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Leaching BehaviourOfAzoxystrobin In Clay Loam SoilUnder Continuous Flow Conditions

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Abstract:

The downward movement of azoxystrobinin clay loam soil was studied under continuous flow conditions in undisturbed soil columns under laboratory conditions. The undisturbed columns containing clay loam soil were leached at two application rates, 50µg and 100µg, with water equivalent to 300 mm rainfall. Residues of azoxystrobin in soil and leachate were estimated by gas–liquid chromatography and confirmed by gas chromatography–mass spectrometry. Results revealed that although majority of the azoxystrobin remained in top 0–5 cm layer, substantial amount moved to 5–10 and 10-15cm depth under continuous flow conditions at both doses. Results indicated that the retention of azoxystrobin residues was more under continuous flow conditions at higher dose, whereas low retention of azoxystrobin residues at low dose. Low mobility of azoxystrobin in soil indicated that it may be safe for ground water. Leachate fractions were free from azoxystrobin residues.

Keywords: *Mobility; Leachate; Azoxystrobin ; clay loam soil ; Column; Residues*

Introduction

Intensive agricultural practices often include the use of pesticides to enhance crop yields. However, the improvement in yield is sometimes concominant with the occurrence and persistence of pesticide residues in soil and water (Ware and Whitacre 2004). Soil, an important component of environment, acts as a sink for majority of pesticides used in agriculture. It acts as filter, buffer and degradation potentials with respect to storage of pollutant with the help of soil organic carbon (Burauel and Bassmann 2005). The fate of pesticides in the soil is greatly influenced by their interaction with soil components, the environment and their transport from one environmental compartment to another (Ismail and Kalithasan 2003; Racke 1993). The rate of degradation of pesticides in the soil is one of the most important criteria that determine the behaviour of pesticides in the environment (Goring and Hamakers 1975). Heavy usage of pesticides in agriculture may cause adverse effects on the environment and consequently on human health. The leaching of pesticides into groundwater is a major environmental concern because it affects the quality of underground water (Lehmann et al. 1993).

A great variety of fungicides is being used to control fungal pathogens of crop plants. Some of them have been reported to affect soil microorganisms and consequently mycorrhizal fungi adversely (Nemec, 1980; Carey *et al*., 1992; Schreiner and Bethlenfalvay, 1997, 1997a). This symbiotic association between plant roots and zygomycete fungi is known to stimulate plant health and growth of shoots and roots by bioprotecting colonized tissues from infections by soil-borne pathogens (Schönbeck, 1979; Linderman, 1994; Harrier and Watson, 2004). It improves plant nutritional status as well as

soil physical and biological properties (Linderman, 1994). Azoxystrobin and kresoxim-methyl are the members of a class of fungicides derived from the fungal secondary metabolite strobilurin A produced by *Strobilurustenacellus*(Anke*et al.*, 1977; Sauter*et al.*, 1995). These fungicides are characterized by a broad spectrum of fungicidal activity against numerous foliar pathogens representing members of oomycetes (chromista), ascomycetes, basidiomycetes and deuteromycetes (fungi), parasitizing different crop plants like pome fruits, grape vine, vegetables and cereal crops (Grossmann and Retzlaff, 1997).

To protect the crop from severe damage, many pesticides have been evaluated to minimize loses (Krishnaiah et al. 1976; Mishra and Singh 1976; Mishra 2002). As the use of pesticides by farmers is the only way to sort out the problem of insects/pests, hence farmers apply the pesticide either at higher dose or give more number of sprays. This way, improper and injudicious use of pesticide, beside posing health threat to the farm workers, also leave harmful pesticide residues on the crop and soil. All fungicides are at risk of losing all or part of their effectiveness due to development of fungicide resistant fungi (Brent and Hollomon1998). 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-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is a new synthetic biodegradable strobilurin fungicide. It is a broad-spectrum systemic soil applied fungicide. Its solubility in water at 25° C is 10 mg l^{-1} (Anonymous, 2003). It is absorbed through the roots and translocated the 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 spore germination, mycelial growth and spore production of fungi. 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.

