before the final application were below the limit of quantification (LOQ) level of 0.05 mg kg^{-1}. Residue levels of spirotetramat in/on mango after 3 applications at the recommended and double dose of 90 and 180 g a.i. ha^{-1} were 0.327 and 0.483 mg kg^{-1}, respectively (Table 3). The residues dissipated very fast. After 1 day 20 % residues had dissipated and by 7 days 80 % residue dissipation was observed from treatment at the recommended dose. From double dose treatment 22.7 % residue dissipation was observed after 1 day and 71 % after 7 days. After 10 days 100 % spirotetramat residues had dissipated from both treatments. The residues dissipated with the half-life of 3.3 and 5.2 days from treatment at the recommended and double dose. Maximum residue limit (MRL) of spirotetramat on mango is not available. However, considering the LOQ of 0.05 mg kg^{-1} the PHI was calculated as 10 and 15 days. Mature mango fruits with or without peel or field soil collected at harvest was free from spirotetramat residues. The major metabolite, spirotetramat-enol was not detected in mango or soil.

Table 3 Residues of spirotetramat, spirotetramat-enol and imidacloprid in/on mango and soil

Days after treatment	Untreated control	Average residues recovered ± SD[a] (mg kg^{-1})					
		Application at 90 g a.i. ha^{-1}			Application at 180 g a.i. ha^{-1}		
		Spirotetramat	Spirotetramat-enol	Imidacloprid	Spirotetramat	Spirotetramat-enol	Imidacloprid
Before last application	ND		BDL	0.050 ± 0.010		BDL	0.120 ± 0.007
0 (1 h)	ND	0.327 ± 0.025	BDL	0.329 ± 0.014	0.483 ± 0.035	BDL	0.536 ± 0.038
1	ND	0.262 ± 0.016	BDL	0.271 ± 0.011	0.374 ± 0.029	BDL	0.424 ± 0.011
3	ND	0.215 ± 0.006	BDL	0.215 ± 0.099	0.324 ± 0.018	BDL	0.353 ± 0.009
5	ND	0.151 ± 0.008	BDL	0.181 ± 0.002	0.259 ± 0.007	BDL	0.302 ± 0.005
7	ND	0.066 ± 0.004	BDL	0.155 ± 0.087	0.171 ± 0.007	BDL	0.256 ± 0.011
10	ND	BDL	BDL	0.076 ± 0.009	BDL	BDL	0.216 ± 0.013
15	ND	BDL	BDL	BDL	BDL	BDL	0.136 ± 0.009
20	ND	BDL	BDL	BDL	BDL	BDL	BDL
Mature whole fruit (at harvest)	ND	BDL	BDL	BDL	BDL	BDL	BDL
Pulp (at harvest)	ND	BDL	BDL	BDL	BDL	BDL	BDL
Pulp (at harvest)	ND	BDL	BDL	BDL	BDL	BDL	BDL

[a] Average residues ± SD from analyses of triplicate laboratory samples taken from a composite field sample

ND not detected

BDL below detectable limit (<0.05 mg kg^{-1})

Residues of imidacloprid before the last application were 0.05 and 0.12 mg kg^{-1} from treatment at the recommended and double the recommended dose of 90 and 180 g a.i. ha^{-1}, respectively. After the last application the residues of imidacloprid on mango whole fruits were 0.329 and 0.536 mg kg^{-1}, respectively as initial residue deposits (Table 3). Imidacloprid residues remained on mango fruits for 10 days from treatment at 90 g a.i. ha^{-1} and reached less than the LOQ of 0.05 mg kg^{-1} after 15 days. From treatment at 180 g a.i. ha^{-1} the residues persisted beyond 15 days but reached LOQ by 20 days. Imidacloprid residues dissipated with the half-life of 5.2 and 8.2 days, respectively from recommended and double dose treatment. The maximum residue limit (MRL) of imidacloprid on mango is fixed at 0.2 mg kg^{-1} by Codex Alimantarius Commission. Based on this MRL value the pre-harvest interval is worked out as 4 and 11 days for recommended and double dose treatment.

