

## Leaching behavior of Kresoxim-Methyl and Acid Metabolite in normal and sludge amended inceptisol soil

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### ABSTRACT

Kresoxim-methyl, a strobilurin fungicide, is a broad spectrum, foliar fungicide. Leaching studies conducted with kresoxim methyl and acid metabolite separately revealed that in column soil >90% of the kresoxim methyl undergo hydrolysis and changed into acid metabolite. Acid metabolite showed more leaching potential than the parent molecule. Residues moved to the lower soil depth with increasing amount of rainfall. Increasing the organic matter content of the soil by sludge amendment (5%) reduced the leaching potential of both the compounds. With the same amount of rainfall, leaching was found to be more under discontinuous flow than continuous flow treatment.

### Highlights

- kresoxim-methyl readily transformed in its acid metabolite
- Acid metabolite shows high leaching potential as compared to kresoxim-methyl
- Mobility of kresoxim-methyl and toxic acid metabolite was highly reduced due to organic carbon amendment

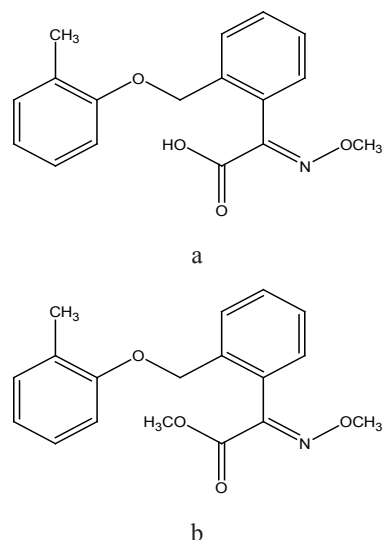
**Keywords:** Kresoxim-methyl; acid metabolite; Sludge; soil column; leaching

Kresoxim-methyl (Figure 1a) (Methyl (E)-2-methoxyimino-2-[2-(o-tolyloxymethyl) phenyl] acetate), a strobilurin fungicide, is a broad spectrum foliar fungicide which is effective against downy mildew in pearl millet (Sudisha *et al.*, 2005), neck blast in paddy (Sunder *et al.*, 2010), powdery mildew and fusarium wilt in wheat (Yang *et al.*, 2011; Pszczolkowska *et al.*, 2013), turicum leaf blight and rust of maize (Kumbhar *et al.*, 2012), botrytis blight in gladiolus (Singh *et al.*, 2011), rusty spot control in peach (Nenad *et al.*, 2010), cercospora leaf spot of sugarbeet (Karadimos and Karaoglandis 2006), late and early blight of potato (Chakraborty and Roy 2012), etc. The molecule binds to quinol oxidation (Qo) site (or ubiquinol site) of cytochrome b in

mitochondria and stop the electron transfer between cytochrome b and cytochrome c. This reduces nicotinamide adenosine dinucleotide phosphate (NADH) oxidation and adenosine triphosphate (ATP) synthesis which are essential for metabolic processes in the fungal cell (Bartlett *et al.*, 2002 Balba 2007). It has been introduced in India by Rallis India Ltd as Ergon 44.3 SC® and is presently registered for control of blast and sheath blight in paddy and downy and powdery mildew in grape at recommended doses of 250-350 g a.i./ha (CIBRC 2013).

A review of literature revealed that kresoxim methyl in soil readily dissipates into its acid metabolite {(E)-2-methoxyimino-2-[2-(o-tolyloxymethyl) phenyl] acetic acid} (figure 1b) (APVMA 2000, EFSA, 2010).

In the present investigation effort has been made to study the leaching potential of kresoxim methyl/ and its acid metabolite in Inceptisol. Effect of sludge amendment on mobility of the parent and the metabolite in soil column was also investigated.



**Figure 1.** Chemical structure of (a) kresoxim-methyl and (b) acid metabolite of kresoxim-methyl.

### Materials and Methods

Analytical grade kresoxim methyl (purity>98.4%) and its commercial formulation Ergon 44.3 SC® were supplied by the Rallis India Ltd. The solvents and chemicals like acetonitrile (HPLC grade), acetone (AR), dichloromethane (AR), anhydrous sodium sulphate, sodium chloride etc. were purchased from Merck Specialities Private Ltd., Mumbai, India.

