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Analysis of tebuconazole residues in coconut water, kernel and leaves using LC–MS/MS

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ABSTRACT

A method was validated for determining tebuconazole residues in coconut water, kernel and leaves using Liquid chromatography-Mass spectrometry/Mass spectrometry (LC–MS/MS) with electro spray ionization in positive ion mode. Samples were extracted with acetonitrile and subsequent clean-up was done using dispersive solid phase extraction. Recovery ranged between 70 and 114.39 % and the RSD was between 0.64 and 10.24 %. Root feeding studies with tebuconazole @ 5 and 10 mL/100 mL of water/tree revealed the presence of tebuconazole residues in coconut leaves until three days after treatment but dissipated to below quantifiable limit on 5th day at single dose while the residues went below quantifiable limit after 10 days at double the dose. Residues were below quantifiable limit in coconut water and kernel until three days. Data obtained from the study were used for estimating the risks associated with the exposures to tebuconazole residues in coconut.

1. Introduction

Coconut (*Cocos nucifera* Linn.) is widely cultivated in coastal areas of India and also in many countries across the world. In India it is cultivated in around 21,000 km^2. The coconut palm is the most useful palm in the world. Coconut water is a refreshing natural drink rich insugars, salts, minerals, vitamins, amino acids and phyto hormones like cytokinins and auxins (Yong et al., 2009) consumed by the children, adults, ailing people and sports persons for quenching the thirst and for instantaneous energy. It is also recommended as an oral rehydration drink. Coconut kernel or solid endosperm is a main cooking ingredient in many countries and also eaten raw. Tebuconazole [1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol] (Fig. S1) is a triazole group of fungicide, which is used in crop protection for control of diseases in field crops, fruits, vegetables and nut crops. It is used as a seed dressing chemical and as foliar spray to control a wide range of diseases such as rusts, smuts, bunt, powdery mildew, leaf spots, and blight. Tebuconazole has a systemic mode of action that helps in effectively controlling the target fungi. Tebuconazole is not registered on coconut in India but it is applied for the management of leaf blight and there is no data on its residue level in coconut products. Its residues were reported in urine and hair samples of farm workers (Fustinoni et al., 2012; Schummer, Salquèbre, Briand, Millet, & Appenzeller, 2012). The interactions between tebuconazole and human serum albumin were reported by Stanicova, Zelonkova, Verebova, Holeckova, and Dianovsky (2018) and Zelonkova et al. (2019). It has been classified by the US EPA as possible Human Carcinogen (US EPA, 2006). Known its adverse effect on human beings, it becomes imperative to study the residues of tebuconazole in coconut water and kernel.

Tebuconazole residues were studied in various matrices like strawberry, cucumber and soil using LC–MS/MS (Zhang et al., 2012; Wang et al., 2018; Lv et al., 2014); in watermelon using GCMS (Dong & Hu, 2014); and in cabbage following spectro photometric method (Omer & Fakhre, 2020). Few researchers employed gas chromatography mass spectrometry and HPLC analysis for multi-residue analysis in coconut water (dos Anjos and de Andrade, 2014; Brito et al., 2002). But more sensitive results can be obtained with LC–MS/MS in spite of strong chromatographic and mass spectrometric interferences caused by co-eluting matrix-compounds, as tebuconazole is a highly polar compound. Ferreira et al. (2015) and Ferreira et al. (2016) developed a method for multi-residue determination of pesticides in coconut trunk and in coconut water and pulp following QuEChERS (Quick, Easy,

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Cheap, Effective, Rugged, and Safety) extraction method (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003) and LC–MS/MS. However, no studies have been done on tebuconazole method validation in coconut matrix and the persistence of tebuconazole in coconut water, kernel and leaves after root feeding which is the preferred mode of pesticide application by coconut farmers.

Hence, this study was planned to develop a sensitive method for accurate determination of tebuconazole residues using LC–MS/MS and assess its persistence and dissipation in coconut water, kernel and leaves after root feeding.

