

*Full Length Research Paper***Leaching behaviour of azoxystrobin in sandy loam soil**Priyanka Dagar¹ and Beena Kumari^{2*}¹Department of Chemistry and Physics, CCS Haryana Agricultural University Hisar-125 004, India.²Department of Entomology CCS Haryana Agricultural University Hisar-125 004, India.

Received 11 April, 2014, Accepted 1 August, 2014

The mobility of azoxystrobin in sandy loam soil was studied under continuous and discontinuous flow conditions in soil columns under laboratory conditions at two application rates (50 and 100 µg), with simulated rainfall of 300 mm. Residues of azoxystrobin in soil and leachate were estimated by gas-liquid chromatography and confirmed by gas chromatography-mass spectrometry. Though maximum concentration of azoxystrobin was found in the top 10 cm layer under both continuous and discontinuous flow conditions but azoxystrobin residues under continuous flow conditions were recorded down to a depth of 15-20 cm and under discontinuous flow conditions residues, were recorded at depths of up to 20 and 25 cm for the single and double dose treatments, respectively. The retention of azoxystrobin residues was greater under continuous flow conditions at the higher dose, whereas a low retention of azoxystrobin residues at the lower dose and under discontinuous flow conditions was observed. The low mobility of azoxystrobin in soil indicated that it represents a low risk to groundwater systems. Leachate fractions were free from azoxystrobin residues.

Key words: Leaching, azoxystrobin, sandy loam soil, column, residues.

INTRODUCTION

Pesticides are one of the major technological developments of twentieth century. Whether natural or synthetic, they have toxicological significance and pose a potential risk when they persist in the environment. The indiscriminate use of pesticides has given rise to many problems viz. persistence of toxic residues in the environment, development of resistance in insect pests and their subsequent resurgence. To protect crops from severe damage, many pesticides have been formulated to minimize any losses (Krishnaiah et al., 1976; Mishra and Singh, 1976; Mishra, 2002). As the use of pesticides by farmers is the main method used to combat the

problem of insects/pests, farmers often apply them either at high doses or using multiple sprays. This way, improper and injudicious use of pesticides, besides posing health threat to the farm workers, can also leave harmful pesticide residues on the crops and in the soil. Recent studies have detected fungicides and their primary transformation products (TPs) in streams, precipitation, groundwater, and bed sediment (McConnell et al., 1998; Wauchope et al., 2004; Scribner et al., 2006). In some areas of the U.S, this may represent the first time that fungicides have been included in crop management practices. All fungicides are at risk of losing

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all or part of their effectiveness due to development of fungicide resistant fungi (Brent and Hollomon, 1998). Soil, an important component of the environment, can act as a sink for agricultural pesticides. Such treatments may suppress soil micro flora and hence affect soil properties. The pesticides present in soil sometimes act as a source of contamination for subsequent crops. From soil, pesticide residues can reach water bodies by leaching and runoff. The main processes potentially affecting the ultimate fate of pesticides in soil are retention by soil materials (involving adsorption/desorption processes), transformation processes (biological and chemical degradation), and transport (through soil, atmosphere, surface water, or ground water) (Saltzman and Yaron, 1986; van der Hoff and van Zoonen, 1999). Fungicidal natural products, which can be obtained from a wide variety of sources, including plants, bacteria and even fungi, are a particularly attractive source of new leads due to their structural diversity (Godfrey, 1995; Copping, 1996). Azoxystrobin (methyl (E)-2-{2-[6-(2-cyano-phenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is a synthetic biodegradable strobilurin fungicide. It is a broad-spectrum systemic soil applied fungicide. At room temperature, it is a white crystal solid with a melting point between 118 and 119°C. Its solubility in water at 25°C is 6 mg l⁻¹ (Anonymous, 2003). It is absorbed through the roots and translocate to stems and leaves via xylem, or through leaf surfaces to the leaf tips and growing edges. The mode of action is by inhibition of mitochondrial respiration in fungi. It also inhibits mycelial growth, along with spore production and germination. It is active at very low doses against a wide range of fungal pathogens. Laboratory studies show that azoxystrobin is moderately persistent in soil in the absence of light and moderately mobile in soil profile. Therefore, the present investigation was carried out to obtain information on the mobility and leaching behaviour of azoxystrobin in packed soil columns of a sandy loam soil from Northern India under different flow conditions.