Kresoxim-methyl acid metabolite was synthesized under laboratory condition by refluxing kresoxim methyl with 10% ethanolic KOH solution. Soil used

in this study was collected from vegetable field of Indian Agricultural Research Institute, New Delhi. The soil was air-dried in shade, ground, sieved through 2 mm mesh screen and then stored in plastic container. To increase the organic carbon content of the field soil, it was amended with sludge at 5% level. Sludge which was used in the study was collected from the water treatment plant located in Keshopur, Delhi. Various physico-chemical properties of the test soils and sludge, determined by the standard procedures (Singh *et al.*, 2005), are given below in Table 1.

Leaching studies with analytical grade kresoxim-methyl and its acid metabolite were carried out in separate laboratory packed columns [25 cm (l) × 2.86 cm (i.d)]. Dry weight of the soil packed in column was ~ 150 g. The lower end of the columns was dipped overnight into water and the water was allowed to rise into the column by capillary action. Next day the columns were hung vertically to allow excess water to drain out. Soil (10 g) fortified with 150 µg of kresoxim methyl or its acid metabolite was spread on top of the column and the leaching was started. Different columns fortified with analytical grade material and acid metabolite were leached with 250, 500 and 1000 mL of water simulating 300, 600 and 1200 mm rainfall under continuous flow conditions. Leaching of Kresoxim methyl was also studied under discontinuous flow condition separately simulating 600 mm rainfall condition. Under discontinuous flow condition, 125 mL water (simulating 150 mm rainfall) was passed through the column on weekly basis. Study continued for total of four weeks. The effect of sludge on leaching of kresoxim-methyl and

**Table 1.** Physico-chemical properties of test soils

Soil	Location	Texture	pH	EC	Organic Carbon (%)	Sand	Silt	Clay
Inceptisol	Delhi	Sandy loam	8.15	0.23	0.37	54.4	23.3	22.3
Sludge amended Inceptisol	Delhi	-	7.9	0.34	2.46	-	-	-
Sludge	Delhi	-	6.25	16.0	47.2	-	-	-



acid metabolite were also studied by packing top 15 cm of the column with 5% sludge amended soil. The column was leached with water under continuous flow condition simulating ~600 mm rainfall. All the leaching experiments were conducted in duplicate.

Leachate fractions (~250 mL under continuous flow and 125 mL under discontinuous flow) from each column were collected, filtered and analysed for kresoxim-methyl/acid metabolite residues. At the end of the leaching experiment, soil columns were cut horizontally into five cores of 5 cm each. Soil from each core was processed and analysed for the kresoxim-methyl/metabolite residue using HPLC-PDA.

Kresoxim-methyl and its acid metabolites both were simultaneously quantified using Shimadzu Ultra High Performance Liquid Chromatograph (UHPLC, Nexera™) equipped with Phenomenax® RP-18 column (250 X 4.60 mm, 5  $\mu$ m) and Photo Diode Array (PDA) detector set at 210 nm. Mixture of acetonitrile and water (80:20 v/v) was used as a mobile phase with a flow rate of 1 mL.min<sup>-1</sup>. The injection volume was 10  $\mu$ L. Under the standardized conditions, kresoxim-methyl and its metabolite were eluted at 4.52 min and 1.83 min respectively. The calibration curve was linear over a range of 0.001 to 10  $\mu$ g mL<sup>-1</sup> with R<sup>2</sup> value of 0.99 for both the compounds. Instrument limit of detection (LOD) of kresoxim-methyl was found to be 0.1 ng (0.01  $\mu$ g mL<sup>-1</sup> with 10  $\mu$ L injection volume) and for its metabolite was 0.05 ng (0.005  $\mu$ g.mL<sup>-1</sup> with 10  $\mu$ L injection volume).

## Results and Discussion

Mobility studies were carried out in lab packed soil columns with kresoxim-methyl and its acid metabolite in Delhi soil separately. Effect of rainfall and sludge amendment was observed for both the compounds. Effect of flow type i.e. continuous flow and discontinuous flow was studied separately for kresoxim-methyl. The amount of kresoxim-methyl residues remaining and formation of acid metabolite during leaching experiment under different treatment are presented in figure 2.

