

ORIGINAL ARTICLE

Uptake, distribution, translocation and metabolism of kresoxim-methyl in paddy

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Kresoxim-methyl; LC-MS/MS; metabolism; paddy; soil; Strobilurin fungicides.

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Abstract

Introduction Kresoxim-methyl is a broad spectrum fungicide used for controlling sheath blight and blast in paddy, also downy and powdery mildew diseases in grapes. **Objectives** A supervised field trial consisting of two experiments viz. soil application and foliar application was conducted in order to study the uptake, distribution, translocation and metabolism of kresoxim-methyl in paddy, treated at the rates of 250 and 500 g a.i. ha⁻¹. **Methods** Depending on the crop growth and stage (40, 60, 80 and 110 days after transplanting), different plant parts like foliage, shoot, roots, panicles, straw and grain, along with soil, were sampled and analysed using liquid chromatography tandem mass spectrometry for parent compound kresoxim-methyl and its metabolites. **Results** The results of the study revealed that there was little translocation of residues of kresoxim-methyl and its acid metabolite to various plants parts, i.e. foliage, roots, soil, shoot and panicle, and very little to straw and grains. **Conclusion** The major route of metabolism was hydrolysis with formation of acid metabolite.

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Introduction

Kresoxim-methyl, (Methyl (E) methoxyimino [2-(o-tolyloxymethyl) phenyl] acetate), is a broad spectrum fungicide structurally related to Strobilurin A, a natural product of the wood-decaying fungus *Strobilurus tenacellus*. It belongs to a recently developed class of biologically active compounds from the Strobilurin family and used for controlling sheath blight and blast in paddy, also downy and powdery mildew diseases in grapes (Tomlin, 2006). It acts on the respiration process by blocking the transport of electron within the mitochondria from cytochrome b to cytochrome c₁ by binding to a specific site (Ammermann *et al.*, 1992). As the mechanism of action of these compounds is different from that of the conventional fungicides, these compounds are important tools for the creation of new strategies against resistance. There is, however, very little information on uptake, persistence and metabolism of kresoxim methyl in plant, and therefore the present study was conducted to

determine the uptake, distribution, translocation and metabolism of kresoxim-methyl in paddy under Indian environmental conditions.

Kresoxim-methyl is toxic to aquatic species, but exposure tests and ecological studies have shown that there is no danger of permanent damage to aquatic organisms when kresoxim-methyl is used as recommended (Tomlin, 2006). Acute exposure of fish to kresoxim-methyl technical and as the formulated product, showed that the chemical was highly toxic. In chronic studies with the technical and formulated product, the chemical was classified as, respectively, moderately and slightly toxic to exposed fish. It is practically non-toxic to birds in acute and sub-acute dietary studies and only of marginal toxicity based on a first-generation reproduction study (Anonymous, 2000). Kresoxim-methyl acute oral LD₅₀ for rats is >5000 mg kg⁻¹. Acute percutaneous LD₅₀ for rats is >2000 mg kg⁻¹. It is non-irritating to skin and eyes. Its inhalation LC₅₀ for rats is >5.6 mg L⁻¹ (Tomlin, 2006).

A suitable analytical method was required for determining kresoxim-methyl, its acid metabolite and Z-isomer concentrations in various substrates of paddy for determining the fate of kresoxim-methyl in plant. Few analytical methods have been reported for determination of these fungicides (Cabras *et al.*, 1997, 1998a, 1998b; Wong & Halverson, 1999; Navickiene & Ribeiro, 2001; Navalon *et al.*, 2002; De Melo Abreu *et al.*, 2005). Capabilities of different liquid chromatography tandem mass spectrometry (LC-MS/MS) systems in determining pesticide residues in food were assessed (Carla & James, 2007). An LC-MS/MS method has been developed and validated for determination of kresoxim-methyl, its acid metabolite and Z-isomer concentrations in soil, paddy foliage, straw and grain. The standardized method was used to study the uptake, distribution, translocation and metabolism of kresoxim-methyl in paddy following soil and foliar applications.