2. Materials and Methods

2.1 Chemicals and reagents

All the solvents used for this study were of analytical grade. The analysis of the formulation in acetone extract with respect to its active ingredient of azoxystrobin was estimated using gas– liquid chromatography (GLC). Solvents like 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 the common solvents were redistilled before use in glass apparatus and their suitability was ensured by running reagent blanks before actual analysis. The stock solution of azoxystrobin fungicide prepared at concentration of 100 µg ml^{-1}; and further diluted to prepare working standards.

2.2 Preparation of standard solution

A standard stock solution of azoxystrobin having concentration of 1mg ml^{-1} 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 4ºC.

2.3 Instruments

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

2.4 Experiment

The leaching experiment was conducted under laboratory condition. Clay loam soil was collected from Karnal with no history of pesticide application. Soil was air dried in shade, ground and sieved through 2-mm sieve. Commercial formulation (Amistrar 23SC) was used for 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 the height of 60 cm to a bulk density of 1.35 g cm^{-1} of clay loam soil. Weighed amount of clay loam soil (137 g) was poured in the column each time. The process was repeated till 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. Azoxystrobin formulation was dissolved in deionized water and simultaneously applied to the last 5 cm of the soil in the column at the dose of 50 µg and 100 µg as single and double dose respectively. After application of azoxystrobin formulation, the columns were irrigated with 50 mm of distilled water (equivalent to 300 mm rain) at the time interval of 24 h under continuous flow conditions. During leaching 2-3 drops of toluene solution was added to each column to check microbial growth in it. Residues of azoxystrobin were estimated at different depths of soil, *i.e.*, 0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 in leachates. Three leachate fractions were collected from each treatment. Columns were then cut into two equal halves and the soil was sampled in 5cm segments. The segments from same column were pooled for use in analyzing residues.

2.5 Confirmation by GCMS

Confirmation of azoxystrobin was achieved by gas chromatography mass spectrometer (GC-MS) in single ion monitoring mode. A capillary column (30m × 250 µm × 0.25 µm film thickness) was used for confirmation of these residues. The GC-MS operating conditions were: Oven (program) initial temperature was 70^0C and held for 2 min ramped 25^0 C min^{-1} to 150^0 C which was held for 0 min, then ramped 3^0C min^{-1} to 200 and held for 0 min then again ramped 8^0C min^{-1} to 280^0C and held for 1min; injector temperature 280^0C. Helium was used as a carrier gas with a flow rate of 1ml min^{-1}. Injection volume was 2µl with split ratio of 1:10. On the basis of above information a program me 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 MS_1 range starting from 150 and MS_2 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 41.63 min.

3. Result and Discussion

3.1 Efficiency of the method

In the present investigations recovery experiments were carried out at different levels to establish the reliability and validity of analytical method and to know the efficiency of extraction and cleanup procedures for soil and water. The control samples of soil and water 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 80.20 to 82.40 per cent and in water ranged from 89.20 to 94.75 per cent (Table 1).The average recovery values from the fortified samples were found to be more than 80 percent. Therefore, the results have been presented as such without applying any correction factor. The parameters like limit of detection (LOD), limit of quantification (LOQ), precision and accuracy were derived keeping in view the guidelines as mentioned by Thompson et al. (2002). Accordingly, the limit of quantification (LOQ) was found to be 0.01 mg kg^{-1} and limit of detection (LOD) being 0.003 mg kg^{-1}.

