

Fate and behaviour of azoxystrobin in chilli by using liquid chromatography with mass spectroscopy

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ABSTRACT

A liquid chromatography mass spectrometry (LC/MS-MS) method for analysis of residual azoxystrobin in chilli was developed in present work. Azoxystrobin is generally a systemic fungicide applied in chilli to control anthracnose and powdery mildew diseases. The method was based on extraction with 1:1 ethyl acetate: 10% aq. NaCl solution, clean-up by PSA, GCB and florisil, evaporated in *N*₂ evaporator, followed by volume make up with Acetonitrile and analysed by LC/MS-MS in multiple reaction monitoring (MRM) mode. Recoveries from fortified samples were found in the range from 90% to 92%. The limit of quantification (LOQ) of the method was 0.01 ppm. The half life value of azoxystrobin in chilli was determined as 0.73 to 0.80 days.

Keywords: Azoxystrobin, half-life, LC-MS/MS, LOQ

Azoxystrobin is one of strobilurin fungicides, which are synthetic analogue of naturally occurring fungal metabolites (Christensen *et al.*, 2001; Anon, 2003). Pesticides that contain azoxystrobin are recommended for protection of garden and greenhouse vegetables against powdery and downy mildew and grey mould. They can be also used to protect cereals against fungal diseases such as powdery mildew, stem rust, leaf rust, and black stem rust, and to protect fruits against blight and mildew (Cabras *et al.*, 1998; Giza *et al.*, 2003). Chilli is one of the most valuable vegetable crops of India. It is predominantly popular for its green pungent fruits, which is used for culinary purpose (Pariari *et al.*, 2013). It is grown almost throughout India. Our country is the largest producer of chilli in the world (Jagtap *et al.*, 2012). There are several countries in the world which are the importer of Indian chilli. Beside this, within the country there is a great demand of chilli. But sometime the price of chilli rises up due to less production of chilli. One of the reason is that chilli is affected by several diseases of which anthracnose and powdery mildew are very devastating (Ganeshan *et al.*, 2011). Azoxystrobin is now applied to control such diseases. In the present study the persistence feature and residue of azoxystrobin in chilli was undertaken to find out the safe waiting period of azoxystrobin in chilli.

MATERIALS AND METHODS

One field trial was conducted at AB Block Seed farm of Bidhan Chandra Krishi Viswavidyalaya,

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Kalyani, Dist- Nadia, WB to estimate the persistence nature and harvest time residues of Azoxystrobin 23% (Supplied by Crystal Crop Protection Pvt. Ltd., Delhi) in/on chilli during June 2013. In field study the formulation was applied with the help of a knapsack sprayer equipped with WFN 62 nozzle @125g ha⁻¹ (T1) and 250g ha⁻¹ (T2) along with untreated control (T3) plots. The experiment was laid in a Randomized Block Design (RBD). 10 mg of analytical standard of azoxystrobin was weighted (Sartorius, model no. CP225D, Germany) and taken in a 100 ml volumetric flask and volume was made up to the mark with acetonitrile. Necessary dilutions were made from this stock standard as and when required.

Mobile phase A: 1 ml of acetic acid was added to 900 ml of millipore water and 385.4 mg of ammonium acetate was added to it. Then the volume was made up to 1000 ml with millipore water to prepare 1000 ml of (H₂O + 0.1% CH₃COOH + 5 mM NH₄COOCH₃).

Mobile phase B: 1 ml of acetic acid was added to 900 ml of methanol and 385.4 mg of ammonium acetate was added to it. Then the volume was made up to 1000 ml with methanol to prepare 1000 ml of (CH₃OH + 0.1% CH₃COOH + 5 mM NH₄COOCH₃)

The samples were homogenized using blender. 10 g of the homogenized sample was taken in a 50 ml centrifuge tube. Then 10 ml of ethyl acetate and 10 ml of aqueous solution of 10% sodium chloride were added in the centrifuge tube. The mixture was then subjected to vortex (Spinix) for 2 min. After that,

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mixture was subjected to roto-spin (Tarson) for 30 min and followed by centrifuge (Eltek; Model no. : TC 4100 D) for 10 min at 5000 rpm speed.

Supernatant liquid (3 ml) collected from the above organic layer and subsequently evaporated to dryness in N₂ evaporator (Turbo Vap, Caliper Life Science, USA) at 25°C. The residue was then reconstituted with 3 ml of acetonitrile. Then part of this sample was taken in 2 ml centrifuge tube and 25 mg each of florisil and PSA and 15 mg of GCB were added to it and again centrifuged for 5 min at 5000 rpm. Finally, the sample was filtered through 0.2µm membrane filter and transferred into a vial for analysis in LC-MS/MS.