### *Kresoxim methyl leaching*

Different columns were leached with 250, 500 and 1000 mL of water simulating 300, 600 and 1200 mm rainfall respectively. The leaching data are presented in Table 2.

In different columns, out of the applied 150  $\mu$ g of kresoxim-methyl, 82.0-91.9% of the residues were recovered in the form of parent molecule or its acid metabolite. The analysis of column soil and leachate showed that on leaching the soil with 250 ml water (equivalent to 300 mm rainfall), 91.9% of applied kresoxim-methyl was recovered. Out of total recovered amount, 6.4% residues were in the form of kresoxim-methyl and 93.6% residues were in the form of acid metabolite, indicating very fast dissipation of parent molecule into acid metabolite in the column soil. Small amount of residues of kresoxim methyl were present in all the soil cores but the highest percent was present in top 5 cm layer.

Soil column leached with 500 ml water (equivalent to 600 mm rainfall) showed that out of the total applied, 87% was recovered in the form of kresoxim methyl and its acid metabolite. Out of total recovered amount, 6.7% was in the form of kresoxim-methyl and 93.3% was in the form of acid metabolite. Small amount of kresoxim- methyl remaining in the soil was found to be distributed throughout the column and also in leachate fraction. Out of the total acid metabolite formed, ~23.8% was recovered from 15-20 cm soil core and ~ 23.0% was recovered from the leachate fraction (Tab 2).

Soil column leached with 1000 ml water (equivalent to 1200 mm rainfall) showed that out of the total applied, 82.0% kresoxim-methyl was recovered in the form of kresoxim-methyl and its acid metabolite. Out of total recovered, only 0.3% residues were accounted for kresoxim-methyl and 99.7% of residues were accounted for acid metabolite. None of the soil core contained any detectable residues of kresoxim methyl. Out of total acid metabolite formed, highest percent (42.5%) was observed in leachate fraction (Tab 2).



Results revealed that as the amount of rainfall increases, amount of kresoxim methyl recovered from the soil core decreases. ~6-7% of the kresoxim methyl residues were recovered with 300 and 600 mm rainfall whereas only 0.3% of the kresoxim methyl residues were recovered with 1200 mm rainfall. In different rainfall treatment 93.3-99.7% of the recovered residues were detected in the form of acid metabolite indicating very fast degradation of kresoxim-methyl into its acid metabolite during leaching experiment. In 300, 600 and 1200 mm rainfall treatment, 9.2%, 23.0% and 42.5% of the acid metabolite were recovered from leachate fractions. Results revealed that with increasing amount of water available for leaching, downward mobility of both parent and the metabolite go on increasing. Similar results have been reported by Gunasekera *et al* (2007).

