Environ Monit Assess (2016) 188: 406
DOI 10.1007/s10661-016-5404-2

Residue level and dissipation of carbendazim in/on pomegranate fruits and soil

Soudamini Mohapatra · Lekha S.

Received: 17 November 2015 / Accepted: 1 June 2016 / Published online: 14 June 2016

Abstract Carbendazim is widely used on pomegranate for control of a large number of fungal diseases. Its residue levels in/on pomegranate fruits and soil were evaluated under field conditions. The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method in conjunction with liquid-chromatography mass spectrometry was used for analysis of carbendazim. Recovery of carbendazim was within 78.92–96.28 % and relative standard deviation within 3.8–10.9 % ($n = 6$). Carbendazim residues on pomegranate fruits dissipated at the half lives of 17.3 and 22.8 days from treatments at 500 and 1000 g active ingredient (a.i.) ha^{-1}, respectively. Its residues in pomegranate aril were highest on the tenth day and reduced thereafter. The residue level of carbendazim on pomegranate whole fruits from standard dose treatment was less than the EU maximum residue limit (MRL) of 0.1 mg kg^{-1} at harvest. The carbendazim residues were <LOQ in the aril and field soil at harvest. The pre-harvest intervals (PHIs) of carbendazim on pomegranate were 65.4 and 103.4 days. The results of this study can be used to determine the judicious use of carbendazim for plant protection of pomegranate crop.

Keywords Analysis · Carbendazim · LC-MS/MS · Pomegranate · Residue

S. Mohapatra (✉) · L. S.
Pesticide Residue Laboratory, Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore 560089, India
e-mail: soudamini_mohapatra@rediffmail.com

Introduction

Pomegranate (*Punica granatum*) is an economically important fruit crop of India. It contains high levels of flavonoids and polyphenols and potent antioxidants and reportedly offers protection against a number of diseases. Pomegranate peel, flower, and seed oil have anti-inflammatory properties (Rinaldi et al. 2013; Harzallah et al. 2016). India is a major producer of pomegranate fruits and occupies a significant position in the world in terms of total production. West Asia and the European Union are the largest markets for Indian pomegranates. Pomegranate crop is affected by a large number of pests and diseases. The major diseases of pomegranate plant are bacterial blight, wilt, leaf and fruit spots, and fruit rot (Anonymous 2007).These diseases caused widespread destruction to pomegranate orchards, and the area under pomegranate crop in India is reducing due to the onslaught of these diseases (Jamadar et al. 2011). The affected fruits are usually non-marketable because of poor quality. For controlling these devastating diseases of pomegranate, repeated application of fungicides become a necessity. However, repeated application of fungicides may result in residue accumulation on the fruits beyond their maximum residue limits (MRL) as well as contamination of the field soil.

Carbendazim (methyl benzimidazol-2-yl-carbamate) is a systemic fungicide with protective and curative action (Tomlin 1994). It is a broad-spectrum fungicide, which is used extensively in agriculture. Carbendazim could control bacterial leaf spot in almond, stem end rot

in mango, sapota leaf spot disease, and dry bubble disease and smoky blight canker in apple (Kjindal et al. 1989; Bhatt and Jadeja 2010; Indra 2013; Jatav et al. 2014; Kumar et al. 2015). It is also widely used for the control of anthracnose, fruit spot, and *Alternaria* rot of pomegranate (Singh and Majumdar 2002; Khosla et al. 2008; Patel 2009; Jamadar et al. 2011; Nargund et al. 2012). Carbendazim is also used to increase post-harvest shelf life of pomegranate fruits (Sarkale et al. 2003; Navale et al. 2010).

Carbendazim persists for a long time in the environment, and it is one of the most frequently occurring pesticides in fruits and vegetables monitored for pesticide residues (Bakirci et al. 2014). Carbendazim residue has frequently been detected in pomegranate, when fresh vegetables and fruits were monitored for pesticide residues (Gurbuz et al. 2014). It is therefore essential to generate information on the persistence of carbendazim residue on pomegranate. The information currently available on the residue dissipation of carbendazim on pomegranate is from a different agro-climatic zone (Utture et al. 2011). Crop species and stage of application have direct impact on pesticide dissipation in addition to environmental conditions (Janaki et al. 2013). Dissipation pattern of flubendiamide on cabbage varied widely under different agro-climatic conditions (Sharma et al. 2014). It is therefore necessary to generate information on the residue persistence of carbendazim on pomegranate at different locations. In this study, we evaluated the residue dissipation of carbendazim in/on pomegranate and soil as per good agricultural practices (GAP) under field conditions.