2. Materials and methods

2.1. Chemical reagents and standards

The reference standard of tebuconazole (98.1% purity) obtained from Sigma Aldrich, Bangalore, India was used for this study. Acetonitrile of HPLC grade, sodium chloride (NaCl), florisil, anhydrous sodium sulphate (Na_2SO_4) and anhydrous magnesium sulphate ($MgSO_4$) of analytical grade were purchased from Merck, India. Primary Secondary Amine (PSA) (Bondesil 40 μm), and Graphitized Carbon Black (GCB) were purchased from Agilent Technologies, USA. Millipore water-purification system (Millipore, USA) was used for ultra purified water production.

2.2. Pesticide standard preparation

Primary stock solution of tebuconazole (419.868 μg mL^{-1}) standard was prepared by dissolving 10.70 mg of analyte in 25 mL HPLC grade acetonitrile in a volumetric flask. From this stock, an intermediate stock solution of 100 μg mL^{-1} was prepared by transferring 4.76 mL into a 20 mL volumetric flask and the volume was made up with HPLC grade acetonitrile. Working standard solutions of tebuconazole (0.001 to 0.25 μg mL^{-1}) was prepared by diluting intermediate stock with acetonitrile. It was used to find the retention time of tebuconazole and for quantitative determination of residues in samples (Bempelou, Kappatos, & Liapis, 2019; Deng, Zhou, Zheng, Bai, & Zhou, 2018). The stock and working standard solutions were stored in the deep freezer at −20 °C.

2.3. Instrumentation

The analysis was carried out using Waters Alliance 2695 separations module coupled with Acquity TQD Mass Spectrometry with electrospray ionisation (ESI) interface in positive mode. The interface conditions that were standardized for higher intensity of the parent and daughter ions are as follows: capillary voltage 3.0 kV; desolvation (drying gas) and cone gas flows were 1000 and 80 L h^{-1}, respectively; flow of collision gas was 0.18 mL min^{-1}; source and desolvation temperatures were 120 and 500 °C, respectively. Nitrogen was used as desolvation and cone gas, while pure argon was the collision gas. Transition m/z 308 > 70 and m/z 308 > 125 were used for quantification and confirmation of tebuconazole.

Chromatographic separation was carried out in Waters XTerra C18 column, of dimension 4.8 × 250 mm and 5 μm pore space in isocratic mode. The mobile phase was MS grade acetonitrile with 0.1% formic acid and water acidified with 0.1% formic acid (80:20, v/v), at a flow rate of 0.5 mL min^{-1}. Infusion technique was done for preliminary tuning. Total run time was 15.0 min. For quantitation and confirmation, multiple reaction monitoring mode was used. Analytical instrument control and acquisition of sample data was done by using the inbuilt Masslynx software (Waters, USA) with ease of operation.

2.4. Sampling

Coconut water, kernel and leaf samples were collected from untreated trees maintained at Coconut Research Station, TNAU, Aliyar. Care was taken to collect samples from top, bottom and middle bunches and fronds for nuts and leaves, respectively. The samples were transported to the laboratory and nuts were broke open to collect coconut water and the kernel was scooped out. Coconut water was collected in the sampling bottles. Coconut kernel and leaf matrices were homogenized thoroughly using a high speed blender (Roubot coupe high volume blade homogenizer- Blixer 6) and all the samples were stored in deep freezer at −20 °C until use.

2.5. Extraction and clean up

A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (Anastassiades et al., 2003) was followed. A representative sample of 10 g of the finely ground coconut kernel sample was transferred into a 50 mL centrifuge tube, to which 20 mL of acetonitrile was added and thoroughly mixed using a vortexer for one min. To this solvent extract, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added and again vortexed and centrifuged at 6000 rpm for 10 min. After centrifuging, 9 mL of clear supernatant solution was transferred to test tube containing anhydrous sodium sulphate (Na_2SO_4). Again, from the supernatant solution, 6 mL was pipetted into a 15 mL centrifuge tube containing 100 mg PSA, 600 mg anhydrous $MgSO_4$ and 10 mg GCB. PSA was added to remove matrix co-extractives like fatty acids and sugars as it has both a primary and a secondary amine while GCB was used to remove some fatty acids and pigments, if any. The mixture was vortexed again for one min. and then centrifuged for 10 min at 3000 RPM. From the centrifuged sample, 4 mL of the upper extract was taken and filtered using a 0.2 μm syringe filter to remove the salts, if any. The filtered extract was then transferred into a glass tube and concentrated to near dryness using nitrogen in a turbovap LV at 40 °C for 30 min. Finally, the sample was reconstituted to 1 mL with acetonitrile and transferred into a 1.5 mL glass auto sampler vial for LC–MS/MS analysis.

