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Simultaneous determination, dissipation and decontamination of fungicides applied on cabbage using LC-MS/MS

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ABSTRACT

A method for simultaneous determination of carbendazim and tebuconazole residues in cabbage was developed and validated in LC-MS/MS. Samples were extracted and purified following the modified QuEChERS procedure, which enabled the elution of carbendazim and tebuconazole at 0.96 and 5.31 min, respectively. LOD and LOQ were 0.0005 and 0.0015 mg kg^{-1}, respectively. Mean recovery was in the range of 78.94 to 104.89% for carbendazim and 76.07 to 98.62% for tebuconazole. The field samples recorded residues of 0.274 and 0.481 mg kg^{-1}; and 0.194 and 0.392 mg kg^{-1} at single and double dose for carbendazim and tebuconazole, respectively. Half-life values were 2.17 and 2.99 for carbendazim and 2.74 and 2.81 for tebuconazole at single and double dose, respectively. Decontamination with saltwater wash followed by cooking and lemon water wash found superior in the removal of residues more than 90%.

1. Introduction

Cabbage (*Brassica oleracea* L.) is a major vegetable crop grown extensively during the winter season for its valued head, which is an edible and rich source of minerals, vitamins, and fiber (Norman, 1992). Presently, cruciferous vegetables formed a major part of the food and were being consumed as either raw or cooked. In the recent past, cabbage and other cruciferous vegetables (Brassicaceae members) have been identified as major sources of chemoprotective phytochemicals in the food. Cabbage production has biotic and abiotic challenges as it is infected by many fungal diseases from seedling to harvest, leading to a reduction in yield and quality (Singh, Jyoti Reddy, Kesavachandran, Rastogi, & Siddiqui, 2007). To contain these diseases, the application of synthetic fungicides is a regular practice among farmers. The carbendazim and tebuconazole are the systemic fungicides being used extensively by the farmers as foliar sprays for protecting the crop against alternaria leaf spot, powdery mildew, and head rot diseases. However, extensive application of these fungicides creates several problems such as fungicide-residue in the cabbage head, and it will further lead to consumer-rejection.

Carbendazim [Methyl *1H*-benzimidazol-2-ylcarbamate] and tebuconazole [(*RS*)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1*H*, 1,2,4-triazol-1-ylmethyl) pentan-3-ol] are the two major fungicides being used worldwide to manage the diseases of different fruits, vegetables, cereals, etc., (Yu et al., 2015; Li et al., 2016; George, Paul, Kumar, Pratheeshkumar, & Xavier, 2016). The carbendazim belongs to the benzimidazole group, which is a broad-spectrum systemic fungicide having prophylactic as well as curative action (Li et al., 2016). In contrast, tebuconazole is a broad-spectrum systemic fungicide belongs to the triazole group, is highly effective against foliar fungal pathogens by inhibiting their fungi ergosterol biosynthesis (Fungicide Resistance Action Committee, 2018). The frequent applications of fungicides not only affect the quality of crop produce but also lead to significant residual levels in soil and affect the soil beneficial micro flora (Virág et al., 2007)). The maximum residual limits for tebuconazole in broccoli (0.2 mg/kg), brussels sprouts (0.3 mg kg^{-1}), cabbage head (1 mg kg^{-1}); and carbendazim in brussels sprouts is 0.5 mg/kg have been set by the European Commission (Pesticides, 2016). However the MRLs in the range of 0.02 to 0.7 and 0.1 to 0.5 mg/kg, respectively, tebuconazole and carbendazim for brassica vegetables for ensuring food safety by the European Commission (Pesticides, 2016).

The analytical techniques for quantification of carbendazim and tebuconazole residues in foods had constantly been explored in compliance with the food safety policy (Dong & Hu, 2014; Mohapatra, 2015). Several studies conducted previously have employed the

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different methods such as HPLC with UV (Phansawan et al., 2015), RP-HPLC-FD (Hazer, Akkbik, Demir, & Turhan, 2017), and mass spectrometry to detect theses fungicides in different brassica vegetables and fruits (Yu et al., 2015; Bhandari et al., 2019; Wang et al., 2012a,b; Kocourek et al., 2017; Dong, Yang, Pang, & Hu, 2018; Zhao, Wang, Gao, Guo, & Zhao, 2019). Analysis of fungicide residues using highly-sensitive techniques will have more advantages in detecting the residue at trace level. There will be a scope to quantify the residue to the lowest possible level to meet the global food standards. This was achieved in this study using LC-ESI-MS/MS technique. Up to now, most of the investigations were primarily focussed on monitoring the tebuconazole and carbendazim residue in various vegetables and fruits. Some of the previous efforts primarily focussed on the analysis of tebuconazole in several edible crops such as watermelon (Dong & Hu, 2014), apple (Patyal, Sharma, Chandel, & Dubey, 2013), onion, mango (Mohapatra, 2015), grape (Dong et al., 2018; Jyot, Arora, Sahoo, Singh, & Battu, 2010), cabbage, cucumber (Wang et al., 2012a)and carbendazim in fruits and vegetables (Hazer et al., 2017), tomato (Li et al., 2016), and brassica vegetables (Yu et al., 2015). In the literature, very limited reports are available on the dissipation kinetics of tebuconazole and carbendazim, which was reported in cabbage (Kocourek et al., 2017;(Wang et al., 2012a) , and brassica vegetables (Yu et al., 2015). However, no reports have described the simultaneous determination, persistence, dissipation, and decontamination of tebuconazole and carbendazim fungicides using household techniques in cabbages.

