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RESEARCH ARTICLE

Residue dissipation kinetics and consumer risk assessment of tebuconazole in tomato fruits and the soil

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Abstract

Tebuconazole residue in tomato fruit and soil was estimated using gas chromatography equipped with a microelectron capture detector (μECD, ^{63}Ni) and the analytical method was validated. The percent recovery at 0.01, 0.02, 0.05, 0.10 and 0.50 mgkg^{-1} was 85.00 to 94.00% in tomato and 81.67 to 89.67% in soil. Tebuconazole is used to control early blight, late blight and powdery mildew. Its residues may remain in the crops, causing the health hazard. Thus, analysis of tebuconazole residue, its dissipation rate and safety evaluations in tomatoes were studied using the validated method. The dissipation of tebuconazole was studied in two doses, i.e., single dose (SD) and double doses (DD). Residues were extracted from tomato fruit with ethyl acetate, and cleanup was given using primary secondary amine (PSA) and magnesium sulfate. The half-life was 3.75 and 1.35 days for SD and DD, respectively. Dietary exposures of the residues were less than MPI of 0.48 mg person^{-1} day^{-1} on all the sampling days for both doses. The terminal residue in the soil was found below LOQ for both doses.

Keywords: Dissipation kinetics, Tebuconazole residue, Consumer risk assessment, tomato.

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables in the world and belongs to the Solanaceae family, which includes species with considerable economic importance (e.g., potato, pepper, eggplant, tobacco, and petunia) (Bergougnoux, 2014). The largest and most likely commercially significant genus in this family, *Solanum*, contains both potatoes and tomatoes (Iris and Spooner, 2001; Peralta and Spooner, 2007). As a salad, tomato fruits are eaten raw as well as also frequently used in soups, pickles, ketchup, sauces, dehydrated powder, and other recipes. In addition to providing various minerals necessary for optimal health, it is a rich source of vitamins C and K (Borguini and Ferraz da Silva Torres, 2009). Potent natural antioxidants carotene & lycopene, found in tomato fruit enhance the skin's defense against damaging ultraviolet radiation. India is a major exporter of tomatoes; in 2010–11, it sent tomatoes valued at Rupees 114.8 billion to Bangladesh, Nepal, Saudi Arabia, Qatar, Kuwait, Bahrain and the United Arab Emirates (Anonymous, 2011b). The populations of Asia and the Mediterranean region regularly eat tomato fruit. According to Zhao et al. (2014), tomato ketchup is a popular addition to a variety of foods since it gives the food a distinct flavor. In India, tomatoes are grown on 789000 hectares of land, yielding 19.7 million tonnes of production with 25.0 t/ha of productivity according to estimates for 2017–2018

(NHB 2018). Tomatoes can grow in cold air, with temperatures at night ranging from 10-20°C and temperatures during the day ranging from 19 to 29℃. The scorching sun of an excessively high temperature may cause damage, while growth disruptions will result from an excessively low temperature. In India, the tomato crop is frequently infested by a number of diseases like early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), White rot (*Sclerotinia sclerotiorum*) and powdery mildew (*Leveillula taurica*) at different developmental stages. According to reports, early blight alone can result in crop losses of up to 78% (Datar and Mayee, 1985). Late blight, caused by the phytopathogenic oomycete (*Phytophthora infestans*,) is one of the most devastating tomato diseases, demanding high chemical input for disease control worldwide. It must provide novel, efficient fungicides and bioagents with action mechanisms that will support a rise in the number and quality of tomatoes produced (Sahu et al., 2013). Currently, fungicides are primarily responsible for the prevention and treatment of many illnesses. Combination formulations of fungicides are available in the Indian market and popular among farmers due to broad-spectrum activity**.** Tebuconazole, [(RS)-1-p-Chlorophenyl)-4, 4 dimethyl3-(1H-1, 2, 4-triazole-1-ylmethyl) pentan-3-ol], belonging to the triazole group, is a broad-spectrum fungicide and is extensively used worldwide for the control of many diseases (Bayer Crop Science., 2012). The chemical structure and physiochemical properties of tebuconazole are given in Figure 1 and Table 1, respectively. However, residues can still be present in the crops and pose a risk to consumers' health. Analyzing the residual pesticide status of the soil and crops is therefore crucial. Thus, maximum residue limits (MRLs) are used to restrict pesticide residues in various countries. The current study aims to estimate the terminal residue in the soil to ensure the contamination level in the following crop, half-life ($t_{1/2}$) of the tebuconazole residue in tomato fruits. Also, day-by-day residue levels were used for safety evaluation based on acceptable daily intake (ADI), taking into account the repeated plucking of tomato fruits.