For coconut water sample analysis, 10 mL of the sample was extracted with 20 mL of acetonitrile and cleaned up similar to kernel sample. In the first step, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added, vortexed and centrifuged at 6000 rpm for 10 min. Further, 9 mL of the supernatant extract was passed through anhydrous sodium sulphate (Na_2SO_4). From this extract, 6 mL was taken in a 15 mL centrifuge tube containing 100 mg PSA, 600 mg anhydrous $MgSO_4$ and 10 mg GCB. From the upper extract, 2 mL was taken and filtered. The extract was concentrated to near dryness and reconstituted to 1 mL with acetonitrile and taken for LC–MS/MS analysis.

For coconut leaf sample analysis, 5 g of the homogenized sample was added with 10 mL water, mixed thoroughly and extracted with 10 mL of acetonitrile. For clean-up step, along with 100 mg PSA and, 600 mg anhydrous $MgSO_4$, GCB @ 100 mg was added to remove the chlorophyll pigment and florisil @ 100 mg was added for removal of polar and low fat co-extracts in the leaves. Finally, 2 mL was concentrated and reconstituted to 1 mL with acetonitrile.

2.6. Method validation

The developed method was validated by means of linearity, specificity, limits of detection and quantification, recovery and precision (SANTE, 2019). For all the analyses with regard to method validation studies, blank samples collected from untreated trees were used.

Linearity was studied based on a five-point standard calibration graph by plotting the mass detector response against concentration of the standards within the range 0.005 to 0.05 μg mL^{-1} making three replicates for each concentration. Matrix matched standards were studied to prove that there is no matrix effect by any of the three matrices. The evaluations of the influence of matrix components (ME) on chromatographic response were performed using the slope of the matrix match calibration curve and the slope of calibration curve in solvent. For determining the LOD and LOQ, 7 independent analyses of coconut water, kernel and leaf samples were performed after spiking