The effectiveness of several household processing methods on the level of pesticide residue is essential in assessing the risk linked with the intake of pesticide residues (Aktar, Sengupta, Purkait, & Chowdhury, 2010). Various processes in decontamination of pesticides *viz.,* water-washing, boiling, cooking, detergent-washing, blanching, drying, peeling in different vegetables have been proved to be effective (Liang, Wang, Shen, Liu, & Liu, 2012; Thanki, Joshi, & Joshi, 2012; Tomer & Sangha, 2013). To ensure secure consumption, there is an urgent need to establish different approaches to extricate fungicide residues in the cabbage following simple household preparations. Since there are no reports referring particularly to the decontamination process for carbendazim and tebuconazole residues from cabbage are available, washing, cooking, and removing the outer layer of the cabbage heads were found effective in reducing the fungicide residues in the present study. An aspect of the absence of comprehensive research to understand the persistence, dissipation and decontamination of pesticides in cabbage, the present investigation was undertaken to establish a rapid, simple, and sensitive analytical method for simultaneous determination of persistence and dissipation kinetics of carbendazim and tebuconazole fungicides in cabbage. An effective decontamination process in cabbage has also been identified.

2. Material and methods

2.1. Chemicals and reagents

Carbendazim (99.0%) and tebuconazole (98.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). LiChrosolv Methanol, acetonitrile (LC-MS grade), formic acid, and ammonium formate (99.9%) were procured from Merck India, Mumbai, India. 18.2 M_Ω Ultrapure water was collected from a Milli-Q water purification system (Merck Millipore, USA). Analytical grade $MgSO_4$ and anhydrous sodium acetate (99.90%) were obtained from Himedia (Bangalore, India). The primary, secondary amine (PSA, 40 μm) was purchased from Agilent Technologies (USA).

The standard stock solution of carbendazim and tebuconazole at 1000 μgmL^{-1} concentration were prepared in solvent methanol and kept at −15°C. Further, an intermediate solution of 100 μgmL^{-1} and standard solutions mixture concentration of 0.5mgL^{-1} was prepared in methanol. Standard solution for calibration ranging from 0.0015 to 0.1μg mL^{-1} was prepared in methanol by serial dilutions.

2.2. LC-MS/MS parameters

A Shimadzu 8040 series triple quadrupole MS/MS system is used for the experiments. A Shimadzu 8040 series LC system has a quaternary pump, degassing unit, an auto-sampler, and a column with a temperature control compartment that was used in the LC-MS/MS system. Chromatographic separation was carried out on a Shimpack XR, octadecylsilyl (ODS III) C18 column (2 mm i.d × 150 mm, particle size of 2.2 μm) with a column oven temperature of 40°C. The mobile phase A was prepared with HPLC water, 5 mM ammonium formate, 0.01% formic acid, and B was prepared with 100% MeOH, 5 mM ammonium formate, 0.01% formic acid. The LC time program was 5% B at the beginning for 0.12 min, followed by 100% up to 6.50 min and then 5% of B for 7 min. The flow rate of 0.4 mL min^{-1} and the injection volume of 2 μL were set. The MS was handled by utilizing a positive (ESI +) mode electrospray ionization (ESI) source. The MS parameters were as follows; 4.5 kV interface voltage, 250°C desolvation temperature, 400°C heat block temperature, 2.9 L/min desolvation gas (N_2), and drying gas at 2.9 L/min. Argon gas was used for collision with different collision energies. Three unique multiple reactions monitoring (MRM) transitions (Table S1) were observed particularly for the test fungicides and were used for confirmation analysis according to the guideline SANTE/12682/2019 (European Commission, 2019). The most abundant product ion transition was utilized for quantification assay.

2.3. Method validation

The proposed method for analysis of carbendazim and tebuconazole residue in cabbage sample was validated based on the SANTE/12682/2019 guideline (European Commission, 2019), which fulfils the requirements for linearity/sensitivity, matrix effect, the limit of detection (LOD), the limit of quantification (LOQ), specificity, trueness in terms of average recovery for the spiked concentration (bias), intraday (RSD_r) and interday (RSD_{wR}) precision in terms of repeatability RSD for each spiking level tested. The blank control matrix of cabbage was extracted and used in the validation process. To detect the presence of possible interferences, six individual non-fortified blank samples were injected. Solvent and matrix-matched linearity was assessed by evaluating the deviation of each linear concentrations injected from actual linear concentration (0.0015, 0.002, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.01, 0.20, 0.30 and 0.5 μg mL^{-1}), on the basis of linear regression equation. The matrix effect was performed by comparing the angular coefficients obtained by the curves in the solvent and matrix according to the following equation:

$$\text{Matrix effect}(\%) = (b_m - b_s)/b_s \times 100$$

where b_m and b_s are the angular coefficients of the curve in the matrix and in the solvent, respectively (Naik et al., 2020).