Materials and Methods

Chemical and reagent

Ethyl acetate, anhydrous sodium sulphate (Na_2SO_4) and glacial acetic acid (Analytical Reagent grade) were obtained from India. HPLC grade water was obtained from the Sartorius water purification system. The supplier of magnesium sulfate and primary secondary amine (PSA, 40µm) was Agilent Technologies, located in Bangalore, India. Among the equipment used were a mixer and grinder, an ultrasonic bath, a centrifuge, a precision balance, a vortex mixer and a centrifuge.

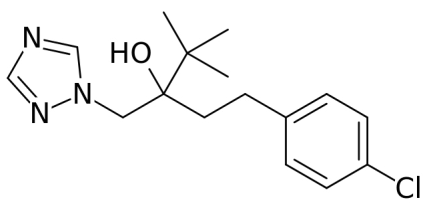

Figure 1: Chemical structure of tebuconazole

Reference standard

The certified reference standard of tebuconazole with the purity of 97.4% was acquired from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standard stock solutions were prepared by accurately weighing 10 (± 0.1) mg reference standards dissolving in 10 ml of ethyl acetate resulting in a final concentration of 1000 µg mL^{-1}. The separate standard stock solutions were appropriately mixed and diluted to create a working standard mixture of 10 µg mL^{-1} in ethyl acetate. This was used to prepare the calibration standard solutions at 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 µg mL^{-1}. The tomato extract obtained through the sample preparation technique outlined in the sample preparation and analysis section was used to prepare the matrix-matched standards at the same concentrations.

Field experiment

The field experiment was conducted in sandy loam soil at the Indian Institute of Vegetable Research's experimental farm near Varanasi (82°52' E longitude and 25°12' N latitude) Uttar Pradesh, India (Majumder et al., 2022) using a randomized block design with three replications of each treatment. Kashi Aman, the promising tomato variety, was raised using standard agronomic techniques. Tebuconazole was sprayed to tomatoes in two different dosages: single doses (SD) and double doses (DD). Three foliar sprays were given at seven days intervals at the early fruit development stage. In the control plot, water was sprayed in the same manner for comparison. In the afternoon, fungicide treatments were applied in sunny conditions with little to no wind. The average maximum and minimum temperatures during the experimental period were 18 and 30℃, respectively with an average relative humidity between 44 to 78% and no rainfall was reported during the crop study period of October to December 2023.

Sampling

After the final spraying, on days 0 (2 hours after spraying), 1, 3, 5, 7, and 10, soil samples were taken for the terminal residue analysis. Tomato fruit samples were randomly taken from each replicate of the treatment and control plots independently at regular intervals. Before being analyzed, the samples were kept at -20^{0}C in polythene bags to prevent any deterioration. No washing or pretreatment was applied to the tomato fruits before they were extracted from the pedicels and examined.

Table 1: Physiochemical properties of tebuconazole

S. No.	*Parameters*	*Properties*	
1.	Molecular Formula	$C_{16}H_{22}ClN_3O$	
2.	IUPAC Name	1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl) pentan-3-ol	
3	CAS Name	α-(2-(4-chlorophenyl) ethyl)-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	
4.	Molecular Weight	307.82 g/mol	
5.	Color / Form	Colorless crystals	
6.	Melting Point	105^0C	
7.	Solubility	In water, 36 mg/L at pH 5-9, 20 °C	
8.	Vapor Pressure	0.00000001 [mmHg]	
9.	LogP	log Kow = 3.7	
10.	Stability / Shelf Life	Stable to elevated temperatures, and to photolysis and hydrolysis in pure water, under sterile conditions.	
11.	Decomposition	When heated to decomposition it emits toxic vapors of /hydrogen chloride and nitrogen oxides	
12.	Pesticide type	Fungicide	
13.	Minimum active substance purity	905 g kg^{-1}	
14.	Mode of action	Systemic with protective, curative and eradicant action. Disrupts membrane function. Sterol biosynthesis inhibitor.	
15.	CAS RN	107534-96-3	
16.	EC number	403-640-2	
17.	CIPAC number	494	
18.	Melting point (°C)	105	
19.	Boiling point (°C)	Decomposes before boiling	
20.	Degradation point (°C)	350	
21.	Fat solubility of residues	Solubility	Likely to be soluble
		Data type	Based on chemical group
22.	Density (g ml^{-1})	1.25	
23.	Vapour pressure at 20 °C (mPa)	1.30 X 10^{-03}	
24.	Earthworms - Acute 14-day LC_{50} (mg kg^{-1})	1381	
25.	Control disease	Rust fungus, sheath blight, leaf spot, and anthracnose etc.	