MATERIALS AND METHODS

Chemicals and reagents

All the solvents used for this study were of analytical grade. The concentration of the azoxystrobin active ingredient present in the acetone extracts were quantified using gas- liquid chromatography (GLC). The analytical solvents used (acetone, dichloromethane and hexane) were procured from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade ≥ 99.9 %) was also obtained from Merck, Darmstadt, Germany. All of the solvents used were redistilled before use in glass apparatus and their suitability was ensured by running reagent blanks before each analysis. The stock solution of azoxystrobin fungicide was prepared at concentration of 100 µg ml⁻¹; and further diluted to prepare working standards.

Preparation of standard solution

A standard 1 mg ml⁻¹ stock solution of azoxystrobin was prepared in

acetone. The standard solutions required for plotting a calibration curve (2.00, 1.50, 1.00, 0.50, 0.25 and 0.10 µg mL⁻¹) were prepared from stock solution by serial dilution using n-hexane. All standard solutions were stored at 4°C.

Instruments

Analysis of azoxystrobin was carried out on gas liquid chromatograph (GLC) Shimadzu Model GC-2010) equipped with ⁶³Ni electron capture detector (ECD) supplied by M/s Shimadzu, Kyoto, Japan. Confirmation of azoxystrobin was carried out on a gas chromatograph (GC-MS/MS Model Agilent 7890) coupled with mass detector (Mass 7000 GC/MS Triple Quadrupole).

Experiment

The leaching experiment was conducted under laboratory conditions. A sandy loam soil was collected from Research Farm, CCS Haryana Agricultural University, Hisar which had no previous history of pesticide application. Soil was air-dried in the shade, ground and sieved through 2-mm sieve. Commercial formulation (Amistar 23SC) was used for the leaching experiment. Plexi glass columns (90 cm × 5 cm internal diameter) fitted with a perforated sieve covered with filter paper (Whatman No. 1) was used. Each column was sequentially filled with soil up to a height of 60 cm with a bulk density of 1.50 g cm⁻¹ of sandy loam soil. This equated to 152 g of sandy loam soil per column each time. The process was repeated until each column was uniformly filled to a height of 60 cm. The experiment was conducted with triplicates and a blank. Before packing, the filter paper was kept at the perforated distal end of the column to allow only the passage of leachates. The azoxystrobin formulation was dissolved in deionized water and simultaneously applied to the top 5 cm of the soil in the column at doses of 50 and 100 µg as single and double dose respectively. After application of the azoxystrobin formulation, the columns were irrigated with 50 mm of distilled water (equivalent to 300 mm rain) at a time interval of 24 h under continuous flow conditions and after one week under discontinuous flow conditions. During leaching 2-3 drops of toluene solution were added to each column to monitor microbial growth. Residues of azoxystrobin were estimated at different soil depths: 0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 cm and in the leachates. Three leachate fractions were collected from each treatment. Columns were then cut into two equal halves and the soil was sampled in 5 cm segments. Segments from the same column were pooled for use of residue analysis.

Estimation of residues

Residues of azoxystrobin were quantified on a gas chromatograph (GC) (Shimadzu Model GC- 2010) equipped with capillary column HP-1 (30 m × 0.32 mm i.d × 0.25 µm film thickness of 5% diphenyl + 95% dimethyl polysiloxane). Other GC parameters were as follows: Temperature (°C): Injection port (300°C), Detector (320°C); Carrier gas (N₂) flow was maintained at 60 and 2 ml min⁻¹ through column with split ratio 1:10. Retention time (R_i) observed for azoxystrobin was 6.442 min.

Confirmation by GC-MS

Confirmation of the presence of azoxystrobin was achieved using a gas chromatograph mass spectrometer (GC-MS) in single ion monitoring mode. A capillary column (30 m × 250 µm × 0.25 µm film thickness) was used for confirmation of these residues. The GC-MS operating conditions were as follows: oven (program) initial

Table 1. Per cent recoveries of azoxystrobin in sandy loam soil and water.

Substrate	Level of Fortification (mg kg ⁻¹)	Azoxystrobin (% Recovery*±SD)	Mean (% recovery)
Sandy loam soil	0.01	84.40±2.40	85.47
	0.10	85.90±2.65	
	0.25	86.10±1.99	
Water	0.01	89.20±2.13	91.58
	0.10	90.80±2.00	
	0.25	94.75±1.97	

*Average of three replicates.