The LOD and LOQ were estimated following the guidance document on the estimation of LOD and LOQ for measurements in the field of contaminants in feed and food (Wenzl, Haedrich, Schaechtele, Robouch, & Stroka, 2016; Harischandra et al., 2021). The mathematical formulae employed as follows,

$$X_{LOD} = 5.2 \text{ x } S_y, \text{ net/b}$$
$$X_{LOQ} = 3.3 \text{ x } X_{LOD}$$

where, X_{LOD}: Limit of detection; S_y,*net*: Standard deviation of the precision data at the lowest spike level; b: Slope of the calibration curve; X_{LOQ}: Limit of quantification

Trueness or bias of the developed method was tested by computing the average recovery for each fortification level tested. Recovery experiments were carried out at 3 fortification levels (0.0015, 0.075, and 0.015 mg kg^{-1}) by spiking homogenized blank cabbage sample (analytical portion) with a standard working solution. Each level of

spiked cabbage samples of six replications was then kept at room temperature of 25°C for 30 min. to reach sample stability. The spiked samples were further extracted by adopting the sample extraction procedure given below and injected to LC-MS/MS. The area response in spiked concentration after extraction and matrix-matched standards was compared to calculate the percent recovery. The method precision was determined with regards to the repeatability relative standard deviation. The repeatability (RSDr) was assessed on the same day, while within-laboratory reproducibility (RSDwR) was measured on two consecutive days by two different analysts. The precision values were calculated as relative standard deviation (RSD) of replicate measurements and percent recovery.

2.3.1. Sample extraction and clean-up

Extraction and clean-up of fungicide residues from cabbage head samples was done by method QuEChERS and its variations elaborated by Anastassiades, Lehotay, Stajnbaher, and Schenck (2013) and Lehotay, Mastovska, and Yun (2005). The collected samples were chopped thoroughly and grounded using high volume homogenizer (Robo-Coup). Ten grams of the prepared sample was weighed into a 50 mL polypropylene tube. A volume of 20 mL of acetonitrile was added and homogenized in a low volume homogenizer at 16,128 g for 5 min. After this, 1.5 g NaCl was added and mixed well by shaking vigorously and centrifugation was done at 16128g for 5 min. to separate the organic layer. A volume of 8 mL supernatant (upper organic layer) was carefully transferred into a test tube, and 3 g anhydrous Na_2SO_4 was added to remove moisture content. The clean-up was done through dispersive solid-phase by use of 0.10 g PSA sorbent and 0.30 g anhydrous $MgSO_4$ into 15 mL centrifuge tubes containing 6mL supernatant. Then tubes were centrifuged at 11,200×*g* for 5 min. and 1 mL of supernatant was delivered to the test tube and evaporated near to dryness in using an N_2 flash evaporator (turbovap). The residue was dissolved with 1 mL methanol and filtered using a 0.22 μm syringe filter and collected in LC vials. Finally, 2 μL of the extract was injected into the LC-MS/MS.

2.4. Field experiment

A field experiment was conducted at the experimental plots of Univerisity of Agricultural Sciences, Raichur (Longitude: 77.3345°E, and Latitude: 16.2043°N) in the randomized block design (RBD) with three treatments and eight replications. The treatment plot size was 30 × 22.5 m^2. A popular cabbage cultivar East-West was planted to study the dissipation behavior of two fungicides by adopting good agricultural practices. The market grade formulations of carbendazim (50% WP) and tebuconazole (25.9% SC) fungicides were purchased from the local authorized pesticide dealers. Two applications (at ten-days interval) of carbendazim 50% WP and tebuconazole 25.9% SC @ at the rate of 250 and 258 g.a.i.ha^{-1} (single) and 500 and 516 g.a.i.ha^{-1} (double dose), respectively, along with untreated control were given during head-development stage of cabbage crop. The water spray was served as control. To avoid the fungicide drift during and after spray, maize crop (cv. DHM 103) was sown as a border-crop. After the second spray, representative four to five cabbage (≈2 kg) were collected at 0, 3, 5, 7, 10, 14, 21, and 25 days for analysis. After picking, the samples were kept in an inert polyethylene bags, and sealed within the dry icebox, and were transported immediately to the laboratory. All samples were labelled and preserved at −20°C for further analysis.

