

Original Research Article

Degradation pattern and safety evaluation of Azoxystrobin in/on Tomato fruit

ABSTRACT

An ethyl acetate method was validated for determining azoxystrobin in tomato using LC-MS/MS. The average recoveries ranged from 79.56% (± 3.28) and 89.28% (± 4.71) %. The dissipation and residue of azoxystrobin in tomato at GAP conditions were investigated. The dissipation results indicated that the half-life of azoxystrobin in tomato fitted in 1st+1st order was 1.818 and 2.32 at recommended dose double recommended dose respectively. The dissipation rates of azoxystrobin in tomato were almost the same at both doses. Terminal residues in tomato (0.004 mg kg^{-1}) were all below the maximum residue limit (3 mg kg^{-1} by EU). The results demonstrated the safety of using azoxystrobin at the recommended dosage to protect tomato from diseases. Moreover, the PHIs estimated will be useful for farmers to ensure their field applications in a judicious manner and, in turn minimizing the residue loads within MRL compliance, at harvest.

Keywords: Tomato, Azoxystrobin, Dissipation, Post harvest interval, Half-life, Dietary Exposure

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important crops grown widely throughout the world and constitutes an important part of human diet pertaining to its antioxidant supplements. The tomato is mainly consumed as raw in most of the countries as salads. Apart from salads, it constitutes an important ingredient in many dishes, sauces, and drinks. In India, tomato is cultivated in 8.12 lakh ha area with the average annual production of 205.73 lakh tons [1]. Being susceptible to a score of diseases, fungicides have been widely used to control fungal pathogens in greenhouse systems as well as the open field. However, in a number of monitoring studies done throughout the world, residues of pesticides in fruits and vegetables have been reported [2,3,4,5].

Azoxystrobin controls key diseases on major crops with a high level of crop safety. It is active against all the major groups of fungi, including Ascomycetes, Basidiomycetes, Deuteromycetes, and Oomycetes. Azoxystrobin is also used for controlling turf diseases such as brown patch (*R. solani*), Pythium blight (*Pythium aphanidermatum*), and Fusarium patch (*Microdochium nivale*). It is also used against black and yellow Sigatoka on banana (*Mycosphaerella fijiensis* and *M. musicola*). It shows good translaminal movement, as well as systemic movement providing foliar protection when applied as a root drench, nursery box granule, paddy granule, or seed treatment. Although it shows excellent curative and eradicator activity, it is most effective when used as a protectant fungicide.

Azoxystrobin is non-mutagenic and non-teratogenic which gives only slight skin and eye irritation. Its NOEL is 18 mg/kg body weight/day [6]. It has no toxicity to birds in

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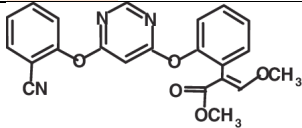
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acute studies ($LD_{50} > 2,000$ mg/kg). Due to its low toxicity and rapid degradation in the environment, it is harmless to other nontarget and beneficial organisms such as honey bees, earthworms, beneficial arthropods. Even though the fungicide has been reported as less harmful to the nontarget organisms and environment, the indiscriminate use by the unaware farmer without proper doses makes it important to establish the PHI of the fungicide in tomato. Moreover, tomato is consumed in various forms such as a raw, cooked and canned product. Therefore, the study was conducted to assess the residual behaviour of azoxystrobin in tomato to establish the PHI and to evaluate the health risk assessment.

Table 1. Physico-chemical profile of Azoxystrobin [7]

Particulars	Remarks
Structure	
CAS Name	methyl(E)-2-([6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl)-3-methoxyprop-2-enoate
Molecular Formula	$C_{22}H_{17}N_3O_5$
Appearance	White crystalline solid
Melting point	118-119°
Density	1.33
Solubility in water (25°)	10 mg/l.
Log P (octanol/water):	4.40
Vapor pressure (20°):	<10-5Pa
LD_{50}	in rats (mg/kg): >5000 orally; >2000 dermally