Materials and methods

Chemicals

Kresoxim-methyl 500 g L⁻¹ SC test material, kresoxim-methyl reference standard of 97.2% purity, methyl (Z)-methoxyimino [2-(o-tolyloxy methyl) phenyl] acetate (Z-isomer), of 99.0% purity, (E)-methoxyimino [2-(o-tolyloxymethyl) phenyl] acetic acid (carboxylic acid metabolite) of 99.0% purity and tebuconazole (internal standard) of 98.28% purity, were sourced from Rallis India Limited (Bangalore, Karnataka, India).

Treatment and sampling

Rabi paddy (variety – Tanu) was field grown in Arakaldoddi village, Mandya District, Karnataka, India. In experiment 1, a single spray of kresoxim-methyl 500 g L⁻¹ SC at 250 and 500 g a.i. ha⁻¹ was applied (using Knapsack sprayer) to paddy soil at the time of transplanting of paddy. The soil samples treated at '0' (control), 250 (low dose) and 500 (high dose) g a.i. ha⁻¹ was collected and analysed immediately after application, i.e. on '0' day. During the experiment, subsequent sampling of soil and whole paddy plant was carried out at 40, 60, 80 and 110 (at harvest) days after treatment (DAT). In experiment 2, three foliar sprays of kresoxim-methyl 500 g L⁻¹ SC at 250 and 500 g a.i. ha⁻¹ were applied to paddy crop at 40, 60 and 80 days after transplanting (DAT). Sampling of whole paddy plants along with roots and of soil was carried out at 40 DAT (i.e. immediately after first spray), 60 DAT (just before the second spray), 80 DAT (just before the

third spray) and 110 DAT (at harvest). The whole paddy plant along with roots were uprooted randomly from 10 to 12 different spots in each plot, pooled separately, collected in separate polythene bags, properly labelled and similarly soil samples were also collected and stored in deep freezer at less than -10 °C until analysis. At each of these sampling intervals, depending on the crop growth stage, different plant parts like foliage, shoot, roots, panicles, straw, grain and soil were analysed for the concentration of kresoxim-methyl, its acid metabolite and its Z-isomer.

Analytical method validation and sample preparation

The analytical methods for analysis of kresoxim-methyl, acid metabolite and Z-isomer of kresoxim-methyl in the substrates viz. soil, paddy foliage, straw and grain were developed and validated prior to their use for sample analysis. The analytical method was validated by establishing linearity, range, accuracy, precision, specificity and limit of quantitation. The samples of soil, paddy foliage, straw and grain were spiked at 0.01, 0.05 and 0.10 µg g⁻¹ level with kresoxim-methyl, acid metabolite of kresoxim-methyl and Z-isomer of kresoxim-methyl. These spiked samples were analysed using the method described below.

The method involved extraction of paddy substrates and soil with acetone, partitioning the residues into dichloromethane after concentration. Dichloromethane extracts were cleaned up by adsorption column chromatography using activated silica; elute was collected and concentrated to dryness using rotary vacuum evaporator at 40 °C. An additional extraction was performed on residual paddy substrate/soil for extracting the carboxylic acid metabolite with 0.1 N NaOH, the extract acidified with HCl (pH ~1), residues partitioned into dichloromethane: ethyl acetate mixture concentrated and quantified by LC-MS with MS detection in multiple reaction monitoring (MRM) mode. The minimum quantifiable limit of the method was 0.01 µg g⁻¹ (in all substrates for each analyte).

The samples (foliage, shoot, roots, panicles, straw, grain and soil) collected at various time intervals of the field study were analysed using the previously described method. Soil samples were also sampled and analysed.