Table 2. Leaching behaviour of azoxystrobin in sandy loam soil under continuous flow conditions.

Soil depth (cm)	Residues* (µg)				
	Single dose (50 µg) ± SD	Per cent retention	Double dose (100 µg) ± SD	Per cent Retention	Mean
0-5	22.105±0.006	52.38	43.093±0.002	50.98	32.599
5-10	18.007±0.006	42.65	27.277±0.006	32.27	22.642
10-15	2.100±0.036	4.98	10.156±0.005	12.01	6.128
15-20	BDL	-	4.005±0.005	4.74	2.003
20-25	-	-	BDL	-	-
Mean	10.553		21.133		

*Average of three replicates. CD at 5% level of significance : Soil depth= 0.011; Dose = 0.008 ; Soil depth x dose= 0.015.

temperature of 70°C holding for 2 min followed by ramping at 25°C min⁻¹ to 150°C and holding for 0 min, then ramping by 3°C min⁻¹ to 200°C and holding for 0 min, then ramping at 8°C min⁻¹ to 280°C and held for 1 min; injector temperature 280°C. Helium was used as a carrier gas with a flow rate of 1ml min⁻¹. The injection volume was 2 µl with split ratio of 1:10. On the basis of above information a programme was developed in product ion monitoring mode with molecular mass 403.4 in azoxystrobin at four different collision energies of 15, 20, 25 and 30 and MS₁ range starting from 150 and MS₂ range ending at 350. The precursor ion of azoxystrobin at m/z 343.8 was found to break completely and showed fragmentation peak of product ion at m/z 329 with retention time R_t of 41.786 min (Figures 1 and 2).

RESULTS AND DISCUSSION

Efficiency of the method

In the present investigations, recovery experiments were carried out to establish the reliability and validity of the analytical method and to determine the efficiency of the extraction and cleanup procedures for the soil and water. The soil and water control samples were spiked at 0.01, 0.10 and 0.25 mg kg⁻¹, respectively, and processed by following the methodology as described above. Mean

recoveries of azoxystrobin in soil were found to range from 84.46 to 86.10% and in water ranged from 89.20 to 94.75% (Table 1). The average recovery values from all the fortified samples were in excess of 85%. Therefore, the results have been presented as such without applying any correction factor. Limit of detection (LOD), limit of quantification (LOQ), precision and accuracy parameters were derived using the guidelines described by Thompson et al. (2002). Accordingly, the limit of quantification (LOQ) was 0.01 mg kg⁻¹ and limit of detection (LOD) was 0.003 mg kg⁻¹. The overall results of azoxystrobin at different soil depths are presented in (Tables 2 and 3). The results show that azoxystrobin leached down to depths of 15 and 20 cm under continuous flow conditions and 20 and 25 cm under discontinuous flow conditions, at single (T₁) and double (T₂) doses under 300 mm rainfall conditions. The highest concentration of azoxystrobin was found at 0-5 cm depth in both the application rates, and was higher in the T₂ dose than the T₁ dose. Azoxystrobin leached down to a depth of 0-10 cm in both the doses. Several factors such as adsorption of the pesticide by the soil particles, water solubility of the pesticide, volume of leachate, pH and soil texture can influence the leaching of the pesticide