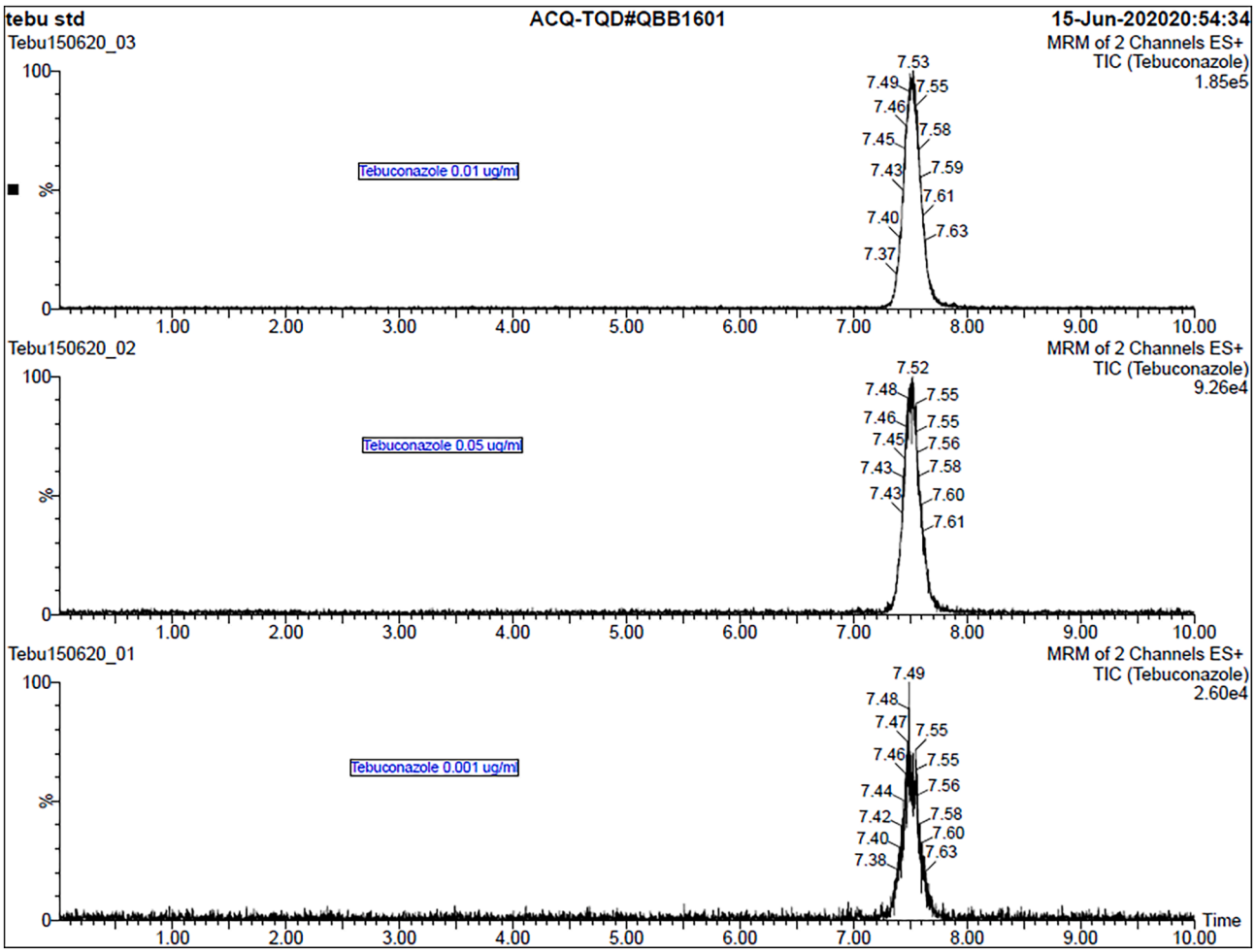

Fig. 1. Standard Chromatogram of tebuconazole.

with tebuconazole at 0.005 μg g^{-1}. The LOD was calculated from the standard deviation associated with the measurement of tebuconazole and student *t* test value (3.14 for seven replicates and six degrees of freedom) (ADLC/1996). The limits of quantification (LOQs) were calculated by considering a value of 3.3 times the LOD.

Method recovery study samples were prepared from the homogenized sample matrices at five concentrations. The homogenized untreated coconut water samples were spiked at concentrations of 0.001, 0.005, 0.025, 0.05, 0.0625, 0.125, and 0.25 μg mL^{-1}, following seven replications. Kernel and leaf samples were spiked in the range from 0.005 to 0.25 μg g^{-1} and 0.005 to 0.1 μg g^{-1}, respectively. The spiked samples were equilibrated and processed by adopting the above said extraction and clean up procedure. Accuracy and precision of the method was determined from the measurements during recovery study carried out by spiked samples. Repeatability of the developed method was estimated through the relative standard deviation (RSD %). The dilution factor and concentration factor involved in the process were considered while calculating the residue level.

The effect of analytical variables such as column temperature, flow, column pressure and desolvation temperature were studied to establish ruggedness of the analytical method developed and estimated through RSD %. The expanded Measurement Uncertainty was calculated using the estimates of the method namely sample repeatability, recovery, concentration of reference standard, sample volume measure (pipette and volumetric flask), mass and calibration curve expressed in terms of expanded uncertainty as per the procedure given by SANTE (2019) guidelines.

2.7. Residue studies

Field experiment was conducted in maintained coconut garden (Variety ALR 1) at Coconut Research Station, Tamil Nadu Agricultural University, Aliyarnagar, during 2019 to 2020 to detect the residues of Tebuconazole 25.9% EC in coconut kernel, coconut water and leaf. The experiments were conducted following Randomized Block Design (RBD) with three replications.

