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Effect of soil amendments on persistence of hexaconazole and tebuconazole in soil and its residues in tomato

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Abstract

A study was performed to determine the effect of soil amendments on persistence of hexaconazole and tebuconazole in soil and its residues in tomato. A typical black cotton soil was amended with FYM, gypsum, biocompost @ 5 tonnes/ha. The amended and unamended soils were treated with hexaconazole and tebuconazole at the rate of 2 mg/kg. Analytical protocol adopted for the analysis of residues of these fungicides from unamended and amended soil, and tomato fruits were validated. The linear range of hexaconazole was 0.05-1.0 mg/kg and for tebuconazole was 0.25-5.0 mg/kg on GC-ECD and GCMS-ITD, respectively. The extraction procedure for soil (amended and unamended) and tomato fruits were accurate and precise as the recovery and % RSD of hexaconazole and tebuconazole in amended and unamended clay soil and tomato fruits were in the range of 74.88 -112.98 and 1.56-16.0%, respectively. The LOD and LOQ of analytical method was less than 0.1 mg/kg for all the matrices analyzed. The persistence of hexaconazole was highest in soil amended with gypsum (DT₅₀, 77 days) followed by biocompost (DT₅₀, 68 days), FYM (DT₅₀, 57 days) and without amendment (DT₅₀, 45 days) soil. However, persistence of tebuconazole was highest in bio-compost (DT₅₀, 69.31 days) amended soil followed by FYM (DT₅₀, 66.01 days), gypsum (DT₅₀, 43.87days) and unamended (DT₅₀, 37.46 days) soil. The terminal residues of hexaconazole and tebuconazole in soil and tomato were correlated but impact of hexaconazole residues in soil on its terminal residues in tomato is quite high with respect to tebuconazole.

Keywords: Biocompost, FYM, gypsum, hexaconazole, persistence, tebuconazole, and tomato

Introduction

Hexaconazole and tebuconazole have been registered to control various fungal diseases such as powdery mildew, sheath blight, early and late blight of potato, scab, leaf spot rust, bunt *etc.* in India. Azoles fungicides are widely used due to their broad spectrum antifungal activities cost effectiveness, systemic action (Hof, 2001)^[8] and their long lasting stability in different domains of environment such as soil, water *etc.* (Tomlin, 1997)^[19]. Consequently, azole residues have been detected in various food items *e.g.* strawberry, (Yamazaki and Ninomiya, 1998)^[20] and environmental matrices (Tomlin, 1997)^[19]. Therefore, these compounds are the potential candidate for environmental and human health concern (Kahle *et al.*, 2008)^[11].

Soil is known as the biggest sink of different agrochemicals in the environment. Therefore, the persistence study of different pesticides is of paramount importance. For most of the pesticides soil organic matter and clay content are the most important properties which affect the sorption and transformation (Durovic *et al.*, 2009^[6]; Osborn *et al.*, 2009)^[13]. Application of organic carbon (OC) in the form of compost, sludge, effluent, and crop residues has been a common agronomic practice followed in agriculture to increase the soil fertility and crop productivity. However, soil amendments also play an important role in the management of pesticides residues in agricultural fields. Therefore, a study entitled "Effect of soil amendments on persistence of hexaconazole and tebuconazole in soil and its terminal residues in tomato" was performed.

Materials and Methods

All the chemicals, reagents and solvents used were of HPLC grade. The certified reference materials (CRMs) of hexaconazole (purity, 99.70%) and tebuconazole (purity, 99.5%), was procured from Sigma-Aldrich India Ltd., Bangalore. The commercial formulation of hexaconazole (Controll Total 5% SC) and tebuconazole (Folicur 25.9% EC) was obtained from Meghmani Industries Pvt. Ltd and Bayer crop science limited, respectively. All the instruments like Gas chromatograph with ECD, Gas chromatograph-mass

spectrometer with ion trap, centrifuge analytical weighing balance *etc.* were subjected to three point calibration

Soil and amendments: The soil (0–15 cm depth) was randomly collected from the Certified Organic Farm, Navsari Agricultural University, Navsari, Gujarat, India. The field, from which the soil samples were collected, is the Government certified organic farm.