Chromatography

The estimation of kresoxim-methyl, carboxylic acid metabolite and Z-isomer of kresoxim-methyl in paddy substrates and soil was carried out by dissolving the concentrated

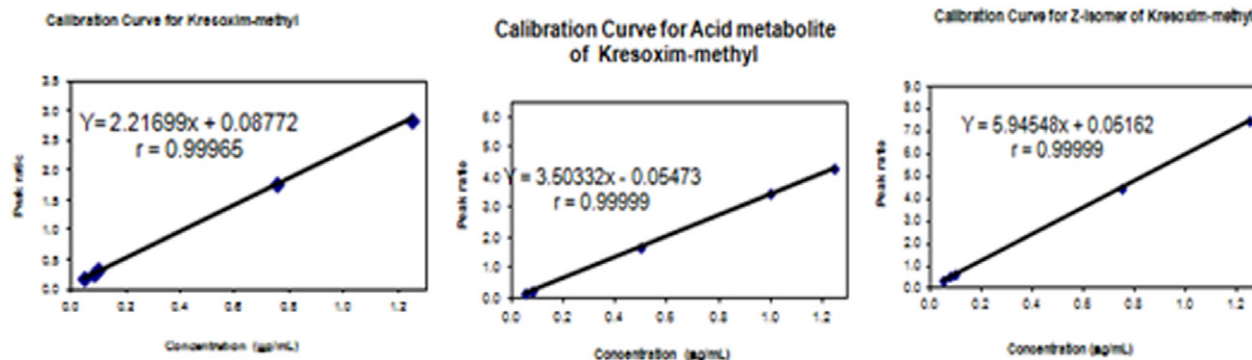


Figure 1 Linearity of instrument response and representative chromatogram for kresoxim-methyl, acid metabolite and Z-isomer of kresoxim-methyl.

residue in acetonitrile and transferring quantitatively into 10 mL volumetric flask using mobile phase; 1 mL of 1 ppm of tebuconazole (internal standard) was added and volume made up. An aliquot of 100 μL was injected into LC-MS/MS consisting of an Agilent 1100 series HPLC (Agilent Technologies, Bangalore, India) equipped with quaternary pumps, column oven, auto sampler and UV-VIS detector with Alltima hp C18 3 μm , 53 mm \times 7 mm stainless steel column interfaced with tandem mass spectrometric detector (Applied Biosystems API 2000 MS/MS, Bangalore, India) operated under the following conditions. The mobile phase was 2 mM ammonium formate (pH 3) + acetonitrile, (25 + 75, v/v) pumped at the rate of 1 mL min^{-1} . The injection volume was 100 μL . Under the said conditions, the retention times of the target analytes were: kresoxim-methyl – 2.5 min; Z-isomer of kresoxim-methyl – 3.5 min; carboxylic acid metabolite – 1.9 min; and tebuconazole (internal standard) – 2.1 min. Parameters were optimized by continuous infusion of individual standard solutions (20 μL) via a syringe pump at a flow rate of 10 $\mu\text{L min}^{-1}$. The MS-MS transition (m/z) for quantification of kresoxim-methyl was 314.2 \rightarrow 116.2 and for Z-isomers of kresoxim-methyl was 314.2 \rightarrow 206.2. Declustering potential (DP) was set at 18 and 16 V; collision energy (CE) was set at 18 and 19 V; and collision cell exit potential (CXP) was set at 3 and 1 V. The MS-MS transitions (m/z) for quantification of carboxylic acid metabolite of kresoxim-methyl was 298.3 \rightarrow 102.0. DP was set at –10 V; CE was set at –18 V; and CXP was set at –2 V. The ion spray voltage was 4500 V, and temperature was 400 $^{\circ}\text{C}$. The quantification of above target analytes was done by LC-MS/MS with MS detection in MRM mode in two experiments, where kresoxim-methyl and Z-isomers of kresoxim-methyl were quantified in the positive ionization mode, and carboxylic acid metabolite of kresoxim-methyl was quantified in negative ionization mode.

Results and discussion

The analytical method validation results are presented in Figure 1 and Table 1. The detector response for each analyte was linear in the concentration range of 0.05 to 1.25 $\mu\text{g mL}^{-1}$ with correlation coefficient values more than 0.999. The accuracy as per cent recovery of the method varied between 85.0 and 109 for all the analytes in all the substrates. The precision of six replicate analysis of each analyte in each substrate as %RSD was between 1.6 and 8.9. The lowest concentration used in the accuracy/precision test which gave acceptable validation results has been taken as limit of quantitation of the method, which was 0.01 mg kg^{-1} in all the cases.