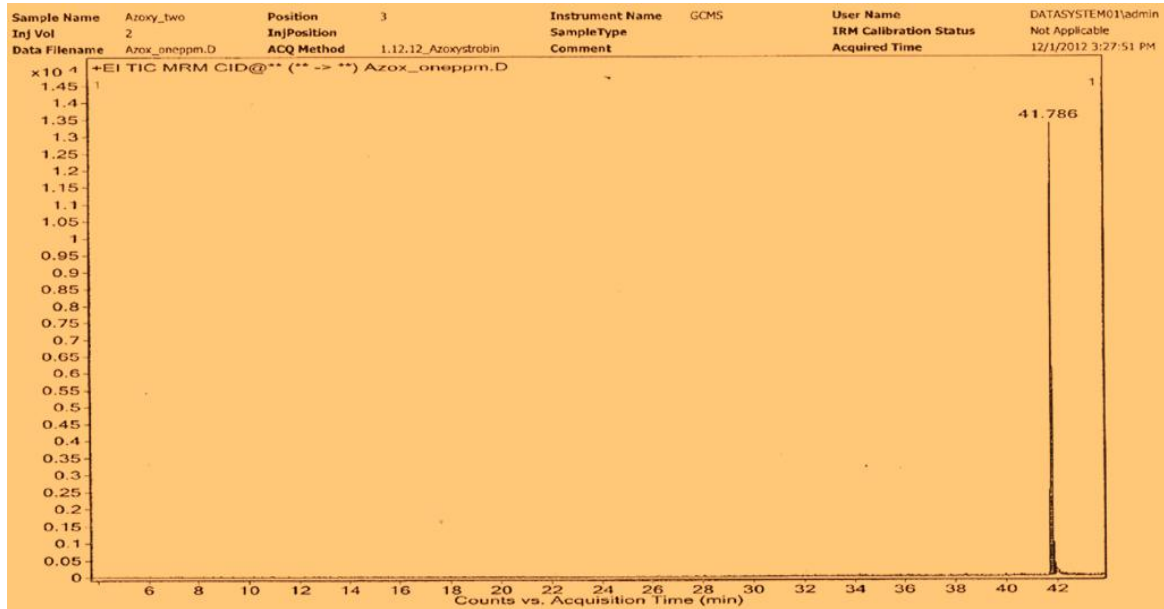


Figure 1. Retention time of azoxystrobin in MRM mode.

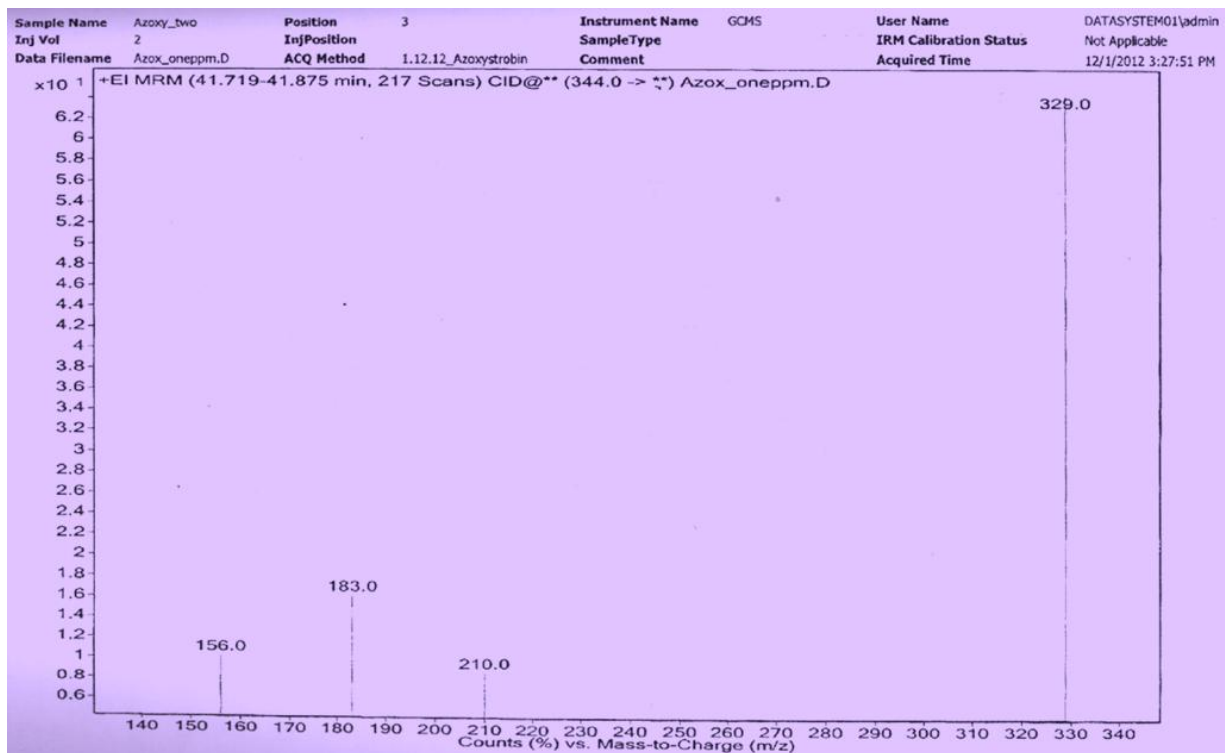


Figure 2. Mass to charge ratio (m/z) counts (%) of azoxystrobin at collision 20 in MRM Mode.