The physico-chemical properties of soil are given in Table 1. The chemical properties of FYM, gypsum, biocompost and soil treated with amendments are given in Table 2.

Table 1: The physico-chemical properties of soil

No	Property	Values	Methods and references
1.	Mechanical analysis		International Pipette Method (Piper, 1966)
(i)	Coarse sand (%)	1.23	
(ii)	Fine sand (%)	15.21	
(iii)	Silt (%)	26.39	
(iv)	Clay (%)	57.17	
	Textural class		Clayey
2.	Chemical Analysis		
(i)	pH(water, 1:5)	7.34	Jackson (1979)
(ii)	EC at 25° C (dS/m)	0.38	Jackson (1979)
(iii)	Organic C (%)	0.58	Walkley and Black Method (Jackson, 1979)
(iv)	CaCO ₃ (%)	3.53	Rapid Titration Method (Jackson, 1979)

Table 2: Chemical properties of FYM, gypsum, biocompost and soil treated with amendments

Sr. No.	Parameter	FYM	Gypsum	Biocompost
1.	pH	7.38	5.7	6.89
2.	pH (soil + amendment)	7.87	6.5	7.73
3.	O.C. (%)	49.75	-	76.5
4.	O.C. (%) (soil + amendment)	1.25	-	1.5

Stock solution: A technical grade fungicide standard (20 mg), were accurately weighed on Ohaus (maximum capacity 210 g and sensitivity 0.001 g). The standards were then transferred to 100 mL capacity volumetric flasks. The content was initially dissolved with *n*-hexane: acetone (9:1, v/v) and final volume was made up with hexane: acetone (9:1, v/v) which gave the concentration of 200 µg/mL. The stock solution was serially diluted to prepare the secondary/intermediate standard and working standards.

Method performance verification studies: Method performance verification studies such as linearity (calibration curve), Trueness (Average recovery for spike levels) Precision (Repeatability %RSD for spike level) and LOQ (Lowest spike level meeting the method performance criteria for trueness and precision) were taken under consideration. The linearity study was performed by plotting the calibration curve between response (height/area) of GC-ECD/GCMS-ITD of seven different concentrations in the range of 0.01- 1.0 µg/mL of the working standard. A correlation coefficient and equation was determined by using linear regression model. Further, the appropriateness of the model was assessed the by defining % residuals. The % residual was determined by calculating the difference between observed value of the dependent variable and the predicted value. Each data point has one residual.

$$\% \text{ Residual} = \frac{(\text{Observed value} - \text{Predicted value})}{\text{Observed value}} \times 100$$

In order to ensure quality assurance information such as accuracy or trueness and precision of the analytical method, the recovery study was carried out for different matrices *viz.*, soil and tomato. A representative soil and tomato sample were fortified with mixture of hexaconazole at 0.05, 0.1 and 0.5 mg/kg level and tebuconazole at 0.5 mg/kg level. The fortified samples were kept at room temperature for 2 hrs and residues were estimated. Prior to quantification of fungicide in two different matrices *viz.*, tomato fruit and soil, the LOD and LOQ were worked out. This was carried out by injecting matrix-match fungicide in gas chromatograph to get signal to noise ratio 3:1 for LOD and 10:1 for LOQ.

Persistence study: Approximately 2.5 kg air dried soil was taken in plastic bowl and sieved with an aluminum sieve of 2 mm diameter. From this, 500 g soil was weighed and treated with different amendments at rate of 5 tonnes/ha (2.2 g/kg; w/w basis). An untreated control sample was also maintained along with the treated soil samples. The amended clay soil was fortified at the rate 2 mg/kg with the mixture of triazole fungicides and analyzed to study the dissipation and persistence behavior of both triazole fungicides. The soil sample (10 g) in duplicate was drawn on 0 day (2 hrs after fortification), 1, 3, 5, 7, 10, 20 and 40 days and analyzed for fungicide residues. The dissipation pattern and DT₅₀ of hexaconazole and tebuconazole were worked out from unamended and amended soils.