Instrumental condition: Column : Xterra, MS-C-18, 2.1 × 50 mm × 3.5µm; Eluent : A: (H₂O + 0.1% CH₃COOH + 5 mM NH₄COOCH₃) and B: (CH₃OH + 0.1% CH₃COOH + 5mM NH₄COOCH₃); Elution: Gradient; Flow rate : 0.3 ml min⁻¹ Run time : 8 min;

Injection volume : 20 µl; Column temperature : 25°C ± 0.8°C.

Instrument: Waters Micro mass Quattro micro API; Ionization mode : ESI (+ ve) mode; Scan type : MRM; Capillary voltage : 1.20 kV; Cone voltage: 18 V; Extractor: 2V; Source temperature: 120°C; Desolvation temperature: 350°C; Desolvation gas flow : 650.0 (l hr⁻¹); Cone : 25 (l hr⁻¹). The ions used to identify and quantify azoxystrobin were m/z 328.94, 343.93, 371.92 and 403.84.

A linearity check study was carried out with the help of standard solution of azoxystrobin.

In this study a calibration curve was prepared by taking the areas of corresponding to different concentrations of standard solution of azoxystrobin. From this study the limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.005 ppm and 0.01 ppm respectively.

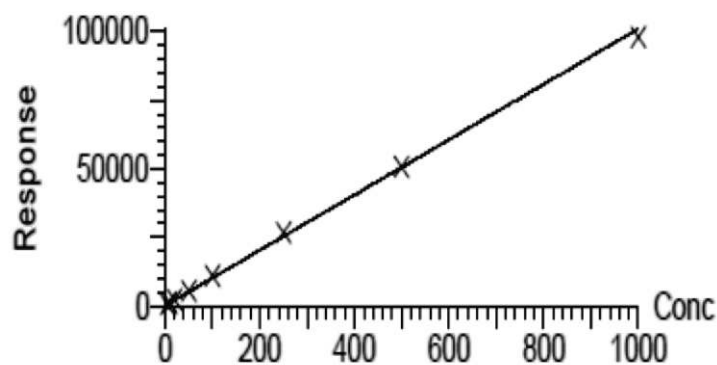


Fig. 1: Calibration curve of azoxystrobin

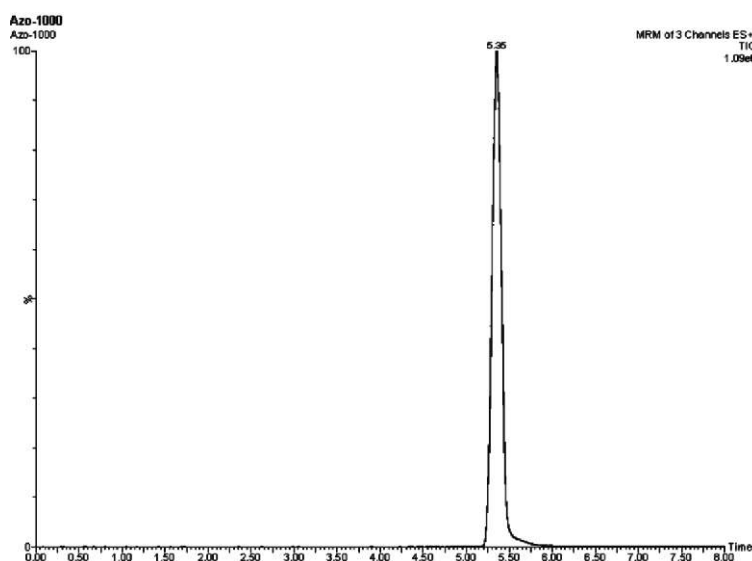


Fig. 2: Chromatogram of azoxystrobin 1 ppm

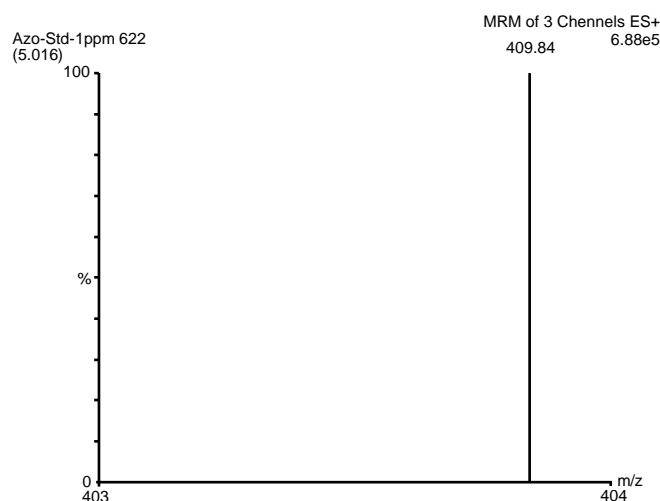


Fig. 3: Mass spectra of azoxystrobin

Recovery studies were carried out in order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up steps employed for the present study by fortifying the chilli and cropped soil samples with 0.01, 0.05, 0.5 ppm of standard azoxystrobin solution. The recovery result is presented in table-1.