The overall results of azoxystrobin at different soil depths are presented in Table 2 and 3, respectively. The results showed that azoxystrobin leached up to the depth of 10 and 15 cm under continuous flow conditions at single (T_1) and double (T_2) dose at 300 mm rainfall condition. The highest concentration of azoxystrobin was found at 0-5 cm depth in both the application rates and it was higher at T_2 dose as compared to T_1 whereas azoxystrobin leached up to depth of 0-10 cm at 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 through the soil (Kidd and James 1991; Crisanto et al. 2000; Halimah et al. 2004). Recovered amount of azoxystrobinresidues

at various soil depths were analyzed statistically. Significant differences on the recovered amount of azoxystrobin at various depths were observed at both application rates. Irrespective of soil depth, residue levels were significantly low in single dose compared to double dose. Percent distribution of azoxystrobin in different soil cores (0-5 and 5-10 cm) was 80.30 and 19.70, respectively at single dose. In case of double dose, percent distribution of azoxystrobin was 74.46, 16.79 and 8.73 at 0-5, 5-10 and 10-15 cm depth, respectively. Azoxystrobin was retained between 100.00 and 99.99 per cent at single and double dose under continuous flow conditions, respectively. None of the leachate fractions contained residues of any insecticides in both the doses. Hence, azoxystrobin fungicide seems to be safe for ground water. The present results are in agreement with earlier reports. Gupta and Gajbhiye (2004) whostudied the leaching behaviour of thifluzamide in alluvial soil under laboratory conditions. The study revealed that thifluzamide was moderately mobile in alluvial soil. Only small amounts (<1%) were recovered from leachate fractions, whereas, major portion remained in 0-15 . Comparing to the present results, Bending *et al.* (2006) reported variability in degradation rate of pesticides isoproturonazoxystrobin and diflufenican, in sites on clay loam and clay loam. Degradation, with no change in the rate of decline after 8 months, the amount of azoxystrobin remaining ranged from 15.4 to 50.9 per cent at wellebourne, and between 29.9 and 49.3 per cent at kirton. Ghosh and Singh (2009) also reported that azoxystrobin was immobile in the clay loam soil but its mobility increased with amount of percolating water. Then after percolating water equivalent to 126 mm of rainfall, azoxystrobin leach down to 5-10 cm depth and nearly 90% of applied fungicide retained in the top 0-5 cm layer, while increasing water (362 mm rainfall) azoxystrobin leached down to 10-15 cm soil depth and 50 per cent azoxystrobin moved down to 5-10 cm soil section.

Table 1: Recovery studies of azoxystrobin in clay loam soil and water

Substrate	Level of Fortification (mg kg^{-1})	Azoxystrobin % Recovery*±SD	Mean % Recovery
Clay loam soil	0.01	80.20±2.04	81.48
	0.10	81.85±1.87	
	0.25	82.40±1.96	
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 clay 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**
0-5	32.465±0.004	80.30	60.33±0.003	74.46
5-10	7.966±0.005	19.70	13.605±0.006	16.79
10-15	BDL	-	7.079±0.008	8.73
15-20	-	-	BDL	-

***Average residues of three replicates; No residues were detected in leachate**

Table 3: Average residues of azoxystrobin in clay loam soil under continuous flow conditions

Soil depth (cm)	Residues* (µg)		Mean
	Single dose (50 µg)	Double dose (100 µg)	
0-5	32.465	60.331	0-5
5-10	7.966	13.605	5-10
10-15	BDL	7.079	10-15
15-20	-	BDL	15-20
Mean	**13.477**	**27.005**	**Mean**