Terminal residues of fungicides in tomato fruits and soil: A pot experiment was performed to determine the terminal residues of hexaconazole and tebuconazole grown in the soil

amended with organic and inorganic amendments. The pots (approx. 10 kg capacity) were filled with unamended (control) soil and soil treated with different amendments @ 5 t/ha. The experiment was carried with four replications along with an untreated control. After properly mixing of amendment to the soil, the soil was irrigated. After this, the tomato seedlings (variety Gujarat Tomato-2, GT 2) procured from Regional Vegetable Research Station, NAU, Navsari were transplanted in the pots. Two seedlings were transplanted in each pot at the beginning of experiment. The two sprays of fungicides viz. hexaconazole (5% S.C.) and tebuconazole (25.9% EC) were performed at 50% flowering stage followed by second spray at 15 days interval, respectively. The soil (50 g/ treatment) and fruit samples (200-250g/treatment) was collected on 3rd day after the last spray. The tomato fruit and soil samples were subjected to processing for the probable presence of residues of fungicides.

Extraction and cleanup procedure

Soil: The method used for the multi-residue analysis of soils is popularly known as Qu ECh ERS method. To a representative 10 g soil sample, 20 mL of acetonitrile was added. The content was shaken vigorously for 1 min, centrifuged at 3500 rpm for 2 min after adding 4 g MgSO₄ and 1.0 g NaCl. From this 10 mL aliquot was transferred to 15 mL centrifuge tube followed by 1.5 g MgSO₄ and 0.25g PSA.

The sample was centrifuged again at 2500 rpm for 2 min. An aliquot of 4 mL was transferred from supernatant to the test tube (weight of sample 2 g) and evaporated to dryness. Finally volume was made up to 2.0 mL using *n*-hexane: acetone (1:1, v/v) and quantitative analysis was performed on GLC-ECD and GC-MS-ITD (AOAC, 2007)^[3].

Tomato fruits: The collected fruit samples were cut and homogenized by homogenizer and a representative sample (15.0 + 0.1 g) was taken in 50 mL capacity polypropylene tubes. To this 1% acetic acid in acetonitrile (15 mL) added and kept in deep freeze for 20-30 min. The mixture of MgSO₄ (6.0 g) and sodium acetate (1.5 g) was added and vortexed for 1.0 min. The content was subjected to centrifugation for 2.0 min at 3500 rpm. The supernatant (6.0 mL) was transferred in 15 mL capacity polypropylene tubes containing mixture of MgSO₄ (0.9 g) and primary secondary amine (PSA) (0.3 g), vortexed for 1.0 min and then centrifuged again for 2.0 min at 2500 rpm. Finally an aliquot (2.0 mL) was transferred to a 15 mL capacity test tube and evaporated to near dryness with nitrogen gas using Turbo Vap. The residues were reconstituted with 2 mL (3:1, v/v) *n*-hexane: acetone and quantitative analysis was performed on GLC- ECD (AOAC, 2007^[3]).

The GC and GC-MS parameter is mentioned below:

Hexaconazole: Thermo made GLC Trace GC-Ultra[®] equipped ECD and Auto sampler

Column	:	DB-5, 30 m, 25 mm id, 0.25 μm FT
Carrier gas	:	Helium
Oven programming	:	180 °C 12 °C/min 270 °C (0.0 min) → (2.0 min)
Column flow mode	:	Constant flow
Column flow	:	1.5 mL/min
Injection mode	:	Split
Split ratio	:	1:5
Injection volume	:	1.0 μL
Injector temp.	:	230 °C
Detector temp.	:	330 °C
Current	:	1.0 Amp
Makeup gas/ flow	:	Nitrogen/45 mL/min

Tebuconazole: GC-MS (Thermo) ITQ-900

Column	:	RTx-5ms 30 m, 0.25mm id, 0.25μm FT
Carrier gas	:	Helium
Oven	:	1205 °C /min 290 °C (3.0 min) → (10.0min)
Column flow mode	:	Constant Flow
Column flow	:	1.0 mL/min
Injection mode	:	Splitless (Splitless Time 1.0 min)
Injection volume	:	1.0 μL
Injector temp	:	250 °C
MS	:	Ion Trap
Ionization mode	:	Electron impact (EI)
Detector temp	:	230 °C (Ion Source)
Transfer line	:	290 °C