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2. MATERIALS AND METHODS

2.1. Chemical and Reagent

The certified reference standards of azoxystrobin (98% pure), was purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). The agricultural formulation of Azoxystrobin 23%SC (Amistar) was obtained from BASF, India. Analysis of LC-MS grade acetonitrile extract of the formulation showed only azoxystrobin, and none of its metabolic products and no interfering peaks were observed under the retention time of the compound being estimated. Moreover, the concentration of azoxystrobin in the formulation was found to be accurate with respect to its purity as claimed by the manufacturers. All other solvents and reagents used were of LC-MS grade or equivalent purity (Sigma-Aldrich India, Bangalore). Formic acid (98% pure), acetic acid (99% pure), methanol, and ethyl acetate were obtained from Merck India Ltd. (Mumbai, India). HPLC grade water was procured from Sartorius water purification system (Gottingen, Germany). Sodium chloride, sodium sulfate anhydrous (AR grade) and magnesium sulphate anhydrous (AR grade) were also obtained from s.d. fine Chemicals, Mumbai. Bondesil-PSA (Primary secondary amine), 40 μ m purchased from Agilent technologies Bangalore, India. A reagent blank before actual analysis was run to ensure the suitability of the solvents and other chemical reagents used.

2.2. Field experiment

Field experiment was conducted on Tomato (*Solanum lycopersicum* L) at Manjri village located in western peninsular of India, in the city of Pune. Azoxystrobin 23%SC

(Amistar) was applied at recommended dose (RD; 0.75 g L⁻¹ on formulation basis) and double the recommended dose (DRD; 1.5 g L⁻¹ on formulation basis) in separate plots @ 1000 L water ha⁻¹ at 30 and 15 days before harvest and a final spray was done on one day before harvest (it is not clear how did you applied chemical. Mentione what are the parts of the crop that chemical applied. Is it on to leaves? flowers> roots etc.. All the treatment, alongwith untreated control, was replicated 3 times in randomized blocks. The average maximum and minimum temperatures recorded during the field experiment were 27.8 and 21.1°C, with average relative humidity ranging between 41-65%. There was no rainfall during the study and the crop was grown under drip irrigation following a recommended package of practice.

2.3. Sampling and sample preparation

For degradation in tomato, after the last spray, representative samples were drawn at 0, 1, 2, 3, 5, 7, 10, days interval. About 2 kg of tomato fruits was randomly collected into plastic polyethylene bags from each plot as representative samples. The Samples were immediately transported to the laboratory and homogenized (How did you homogenized them?). Two representative subsamples of 4 g were taken for each plot. One subsample was prepared for chromatographic analysis and the other was frozen at -40°C to slow down the enzymatic degradation of pesticide residues prior to use; the frozen samples were thawed at 4°C overnight.

For sample preparation, the ethyl acetate method for grapes reported by Banerjee et al. 2007 [8] was used. Laboratory sample of tomatoes were crushed thoroughly in a blender. From this blended tomato sample, 200 g was further homogenized. From this homogenised sample, a 10 g sample was drawn and extracted with 10 mL ethyl acetate plus 10 g anhydrous sodium sulfate by homogenization followed by centrifugation at 5000 rpm for 5 min. An aliquot of supernatant (5 mL) was cleaned using 50 mg primary secondary amine (PSA) and 150 mg MgSO₄. For final analysis by LC-MS/MS, 2 mL of the aliquot was evaporated to near dryness under a gentle stream of nitrogen in a low volume concentrator (TurboVap LV; Caliper Life Sciences, USA) at 35°C. The residues were re-dissolved in 0.5 mL methanol + 0.5 mL water and analyzed by LC-MS/MS.

2.4. LC-MS/MS analysis

The analysis was carried out using high performance liquid chromatography (HPLC) (Waters 2695 separation module) hyphenated to triple quadrupole (Quattro Premier, Waters Corporation, Milford, USA) mass spectrometer equipped with electron spray ionization (ESI) probe. An aliquot of 10 µL was injected via autosampler. Azoxystrobin eluted at retention time (RT) of 7.9 min through a C₁₈ column (LiChroCART 55 mm × 2 mm, 3 µm; Merck India Ltd., Mumbai) with mobile phase flow rate of 0.4 mL/min (figure 1). The mobile phase was composed of A- methanol: water (20:80, v/v) with 5 mM ammonium formate and B- methanol: water (90:10, v/v) with 5 mM ammonium formate; gradient 0-0.5 min/30%B, 3.0-5.0 min/98%B, 6.0-10.0 min/30%B. The column oven temperature was maintained at 25°C. The LC-MS/MS analysis was performed in positive polarity by multiple reaction monitoring (MRM) with 404[M+H]⁺>329 (dwell time, 0.1 s) for quantification and 404>372 for confirmation. The ratio of the peak area of these two daughter ions was 1.26. The corresponding ion ratio in the positive samples was determined and confirmed in accordance with the European Commission (EC) guidelines[9]. Samples showing the ion ratios within a range of ± 20% were accepted as confirmatory presence of azoxystrobin residues. Analyte dependent mass parameters are given in Table 2.