The tebuconazole solution was given as root feeding at recommended dose (5 mL/100 mL water/tree) and double recommended dose (10 mL/100 mL water/tree) for 10 coconut palms per treatment. A fresh and live root was selected, cut sharply at an angle and was inserted into the fungicide solution containing tebuconazole in a polythene bag and secured tightly with a cotton thread. The absorption was checked eight hours later. Two rounds of root feeding were given following 30 days interval. Three nuts were collected from each treated tree representing top, middle and lower bunch after 1, 5 and 10 days after second application of tebuconazole for both the doses. Leaf samples of 250 g were collected from top, middle and lower fronds separately from the treated trees and all the collected samples were transported to laboratory and processed immediately and stored at −20 °C before analysis.

2.8. Analysis of data

A final sample volume of 10 μL was injected by an autosampler into the LC–MS/MS. The final quantification was worked out using the following formula with the parameters from chromatogram as

Table 1
LOD and LOQ for tebuconazole in Coconut.

Matrix	Signal Mean*± SD	LOD	LOQ
Coconut kernel (μg g^{-1})	0.004 ± 0.00044	0.0014	0.0046
Coconut water (μg mL^{-1})	0.005 ± 0.0013	0.0004	0.001
Coconut leaves (μg g^{-1})	0.0048 ± 0.00048	0.0015	0.005

$$Residues\left(ug\ g^{-1}\right) = \frac{As}{Astd}\chi\frac{Wstd}{Ws}\chi Vs$$

As – Sample peak area
Astd – Standard Peak area
Wstd – Weight of standard (μg mL^{-1})
Ws – Weight of the sample (g)
Vs – Volume of the final extract in ml

2.9. Dietary risk assessment of tebuconazole in coconut

In the assessment of dietary exposure to tebuconazole, expected daily coconut consumption data (kg day^{-1}) was multiplied with data on mean residual tebuconazole concentration from supervised field experiments (mg kg^{-1}) and divided by a body weight of 60 kg for an adult person. As part of the risk characterization, the acute dietary exposure estimate was then compared with the relevant health-based guidance ADI value of 0.03 mg kg^{-1} body weights for tebuconazole as given by EFSA (2012). Then Hazard Risk Index of the residue was computed using the following equation.

$$HRI = EDI/ADI$$

where EDI is estimated daily intake and ADI is acceptable daily intake. HRI value more than 1 is considered as not safe for human health.

3. Results and discussion

3.1. Method validation

The parent ion, quantification and confirmation ions of tebuconazole are 308.24, 70.13 and 125.13 *m/z* (Fig. 1 and S2 a-b). The linearity of the calibration curve for tebuconazole was established in the range of 0.005 to 0.05 μg mL^{-1} (5 levels) with a correlation coefficient (r) of 0.9968 and r^2 of 0.9935 (Fig. S3). The Limit of Detection (LOD) was 0.0004 μg mL^{-1} and Limit of Quantification (LOQ) was 0.001 μg mL^{-1} for tebuconazole standardized in coconut water matrix using LC–MS/MS. The LOD and LOQ established for coconut kernel and leaves are 0.0014 and 0.0046, and 0.0015 and 0.005 μg mL^{-1}, respectively (Table 1). As regulatory limit is not prescribed for coconut kernel by CODEX, the MRL for tree nuts was taken. The LOQ of this method for kernel was lower than the codex MRL of 0.005 μg g^{-1} for tree nuts. In calculating LOD and LOQ based on signal-to-noise ratio, only instrument sensitivity is taken into consideration. In this study, fortified blank samples were analyzed for calculating the LOD and LOQ, as all steps of sample preparation, extraction and analysis procedure were included to recognize the method detection values. Lawal and Koki (2019) did multiresidue analysis in coconut water and reported limit of detection (LOD) and quantitation (LOQ) from 0.08 to 0.92 and 0.28 to 3.08 μg kg^{-1}, respectively. Isocratic analysis was carried out by means of high-performance liquid chromatography to analyze insecticide residues in coconut water by Brito et al. (2002) and their limits of detection ranged from 0.002 to 2.0 mg kg^{-1}. Lv et al. (2014) did separation of tebuconazole on a C18 column with a mobile phase of acetonitrile - water containing the buffer 5 mM ammonium acetate and ended up with a higher LOD level of 0.003 mg kg^{-1} when compared to our studies. Zhang et al. (2012) used a mobile phase of 70% (v/v) methanol and 30% water (v/v) + 0.1% formic acid solution and achieved LOQ level of 2.5 μg kg^{-1} for tebuconazole in strawberry matrix.