The results of the study revealed that soil and foliar application of kresoxim-methyl to transplanted paddy resulted in acropetal and basipetal uptake, distribution, translocation and metabolism of the chemical to different plant parts, i.e. roots, shoots, foliage, panicle, grain and straw. The residues present were mainly in the form of parent compound, i.e. kresoxim-methyl or its acid metabolite. At 80 days after soil application of kresoxim-methyl, 0.02 and 0.08 $\mu\text{g g}^{-1}$ residues in soil; 0.04 and 0.06 $\mu\text{g g}^{-1}$ in roots; 0.23 and 0.27 in shoots and none detected and 0.31 $\mu\text{g g}^{-1}$ in panicles were detected in the form of parent compound, i.e. kresoxim-methyl following application at 250 and 500 g a.i. ha^{-1} , respectively. There were also 0.11 and 0.09 $\mu\text{g g}^{-1}$ residues in soil; 0.37 and 0.37 $\mu\text{g g}^{-1}$ in roots; 0.49 and 1.08 $\mu\text{g g}^{-1}$ in foliage; and 0.10 and 0.22 $\mu\text{g g}^{-1}$ in shoots found in the form of acid metabolite following the aforementioned two applications (Table 2, Figure 2).

Similarly, at 80 days after foliar application of kresoxim-methyl at 250 and 500 g a.i. ha^{-1} . There were also 13.83 and 23.73 $\mu\text{g g}^{-1}$ residues in shoots; 1.16 and 4.89 $\mu\text{g g}^{-1}$ in foliage and 0.05 and 2.14 $\mu\text{g g}^{-1}$ in panicles; 0.08 and

Table 1 Recovery of kresoxim-methyl, acid metabolite and Z-isomer of kresoxim-methyl from paddy soil, foliage, straw and grains