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Table 2. LC-MS/MS conditions for the analysis of fungicides

Pesticide	RT (min)	Q	Q1	CE (V)	Q2	CE (V)	C (V)
Azoxystrobin	7.9	404	329	30	372	15	22

RT, retention time (min); Q, protonated parent ion; Q1, quantifier ion; Q2, qualifier ion, DP, declustering potential; CE, collision energy; CXP, collision cell exit potential.

■ XIC of +MRM (2 pairs): Exp 2, 406.000/251.000 Da

Max: 5.2e4 cps.

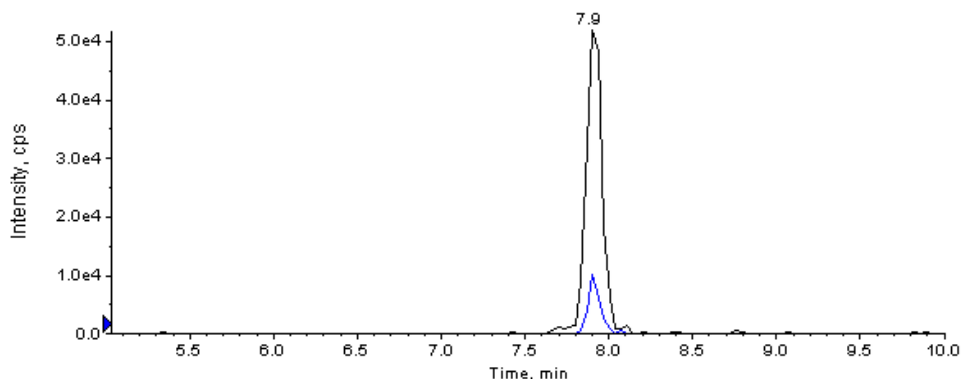


Fig. 1. Total ion chromatograph of azoxystrobin at 0.05 mg kg^{-1} in tomato fruits.

2.5. Method Validation

A single laboratory method validation was performed [9] with respect to calibration, limit of detection (LOD), limit of quantification (LOQ), matrix effect, accuracy and recovery.

2.5.1. Calibration curves and linearity

The calibration curves and linearity were evaluated based on injections of the standard solutions prepared in methanol:water (1:1, v/v) and also in blank matrix extract, at the concentrations in the range of 0.0025–0.25 $\mu\text{g mL}^{-1}$. These solutions were injected in 5 replicates ($n = 5$) and the calibration curve was obtained by plotting the peak area against the concentration.

2.5.2. Selectivity and sensitivity

The method selectivity was evaluated by comparing the chromatograms obtained from fortified matrix samples with those of blank samples and the sensitivity of the method was determined in terms of limit of quantification (LOQ) of the test analyte. The limit of detection (LOD) of azoxystrobin was established by considering a signal to noise ratio (S/N) of 3 with reference to the background noise obtained for the blank sample, whereas, LOQs of the method were established at the lowest fortified level in matrix, checking that this concentration yielded an $S/N \geq 10$ pertaining to the quantifier MRM.

2.5.3. Matrix effects

Matrix effects were evaluated by comparing the peak area response of matrix match standard with that of the solvent standards. The matrix effect was determined using the equation: (Peak area of post extraction spike X 100/Peak area of solvent

standard). The ME values above 100% indicated matrix induced signal enhancement whereas, below 100% indicated signal suppressions.

2.5.4. Accuracy and precision

The accuracy of the method was tested by analyzing tomato samples free of azoxystrobin fortified at three concentration levels (0.01, 0.05 and 0.1 mg kg⁻¹). Each concentration level was extracted and analyzed six times. Precision was evaluated in terms of repeatability by measuring % relative standard deviation (% RSD) at each spiked levels (n=6).

RSD= (SD/mean)*100, where SD=standard deviation of replicates

2.5.5. Data analysis

The time-wise residue dissipation data of azoxystrobin was analyzed using the curve fitting software Table curve 2D v5.01. The software calculated the equation parameters, regression equation and half-life. The different models used are listed below:

$$1^{\text{st}} \text{ order: } [A]_t = [A]_0 \cdot \exp(-k_1 \cdot t) \quad (1)$$

$$1^{\text{st}} + 1^{\text{st}} \text{ order model: } [A]_t = [A]_0 \cdot \exp(-k_1 \cdot t) + [A]_1 \cdot \exp(-k_2 \cdot t) \quad (2)$$

In the above equations, $[A]_t$ is the concentration ($\mu\text{g kg}^{-1}$) of A at time t (days) and $[A]_0$ are the initial concentrations of A at time 0 degraded through 1st or 1st+1st order process. The symbols k_1 and k_2 are the degradation rate constants 1 and 2 respectively. Since the 1st+1st order model cannot be described in a differential form, DT_{50} could only be calculated by an iterative procedure. The equation parameters and DT_{50} for all kinetic models were calculated by use of a commercially available program Table Curve 2D (v 5.01).