The method accuracy was calculated as percentage recovery of tebuconazole from the spiked samples. Acetonitrile in the pure form as extraction solvent gave good recovery for 10 mL of coconut water, 10 g of kernel sample and 5 g of leaf sample.

Coconut kernel sample is the white edible endosperm that contains 35 per cent lipids and also sugars. Coconut water is a turbid liquid that contains lipids, glucose, amino acids and electrolytes. In the clean up step, PSA was used to remove sugar and fatty acids and GCB to remove the pigments from the samples. Florisil was used in the clean-up of leaf samples to separate target analytes from potential interferences. It is considered good for the separation of fats, oils, waxes, alkaloids and carbohydrates. Results showed that the mean recoveries of tebuconazole were 79.45 to 114.39, 70.00 to 92.36 and 72.12 to 107.09% in coconut water, kernel and leaves, respectively (Tables 2–4). Precision in case of repeatability was determined from five concentrations following seven replications on the same day. A very good precision (<20%) was found and the relative standard deviation (RSD) was in the range of 0.64 to 10.24% for all the three matrices. Specificity of tebuconazole in terms of area and retention time was within the acceptable limit of RSD < 2 and 5 percent, respectively (Fig. S4).

Lawal and Koki (2019) used higher analytical sample volume of 20 mL coconut water for residue analysis which was extracted using 15 mL of acetonitrile. Their sample volume was two times higher which would require more salts for clean-up. Brito et al. (2002) used solid-phase extraction technique with methanol as eluting solvent and liquid–liquid extraction with hexane and dichloromethane mixture followed by gas chromatographic analysis for detecting tebuconazole residues. Ma et al. (2008) employed C(18) solid-phase extraction cartridges for clean-up and eluted with ethanol- water -acetic acid mixture and finally dissolved the extract in methanol for HPLC determination of phytohormonesin coconut water. In our study, acetonitrile was used as extraction solvent and dispersive solid phase clean-up was done which is cheap and easy, when compared to solid-phase extraction cartridges or liquid – liquid extraction method.

In case of coconut kernel and leaf samples, the recovery above the level of 63% at few concentrations may be due to interference by organic matters but acceptable as the RSD was less than 20% and mean recovery was ≥ 70%. The results indicate that the present method has a good recovery rate within the criteria set by SANTE (2019) for coconut water, kernel and leaf samples.

Table 2
Recovery percentage of tebuconazole in coconut kernel.

Spiking levels (μg g^{-1})	Recovery %								RSD (%)
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Mean ± SD	
0.005	99.08	90.57	91.15	84.51	78.43	83.58	72.64	85.71 ± 8.77	10.24
0.025	83.56	84.16	78.51	78.67	80.59	80.40	76.45	80.33 ± 2.78	3.46
0.05	80.13	86.57	94.66	93.99	103.74	93.46	93.96	92.36 ± 7.35	7.96
0.625	73.15	69.95	66.69	70.91	71.44	65.26	66.61	70.00 ± 2.96	4.29
0.125	89.31	81.63	79.03	73.67	83.97	80.45	81.10	81.31 ± 4.75	5.85
0.250	69.66	69.40	69.69	70.67	69.64	69.62	70.25	70.00 ± 0.44	0.64

Table 3
Recovery percentage of tebuconazole in coconut water.