CD at 5% level of significance

Soildepth = 0.136
Dose = 0.111
Soildepth x dose = 0.193

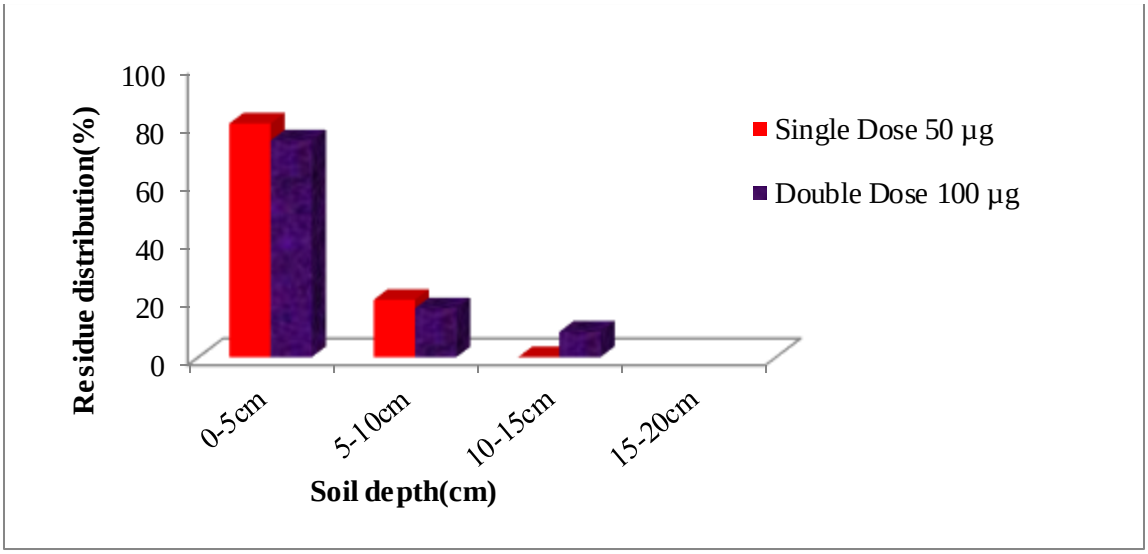

Figure:1Percent distribution of azoxystrobin in clay loam soil by continuous flow conditions

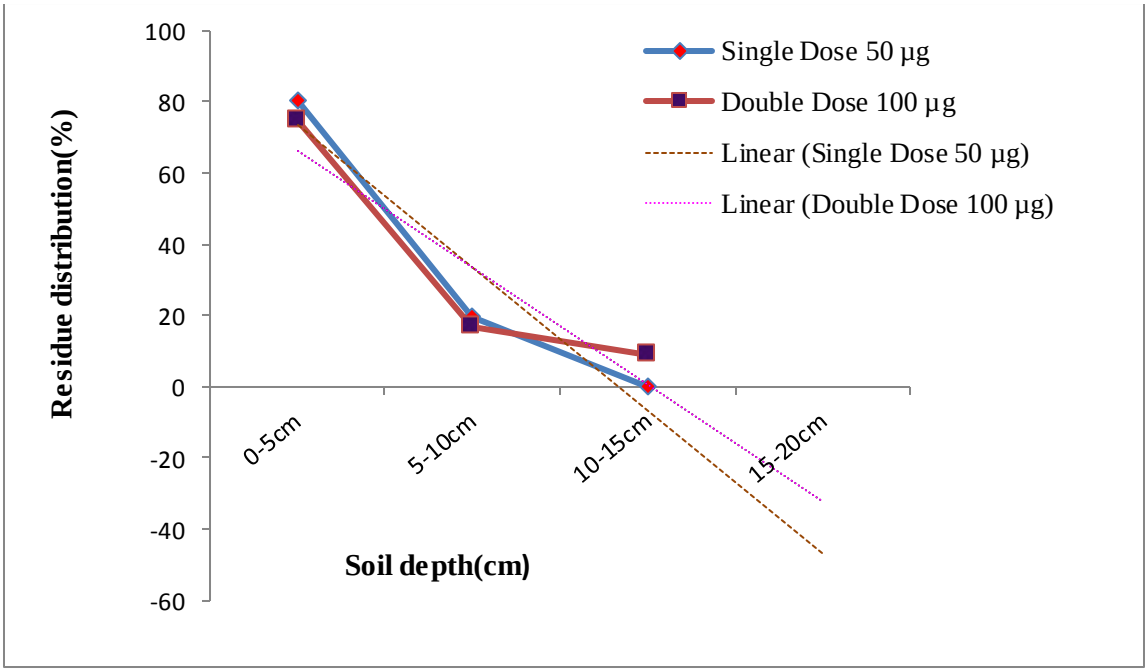

Figure: 2Linear distribution curve of azoxystrobin in clay loam soil under continuous flow conditions

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