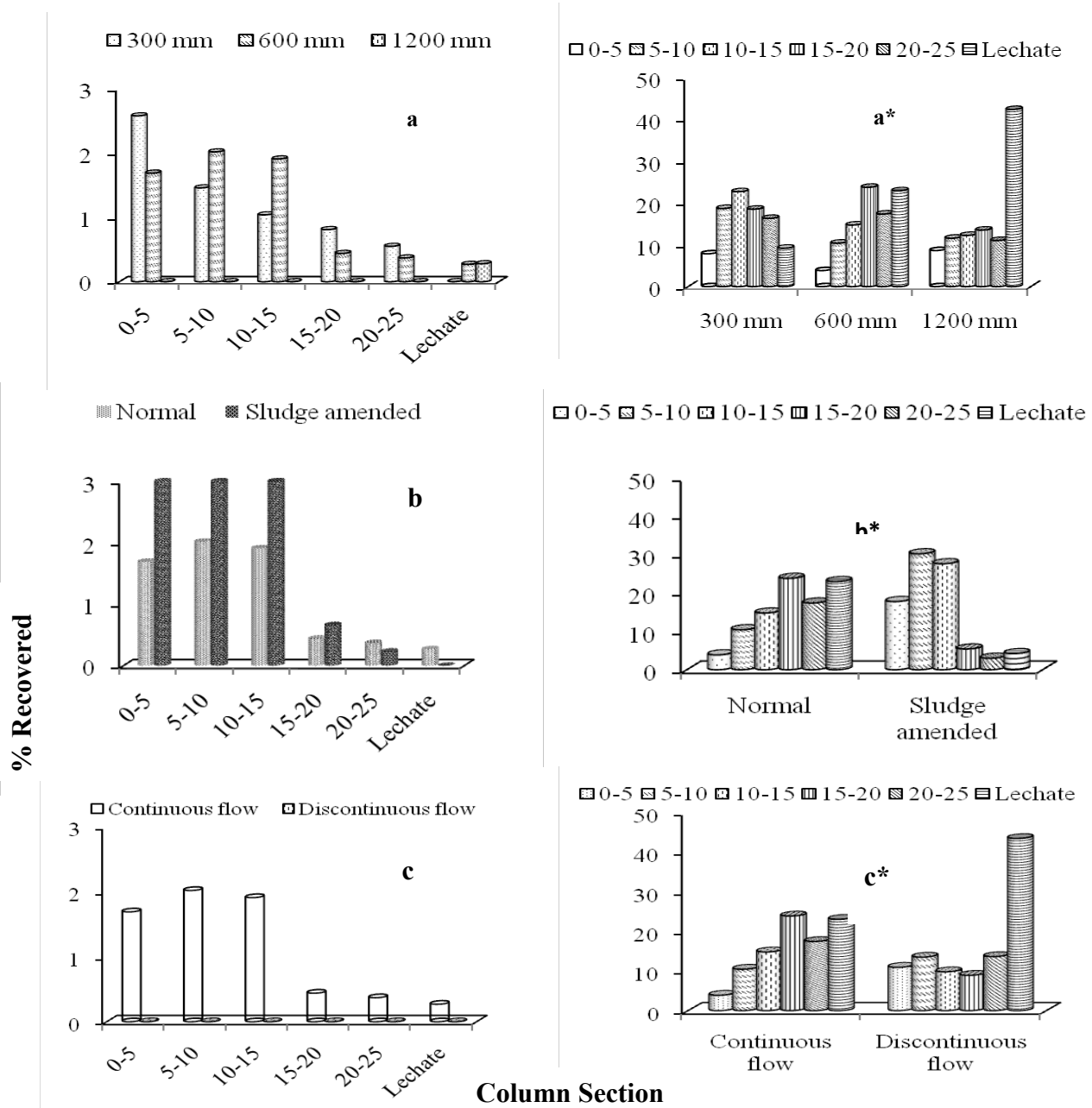
Effect of sludge amendment on leaching of kresoxim methyl and acid metabolite was studied simulating 600 mm rainfall (Tab 2). In sludge amended treatment, after leaching the column with 500 ml water (equivalent to 600 mm rainfall), 73.1% of the applied kresoxim methyl was recovered in the form of kresoxim methyl and its acid metabolite from soil cores and leachate. Out of total recovered amount, 11.7% was present in the form of intact kresoxim-methyl and 88.3% was present as acid metabolite. None of the leachate fraction contained the residues of intact kresoxim methyl, whereas leachate of unamended column showed ~ 0.3% of intact kresoxim methyl residues. Amending the soil with sludge restricts the downward mobility of both the parent compound and acid metabolite. Probably high organic matter content of the amended soil helps in the stronger adsorption on the soil surface and this may be responsible for the reduced leaching.

Effect of continuous and discontinuous flow on leaching of kresoxim methyl revealed that under discontinuous flow, at 600 mm equivalent rainfall, only 37.3% of the added was recovered from the soil cores and leachate. The residues of kresoxim-methyl were found to be uniformly distributed throughout the column and in different cores 8.9 to