Spiking levels (μg mL^{-1})	Recovery %								RSD (%)
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Mean ± SD	
0.001	113.71	117.10	108.51	120.00	113.71	108.51	118.62	114.39 ± 4.62	4.04
0.005	100.23	102.49	101.02	98.19	103.35	104.91	97.89	101.16 ± 2.62	2.59
0.025	80.87	75.25	88.32	83.57	78.29	92.43	95.86	84.94 ± 7.56	8.90
0.05	95.63	100.68	109.23	103.87	103.93	89.52	86.30	98.45 ± 8.32	8.46
0.625	85.93	90.78	85.86	84.73	87.19	85.34	86.48	86.62 ± 2.00	2.31
0.125	85.16	73.82	82.76	82.31	70.50	83.71	77.90	79.45 ± 5.54	6.97
0.250	95.75	88.61	87.16	89.89	93.36	93.41	90.60	91.26 ± 3.04	3.33

Table 4
Recovery percentage of tebuconazole in coconut leaves.

Spiking levels (μg g^{-1})	Recovery %								RSD (%)
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Mean ± SD	
0.005	109.59	95.84	108.90	95.25	97.57	95.34	81.28	97.68 ± 9.59	9.82
0.010	78.72	76.46	74.04	75.84	65.94	66.58	86.07	74.81 ± 6.98	9.34
0.025	71.27	63.77	71.27	84.70	74.83	69.15	69.86	72.12 ± 6.46	8.96
0.050	115.90	100.85	97.66	104.98	111.36	111.48	107.40	107.09 ± 6.42	6.00
0.075	96.24	100.48	107.96	114.10	109.27	94.34	95.58	102.57 ± 7.83	7.64
0.1	99.76	100.08	100.54	101.63	100.32	96.97	105.90	100.74 ± 2.69	2.67

Table 5
Persistence and dissipation pattern of tebuconazole in coconut.

Days after application	Residues (μg g^{-1})*					
	Coconut leaf (μg g^{-1})		Coconut kernel (μg g^{-1})		Coconut water (μg mL^{-1})	
	Tebuconazole 25.9% EC @ 5 mL/tree	Tebuconazole 25.9% EC @ 10 mL/tree	Tebuconazole 25.9% EC @ 5 mL/tree	Tebuconazole 25.9% EC @ 10 mL/tree	Tebuconazole 25.9% EC @ 5 mL/tree	Tebuconazole 25.9% EC @ 10 mL/tree
1	0.015 ± 0.0009	0.02 ± 0.0004	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ
3	0.0079 ± 0.0008	0.0158 ± 0.002	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ
5	≤ LOQ	0.0126 ± 0.005	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ
10	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ
15	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ
Control	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ	≤ LOQ

The demonstration of the ruggedness is performed as one aspect of the method validation, to give confidence that the method should perform well under normal variations in conditions in routine application. Four experimental factors namely, column temperature, flow, pressure and desolvation temperature were studied along with the standard analytical procedure to prove the ruggedness of the method and the results were found satisfactory with RSD less than 20% (Table S1). Change in column flow resulted in varying area. The measurement uncertainties ranged from 2 to 12.69% and the uncertainty values did not exceed 50% of the residue value which were satisfactory (Table S2).

3.2. Field experiment

Tebuconazole residue after root feeding experiments were detected only in leaf sample and were below quantification limit in coconut kernel and water. Only leaflets collected from top fronds were detected with residues and in leaves collected from bottom and middle fronds, residues were below quantification limit. The residues detected in leaves after 1 and 3 days of root feeding ranged from 0.008 to 0.02 μg g^{-1} (Table 5) (Fig. S5). The coconut tree variety ALR 1 subjected to this experiment was a tall variety and the presence of residues in the leaves proved the acropetal movement of tebuconazole within a day from root to top of tree and its potential for leaf blight management. On five days after treatment, the residues dissipated to 0.0126 μg g^{-1} at double the dose and was below quantification limit in single dose treatment. The translaminar redistribution, and systemic movement of pesticides for long distances in xylem and phloem may have affected the fungicide performance to unfixed degrees, and inturn its residual presence in edible parts of coconut tree. Residues were below quantifiable level in coconut kernel and water in 1st and 3rd day samples but was below quantification limit afterwards.