Materials and methods

Chemicals used

Reference standard of carbendazim (purity 99.2 %), LC-MS/MS grade acetonitrile, acetic acid, methanol, ammonium formate, and formic acid were procured from Sigma Aldrich Pvt. Ltd. (Bangalore, India). Formulation, carbendazim 50 WP, was purchased from the local market. Primary secondary amine (PSA) and C-18 sorbent of mesh size of 40 μm were procured from Agilent Technologies (Bangalore, India). Magnesium sulfate, sodium sulfate, and sodium acetate used were of analytical grade and procured from Rankem Avantor Performance Materials India Ltd (Bangalore, India). PTFE membrane filter (0.2 μm) was procured from Phenomenex (Bangalore, India). The deionized water used in the mobile phase was obtained from Millipore Water Purification System (ELIX, Merck Millipore, India Pvt. Ltd.).

Preparation of standard solutions

Preparation of stock solutions, working standards, and matrix-matched standards were carried out as described under Mohapatra (2015). Carbendazim stock solution (200 μg mL^{-1}) was prepared in LC-MS/MS grade acetonitrile and diluted to obtain calibration standards in the concentration range of 0.0015–0.1 μg mL^{-1}. Carbendazim free pomegranate fruits grown in the experimental station of Indian Institute of Horticultural Research (IIHR) was used for preparation of blank sample. Preparation of matrix-matched standards was carried out as per Mohapatra (2014). Blank sample (1 mL) was evaporated to dryness using a TurboVap LV Concentration Workstation (Zymark Corporation, Hopkinton, USA) and reconstituted with 1 mL of working standard at different concentrations.

Field study

Residue study of carbendazim was carried out on pomegranate variety, Bhagwa, at the experimental station of IIHR, Bangalore, India, during September 2014–January 2015 as per Mohapatra (2014). Five pomegranate plants with three replications were selected for each treatment. Untreated control plants were kept for comparison. Carbendazim (50 WP) spray application was given at the standard and double doses of 500 and 1000 g a.i. ha^{-1} during fruit growth stage. The second spray application was given after 15 days. Analysis of carbendazim residues in pomegranate whole fruit and aril was carried out on 0 (2 h), 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 60 days after the second spray. The field soil was analyzed before the second application on 0 (2 h), 15, 30 days, and at harvest (60 days). The climatic conditions (atmospheric temperature, humidity, and rainfall) recorded during the study period is presented in Table 1.

Sample preparation

Sample preparation of pomegranate fruits and soil was carried out as per Mohapatra (2015). Pomegranate

Table 1 Environmental parameters during the study period

Period	Average maximum temperature (°C)	Average minimum temperature (°C)	Average maximum relative humidity (%)	Average minimum relative humidity (%)	Total rainfall (mm)
September 2014	29.7	20.8	69.2	45.4	160.5
October 2014	29.6	21.7	72.4	46.2	197.5
November 2014	28.6	21.6	71.9	43.4	26.0
December 2014	27.8	20.2	71.0	52.1	–
January 2015	28.0	17.3	70.6	44.6	–

samples (approximately 6 kg) were collected from each treated field and brought to the laboratory in plastic bags. "Approximately 3 kg of pomegranate fruits were cut into two parts. One part of each fruit was pooled together and cut into small pieces. The samples were homogenized in a Waring blender and 15 g representative samples in triplicate were then taken for analysis. The remaining pomegranate fruits (3 kg) were washed under running water and peeled to obtain the aril samples. The aril samples were homogenized in a blender and 15 g of sample (three replicates) was taken for analysis. Soil samples were collected at a depth of 0–15 cm from the periphery of each pomegranate plant. The soil samples were air-dried under room temperature (25±2 °C) in the laboratory, powdered, and passed through a sieve (2 mm). A representative 20 g soil sample (in triplicate) was analyzed for carbendazim residues" Mohapatra (2015).

Extraction and clean up

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) analytical method (Anastassiades et al. 2003) was used for extraction and clean up of pomegranate samples. "The samples (15 g) were placed in 50 mL polypropylene tubes, 15-mL LC-MS/MS grade acetonitrile was added and mixed for 1 min. Next, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium acetate were added and mixed for 2 min. The tubes were centrifuged at 4470×*g* for 10 min using a Restek Centrifuge (Q-Sep 3000, Bellefonte, PA, USA). The upper acetonitrile extract (4 mL) was placed in 15 mL centrifuge tubes and subjected to dispersive solid phase extraction clean-up. Then 200 mg primary secondary amine (PSA) sorbent, 100 mg C-18 sorbent and 600 mg anhydrous magnesium sulfate was added to each tube containing the samples. The tubes were mixed for 2 min and centrifuged at 4470×*g* for 10 min. Then 2 mL from the supernatant acetonitrile phase was taken for LC-MS/MS analysis" Mohapatra (2015).

"Soil samples (20 g) in triplicate were extracted with 30 mL acetonitrile: water (2:1, *v*/*v*) by volume as per the method described above. The extracts obtained after centrifugation was transferred to a measuring cylinder with stopper, 10 mL saturated sodium chloride solution was added and mixed thoroughly. After separation of the water and acetonitrile layer, 4 mL of acetonitrile was taken and passed through anhydrous sodium sulfate to remove any remaining moisture. The acetonitrile extract was cleaned up using PSA and magnesium sulfate as described above and analyzed by LC-MS/MS" Mohapatra (2015).