13.6% residues were recovered. In case of continuous flow treatment 23.3% of total recovered (~87%) was recovered from leachate fraction whereas major proportion (76.7%) remained in soil core. Under discontinuous flow, intact kresoxim-methyl was not detected in any of the soil cores and leachate fractions in contrast to ~6.7% intact kresoxim methyl recovered from continuous flow treatment (Tab 2). The leaching studies conducted under continuous flow conditions completed in 2 days, whereas under discontinuous flow the study lasted for one month. Kresoxim methyl is known to undergo fast degradation in soil and in one month time probably 100% dissipation of parent molecule has taken place and this could be the reason for non-detection of any residues of kresoxim methyl under discontinuous flow conditions. Overall low recovery of only 37.3% in discontinuous flow condition in comparison to 87.0% recovery in continuous flow revealed that during one month study period even acid metabolite undergo some degradation. More leaching of metabolite was observed under discontinuous flow condition. Increased leaching under discontinuous flow condition can be attributed to the cracks and natural channels formed due to alternate drying and wetting of the columns. Similar result for azoxystrobin and azoxystrobin acid were reported by Ghosh and Singh (2009).

#### **Kresoxim-methyl acid metabolite leaching**

The effect of rainfall on leaching of kresoxim methyl acid metabolite was studied in the lab packed columns simulating 300, 600 and 1200 mm rainfall. The data has been presented in Table 3. It was observed that on leaching the column with 250 ml water (equivalent to 300 mm rainfall), 82.0% of the added amount was recovered in soil cores and leachate fractions. The highest amount (~33.4%) was present in 10-15 cm column depth and ~7.2% of the compound was recovered from leachate fractions. In 500 ml water treatment (equivalent to 600 mm rainfall), 88.7% of applied amount was recovered from soil cores and leachate. Among this, the highest amount (31.8%) was present in leachate fraction and ~21.3% was detected in 20-25 cm core. The analysis of column soil and leachate fraction showed that on



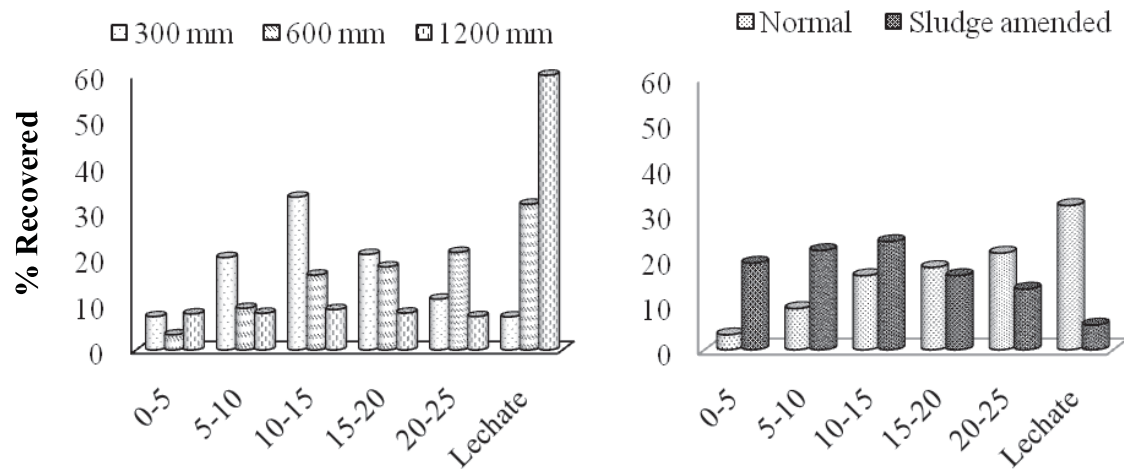
**Figure 2.** Leaching behaviour of kresoxim-methyl and formation of acid metabolite\* a) effect of rainfall, b) effect of organic carbon, c) effect of flow type

**Table 2. Effect of rainfall, organic carbon and flow on the mobility behaviour of kresoxim-methyl in lab paced column**