Fortification level (μg per g)	Paddy Substrates, Accuracy as % recovery																							
	Soil						Foliage						Straw						Grain					
	Replication	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c					
0.01	R1	102.0	95.5	109.0	97.5	89.5	109.0	102.0	96.6	98.1	105.0	88.5	104.0											
	R2	109.0	95.3	108.0	102.0	102.0	104.0	91.6	93.8	102.0	109.0	88.6	98.3											
	R3	108.0	99.4	107.0	95.6	96.8	102.0	106.0	106.0	96.0	90.8	91.0	85.0	85.4										
	R4	106.0	99.6	103.0	110.0	102.0	107.0	107.0	107.0	96.7	93.3	106.0	92.8	89.7										
	R5	107.0	102.0	109.0	109.0	107.0	107.0	109.0	109.0	99.1	105.0	89.3	93.4	87.3										
	R6	107.0	99.6	105.0	92.2	89.1	95.0	105.0	105.0	96.7	106.0	109.0	97.3	99.6										
	Mean \pm SD	106.5 \pm 2.4	98.6 \pm 2.6	106.8 \pm 2.4	101.1 \pm 7.3	97.7 \pm 7.3	104.0 \pm 5.1	103.4 \pm 6.2	96.5 \pm 1.7	99.2 \pm 6.2	101.6 \pm 9.0	90.9 \pm 4.4	94.1 \pm 7.6											
Precision (rsd %)	2.3	2.6	2.2	7.2	7.5	4.9	6.0	1.8	6.3	8.9	4.8	8.1												
0.05	R1	108.0	102.0	107.2	106.2	91.0	109.4	102.4	98.0	100.0	106.8	99.4	97.2											
	R2	107.8	96.0	105.4	107.8	97.6	106.0	105.0	103.6	108.8	104.6	105.0	109.2											
	R3	109.0	106.0	105.8	107.2	99.4	105.8	109.6	109.6	101.4	102.6	109.2	102.2	108.0										
	R4	107.8	100.0	107.8	99.4	100.6	107.8	108.8	108.8	95.6	108.6	107.4	101.0	95.6										
	R5	104.6	99.8	105.0	104.6	96.4	109.4	109.4	109.4	95.6	100.8	105.2	108.8	109.0										
	R6	105.2	92.8	102.2	104.2	103.8	108.4	108.2	108.2	92.2	102.2	104.4	99.2	97.2										
	Mean \pm SD	107.1 \pm 1.7	99.4 \pm 4.6	105.6 \pm 2.0	104.9 \pm 3.0	98.1 \pm 4.3	107.8 \pm 1.6	107.2 \pm 2.9	97.7 \pm 4.2	103.8 \pm 3.9	106.3 \pm 1.9	102.6 \pm 3.7	102.7 \pm 6.6											
Precision (rsd %)	1.6	4.6	1.9	2.9	4.4	1.5	2.7	4.3	3.8	1.8	3.6	6.4												
0.10	R1	91.2	98.0	92.7	104.0	109.0	105.0	91.6	89.4	89.4	98.2	107.0	96.0											
	R2	101.0	102.0	103.0	89.3	86.9	89.9	91.4	91.8	90.6	102.0	107.0	98.0											
	R3	86.6	96.0	86.4	92.2	89.8	94.8	94.5	89.9	92.7	96.5	104.0	92.7											
	R4	102.0	102.0	102.0	95.7	97.6	101.0	97.0	102.0	99.6	89.1	92.0	89.1											
	R5	101.0	107.0	105.0	89.5	96.1	97.5	97.5	102.0	97.2	92.9	93.8	92.4											
	R6	99.9	108.0	101.0	99.1	99.2	99.6	99.1	94.0	97.5	92.5	93.1	91.9											
	Mean \pm SD	97.0 \pm 6.4	102.2 \pm 4.8	98.4 \pm 7.2	95.0 \pm 5.8	96.4 \pm 7.8	98.0 \pm 5.2	95.2 \pm 3.2	94.9 \pm 5.8	94.5 \pm 4.2	95.2 \pm 4.6	99.5 \pm 7.2	93.4 \pm 3.2											
Precision (rsd %)	6.6	4.7	7.3	6.1	8.1	5.3	3.4	6.1	4.4	4.8	7.2	3.4												

a = kresoxim methyl, b = acid metabolite, c = Z-isomer. Bold numbers indicate mean and precision.

Table 2 Uptake, translocation, distribution and metabolism of kresoxim-methyl following its soil application

Substrate	Days after one application of kresoxim-methyl 500 g L ⁻¹ SC at 250 (and 500) g a.i. ha ⁻¹ and analysed concentration in ppm (µg per g)															
	0			40			60			80			110			
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	
Soil	52.2 (85.3)	0.94 (1.13)	ND (ND)	0.08 (0.12)	0.23 (0.55)	ND (ND)	ND (ND)	0.02 (0.08)	0.11 (0.09)	ND (ND)	0.02 (0.08)	0.11 (0.09)	ND (ND)	0.02 (0.08)	0.11 (0.09)	ND (ND)
Roots	1	1	1	ND	0.06	ND	ND	0.04	0.37	ND	0.04	0.37	ND	0.01	0.01	ND
Foliage	1	1	1	0.44 (7.46)	1.91 (2.46)	ND (ND)	0.43 (4.07)	ND (0.49)	0.27 (0.49)	ND (ND)	ND (ND)	0.49 (1.08)	ND (ND)	0.02	0.01	ND
Shoot	1	1	1	2	2	2	0.11 (5.89)	ND (ND)	0.10 (0.22)	ND (ND)	0.23 (0.27)	0.10 (0.22)	ND (ND)	2	2	2
Panicle	1	1	1	2	2	2	0.22 (1.41)	ND (ND)	ND (ND)	D (ND)	ND (0.31)	ND (ND)	ND (ND)	2	2	2
Grains	1	1	1	2	2	2	2	2	2	2	2	2	2	0.02 (0.03)	ND (ND)	ND (ND)
Straw	1	1	1	2	2	2	2	2	2	2	2	2	2	0.04 (0.06)	0.03 (0.05)	ND (ND)

¹Only soil was sampled after spray application of kresoxim-methyl 500 g L⁻¹ SC and just before transplanting of paddy sampling.