2.5. Decontamination of fungicides

Four medium-sized cabbage heads (≈2 kg) were collected from the treated field (see Section 2.4) after a day of 2nd application. The samples were cut into two equal sized pieces and mixed thoroughly to get a true representation of the collected sample. For the decontamination of fungicides, different household techniques were followed (Table 3). Treated cabbages without any decontamination were kept as a control samples. Different decontamination solutions (2%) were prepared by dissolving 40 g tamarind, turmeric, salt each in 2 L of water in a plastic tub of 6 L capacity. Cabbage samples were processed individually with each household technique for 15 min, followed by washing in tap water for 30 *sec* and air drying on blotting papers for 5 h. Further, samples were subjected into residue analysis according to sample extraction procedure mentioned above. Ten gram of sample was taken from each treatment in three replicates for extraction and analysis. The sample aliquot was subjected to LC-MS/MS estimation following the validated method. The percent removal was calculated using the below-mentioned equation,

$$\text{Per cent removal} = \frac{\text{Initial deposit}\left(\text{mg kg}^{-1}\right) - \text{Residues after treatment}\left(\text{mgkg}^{-1}\right)}{\text{Initial deposit}\left(\text{mgkg}^{-1}\right)} \times 100$$

2.6. Statistical analysis

The persistence kinetics, half-life, and the theoretical dissipation time to reach the level of 0.01 mg kg^{-1} were computed using the equations of first-order kinetics (Dong & Hu, 2014):

$$Ct = C_0 e^{--kt}$$

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

where Ct (mg kg^{-1}) is the residual levels of carbendazim and tebuconazole at time t (days), C_0 (mg kg^{-1}) is the initial deposits, and k is the rate constant (day^{-1}).

3. Results and discussion

3.1. Method optimization

Initially, a full scan mass spectrum for both fungicides was noted in array to choose the most abundant *m/z* value. For carbendazim and tebuconazole, the parent ion $(M + H)^+$ of 191.95 and 307.95, respectively, were recorded as precursor ions. Further, based on the known molecular ion, multiple reaction monitoring (MRM) transitions with different collision energies and related acquisition conditions were optimized for the high abundance of product ion with ESI positive mode by injecting 2 μL of 0.5μgmL^{-1} standard into the tandem mass spectrometer. Based on the mass spectra of carbendazim and tebuconazole, the most intense ions 160.0, 70.10, were selected for quantification; 132.0, 105.05, and 125.15, 151.00 respectively, were used for confirmation. The qualifier and quantifier transitions and their collision energies of carbendazim and tebuconazole are given in Table S1 and Fig. S2. After determining the MRM transitions, it is essential to discover the chromatographic parameters for better resolution of the target analytes. Whereas, it should be emphasized that the total ion chromatogram (TIC) has good separation resolution (Fig. 1). The developed MRM positive mode has enhanced the sensitivity and accuracy for the quantification of carbendazim and tebuconazole at their low

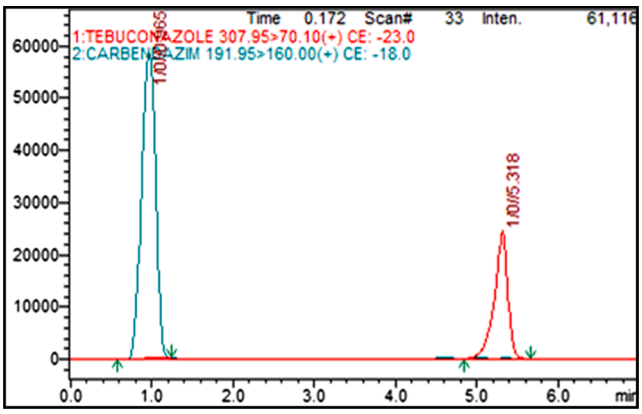

Fig. 1. Total ion chromatogram of pesticide standards for Carbendazim (left) and Tebuconazole (right).

concentrations (0.0015 mg kg^{-1}) in the cabbage matrix. In this method, both carbendazim and tebuconazole were found to be eluted at a retention time of 0.965 ± 0.1 min and 5.31 ± 0.1 min, respectively (Fig. 1). The developed method can identify analytes within a short period of 7.00 min.

The LOD and LOQ of 0.003 mg/L and 0.01 mg/kg for tebuconazole in watermelon (Dong & Hu, 2014), 0.07 mg/kg and 0.22 mg/kg for carbendazim in vegetables, (Hazer et al., 2017), 0.015 μgmL^{-1} and 0.05 mgkg^{-1} for tebuconazole in mango (Mohapatra, 2015), 0.025 μgg^{-1} and 0.01 μgg^{-1} for tebuconazole in cabbage (Wang et al., 2012a)were reported in previous studies. However, the present method in line with the LOD and LOQ of 0.0005 mg/kg and 0.0015 mg/kg, respectively for both the fungicides in cabbage and which was lesser than the MRL. The flow rate of 0.25 to 0.8 mL/min with an injection volume of 5 to 10 μL (George et al., 2016, Hazer et al., 2017, Mohapatra, 2015) was reported in previous studies is also in accordance with the proposed method having similar flow rate (0.3 mL/min). Isocratic gradient with acetonitrile and water (Dong et al., 2018; Li et al., 2015; Wang et al., 2012b) was followed in most of the studies, whereas the present method was standardized with binary gradient with methanol and water combination which could prove the better separation of the fungicides from cabbage matrix.