Effect Particular	Rainfall												Organic carbon (600 mm rainfall)						Flow (600 mm rainfall)					
	300 mm			600 mm			1200 mm			Normal			Sludge amended			Continuous			Discontinuous					
	A	B	Total (%)	A	B	Total (%)	A	B	Total (%)	A	B	Total (%)	A	B	Total (%)	A	B	Total (%)	A	B	Total (%)			
0-5	3.6 (2.6)	10.9 (7.9)	14.4 (10.5)	2.2 (1.7)	5.0 (3.9)	7.2 (5.5)	0.0 (0.0)	10.7 (8.7)	10.7 (8.7)	2.2 (1.7)	5.0 (3.9)	7.2 (5.5)	3.7 (3.4)	19.5 (17.8)	23.2 (21.1)	2.2 (1.7)	5.0 (3.9)	7.2 (5.5)	0.0 (0.0)	6.1 (10.9)	6.1 (10.9)			
5-10	2.0 (1.5)	25.9 (18.8)	27.9 (20.2)	2.6 (2.0)	13.6 (10.4)	16.2 (12.4)	0.0 (0.0)	14.3 (11.6)	14.3 (11.6)	2.6 (2.0)	13.6 (10.4)	16.2 (12.4)	4.0 (3.6)	33.1 (30.2)	37.1 (33.9)	2.6 (2.0)	13.6 (10.4)	16.2 (12.4)	0.0 (0.0)	7.5 (13.5)	7.5 (13.5)			
10-15	1.4 (1.0)	31.4 (22.8)	32.8 (23.8)	2.5 (1.9)	19.3 (14.8)	21.8 (16.7)	0.0 (0.0)	15.1 (12.3)	15.1 (12.3)	2.5 (1.9)	19.3 (14.8)	21.8 (16.7)	4.2 (3.8)	30.3 (27.6)	34.5 (31.5)	2.5 (1.9)	19.3 (14.8)	21.8 (16.7)	0.0 (0.0)	5.5 (9.8)	5.5 (9.8)			
15-20	1.1 (0.8)	25.6 (18.6)	26.7 (19.4)	0.6 (0.4)	31.1 (23.8)	31.7 (24.3)	0.0 (0.0)	16.7 (13.6)	16.7 (13.6)	0.6 (0.4)	31.1 (23.8)	31.7 (24.3)	0.7 (0.7)	5.9 (5.4)	6.7 (6.1)	0.6 (0.4)	31.1 (23.8)	31.7 (24.3)	0.0 (0.0)	5.0 (8.9)	5.0 (8.9)			
20-25	0.76 (0.6)	22.6 (16.4)	23.3 (16.9)	0.5 (0.4)	22.7 (17.4)	23.2 (17.8)	0.0 (0.0)	13.6 (11.1)	13.6 (11.1)	0.5 (0.4)	22.7 (17.4)	23.2 (17.8)	0.3 (0.2)	3.3 (3.0)	3.5 (3.2)	0.5 (0.4)	22.7 (17.4)	23.2 (17.8)	0.0 (0.0)	7.6 (13.6)	7.6 (13.6)			
Leachate	0.0 (0.0)	12.7 (9.2)	12.7 (9.2)	0.4 (0.3)	30.0 (23.0)	30.4 (23.3)	0.3 (0.3)	52.3 (42.5)	52.7 (42.8)	0.4 (0.3)	30.0 (23.0)	30.4 (23.3)	0.0 (0.0)	4.6 (4.2)	4.6 (4.2)	0.4 (0.3)	30.0 (23.0)	30.4 (23.3)	0.0 (0.0)	24.2 (43.4)	24.2 (43.4)			
Total recovered	8.9 (6.4)	129 (93.6)	137.8 (100.0)	8.7 (6.7)	121.7 (93.3)	130.5 (100.0)	0.3 (0.3)	122.7 (99.7)	123.1 (100.0)	8.7 (6.7)	121.7 (93.3)	130.5 (100.0)	12.9 (11.7)	96.8 (88.3)	109.6 (100.0)	8.7 (6.7)	121.7 (93.3)	130.5 (100.0)	0.0 (0.0)	55.9 (100.0)	55.9 (100.0)			
% of total applied	5.9	86	91.9	5.8	81.2	87	2	81.8	82.0	5.8	81.2	87	8.6	64.5	73.1	5.8	81.2	87	0	37.3	37.3			

A-Kresoxim-methyl:  $\mu\text{g}$  (% of total recovered)

B-Kresoxim-methyl acid metabolite formed during leaching study:  $\mu\text{g}$  (% of total recovered)  
 % of total applied = (amount of total recovered/amount applied)  $\times$  100