Mathematical and statistical analysis: Data obtained in the study was subjected to regression analysis for the persistence study. The formulae used were as follow

1. RSD (%) = (SD in response / Mean response) X 100
2. Recovery (%) = (Recovered value / Fortified value) X 100
3. Residues concentration (mg/kg) = (A₁/A₂) X (V/W) X C

Where,

A₁ = Peak area/height of sample (mV), A₂ = Peak area/height of standard (mV)

V = Volume of sample extract (ml), W = Wt. of soil sample for extraction (g)

C = Concentration of pesticide (μg mL⁻¹) standard

4. Dissipation Half-life (DT₅₀) = 0.693 / Slope of regression equation

Results and Discussion

Method performance verification studies: The linear range of hexaconazole was 0.05-1.0 mg/kg while that for tebuconazole was 0.25-5.0 mg/kg on GC-ECD and GCMS-ITD, respectively. The % residuals between the actual concentration and the concentration extrapolated from linearity equation of hexaconazole and tebuconazole was found in the range of 0.69-18.64 and 0.07-19.13, respectively which are under acceptable range i.e. <20% specified by SANTE (2017). The recovery of hexaconazole in clay soil amended with FYM, gypsum and bio-compost was in the range of 74.88 to 98.56% while that for unamended control soil was 95.73 to 97.45% when soil samples were spiked at 3 different levels. However, the recovery of hexaconazole from tomato was in the range of 85.62 to 94.11% at different spiking levels. The % RSD obtained from recovery of hexaconazole and tebuconazole from different matrices were in the range of 1.56-8.08 %. The LOQ worked out for hexaconazole was in the range of 0.03 to 0.05 mg/kg for all the matrices including unamended, amended soil and tomato

fruit. In case of tebuconazole, % recovery, %RSD and LOQ obtained in the study for unamended, amended soil and tomato fruit were in the range of 74.88-103.24%, 3.40-16% and 0.03 to 0.15 mg/kg, respectively (Table 3). The results obtained in method performance verification studies reflects that the analytical method applied for the residue analysis of hexaconazole and tebuconazole for amended and unamended clay soil and tomato fruits was accurate (recovery, 70-120 %), precise (RSD; <20 %), sensitive (LOQ> MRL (0.1, 3.0 and 0.7 mg/kg for hexaconazole and tebuconazole) (Table 4). Several other workers had also employed this QuEChERS based pesticide extraction techniques and found that this analytical approach offered a potential alternative technique for extraction of fluchloralin from soil (Temur *et al.*, 2012)^[18] with acceptable method performance criteria such as, recovery, LOD, LOQ repeatability, precision, and all found to be within the SANTE (2017)^[16] which is in agreement with the findings of our investigation regarding the method validation.

Table 3: Method performance verification study for hexaconazole and tebuconazole in from soil and tomato

Particular	Fungicides												
	Spiking level (mg kg ⁻¹)	Hexaconazole					Tomato	Spiking level (mg kg ⁻¹)	Tebuconazole				Tomato
		Soil							Soil				
		Without amendment	With Amendments			Tomato			Without amendment	With Amendments			
FYM	Gypsum	biocompost	FYM	Gypsum	biocompost								
Accuracy (% Mean recovery*)	0.05	96.08	87.43	77.44	89.90	85.62	0.5	96.26	74.88	97.03	92.74	103.24	
	0.1	95.73	94.10	97.41	92.11	89.64							
	0.5	97.45	90.76	98.56	85.24	94.11							
Precision (% RSD*)	0.05	3.08	6.15	5.98	4.75	6.42	0.5	3.40	15.82	13.58	16.00	5.68	
	0.1	7.72	8.08	3.44	6.38	5.21							
	0.5	2.00	3.41	1.56	5.44	6.94							
LOQ (mg kg ⁻¹)	0.04	0.05	0.03	0.05	0.05	0.05	LOQ (mg kg ⁻¹)	0.03	0.12	0.13	0.15	0.06	
Linearity (R ²)	y = 209921x + 17036 (R ² , 0.99)					Linearity (R ²)	y = 24896x - 1101.7 (R ² , 0.99)						

* n=7

Persistence study

The residues of hexaconazole were built up in the first phase up to 7 days and then a gradual reduction in residues was observed in all amended soil samples. However, the decline in residues of all fungicides started from the 0 day samples in un-amended clay soil.