3.2. Method validation

The matrix injection revealed that no interfering peaks were noticed at carbendazim and tebuconazole (Fig. 2a). Since the effects of a matrix are an instinctive condition of ESI, few techniques have been adopted to take care of its effects, wherein calibration with matrix-match is a familiar way due to its homogeneity and better performance (Rimayi, Odusanya, Mtunzi, & Tsoka, 2015). Therefore, an external matrix-matched standard was used for quantification.

Linearity for both carbendazim and tebuconazole was studied both in the cabbage matrix and in methanol solvent in a range of 0.0015 to 0.5 μgmL^{-1}. Satisfactory linearity and a strong correlation between peak area and concentrations in terms of residuals were obtained at ≤ ±20% with the coefficient of determination (R^2) higher than 0.998 (Table 1, Figs. S3 and S4). The matrix effect was calculated based on angular coefficients obtained by the curves in the solvent and cabbage matrix and found negligible matrix effect. As the calculated matrix effect is −2.020% for carbendazim and 2.592% for tebuconazole, it can be concluded as non-significant due to the fact that the matrix effect is significant if the values are > ±15%.

The estimated LOD was 0.0005 mg $kg^{-1}$for both the fungicides (Table 1, Fig. 2b). The LOQ of the method which refers to the minimum spiked concentration with the acceptable recovery of 70–120% was 0.0015 mg kg^{-1}, which was well below the maximum residue levels (MRLs) established by European Union (0.1 mg kg^{-1} for carbendazim and 0.7 mg kg^{-1} for tebuconazole.

The mean recovery was ranged from 78.94 to 104.89% for carbendazim, and 76.07 to 98.62% for tebuconazole (Table 1). The RSDr and RSDwR for carbendazim were ranged from 5.175 to 9.104% and 1.217 to 12.347%, respectively (Table S2), whereas it was 6.427 to 11.703% and 1.508 to 12.106%, respectively for tebuconazole (Table S3). Recoveries and precision data compliance the SANTE/12682/2019 that suggests a 70–120% recovery rate, RSDr, and RSDwR of ≤20% for pesticides.

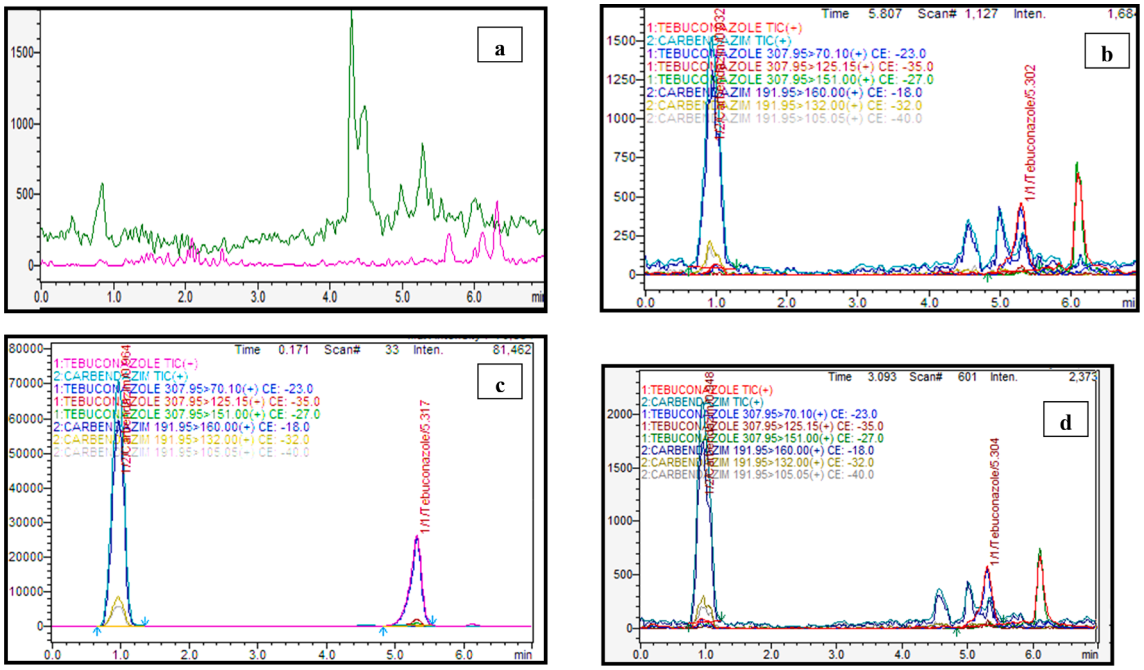

Fig. 2. The representative chromatograms of carbendazim and tebuconazole (a) cabbage blank matrix, b (at LOD level), c (zero day), d (21 DAS).

Table 1
Analytical Curve, Coefficient of determination (R^2), LOD, LOQ, Matrix effect and Accuracy of the proposed method in the fortified samples of cabbage.