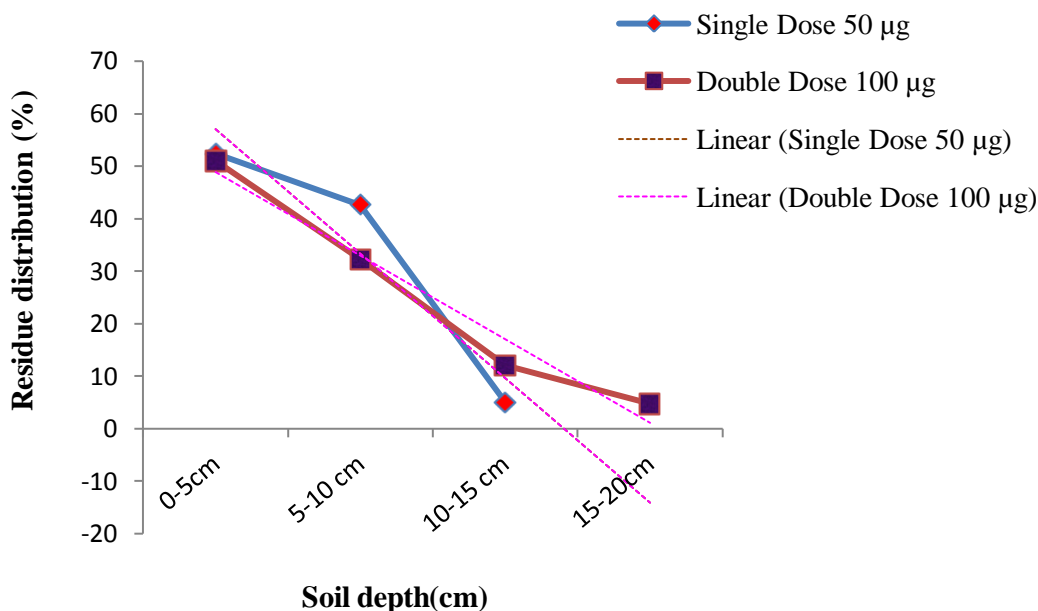
through the soil (Kidd and James, 1991; Crisanto et al., 2000; Halimah et al., 2004). Recovered amount of azoxystrobin residues at various soil depths were analyzed statistically.

Significant differences in the recovered amount of azoxystrobin at various depths were observed at both application rates. Irrespective of soil depth, residue levels were significantly lower following single dose applications

Table 3. Leaching behaviour of azoxystrobin in sandy loam soil under discontinuous flow conditions.

Soil depth (cm)	Residues* (μg)				Mean
	Single dose (50 μg) \pm SD	Per cent retention	Double dose (100 μg) \pm SD	Per cent Retention	
0-5	20.538 \pm 0.007	48.74	41.131 \pm 0.003	48.70	30.835
5-10	13.107 \pm 0.006	31.11	24.200 \pm 0.005	28.66	18.654
10-15	6.421 \pm 0.003	15.24	12.605 \pm 0.006	14.92	9.513
15-20	2.071 \pm 0.004	4.92	5.017 \pm 0.002	5.94	3.544
20-25	BDL	-	1.498 \pm 0.007	1.77	0.749
25-30	-	-	BDL	-	-
Mean	8.427		16.890		

*Average of three replicates. CD at 5% level of significance : Soil depth = 0.010; Dose = 0.006; Soil depth x dose = 0.014.

**Figure 3.** Per cent distribution of azoxystrobin in sandy loam soil under continuous flow conditions.

compared to double dose applications. Per cent azoxystrobin retention at 0-20 and 0-25 cm depth in sandy loam soil is shown in Figures 3 and 4. Azoxystrobin was retained between 100.00 and 99.99% in the single and double dose treatments, respectively under continuous flow conditions. In contrast, approximately 48.74 and 48.70% of the compound was retained in the 0-5 cm core of soil in the single and double dose treatments under discontinuous flow conditions, showing very low mobility of this fungicide in sandy loam soil. The retention of azoxystrobin in soil cores was higher under discontinuous flow conditions than under continuous flow condition at both azoxystrobin dosages. None of the leachate fractions contained any pesticide residues at

both dosages. Hence, azoxystrobin seems to be of minimal risk to groundwater.