Sample preparation and analysis

The complete tomato sample for each replica was divided into four subsamples, thoroughly mixed, and then selected at random to aid in future research. With only minor adjustments, all of the samples were extracted using the given techniques (Majumder et al., 2024). All the tomato samples were crushed extensively in a blender without any addition of distilled water. About 10 g of the sample was extracted using 10 mL of 1% glacial acetic acid in ethyl acetate and 10 g of anhydrous sodium sulfate. The sample was then vortexed for two minutes and centrifuged repeatedly for five minutes at 6000 rpm. An aliquot of the supernatant ethyl acetate layer (1.5 mL) was cleaned by dispersive solid-phase extraction followed by 75 mg PSA, 225 mg $MgSO_4$, and 15 mg of graphite carbon black (GCB). It's challenging to extract lycopene from the matrix because tomatoes have a lot of it. Thus, different concentrations of GCB, such as 8 mg, 12 mg, and 15 mg, were used to clean the extract. Only at a concentration of 15 mg were satisfactory results obtained. The extract was centrifuged at 10,000 rpm for five minutes, and then it was run through a nylon 6, 6 membrane filter with a 0.2 µm filter. The extraction process for soil samples was the same as that for tomato fruits, with the exception that 5 mL of water had to be added to 10 g of samples prior to the addition of ethyl acetate, the extraction solvent.

GC-µECD analysis

Tebuconazole was detected using a gas chromatography system from Agilent (Model 7890B) with an autosampler and electron capture detector (µECD, 63Ni). In the split injection mode, a standard syringe split/splitless injector was used at a ratio of 10:1 at 260℃ with a volume of 1-µL. A capillary column HP-5 (30 m length, 320 µm id, 0.25 µm) film with nitrogen gas flowing at 1-mL/min thickness was used at a pressure of 7.33 psi for separation. In this experiment, a 300℃ detector was maintained with makeup gas, i.e., (N_2) flowed at 35 mL/min. The oven temperature was set to 150℃ for 3 min, which was then ramped to 325 at 15℃/min and retained for 2 min. The pesticide residue concentration was calculated using a formula (Majumder et al., 2022).

Residue (mg kg^{-1}) = ($A_1 \times V_1 \times C$) / ($A_2 \times V_2 \times W$)

Where A_1=area of field sample in the chromatogram, A_2=area of analytical standard in the chromatogram, V_1=total volume of sample in ml, V_2=injected volume in µL, C=concentration of analytical standard in mg kg^{-1}, and W=weight of the sample in gm.

Method validation

According to the SANTE guidelines, a single laboratory technique validation was carried out with respect to linearity, accuracy, matrix effect, limit of quantification (LOQ), and recovery (SANTE 2021).

Calibration curves and linearity

Using six-point calibration curves constructed in ethyl acetate solvent and tebuconazole matrix (control) extract, calibration standards in the range of 0.01 to 0.1 µg mL^{-1} were used to assess the linear response with regard to concentration. (Majumder et al., 2023).

The calibration curve was obtained by plotting the peak area response against the concentration.

Selectivity and sensitivity

The smallest measured quantity in the tomato matrix was determined as the limit of quantification (LoQ), respectively, at which the signal to noise ratio (S/N) was 3:1 and 10:1. Calibration linearity was observed within the range of 0.01 to 0.1 µg mL^{-1}. Recovery studies were conducted with six replicates at concentrations of 0.01, 0.02, 0.05, 0.10 and 0.50 µg mL^{-1}.