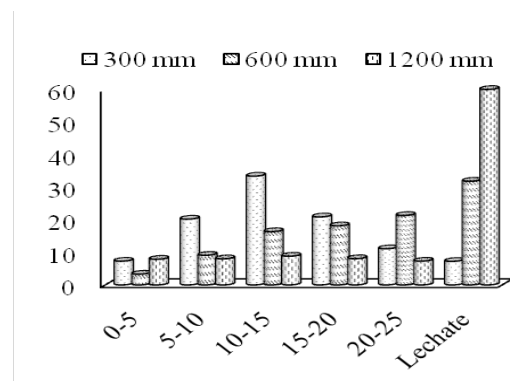
leaching the column with 1000 ml water (equivalent to 1200 mm rainfall), 81.5% of applied kresoxim-methyl acid metabolite was recovered from soil cores and leachate fraction. Out of the total recovered, ~60% of the compound was present in the leachate fraction, whereas 40% of the remaining compound was found to be distributed uniformly in different soil cores (Figure 3a).

Leaching behavior of acid metabolite alone also showed that as the amount of water available for leaching increases, more residues moved towards lower soil cores and leachate. With 300, 600 and 1200 mm rainfall, ~ 7.2%, 31.8% and 60% of the residues were recovered in leachate respectively. Weak adsorption of the acid metabolite in soil and high water solubility might be responsible for the increased leaching with increasing rainfall.

Leaching studies with sludge amendment revealed that out of total acid metabolite added, ~88.7% and ~78.9% was recovered from the normal and sludge amended treatments. In normal soil, amount of acid metabolite present in soil increases with the column depth and highest amount (~21.3%) was detected in 20-25 cm core. ~31.8% of residues were recovered from leachate also. In sludge amendment treatment, out of total recovered amount, 23.8% was present in 10-15 cm layer of soil core. In leachate, only 5.5%

of the residues were recovered (Tab 3, figure 3b). Amending the soil with sludge reduced the leaching potential of metabolite probably due to the strong adsorption of the metabolite on the organic matter surface.

Kresoxim methyl undergoes very fast dissipation in soil and readily forms acid metabolite. The acid metabolite has much more leaching potential as compared to parent molecule. Amending the soil with sludge reduces the leaching potential of acid metabolite. Because of the easy transformation of parent molecule into acid metabolite and high leaching potential of acid metabolite, it must be used cautiously in the high rainfall areas.



**Figure 3.** Effect of (a) rainfall and (b) organic carbon on mobility behaviour of acid metabolite in lab packed column

**Table 3. Effect of rainfall and organic carbon on mobility behavior of acid metabolite in lab packed column**

Effect	Rainfall			Organic carbon	
	300 mm	600 mm	1200 mm	Normal	Sludge amended
Particular	KA(*)	KA(*)	KA(*)	KA(*)	KA(*)
0-5	8.9 (7.3)	4.4 (3.3)	9.6 (7.9)	4.4 (3.3)	22.6 (19.1)
5-10	24.8 (20.2)	12.1 (9.1)	9.8 (8.0)	12.1 (9.1)	25.9 (21.9)
10-15	41.4 (33.4)	21.7 (16.3)	10.8 (8.8)	21.7 (16.3)	28.2 (23.8)
15-20	25.7 (20.9)	24.1 (18.1)	9.8 (8.0)	24.1 (18.1)	19.4 (16.4)
20-25	13.6 (11.1)	28.4 (21.3)	8.9 (7.3)	28.4 (21.3)	15.8 (13.4)
Leachate	8.87 (7.2)	42.4 (31.8)	73.4 (60.0)	42.4 (31.8)	6.5 (5.5)
Total	123.0 (100.0)	133.1 (100.0)	122.3 (100.0)	133.1 (100.0)	118.4 (100.0)
% of total applied	82.0	88.7	81.5	88.7	78.9

\* Percent of total recovered

% of total applied= (amount of total recovered/amount applied)×100

Applied amount=150 µg

KA=Kresoxim-methyl acid metabolite

### Conclusion

Kresoxim methyl undergoes very fast dissipation in soil and form acid metabolite. Acid metabolite has much more leaching potential as compared to parent molecule. Amending the soil with sludge reduces the leaching potential of acid metabolite. Because of the easy transformation of parent molecule into acid metabolite and high leaching potential of acid metabolite, it must be used cautiously in the high rainfall areas.

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