²No samples were available for analysis at sampling interval depending on crop growth and stage.

a = kresoxim methyl, b = acid metabolite, c = Z-isomer; ND ≤ 0.01 ppm.

Note: Values in the parentheses are results of application of kresoxim-methyl 500 g L⁻¹ SC at 500 g a.i. per treatment.

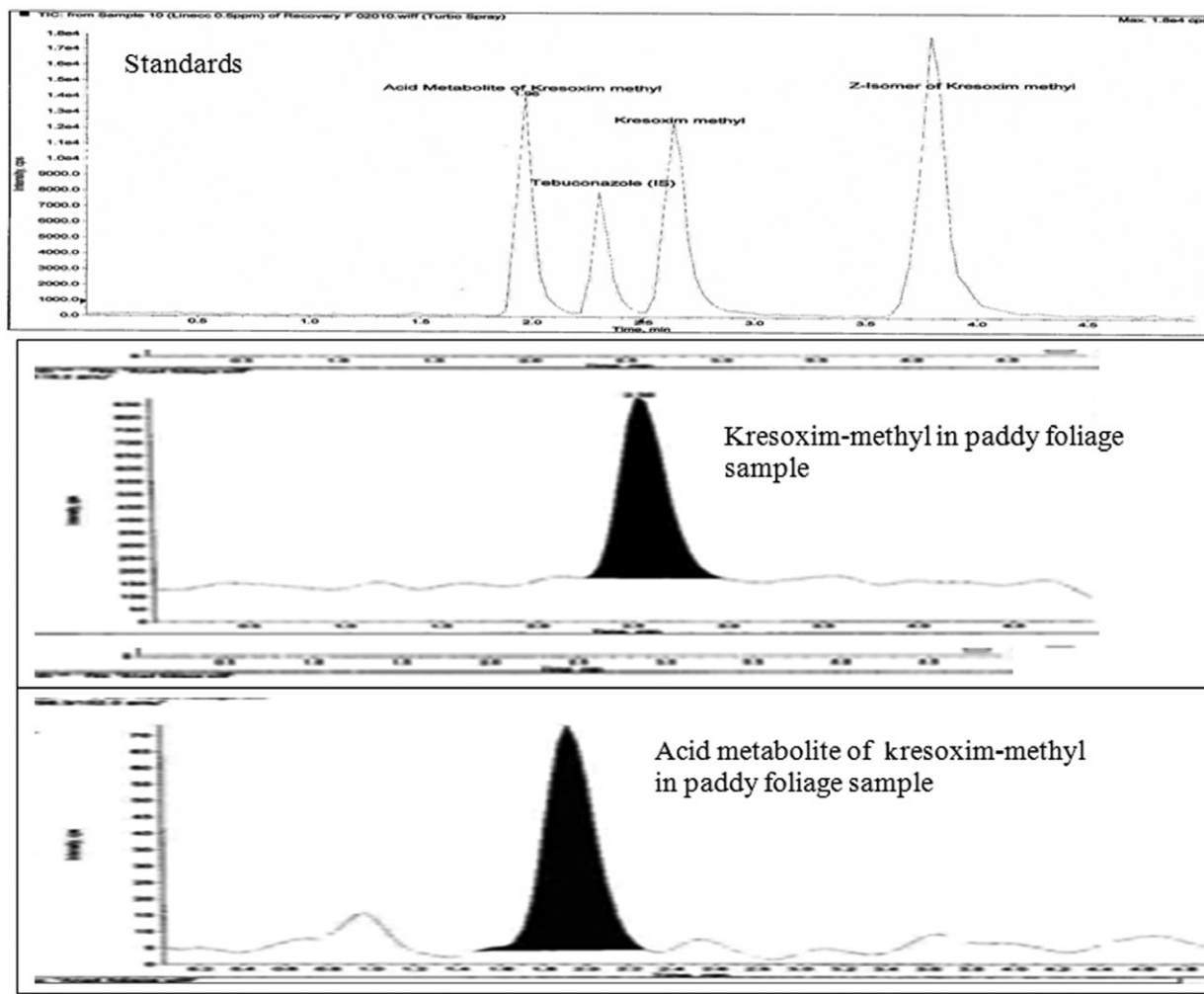


Figure 2 Representative MRM chromatogram of experiment 1 – soil application of kresoxim-methyl 500 g L⁻¹ SC at 500 g a.i. ha⁻¹ (after 80 days) – paddy foliage sample analysed for kresoxim-methyl, Z-isomer of kresoxim-methyl and acid metabolite kresoxim-methyl.

1.06 µg g⁻¹ in roots and 0.02 and 0.03 µg g⁻¹ in soil were found in the form of parent compound, i.e. kresoxim-methyl. There were also 0.05 and 0.25 µg g⁻¹ residues in shoots, 0.04 and 0.05 µg g⁻¹ in foliage, 0.25 and 2.01 µg g⁻¹ in roots, and 0.23 and 1.47 µg g⁻¹ residues in soil found in the form of acid metabolite. This clearly indicated the translocation of residues to various parts of plants, i.e. roots, shoots, panicle, foliage and soil following application to soil as well as plant.

At the time of harvest, very low levels of kresoxim-methyl (0.02–0.07 µg g⁻¹) and its acid metabolite (0.02–0.06 µg g⁻¹) were observed in paddy grain and straw, which indicated that there was very little translocation of residues from panicle to straw and grains (Table 3, Figure 3). Similarly, a

trial conducted in grapes to determine the fate of five fungicide residues from vine to wine had earlier shown low level of residues of kresoxim-methyl in grapes after treatment, and within 2 weeks its residues were below the limits of detection (Cabras *et al.*, 1998a, 1998b).