Recovery study and Matrix effect

The recovery study was carried out at 0.01, 0.02, 0.05, 0.10 and 0.50 µg mL^{-1} levelswith six replicates each. The Matrix effects (ME) was evaluated by comparing the peak area response of the solvent standard with that of matrix matched standard at 0.1 µg mL^{-1}. The matrix effect was calculated using the equation (Majumder et al., 2024).

$$ME\ (\%) = \frac{(\text{Peak area of matrix matched standard} - \text{Peak area of solvent standard})}{\text{Peak area of matrix matched standard}} \times 100$$

The ME values above 100% indicated induced signal enhancement, whereas below 100% induced signal suppression.

Dissipation kinetics

The dissipation of tebuconazole in tomato was studied by subjecting the data to first-order kinetic equation 1 (Majumder et al., 2022).

$$C_t = C_0 e^{-kt} \quad (1)$$

Where C_t is the concentration at time t, C_0 is the initial concentration, k is the rate constant for fungicide dissipation, and t is the time. The residue data were statistically analyzed using Equation 2 in order to determine the half-life ($t_{1/2}$) of the parent compounds (Majumder et al., 2022).

$$t_{1/2} = \ln 2/k \quad (2)$$

Consumer risk assessment

The food safety of tebuconazole was evaluated by comparing the dietary exposure (theoretical maximum daily intake (TMDI)) against the maximum permissible intake (MPI). An average child's body weight (16 kg) was multiplied by the Acceptable Daily Intake (ADI) to determine the MPIs. Tebuconazole's acceptable daily intake (ADI) was 0.03 mg kg^{-1} body weight per day (Anonymous 2012a). Tebuconazole's MPI was calculated to be 2 mg $person^{-1}$ day^{-1}. The calculation of dietary exposures involved multiplying the residue levels in each sample (mg kg^{-1}) by the average per capita consumption of tomato, which was 0.0269 kg day^{-1} in urban areas and 0.0195 kg day^{-1} in rural areas.

Results and Discussion

Sample preparation

After the tomato samples were crushed, more extraction was carried out water was not required to provide a smooth grinding operation. There were barely 5 milliliters of water used in the extraction process. Tebuconazole showed increased matrix-induced signal amplification when tomato ethyl acetate extract was employed, even with just 75 mg of PSA or without cleaning. The matrix effect could be reduced to less than 25% by cleanup with 75 mg PSA. Consequently, only 75 mg of PSA was needed for the purification of the 1.5 mL ethyl acetate extract. The green chili matrices underwent a similar preparation procedure (Majumder et al., 2024).

Method validation

The coefficient of determination (R^2) was 0.989 and 0.906 for SD and DD, respectively, all tested analytes were within the calibration range of 0.01 and 0.1 µg mL^{-1} for both solvent and matrix-matched standards (Figure 2). The LoQ of tebuconazole was found 0.01 mg kg^{-1}. Within the tomato matrix, the average %recoveries of tebuconazole at 0.01,

0.02, 0.05, 0.10, and 0.50 µg kg^{-1} ranged from 85.00 to 94.00%, whereas in the soil sample, it was 81.67 to 89.67% (Table 2). The matrix effects were less than 20%.

Persistence and dissipation

Tebuconazole dissipation behavior at single dose (SD) and double dosage (DD) treatments is shown in Figures 3 & 4. Tebuconazole was first found in tomato fruits at 3.600 and 6.721 mg kg^{-1} in SD and DD, respectively. The residue dissipation data shows that after ten days of spraying, the initial tebuconazole concentration dropped by 100% in SD and 99.747% in DD (Table 4). The dissipation kinetics of pesticide residues are a composite of the rates of physical degradation, volatilization, photolysis, washing off, leaching, hydrolysis, microbial degradation, and other processes. If the substrate (such as the leaf surface, fruit, plant, or soil) is treated with a pesticide foliarly, the residue level on/in the substrate dissipates at an overall rate (Jian-Zhong et al., 2006). In Phase I: the linear plot with R^2> 0.85; in Phase II: two or more nonlinear plots with R^2≤0.85; and in Phase III: tiny or extended half-lives, the rate of degradation kinetics could be pseudo-first, first, or second-order (Malhat et al., 2013). Regulatory authorities have traditionally applied first-order chemical kinetics over the whole dissipation period, even though there is no scientific foundation for doing so when interpreting dissipation data. Pesticides with biphasic dissipation kinetics should be given particular attention, according to federal recommendations (Kumari 2008). The dissipation kinetics of tebuconazole in tomatoes in the current investigation demonstrated first-order