Information on the residue persistence of spirotetramat on mango is not available to our knowledge. However information on other stone fruits (apricot, peach) is available. In study conducted in Northern Europe two applications of spirotetramat at 144 g a.i. ha^{-1} resulted in the residue deposit of 0.14–0.17 mg kg^{-1} on apricot after a period of 7 days (Anonymous 2008b). In Southern Europe from similar treatment residue levels of spirotetramat was in the range of 0.05–0.07 mg kg^{-1} after 7 days. On peach spirotetramat treatment at 180 g a.i. ha^{-1} resulted in residue level of 0.14 mg kg^{-1} in Northern Europe after 7 days. In Southern Europe from a similar treatment the residues were in the range of 0.09–0.15 mg kg^{-1} after 7 days. In the present study similar results were obtained where residues of spirotetramat after 7 days was 0.14 mg kg^{-1} from treatment at 180 g a.i. ha^{-1}. From lower treatment (90 g a.i. ha^{-1}) it was 0.066 mg kg^{-1}. In all the above studies conducted in Europe a significant amount of metabolites of spirotetramat were detected especially the enol. In the present study no enol metabolite was detected. Metabolism study of spirotetramat in plants showed that the major residue in apple fruits and leaves was the unchanged parent compound (Sur 2008). The enol metabolite is known to oxidize to BY108330-ketohydroxy and subsequently to BY108330-MA-amide (Babczinski and Hellpointer 2008). The tropical climatic conditions of Bangalore, India where the experiment was conducted might have facilitated dissipation of enol to other metabolites. During the experimental period the temperature was in the range of 14–34°C, relative humidity in the range of 37 %–76 % and total rainfall was 480 mm. In field soil analyzed at harvest no residue of spirotetramat or its enol metabolite was detected. Spirotetramat is known to degrade quickly in soil. The metabolites generated from spirotetramat are not expected to accumulate in the environment since they are further degraded to form non-extractable residues and CO_2. In a study using combination formulation of beta cyfluthrin and imidacloprid at 75 and 150 g a.i. ha^{-1} residues of imidacloprid on mango fruits were 0.14 and 0.18 mg kg^{-1}, respectively (Mohapatra et al. 2011). The residues persisted for 7 and 10 days. In the present study from treatment at 90 and 180 g a.i. ha^{-1} imidacloprid residues persisted for 10 and 15 days, respectively. Higher residue levels in the present study may be due to higher application rate as well carry-over of residues from previous treatments. The PHI of spirotetramat was 10 days and imidacloprid 4 days at the recommended dose. Therefore for the combination formulation of spirotetramat and imidacloprid a PHI of 10 days is recommended. For the double dose treatment 15 days PHI is recommended. Since matured mango fruits were free from residues of spirotetramat, its metabolite and imidacloprid, at harvest mango fruits are likely to be residue free. However, for consumption of raw mangoes a PHI of 15 days is recommended.

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References

Anastassiades 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 Off Anal Chem 86:412–431

Anonymous (2008a) Biological profile of spirotetramat (Movento)-a new two-way systemic (ambimobile) insecticide against sucking pest species. http://www.bayercropscience.com/bcsweb/cropprotection.nsf/id/EN_8thArticle022008. Accessed 2 Feb 2012

Anonymous (2008b) Spirotetramat, US Environmental Protection Agency, USA 234: 1890–1893

Babczinski P, Hellpointer E (2008) Environmental fate of spirotetramat (MoventoReg.). Bayer Crop Sci J 61:181–202

Hoskins WM (1961) Mathematical treatment of loss of pesticide residues. Plant Prot Bull (FAO) 9:163–168

Lozano F, Kemper K, Tundisi H (2008) Field development of Movento® Plus for sucking pest insect control in Brazil. Bayer Crop Sci J 61:329–341

Mohapatra S, Deepa M, Jagadish GK (2011) Residues of beta cyfluthrin and imidacloprid in/on mango (*Magnifera indica* L). Bull Environ Contam Toxicol 87:202–207

Mohapatra S, Deepa M, Jagadish GK (2012) A simple analytical method for analysis of spirotetramat and spirotetramat-enol by HPLC. Bull Environ Contam Toxicol 88:124–128

Salles LA (2002) Os insetos como vetores de patógenos de plantas. Cultivar 13:3–6

Singh M, Gupta D, Gupta PR (2010) Evaluation of imidacloprid and some biopesticides against mango hoppers, *Idioscopus Clypealis* (Lethierry) and *Amritodus tkinsoni* (Lethierry). Indian J Entomol 72:262–265

Sur R (2008) Metabolism of spirotetramat (MoventoReg.) in plants. Bayer Crop Sci J 61:227–244

van Waetermeulen X, Brück E, Elbert A, Fischer R, Krueger S, Kühnhold J, Nauen R, Niebes JF, Reckmann U, Schnorbach HJ,

Steffens R (2007) Spirotetramat, an innovative fully systemic insecticide for sucking insect pest control in agriculture: biological profile and field performance. In: Proceedings of the XVI International Plant Protection Congress, Glasgow, UK Vol 1, pp 60–67

Verghese A (2000) Effect of imidacloprid, lambda cyhalothrin and azadirachtin on the mango hopper. *Idioscopus niveosparsus* (Leth.) (Homoptera:Cicadellidae). VI international symposium on mango. Acta Hortic 502:733–736