Ferreira et al. (2015) studied recoveries from 40 μg kg^{-1} of coconut tree trunk sample and reported concentrations of pesticides between 44.7 ± 5 and 938.3 ± 20 μg kg^{-1}and proved the translocation of pesticides in different heights in the coconut tree trunk after trunk injection treatment. The researchers also reported acropetal movement of imidacloprid near the leaves at 61 ± 6 μg kg^{-1}. Though residues were present in the leaves, their penetration inside the shell could have been prevented by some means. Lehoczki-Krsjak, Varga, Szabó-Hevér, and Mesterházy (2013) also reported that only traces (<1%) of the tebuconazole residues translocated from wheat flag leaves to the ears.

Coconut husk contains carbon which acts an adsorbent and this might have prevented the movement of tebuconazole inside the shell. This might be a reason for the non-quantifiable trace level of residues in kernel and water, even 1 day after root feeding. Kumrić et al. (2019) too reported the adsorption behavior of coconut shell activated charcoal and successfully used it as SPE adsorbent for the pre concentration of pesticides in the residue analysis process.

A faster dissipation of tebuconazole in mango and strawberry fruit was observed by Mohapatra (2015) and Zhang et al. (2012), respectively. Half-life of 4.75 and 5.42 days was reported for tebuconazole in pomegranate by Patil et al. (2018). Saha (2017) also found that harvest time plant and peanut kernel samples were free of tebuconazole

Table 6
Health risk assessment in coconut samples.

Matrix	Residues at LOQ level (mg/kg)	EDI mg/kg	ADI mg/kg	HRI
Coconut kernel	0.005	1.16E−06	0.03	3.85185E−05
Coconut kernel	0.005	1.66E−06	0.03	5.53704E−05
Coconut water	0.001	5.51E−08	0.03	1.83704E−06
Coconut water	0.001	7.92E−08	0.03	2.64074E−06

residues. The short half-life of tebuconazole molecule might also be a reason for its absence of residues in coconut water and kernel, implying its safety for human consumption. More research work will throw light into its persistence and dissipation with regard to tree age, variety and season.

Though the samples of coconut water and kernel collected on 1 and 3 day after root treatment were found to have detectable but unquantifiable residues, the situation posed a risk involved. Hence risk assessment was done considering residues from the edible portions at LOQ level as worst case estimate. Codex commodity classification was available separately for coconut water, tender coconut kernel or matured coconut kernel. Maximum kernel (Pulp) weight was reported from tall coconut varieties while more tender coconut water was estimated in dwarf varieties. The ratio of tender coconut kernel to tender coconut water varies with maturity of the palm as well as with varieties. As per Mathew and Baby (2011), the per capita consumption of coconut at the all India level ranges from 0.32 to 0.46 nuts/month. This is equivalent to 416 to 598 g kernel and 99.2 to 142.6 mL of coconut water considering the maximum kernel weight of 1300 g (Solangi & Iqbal, 2011) and coconut water of 310 mL/nut. From this, daily average coconut kernel and water intake for adult was arrived as 13.87 to 19.93 g and 3.31 to 4.75 0.168 mL per day per Kg body weight (Table 6). The hazard index calculated using the above data showed that dietary exposure to residues of tebuconazole from root treated coconut tree nuts considered by the present research findings is unlikely to present a public health concern.

4. Conclusion

The finding of the study indicates the reliability of the sample preparation, extraction and clean up method for tebuconazole residue analysis in coconut water, matured kernel and leaves with accuracy and precision. Also, the concentration level of pesticide analyzed was found below the MRL (0.05 μg g^{-1}) fixed for tree nuts and coconut by European Union, which presumes that the analyzed coconut kernel is safe for consumption to avoid health related issues. Though residues were below quantifiable limit in water and kernel samples, it indicates the necessity to study and fix the MRL more specific to coconut, as the produce is used as an important drink and as food.

CRediT authorship contribution statement

A. Suganthi: Conceptualization, Methodology, Validation. **E. Rajeswari:** Methodology, Investigation. **V. Sivakumar:** Methodology, Investigation. **K. Bhuvaneswari:** . **E. Madhu Sudhanan:** Investigation, Writing - review & editing. **N. Sathiah:** . **K. Prabakaran:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129920.

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