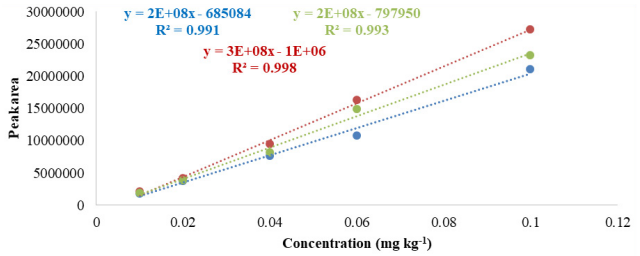

Figure 2: Linearity graph for solvent standard and matrix matched standard (MMS)

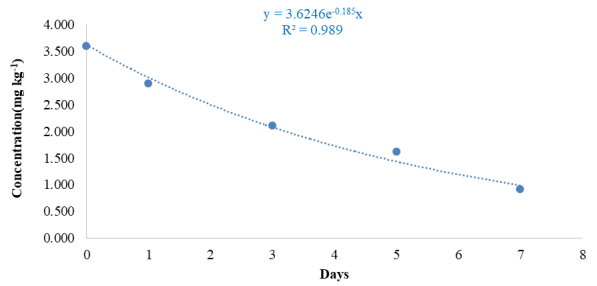

Figure 3: Degradation pattern of tebuconazole of tomato fruit at single dose (SD)

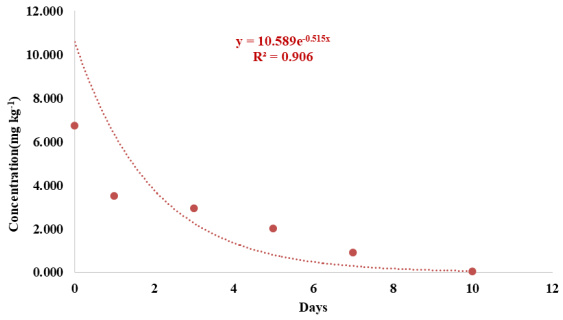

Figure 4: Degradation pattern of tebuconazole of tomato fruit at double dose (DD)

Table 2: Percentage recovery of tebuconazole in tomato cropping field

Level of fortification (mg kg^{-1})	*% Recovery*	*% RSD*
Tomato fruit		
0.01	85.00	3.113
0.02	88.33	18.196
0.05	92.00	14.255
0.10	89.67	0.644
0.50	94.00	13.287
Tomato field soil		
0.01	82.00	3.614
0.02	81.67	8.183
0.05	85.33	6.594
0.10	89.67	2.222
0.50	88.00	5.393

Table 3: Coefficient of determination and half-life of tebuconazole in tomato matrix

Doses	*Regression equation*	*Coefficient of determination (R^2)*	*Half-lives ($t_{1/2}$) days*
Tomato fruit			
SD	$y = 3.624e^{-0.185x}$	0.989	3.75
DD	$y = 10.589e^{-0.515x}$	0.906	1.35

Table 4: Percentage reduction of tebuconazole residue on different days of sampling in tomato

Days after spray	*Single dose (SD)*		*Double dose (DD)*	
	Residues (mg kg^{-1})	*%decrease residue*	*Residues (mg kg^{-1})*	*%decrease residue*
0	3.600	0.000	6.721	0.000
1	2.890	19.722	3.511	47.761
3	2.110	41.389	2.930	56.405
5	1.610	55.278	2.010	70.094
7	0.910	74.722	0.910	86.460
10	0.000	100.000	0.017	99.747

Table 5: Safety evaluation of day wise residues of tebuconazole in tomato fruit

Days after spray	Single dose (SD)			Double dose (DD)		
	Residues (mg kg^{-1})	Dietary exposure (mg $person^{-1}$ day^{-1})		Residues (mg kg^{-1})	Dietary exposure (mg $person^{-1}$ day^{-1})	
		Rural	Urban		Rural	Urban
0	3.600	0.0720	0.0972	6.721	0.1344	0.1815
1	2.890	0.0578	0.0780	3.511	0.0702	0.0948
3	2.110	0.0422	0.0570	2.930	0.0586	0.0791
5	1.610	0.0322	0.0435	2.010	0.0402	0.0543
7	0.910	0.0182	0.0246	0.910	0.0182	0.0246
10	0.000	0.0000	0.0000	0.017	0.0003	0.0005