Table 1: Recovery result

Substrate	Amount fortified (ppm)	Average amount recovered (ppm)*	Recovery %	Average recovery %
Chilli fruit	0.01	0.009	90	90.66
	0.05	0.046	92	
	0.5	0.450	90	

Note: * average of three replications

RESULTS AND DISCUSSION

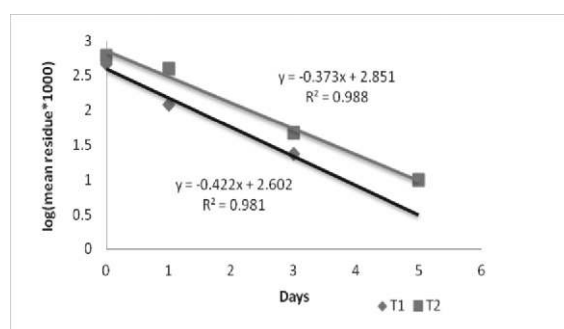
The initial deposits of azoxystrobin in chilli after two hours of spray were found to be 0.47 ppm and 0.61 ppm corresponding to the T₁ and T₂ doses respectively which were dissipated to 0.12 ppm and 0.40 ppm respectively after 1 day. In case of T₁, the residue was 0.02 ppm at 3 days after application and then became below detectable limit (BDL). In case of T₂ the residues dissipated to 0.05 ppm and 0.01 ppm at 3 days and 5 days after application respectively and after 5 days it became BDL. No residue was detected in the control samples of chilli fruits. The half life values of azoxystrobin in chilli were determined as 0.71 and 0.80 days for T₁ and T₂ respectively. In both the cases the degradation dynamics of azoxystrobin residue follows first order kinetics. All the data are presented in table 2.

Table 2: Persistence of azoxystrobin in chilli fruit

Days after treatment	Single dose (T : 125 g a.i. ha ⁻¹)	Dissipation (%)	Double dose (T : 250 g a.i. ha ⁻¹)	Dissipation (%)
	Mean* ±S.D.		Mean* ±S.D.	
0	0.47±0.02	-	0.61±0.02	-
1	0.12±0.01	74.29	0.40±0.02	34.07
3	0.02±0.00	95.00	0.05±0.01	92.31
5	BDL	-	0.01±0.00	98.35
7	BDL	-	BDL	-
Regression Equation	Y = 2.602 - 0.422x		Y = 2.851 - 0.373x	
Half life (T_{1/2})	T_{1/2} = 0.73 days		T_{1/2} = 0.80 days	

BDL= Below Detectable Limit (< 0.01 ppm),

*Average of three replications



Linear plot of dissipation of azoxystrobin in chilli fruit

Many researchers have reported the degradation behaviour of azoxystrobin in or on different fruits, vegetables and field soils. Some scientists reported that the azoxystrobin was applied for 5 times in grapes @ 150 g ai ha⁻¹ (recommended dose) and 300 g ai ha⁻¹ (double the recommended dose) and the samples were analyzed after 5th spray. Here the residual half life of azoxystrobin on grapes varied from 5.4 – 11.2 days and in soil it was 8.1 days (Dureja *et al.*, 2011). Another group of scientists reported that azoxystrobin residue on green garlic became below the MRL at 0 days and the half life was varied from 1.2- 1.4 days (Kang *et al.*, 2011). It was also reported that half life of azoxystrobin in tomato leaves under green house condition was 13 days (Szpyrka *et al.*, 2009). The present results are different from the earlier reports. It may be due to the favourable climatic condition like high temperature, some other factors such as volatilization, leaching and absorption by the crop can be considered to have played some role leading to faster dissipation of azoxystrobin.

Here the average initial deposit of azoxystrobin in chilli after two hours of spray were found to be 0.47 ppm and 0.61 ppm corresponding to the T₁ and T₂ respectively. More than 50% of the initial deposit was dissipated within three days irrespective of any doses. The half-life values of azoxystrobin in chilli sample were determined as 0.71 and 0.80 days for T₁ and T₂ respectively. Azoxystrobin residue was found to be below detectable limit in all the harvest time samples of chilli and field soil as well as in all the control samples. Therefore, it might be stated that azoxystrobin may not create any residual toxicity problem in chilli. It is also observed that the initial concentration of Azoxystrobin at 0 day in chilli fruit was below MRL value *i.e.* 1 ppm as per FSSAI, Govt. of India recommendation irrespective of any doses. So, it may be stated that chilli may safely be consumed without any hazard.

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