Compounds	Parameter	Solvent standard				Matrix matched standard				Fortification level (mg kg^{-1})	Recovery (%) (mean ± SD), n = 6
			tcalc	tcrit	T test		tcalc	tcrit	T test		
Carbendazim	Final Analytical Curve	y = 9423.1x + 10186	2.878	2.262	S	y = 8742.8x + 1062.9	2.889	2.262	S	0.0015	104.89 ± 9.962
	R2 and Residuals	0.997 ± 20%				0.998 ± 20%				0.0075	98.76 ± 3.086
	LOD (mg kg^{-1})	0.0005				1.611					
	LOQ (mg kg^{-1})	0.0015				1.586				0.015	78.94 ± 1.211
	Matrix effect (%)	−2.020									
Tebuconazole	Final Analytical Curve	y = 2936.4x -1234.3	2.844	2.262	S	y = 2970.6x-1337.3	2.843	2.262	S	0.0015	76.07 ± 6.648
	R2 and Residuals	0.998 ± 20%				0.999 ± 20%				0.0075	98.62 ± 6.496
	LOD (mg kg^{-1})	0.0005				1.414					
	LOQ (mg kg^{-1})	0.0015				1.720				0.015	94.34 ± 2.893
	Matrix effect (%)	2.592									

R^2-Coefficient of determination; LOD-Limit of Detection; LOQ-limit of quantification; t_{calc}-t student calculated; t_{crit}-t student critical; S-Significant; SD-standard deviation; n number of replications.

Table 2
Dissipation of carbendazim and tebuconazole fungicides studied in Cabbage.

Residue (mgkg^{-1})								
	Carbendazim				Tebuconazole			
Days after treatment	Recommended Dose (250 g ai ha^{-1})		Double Recommended Dose (500 g ai ha^{-1})		Recommended Dose (258 g a.i ha^{-1})		Double recommended Dose (516 g a.i ha^{-1})	
	Mean (C ± SD)(n = 8)	% Dissipation	Mean (C ± SD) (n = 8)	% Dissipation	Mean (C ± SD) (n = 8)	% Dissipation	Mean (C ± SD) (n = 8)	% Dissipation
0 (1 h)	0.274 ± 0.043	–	0.481 ± 0.079	–	0.194 ± 0.003	–	0.392 ± 0.002	–
1	0.136 ± 0.028	50.36	0.239 ± 0.053	70.89	0.089 ± 0.015	53.40	0.208 ± 0.013	46.94
3	0.063 ± 0.029	77.01	0.120 ± 0.058	75.05	0.065 ± 0.002	65.97	0.102 ± 0.002	73.98
5	0.030 ± 0.004	88.72	0.059 ± 0.005	87.73	0.039 ± 0.023	79.58	0.056 ± 0.001	85.71
7	0.015 ± 0.001	94.53	0.031 ± 0.038	93.56	0.019 ± 0.004	90.05	0.03 ± 0.018	92.35
10	0.007 ± 0.002	97.23	0.015 ± 0.025	96.72	0.0092 ± 0.058	95.18	0.019 ± 0.001	95.15
15	0.002 ± 0.001	98.94	0.008 ± 0.017	98.34	0.0039 ± 0.003	97.96	0.0088 ± 0.012	97.76
21	BQL	–	0.003 ± 0.002	99.25	BQL	98.95	0.0043 ± 0.002	98.90
25	–	–	BQL	–	–	–	BQL	–
k	0.328	–	0.241	–	0.258	–	0.2148	–
$t_{1/2}$	2.17	–	2.99	–	2.74	–	2.81	–

Mean C concentration (mgkg^{-1}), SD standard deviation, n number of replications, D dissipation, k rate constant (day^{-1}), $t_{1/2}$ half-life time (days), $t_{0.01}$ theoretical time to reach the level of 0.01 mgkg^{-1}.

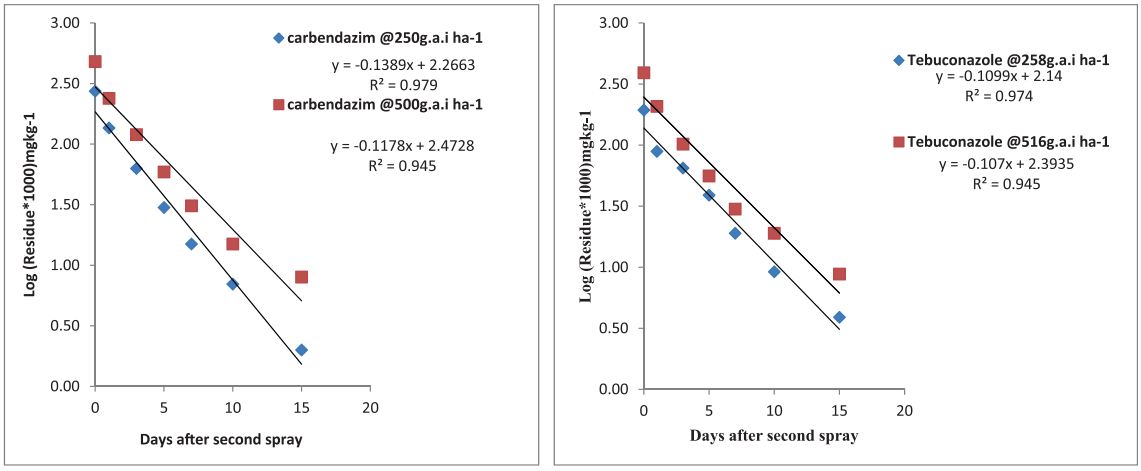

Fig. 3. Logarithm graph showing dissipation pattern of carbendazim (Left) and tebuconazole (Right) in cabbage.