Hexaconazole: The hexaconazole residues detected on 0 day were 2.38, 1.27, 1.30 1.87 mg/kg in unamended soil and soil amended with FYM, gypsum and biocompost. The residues of hexaconazole built-up upto seven days in the soil amended with FYM, gypsum and thereafter a steep decline in hexaconazole residues were observed. However, steady decline in hexaconazole residues was observed since 0 day in unamended soil. The residues of hexaconazole detected on 40th day from FYM, gypsum, biocompost amended and unamended clay soil were 0.64, 0.82 1.02 and 0.53 mg/kg, respectively. The half-life of hexaconazole determined in clay soil amended with FYM, gypsum, bio-compost and un-amended control soil were 57.76, 77.01, 69.3 and 46.2 days, respectively (Table 5). In terms of DT₅₀, Firstly it is clearly evident that persistence of hexaconazole varied in range of 25.02-66.69% in amended soil with respect to unamended soil and secondly the hexaconazole residues were more persistent in amended soil. Hexaconazole is somewhat persistent in soil as its half-life varies between 49-220 days in different sandy

loam soil and very less volatile in nature. Although application of organic amendments had increased the organic carbon multifold in amended soil with respect to un-amended clay soil but in case of inorganic amendment, application of gypsum had not provided any such variation. However, the soil amended with gypsum had recorded a noticeable decline in pH which had a role in increasing the persistency of hexaconazole in clay soil.

Tebuconazole: The results obtained in the study revealed a similar pattern of initial buildup and decline in later stage was also observed in case of tebuconazole. The tebuconazole residues detected on 0 day were 2.03 and 1.57, 1.48, 1.30 1.87 mg/kg in unamended soil and soil amended with FYM, gypsum and biocompost, respectively. The tebuconazole residues detected on 40th day from FYM, gypsum, biocompost and unamended clay soil were 0.81, 0.47, 1.13 and 0.32 mg/kg, respectively. The half-life of tebuconazole determined in clay soil amended with FYM, gypsum, biocompost and un-amended control soil were 69.3, 43.87, 69.31 and 37.46 days, respectively (Table 5).

The persistence data obtained in the study were quite similar to hexaconazole as the persistency of tebuconazole was increased in organically amended soil while there was no much difference was observed in the soil treated with gypsum and unamended control. Incubation of organic amendment

and dissolved organic matter (DOM) affect the pesticide sorption and movement. High organic matter or organic carbon could increase or drastically reduce the persistence of pesticide. But this could be varying with pesticide to pesticide and soil to soil (Cox *et al.*, 2001) [5]. Many researchers had reported that application of organic amendment drastically reduced the persistence of pesticides but particularly in case of hexaconazole, in our investigation, less pronounced effect of organic amendment was observed which has been evident from their half-lives (FYM; 57.76 days and biocompost; 69.3 days). Briceno *et al.* (2007) [4] stated that pesticides in amended soil have different responses and diverse influences. In fact, in present investigation, the application of organic and inorganic amendments had increased the persistency of hexaconazole with respect to unamended control soil which recorded the least half-life of hexaconazole (46.2 days). Singh and Dureja (2000) [17] also reported that the persistence of hexaconazole was not related to the organic carbon content of the soils as about 24% of initially applied hexaconazole was recovered from black soil after 30 days of incubation of organic amendments.

Several studies suggest that soil pH most competent for the best grade of degradation is around pH 7 or neutral pH (Muller *et al.*, 2007) [12] and usually below this range the breakdown is slowed down (Andrea *et al.*, 1994) [2]. Soil pH may affect pesticide adsorption, abiotic and biotic degradation processes. It influences the sorptive behavior of pesticide molecule on clay and organic surfaces and thus, the chemical speciation, mobility and bioavailability (Hussain *et al.*, 1994) [9]. The effect of soil pH on degradation of a given pesticide depends greatly on whether a compound is susceptible to alkaline or acid catalyzed hydrolysis (Reddy and Sethunathan, 1985) [15]. Therefore, decrease in soil pH due to addition of amendments might be potential reason of comparatively higher persistence of hexaconazole in clay soil.