Thus, the results of the study revealed acropetal and basipetal uptake, distribution, translocation and metabolism of kresoxim-methyl in paddy plant. There was little translocation of residues to various plants parts, i.e., foliage, roots, soil, shoot and panicle, and very little to straw and grains in the form of parent compound, i.e. kresoxim-methyl and its acid metabolite. The major route of metabolism was hydrolysis with formation of acid metabolite. No residue of Z-isomer of kresoxim-methyl was observed in any plant part

Table 3 Uptake, translocation, distribution and metabolism of kresoxim-methyl following its foliar application

Substrate	Days after three application (at 40, 60 and 80 DAT) of kresoxim-methyl 500 g L ⁻¹ SC at 250 g a.i. ha ⁻¹ and analysed concentration in ppm (µg per g)											
	40			60			80			110		
	a	b	c	a	b	c	a	b	c	a	b	c
Foliage	58.05 (106.9)	0.07 (0.10)	ND (ND)	9.03 (18.87)	0.52 (4.96)	ND (ND)	1.16 (4.89)	0.04 (0.05)	ND (ND)	1	1	1
Roots	1.14 (3.77)	0.08 (0.08)	ND (ND)	0.12 (0.42)	ND (ND)	ND (ND)	0.08 (1.06)	0.25 (2.01)	ND (ND)	0.20 (0.28)	0.08 (0.12)	ND (ND)
Soil	0.30 (1.03)	0.15 (ND)	ND (ND)	ND (0.08)	ND (ND)	ND (ND)	0.02 (0.03)	0.23 (1.47)	ND (ND)	0.11 (0.32)	ND (ND)	ND (ND)
Shoot	1	1	1	1.26 (7.97)	0.17 (0.48)	ND (ND)	13.83 (23.73)	0.05 (0.25)	ND (ND)	1	1	1
Panicle	1	1	1	0.79 (2.67)	ND (0.01)	ND (ND)	0.05 (2.14)	ND (ND)	ND (ND)	1	1	1
Grain	1	1	1	1	1	1	1	1	1	0.02 (0.03)	0.02 (0.05)	ND (ND)
Straw	1	1	1	1	1	1	1	1	1	0.05 (0.07)	0.03 (0.06)	ND (ND)

¹No samples were available for analysis at sampling interval depending on crop growth and stage.
a = kresoxim methyl, b = acid metabolite, c = Z-isomer; ND ≤ 0.01 ppm.
Note: Values in the parentheses are results of application of kresoxim-methyl 500 g L⁻¹ SC at 500 g a.i. per treatment.