3.3. Persistence and dissipation

The initial deposits of carbendazim and tebuconazole in cabbage were found to be 0.274, 0.194 for single-dose and 0.481, 0.392 for a double dose, respectively (Table 2, Fig. 3). The dissipation of carbendazim and tebuconazole has ensured the first-order kinetic model, which analogous to a correlation coefficient of 0.945, 0.958 for single-dose and 0.979, 0.934 for a double dose, respectively. At the end of the experiment, the concentration of carbendazim and tebuconazole decreased to 0.002, 0.003 mg kg^{-1} for single-dose and 0.003, 0.004 mg $kg^{-1}$for double dose, respectively, which indicated that about 99% of the initial deposits was dissipated over in 21 days after the second application (Fig. 2d). Wang et al. (2012a) reported that the degradation of tebuconazole enantiomers had followed the first-order kinetics in the soils and vegetables. It has also been shown that degradation of tebuconazole was *enantio*-selective and reported the faster degradation of (+)-S-tebuconazole in the cabbage (Li et al., 2015). The dissipation degree of carbendazim and tebuconazole residues was slower in the beginning with 50% dissipation at the recommended dose. In double, the recommended dose for carbendazim and tebuconazole dissipation rate was 70.89 and 46.94%, respectively. These two fungicides showed faster dissipation pattern over period of time, showing a non-linear pattern that fitted the first-order kinetic model. This may be associated with the evidence that during the first spray, the curd (head) is wrapped by the leaves of cabbage, which will open up steadily, leading to the degradation of pesticide residues as the days progress (Mukherjee & Gopal, 2000).

Half-life values were 2.17 and 2.99 for carbendazim and 2.74 and 2.81 for tebuconazole at single and double dose, respectively. The results of the present study with respect to carbendazim were in accordance with its half-life of 1.6–5.0 days in cabbage, red cabbage, kale, kohlrabi, brussells sprouts (Yu et al., 2015). The half-life of 2.1, 2.2, and 3.4 days was reported in tomato samples collected from Jinan, Zhengzhou, and Hefei provinces of China, respectively (Li et al., 2016). Wang et al. (2012a) reported the t ½ of 1.68 in (+)-S-tebuconazole and 0.93 in (−) R-tebuconazole in cabbage. Further, the half-life of 6 days in onion (Mohapatra, 2014); 5.87 in mango (Mohapatra, 2015); 6.93 days in watermelon (Dong and Hu, 2014) and 0.9 days in tomato (Singh & Singh, 2014) was reported for tebuconazole. Since the tebuconazole is a chiral pesticide, further studies should be carried out to clarify the possible reason for the (*enantio*-selective) dissipation in cabbage plant. From the present study, the residue of carbendazim and tebuconazole reached the below quantification limit at 21 days and less than MRL of 0.1 and 0.7 mg kg^{-1}, respectively established by European Commission (European Commission, 2017).

Practically, the dissipation of a particular pesticide relies upon the time interim between usage and crop-harvesting, which is affected by crop species, climate conditions, insect-pest occurrence, and the activity of pesticide. Several reports have demonstrated that the pesticide degradation is reliant on the abiotic conditions (climate, temperature, dampness, soil pH, etc.,), plant species variation and/or associated microbial community, pesticide characteristics (hydrophobicity, pH, etc.), and other biochemical activities take place at the soil and on plant surface (Van Eerd, Hoagland, Zablotowicz, & Hall, 2003).

3.4. Decontamination of carbendazim and tebuconazole

The effect of different decontamination processes in cabbage indicated that 2% saltwater-wash followed by cooking was found to be superior in removing the carbendazim and tebuconazole residues to the extent of 93.07 and 91.27, respectively; which is followed by lemon water-wash treatment, which removes carbendazim and tebuconazole residues up to 91.75 and 91.24%, respectively from the cabbage head (Table 3). Other processes, such as dipping in 2% tamarind, 2% salt solution, direct cooking, and dipping 2% turmeric solution for 15 min, have recorded the >70% removal of both the fungicides. The different chemical properties of these solutions such as pH, electrolytes profile, surfactant activity, and negatively or positively charged ions interference would have influenced the fungicide binding process on the cabbage, leading to the removal of fungicides during the washing as described in the previous reports (Rasolonjatovo et al., 2017). However, processes such as 2% saltwater-wash with cooking and lemon water-wash showed the >90% removal of both the fungicides. The strong electrolyte property of salt solutions having the charge that can interact with the pesticides and create an attractive force to ensure their removal from vegetable matrix (Rasolonjatovo et al., 2017). Some pesticides will be translocated into the cooking water due to their water miscibility properties. Lemon water-wash was also superior in reducing more than 90% of the fungicide residue from the cabbage, and this could be due to the presence of high citric acid. Our study establishes that washing with

Table 3
Effect of different decontamination processes in the removal of carbendazim and tebuconazole in cabbage.