The present results are in agreement with earlier reports. Gupta and Gajbhiye (2004) studied the leaching behaviour of thifluzamide in alluvial soil under laboratory conditions. The study reveals that thifluzamide was moderately mobile in alluvial soil. Only small amounts (<1%) were recovered from leachate fractions, whereas, major portion remained in 0-15 cm soil depth. However, our findings differ from some previously published observations. These differences may result from a number of different parameters including different matrices and conditions like field capacity moisture, submerged conditions, flooded and non-flooded, organic

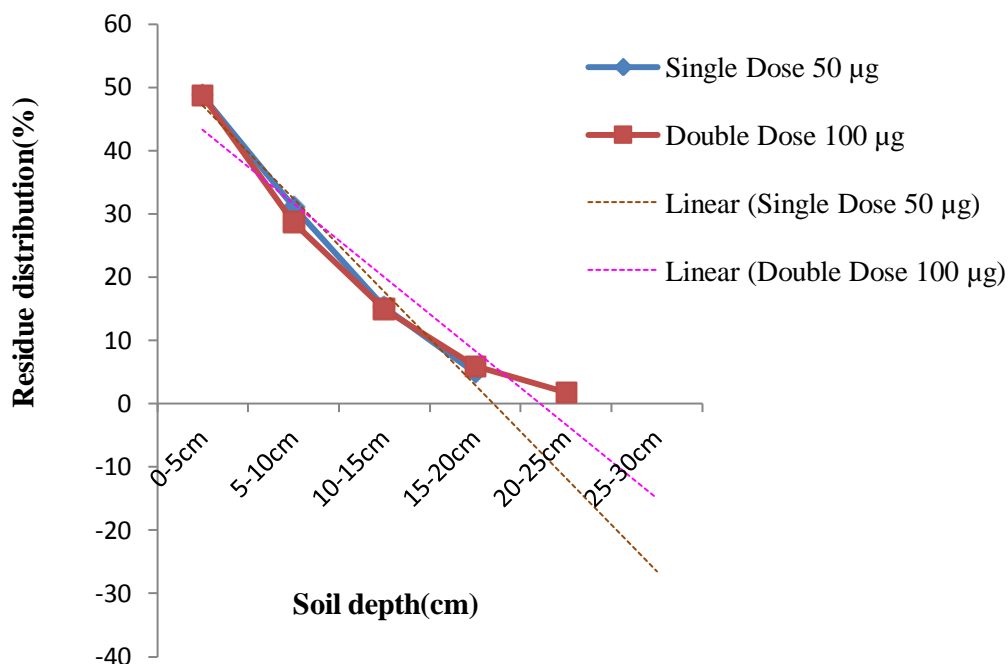


Figure 4. Per cent distribution of azoxystrobin in sandy loam soil under discontinuous flow conditions.

matter.

Bending et al. (2006) reported variability in the degradation rates of the pesticides like isoproturon, azoxystrobin and diflufenican, in sandy loam and clay loam soils. No change in the rate of degradation after 8 months, and the amount of azoxystrobin remaining in the soils ranged from 15.4 to 50.9% at Wellesbourne, and between 29.9 and 49.3% at Kirton. Ghosh and Singh (2009) also reported that azoxystrobin was immobile in a sandy loam soil but its mobility increased with the amount of percolating water added. Following water percolation equivalent to 126 mm of rainfall, azoxystrobin leached down to 5-10 cm depth and nearly 90% of applied fungicide was retained in the top 0-5 cm layer, whilst percolation equivalent to 362 mm rainfall resulted in azoxystrobin leaching down to 10-15 cm soil depth and 50% azoxystrobin leached down to the 5-10 cm soil section. However, under discontinuous flow conditions, a major portion of azoxystrobin (47% of the time zero applied azoxystrobin) was recovered from the 0-5 cm layer, whilst 13% (22% of the azoxystrobin recovered at the end of study) was present in the 5-10 cm layer.

Jørgensen et al. (2012) carried out the study to estimate leaching of the fungicide azoxystrobin and one of its primary degradation products R234886 major fraction at four agricultural research fields (one sandy and three loamy) in Denmark and observed that neither azoxystrobin nor R234886 were detected at the sandy site, but did leach through loamy soils. Azoxystrobin was generally only detected during the first couple of months

following application and R234886 leached for a longer period of time and at higher concentrations (up to 2.1 $\mu\text{g L}^{-1}$).

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Head, Department of Entomology for providing research facilities.

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