kinetics, with tebuconazole (R^2) of SD and DD being 0.989 and 0.906, respectively (Figures 3 & 4). Generally, half-life ($t_{1/2}$) is used to describe how a pesticide dissipates. What represents the rate of degradation is the amount of time needed for the pesticide residue to dissipate 50% from its initial concentration. According to (Table 3), the half-life of tebuconazole was 3.75 days at the SD and 1.35 days at the DD. According to an early study, kresoxim methyl half-lives for SD and DD in green chili were 6.3 and 5.33 days, respectively (Majumder et al., 2022). Two hours following the final spray, the initial deposits for the SD and DD were recorded at 3.60 and 6.72 mg kg^{-1}, respectively (Table 4). Tebuconazole dissipated quickly in tomato fruits at both application doses, reaching the EU-MRL value without the need for further time, and no terminal residue was detected in the soil. Therefore, this chemical can be utilized to manage tomato crop diseases in a safe manner.

Consumer risk assessment

The residues at both doses showed a similar pattern of dissipation to below the default MRL of 2 mg kg^{-1}. The safety of tebuconazole residues in vegetables has to be evaluated because there is a lack of information available on their use. Tebuconazole's acceptable daily intake (ADI) is 0.03 mg kg^{-1} body weight day^{-1}. The MPI of tebuconazole was calculated as 0.48 mg $person^{-1}$ day^{-1} by multiplying the ADI by the typical child's body weight of 16 kg. The MPI of 0.48 mg $person^{-1}$ day^{-1} on all the sampling days for both the single and the double dose was less than the dietary exposures of the residues on each sampling day based on the average daily consumption of 0.1344 for rural and 0.1815 kg tomato per day (Table 5). Tebuconazole has been determined to have a low risk of acute toxicity when used to manage tomato pests.

Conclusion

Ultimately, the approach of ethyl acetate extraction-based residue analysis proved to be highly efficacious in determining the tebuconazole residues in tomato samples using GC-ECD analysis. The half-lives of the tebuconazole residues were 3.75 and 1.35 days for SD and DD, respectively, and the recoveries ranged from 85.00 to 94.00%, with an RSD of 3.113 to 13.287%. The safety review indicates that following the anticipated PHIs, tomato fruits might be deemed safe for ingestion by humans. Given that no terminal soil residue was found following the removal of the tomato crop, it is also safe for the upcoming harvests.

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सारांश

टेबुकोनाज़ोल का उपयोग अगेती झुलसा, पछेती झुलसा और ख़स्ता फफूंदी को नियंत्रित करने के लिए किया जाता है। इसके अवशेष फसलों में रह सकते हैं जिससे स्वास्थ्य को खतरा हो सकता है। इस प्रकार, वैध विधि का उपयोग करके टेबुकोनाज़ोल अवशेषों का विश्लेषण, इसकी अपव्यय दर और टमाटर में सुरक्षा मूल्यांकन का अध्ययन किया गया। टेबुकोनाज़ोल के अपव्यय का अध्ययन दो खुराकों यानी एकल खुराक (एसडी) और दोहरी खुराक (डीडी) में किया गया। एथिल एसीटेट के साथ टमाटर के फल से अवशेष निकाले गए, और प्राथमिक माध्यमिक अमीन (पीएसए) और मैग्नीशियम सल्फेट का उपयोग करके सफाई की गई। टमाटर के फल और इसके खेत की मिट्टी में टेबुकोनाज़ोल अवशेषों का अनुमान माइक्रोइलेक्ट्रॉन कैप्चर डिटेक्टर (μECD, 63Ni) से लैस गैस क्रोमैटोग्राफी का उपयोग करके लगाया गया और विश्लेषणात्मक विधि को मान्य किया गया 0.01, 0.02, 0.05, 0.10 और 0.50 मिलीग्राम किग्रा$^{-1}$ पर प्रतिशत रिकवरी टमाटर में 85.00 से 94.00% और मिट्टी में 81.67 से 89.67% थी। एसडी और डीडी के लिए आधा जीवन क्रमशः 3.75 और 1.35 दिन था। दोनों खुराकों के लिए सभी नमूना दिनों में अवशेषों का आहारीय एक्सपोज़र 0.48 मिलीग्राम व्यक्ति$^{-1}$ दिन$^{-1}$ के एमपीआई से कम था। दोनों खुराकों के लिए मिट्टी में अंतिम अवशेष एलओक्यू से नीचे पाया गया।