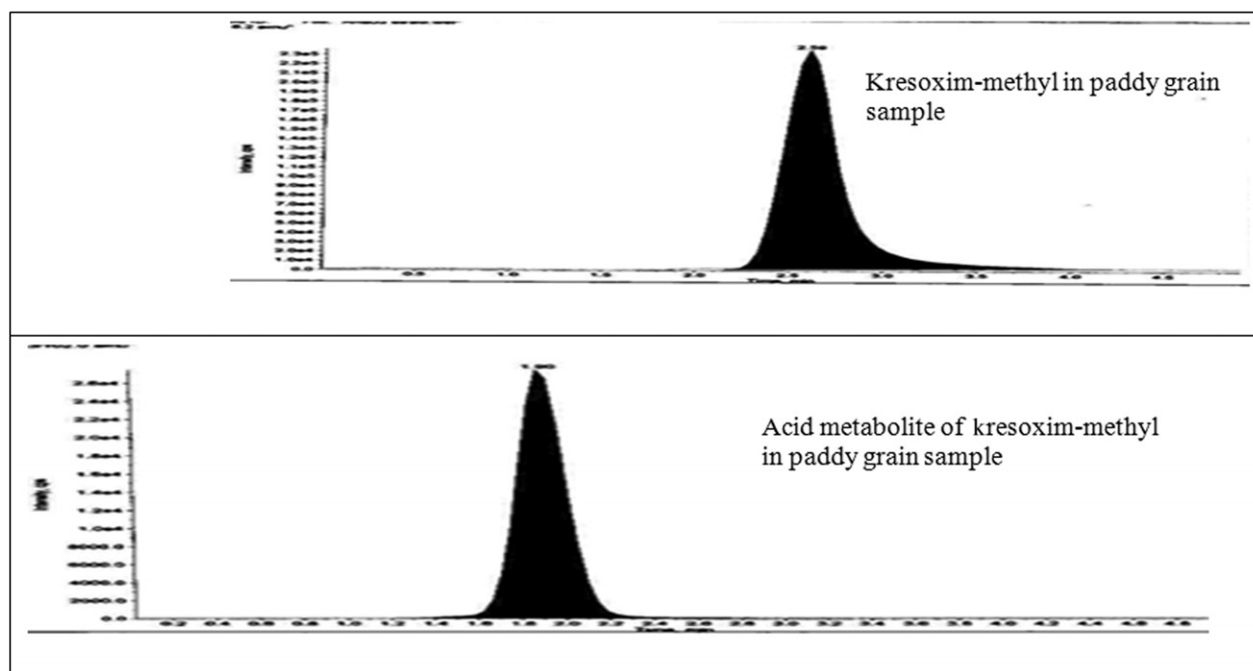


Figure 3 Representative MRM chromatogram of experiment 2 – foliar application of kresoxim-methyl 500 g L⁻¹ SC at 250 g a.i. ha⁻¹ (at 110 days after treatment) – paddy grain sample (sampled after 30 days of 3rd application of test item) analysed for kresoxim-methyl, Z-isomer of kresoxim-methyl and acid metabolite kresoxim-methyl.

or in soil during the study. This is in conformation with earlier studies by Robert & Hutson, 1998.

As per the Joint Meeting on Pesticide Residues (JMPR) definition, the residue for compliance with Maximum Residue Limit (MRL) for kresoxim-methyl for rice in cereals is 0.05 mg kg⁻¹ (EEC, 2004). In this study, the kresoxim-methyl residue levels in grains were 0.02–0.03 mg kg⁻¹ which were less than the recommended MRLs. This indicated that there were no toxic levels of kresoxim-methyl residues in the grains and the compound is safe to be used on paddy crop for control of fungal diseases like sheath blight and blast.

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