Further, the findings of our investigation in close proximity of the study of Fernandes *et al.* (2006) [7] who studied the effects of organic amendments the dissipation of fungicides in soils and found that tricyclazole fungicides (tricyclazole and metalxyl) were more persistent in amended soil than in unamended soil.

The hydrophobic nature of tebuconazole fungicide might be a probable reason of increase in persistency of tebuconazole in clay soil. Alvarez-Martín *et al.* (2013) [1] stated that for hydrophobic pesticides such as tebuconazole and triadimenol, the linearity of the adsorption isotherms increases with the application of Spent Mushroom Substrate (SMS) as organic amendment in soil. Therefore addition of organic amendments might have increased the adsorption of tebuconazole in soil

which in turn increased the persistence of tebuconazole in soil.

Terminal residues of fungicides in tomato and soil

Tomatoes: The harvest time residues or terminal residues of hexaconazole and tebuconazole in tomato 3 day after the second spray varied in the range of 0.56-0.62 and 0.89-0.94 mg/kg, respectively (Table 6). The results obtained in the study reveals that the maximum terminal residues in tomato was observed in tebuconazole grown in either amended or unamended clay soil while minimum terminal residues was detected in of hexaconazole. The terminal residues of tebuconazole and hexaconazole were either on higher side or equal to their respective MRLs (0.7 and 0.1 mg/kg).

Soil: The harvest time residues of hexaconazol and tebuconazole were varied in the range of 0.63-0.72 and 0.88-0.92 mg/kg, respectively in clay soil amended with FYM, gypsum and biocompost. Maximum terminal residues (0.92 mg/kg) were recorded in the FYM amended clay soil fortified with tebuconazole. Overall, tebuconazole recorded the maximum harvest time or terminal residues in clay soil either amended with FYM, gypsum and biocompost or unamended, respectively (Table 4).

The terminal residues quantified from soil amended with different amendments and tomato fruits grown in amended soil were higher which might be due to higher persistency in such amended soil which has been also reflected from dissipation study of hexaconazole and tebuconazole. In case of hexaconazole, there was a positive correlation found between the harvest time residues in soil and terminal residues detected in tomato fruits grown in amended and unamended soil which has been reflected from correlation coefficient ($r=0.89$). However, in case of tebuconazole, terminal residues detected from soil and tomato fruits are also some correlated but their strength is quite low ($r=0.2$). Further, regression study was performed to determine the possibility of cause and effect relationship between terminal residues detected from soil and tomato fruits. The regression coefficient (R^2) thus obtained were 0.8 and 0.04 for hexaconazole and tebuconazole, respectively.

This reflects that the residues of hexaconazole have direct impact on the terminal residues of tomato grown in such soil but this could not be established with tebuconazole. The hydrophobic nature and strong conjugation with soil particles might be a probable reason that poor transmittance of tebuconazole residues from soil to tomato fruit. However, it could be possible that tebuconazole residues could be easily transmitted at later pickings which need further investigation.

Table 4: Evaluation of method performance parameter of hexaconazole and tebuconazole in FYM, gypsum biocompost treated clay soil and tomato with acceptance criterion

Validation parameter	Criterion	Fungicide (Country-MRL (mg/kg))	Range of method performance (Soil and tomato fruits)
Linearity	Per cent Residuals <±20 %	Hexaconazole	0.69-18.64 %
		Tebuconazole	0.07-19.13 %
Trueness (% recovery)	70-120%	Hexaconazole	77.44-98.56%
		Tebuconazole	74.88-103.26%
Precision (% RSD)	≤ 20%	Hexaconazole	1.56-8.08 %
		Tebuconazole	3.40-16.0 %
LOQ (mg/kg)	≤MRL (Tomato)*	Hexaconazole (Japan-0.1 mg/kg)	0.05
		Tebuconazole (Codex-0.7 mg/kg)	0.06