Treatment No.	Treatment details	Carbendazim at recommended dose (250 g ai ha^{-1})		Reduction (%)	Tebuconazole at recommended dose (258 g ai ha^{-1})		Reduction (%)
		Initial Residues levels (mgkg^{-1}) ± SD	Residue after treatment (mgkg^{1}) ± SD		Initial Residue levels (mgkg^{-1}) ± SD	Residue after treatment (mgkg^{-1}) ± SD	
T1	Dipping in 2% Tamarind solution for 15 min	0.274 ± 0.043	0.035 ± 0.16^{e}	87.23	0.194 ± 0.003	0.052 ± 0.005^{ef}	73.20
T2	Hot water (45–50°C) wash for 30 s	0.274 ± 0.043	0.134 ± 0.06^{e}	51.09	0.194 ± 0.003	0.168 ± 0.016^{a}	13.40
T3	Dipping in 2% Turmeric solution for 15 min	0.274 ± 0.043	0.059 ± 1.29^{a}	78.47	0.194 ± 0.003	0.058 ± 2.16^{e}	70.10
T4	Dipping in tap water for 15 min and washing under tap water for 30 *sec*	0.274 ± 0.043	0.128 ± 2.68^{c}	53.28	0.194 ± 0.003	0.105 ± 1.37^{d}	45.88
T5	T5-Removal of outer layer	0.274 ± 0.043	0.129 ± 1.66^{e}	52.92	0.194 ± 0.003	0.153 ± 0.42^{b}	21.13
T6	Dipping in 2% Salt water solution for 15 min followed by cooking for 5 min	0.274 ± 0.043	0.019 ± 0.09^{f}	93.07	0.194 ± 0.003	0.016 ± 0.58 ^{g}	91.75
T7	Dipping in lemon water (1 Lemon in 1lt water) for 15 min	0.274 ± 0.043	0.026 ± 3.20^{d}	90.51	0.194 ± 0.003	0.017 ± 0.63 ^{g}	91.24
T8	Dipping in 2% Salt water solution for 15 min	0.274 ± 0.043	0.039 ± 3.24^{c}	85.77	0.194 ± 0.003	0.118 ± 1.29^{c}	39.18
T9	Direct cooking in pressure cooker for 5 min	0.274 ± 0.043	0.052 ± 1.69^{b}	81.02	0.194 ± 0.003	0.048 ± 1.63^{f}	75.26
T10	Control (No treatment)						

SD: Standard deviation; a, b, c, d, e, ef, g: Significant Differences ($p < 0.05$, student's paired *t*-test) are mentioned with different alphabets in the same test item.

several home-made solutions can get rid of the fungicide residues from cabbage. In this study, we report the effectiveness of solutions of salt, tamarind, and turmeric in removing more than 70% of both carbendazim and tebuconazole residues from cabbage.

Although residues have been partially eliminated through various culinary packages, however; comfortable and complete elimination of the residues has not been achieved yet. Using synthetic chemical solvents as washing solutions to remove pesticide residues will again lead to exposure of foods to the toxic chemicals after removing pesticides. Additionally, to administer the elimination methods in everyday life, the complications to access these chemical solutions becomes a limiting factor. On the other hand, many non-toxic solutions regularly accessible at home will also possess similar washing potential as that of synthetic chemical solvents. Therefore, in this report, various simple, cheaper, and non-toxic household washing solutions were tested, and their comparative efficiency in removing the residues of two fungicides was also studied. This indicates the significance of this study to report easily adoptable and effective household washing techniques for reducing pesticide residues in order to safeguard the consumer's health and food safety.

4. Conclusion

A sensitive, simple, and rapid analytical method was developed in LC-MS/MS and was validated for the simultaneous determination of carbendazim and tebuconazole fungicides in cabbage. The process was satisfactory for quantitative analysis according to the SANTE/12682/2019 guideline. The different decontamination procedures that are easily prepared at home (salt solution, lemon water, etc.) to remove carbendazim and tebuconazole residues from cabbage were found effective in removing more than 90 %of fungicides residues from cabbage. The above-stated household decontamination strategies can function as a powerful tool for the reduction of residues within safe limits.

CRediT authorship contribution statement

M.S. Pallavi: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing - review & editing. **R. Harischandra Naik:** Conceptualization, Methodology, Validation, Supervision, Writing - review & editing, Visualization. **Ratnamma:** Investigation, Data curation, Writing - review & editing. **Udaykumar Nidoni:** Investigation, Methodology, Validation, Writing - review & editing. **M. Bheemanna:** Methodology, Validation, Supervision, Writing - review & editing. **D. Pramesh:** Investigation, Methodology, Validation, Writing - review & editing.

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

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