2.5.6. Safety evaluation

The food safety of azoxystrobin was evaluated by comparing their dietary exposure (theoretical maximum daily intake) vis-a-vis the maximum permissible intake (MPI). Multiplying the acceptable daily intake ($0.2 \text{ mg kg}^{-1} \text{ body weight day}^{-1}$) [10] by the body weight of an average child (16 kg), the MPIs were estimated at $3.2 \text{ mg person}^{-1} \text{ day}^{-1}$. The values of the dietary exposure were calculated by multiplying the residue levels in each sample (mg kg^{-1}) with an average per capita consumption of $0.0179 \text{ Kg of person child}^{-1} \text{ day}^{-1}$ of tomato [11].

3. RESULTS AND DISCUSSION

3.1. Method validation

The linearity of the calibration curve was established in the range $0.0025\text{--}0.25 \mu\text{g mL}^{-1}$ with correlation coefficient (R^2) of the calibration curve >0.99 for both solvent and matrix match standard. The LOD and LOQ were decided as 0.004 and 0.01 mg kg^{-1} . The matrix effect study indicated very light signal suppression of 3%. The average recovery (%) of azoxystrobin at 0.01, 0.05 and 0.1 mg kg^{-1} fortification levels (n=5) were 88.48% (± 7.64), 89.28% (± 4.71) and 79.56% (± 3.28) respectively. Where are the data?

3.2. Dissipation of azoxystrobin residue in field experiment

The dissipation pattern for the extractable residues of azoxystrobin in tomato are presented in figure 2. The initial residues (0 day residue, 2 h after application) of azoxystrobin were 0.9445 and $1.21065 \text{ mg kg}^{-1}$ at RD and DRD, respectively. The relative standard deviations (RSDs) for the concentration of the samples collected in replicates were within 20% for both the RD and DRD of applications. The dissipation rate of azoxystrobin was initially faster, and the speed slowed down with time. So, the

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kinetics of the residue data was better evaluated by fitting the data set into a nonlinear $1^{st} + 1^{st}$ order model for estimation of half-life (DT_{50}). An excellent fit of residue data to $1^{st} + 1^{st}$ order model compared to 1^{st} order model was observed for both the RD and DRD treatments with respective R^2 value of 0.974 and 0.993 in $1^{st} + 1^{st}$ order model as compared to 0.926 and 0.98 in 1^{st} order model (table 1). Similar kind of reporting of $1^{st} + 1^{st}$ order dissipation kinetics model was done in previous studies such as fenamidone + mancozeb and iprovalicarb + propineb in tomato [12], fipronil and difenconazole in okra [13] kresoxim methyl in grapes [14] and dimethomorph, famoxadone, cymoxanil, pyraclostrobin, metiram in raisin preparation [15, 16]. The DT_{50} values according to the $1^{st} + 1^{st}$ order model was 1.81 and 2.32 days at RD and DRD, respectively (Table 5). As reported by earlier researchers, the dissipation rates of azoxystrobin varied in different matrices, such as in Zhang et al. 2008 [17] reported half-life of 12.2 days in *Pleurotus ostreatus*, 5.8 to 8.8 days in mango [18], half-life of 0.8 to 3.6 days in soybean [19], 3.5 to 4.7 days in Panax ginseng [20], and 14.0 to 14.1 days in orange [21]. These indicate that the dissipation rate of azoxystrobin depend upon the complexity of matrices, in addition to the climatic and the physico-chemicals properties of the experimental plot. No PHI was applicable to azoxystrobin for both RD and DRD pertaining to the residues less than MRL value of 3.0 mg kg^{-1} from zero day onwards. However, a PHI of 1 day can be recommended to be on the safe side avoiding any harmful exposure.