Table 5: Effect of different amendments on the dissipation of hexaconazole and tebuconazole in clay soil

Days after Application	Residues (mg/kg)							
	Hexaconazole				Tebuconazole			
	Unamended control	FYM	Gypsum	Biocompost	Unamended control	FYM	Gypsum	Biocompost
0 (after 2 hrs.)	2.38	1.27	1.30	1.86	1.87	1.57	1.48	2.03
1	2.10	2.49	1.93	2.15	1.64	1.88	2.06	2.89
3	1.85	2.65	2.37	2.64	1.44	2.32	2.86	3.16
5	1.62	2.67	2.76	2.79	1.31	2.88	1.81	3.50
7	1.55	2.61	2.63	2.61	1.15	2.28	1.68	3.25
10	1.45	2.35	2.14	1.45	0.88	1.66	1.37	2.56
20	0.96	1.85	1.62	1.20	0.64	1.35	1.16	2.06
40	0.53	0.64	0.82	1.02	0.32	0.81	0.47	1.13
LOQ (mg/kg)	0.04	0.05	0.03	0.05	0.03	0.12	0.13	0.15
Dissipation eq. (R ²)	$y = -0.015x + 2.320(0.98)$	$y = -0.012x + 2.410(0.58)$	$y = -0.009x + 2.362(0.51)$	$y = -0.010x + 2.375(0.65)$	$y = -0.018x + 2.210(0.97)$	$y = -0.010x + 2.352(0.68)$	$y = -0.015x + 2.330(0.84)$	$y = -0.010x + 2.494(0.69)$
DT50 (days)	46.2	57.76	77.01	69.3	38.5	69.3	46.2	69.3
% variation in persistence over unamended	-	25.02%	66.69%	50 %	-	80%	20%	80%

Table 6: Terminal residues of hexaconazole and tebuconazole in soil and tomato treated with FYM, gypsum and biocompost

Amendment	Hexaconazole				Tebuconazole			
	LOQ (mg/kg)		Residues (mg/kg) [@]		LOQ (mg/kg)		Residues (mg/kg) [@]	
	Soil	Tomato fruit	Soil [#]	Tomato fruit ^{\$}	Soil	Tomato fruit	Soil [#]	Tomato fruit ^{\$}
Unamended control	0.04	0.05	0.64	0.56	0.03	0.05	0.91	0.94
FYM	0.05		0.72	0.62	0.12		0.92	0.94
Gypsum	0.03		0.67	0.6	0.13		0.91	0.89
Bio-compost	0.05		0.63	0.58	0.15		0.88	0.92
Correl. Co-eff.(r)			0.89		Correl. Co-eff.(r)			0.20
Reg. equation			$y = 0.5714x + 0.21$		Reg. equation			$y = 0.2778x + 0.6711$
Reg. Co-eff (R ²)			0.8		Reg. Co-eff (R ²)			0.04

@-mean of 4 replicates, #-At the time of 2nd spray, \$-3 days after the 2nd spray

Conclusion

The method performance verification studies reveals that the analytical method adopted for the analysis of hexaconazole and tebuconazole from unamended and amended clay soil and tomato fruits was satisfactorily accurate (recovery; 70-120%), precise (RSD- \leq 20%), instruments responds proportionately as % residual between actual and extrapolated values were- \leq 20%. The initial concentration of both fungicides was built up to 7 days and thereafter a steady decline in residues was observed. On the basis of DT₅₀, the persistency of hexaconazole was 25.02-66.69% higher in amended soil with respect to unamended soil while that was 20-80% in case of tebuconazole. The maximum persistence of hexaconazole was observed in soil amended with gypsum that tebuconazole was FYM and biocompost. Appreciable decline in soil pH due to addition gypsum might be potential reason increase of persistence of hexaconazole in soil while that for tebuconazole might be its hydrophobic nature and its ability to form strong conjugates with soil matrices. It has been also being reflected from poor transmittance of residues of tebuconazole from soil to tomato fruit with respect to hexaconazole.

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