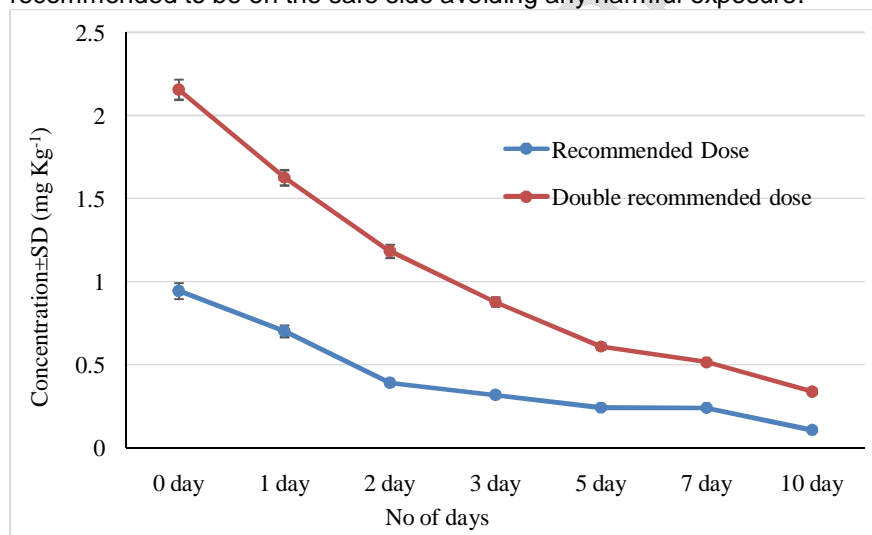


Fig.2. Dissipation of azoxystrobin in tomato at recommended and double recommended dose.

Table 3. Dissipation rate kinetics data of azoxystrobin in tomato

Order	Parameters	Azoxystrobin	
		EU-MRLs = 3.0 mg kg^{-1}	
		RD	DRD
$1^{st} + 1^{st}$	R^2	0.974	0.993
	a (mg kg^{-1})	0.659	1.053
	b (day^{-1})	0.628	0.307

	c (mg kg ⁻¹)	0.303	0.163
	d (day ⁻¹)	0.076	1e-12
	DT ₅₀ (days)	1.818	2.32
	PHI (days)	-	-
1 st	R ²	0.926	0.98
	A (mg kg ⁻¹)	0.902	1.179
	b(day ⁻¹)	0.290	0.215
	DT ₅₀ (Days)	2.32	3.23
	PHI(days)	-	-

RD: Recommended dose; DRD: Double recommended dose

3.3. Safety evaluation

The MPI for azoxystrobin was estimated at 3.2 mg person⁻¹ day⁻¹, calculated by multiplying the acceptable daily intake (0.2 mg kg⁻¹ body weight day⁻¹, EU Pesticides Database, 2024) by the body weight of an average child (16 kg). The dietary exposure of azoxystrobin on each sampling day based on average daily consumption of 0.0179 Kg tomato per day were in general less than the MPI both at the RD and DRD of applications (Table 4).

Table 4. Safety evaluation of day-wise residues of Azoxystrobin in tomato

Sampling days	Recommended dose		Double Recommended Dose		Maximum permissible intake, MPI (mg person ⁻¹ day ⁻¹)
	Residues (mg kg ⁻¹)	Dietary exposure (mg person ⁻¹ day ⁻¹)	Residues (mg kg ⁻¹)	Dietary exposure (mg person ⁻¹ day ⁻¹)	
0 day	0.94455	0.016907	1.21065	0.021671	3.2
1 day	0.7026	0.012577	0.9231	0.016523	
2 days	0.39205	0.007018	0.7915	0.014168	
3 days	0.3185	0.005701	0.5601	0.010026	
5 days	0.2423	0.004337	0.3693	0.00661	
7 days	0.2418	0.004328	0.27405	0.004905	
10 days	0.1084	0.00194	0.2313	0.00414	

4. CONCLUSION

Dissipation rate and half-life after second application with recommended and double recommended dose for azoxystrobin on tomatoes under field conditions were conducted using validated ethyl acetate method for sample preparation and determination using LC-MS/MS. The dissipation rate of azoxystrobin was explained by using 1st + 1st order kinetics. Half-life (DT₅₀) was found to be from 1.818 to 3.23 days less than EU-MRL value. Risk assessment found dietary intake at zero day for both doses are less than maximum permissible intake which is humanly safe. Considering the multi-harvesting nature of the crop, the calculated PHI (1 day) will help the farmer from any crop loss. This study will be useful for promoting effective residue management and to ensure safe use of azoxystrobin in tomato.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that no generative ai technologies such as large language models (chatgpt, copilot, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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