LC-MS/MS analysis

Analysis of carbendazim was carried out using a LC-MS/MS system, 1290 infinity LC coupled with 6460 triple quadrupole mass spectrometer. A Zorbax eclipse plus C-18 column (2 × 100 mm id, 1.8 μm particle size) was used for separation of the analyte. "The mobile phase was composed of (A) water with 0.1 % ammonium formate (5 mM) and 0.01 % formic acid, $v\ v^{-1}$ and (B) methanol with 0.1 % ammonium formate (5 mM) and 0.01 % formic acid, $v\ v^{-1}$. The gradient programme started with 85 % A and 15 % B phase (0–1 min). A linear gradient was established in order to reach 50 % A and 50 % B composition at 6 min, 5 % A and 95 % B at 12 min; return to the initial conditions at 18 min. The column temperature was maintained at 40±0.8 °C and the column flow rate was 0.4 mL min^{-1}. The sample (2 μL) was injected using an auto-sampler. Determination of carbendazim residues using multiple reactions monitoring (MRM) mode and the electro spray ionization (ESI) probe was operated in the positive mode. The

instrument parameters were optimised and two most abundant MS/MS (precursor–product) ion transitions were monitored. Out of the two ion transitions, one was used for quantification and another for confirmation (quantifier and qualifier). For confirmation, the ion ratio 15.9 (calculated as percent ratio of peak areas of the qualifier and quantifier MRMs) was used" (Siddamallaiah and Mohapatra 2016). The chromatogram of carbendazim standard solution, pomegranate control sample, and pomegranate field sample is given in Fig. 1. The extracted ion chromatograms of carbendazim analysis by LC-MS/MS are presented in Fig. 2.

Method validation

The method used to determine carbendazim residues in pomegranate fruit and soil was validated as SANCO (Santé et Consommateurs 2013). The parameters studied were recovery, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, and precision and selectivity (SANCO 2013). To obtain the recovery percent, pomegranate fruits and soil were spiked with carbendazim at 0.005, 0.01, 0.025, 0.05, and 0.1 mg kg^{-1}. For each spiking level, six replications were taken. The LOQ of the method was considered the lowest concentration of carbendazim that could be quantified in pomegranate fruits and soil with acceptable precision and accuracy.

To determine the LOQ, carbendazim analysis in the matrices (pomegranate fruits and soil) was carried out at five levels in the concentration range of 0.005–0.1 mg kg^{-1}. For LOD determination, carbendazim standard solution analysis was carried out at 0.0015, 0.0025, 0.005, 0.01, 0.025, 0.05, and 0.1 μg mL^{-1}. The linearity curve was obtained by plotting the concentration versus the peak area. The accuracy of the method was determined to evaluate the closeness of test results to the true value. To determine the precision of the method, the spiked sample analysis was carried out in 1 day (intraday) and over a period of 6 days (interday). The precision was expressed as relative standard deviation (%RSD). "Selectivity of a method is the extent to which analyte/analytes can be determined in a complex matrix without interference from other components in the matrix" Mohapatra (2015). To determine the selectivity, we analyzed blank and spiked (five concentrations) pomegranate whole fruit, aril, and soil samples.

Matrix effect

Matrix effect from co-eluting matrix components can affect ionization efficiency of target analytes, leading to quantification errors of the analytes of interest (Wang et al. 2016). For accurate quantification of the target, analytes matrix matching of the calibration solution is required (Yarita et al. 2015). In this study, we prepared matrix matched calibration standards by adding carbendazim (0.0015–0.1 μg mL^{-1}) to blank samples. Quantification of carbendazim in pomegranate fruits and soil was carried out using the matrix-matched standards.

Data analysis

Carbendazim dissipation in/on pomegranate fruits and soil was studied by fitting the residue data into the first order rate equation:

$$\mathrm{In}\frac{[A]t}{[A]0} = -kt$$

where [A] is the concentration at time t and $[A]_0$ is the concentration at time 0, and k is the first-order rate constant (Petrucci 2007).

The half-life, $t_{1/2}$, was calculated using the equation:

$$t_{1/2} = \frac{\mathrm{In}(2)}{k} \approx \frac{0.693}{k}$$

The pre-harvest interval was calculated as per Hoskins (1961).

Results and discussion

Method validation

Satisfactory results were obtained for the method validation parameters studied based on SANCO (2013). The calibration curve was linear, and the correlation coefficient $R^2 = 0.999$ within a concentration range of 0.0015–0.1 μg mL^{-1} (Fig. 3). For quantification of carbendazim, matrix-matched calibration curve was used to nullify the matrix effect. The LOD of the method was 0.0015 μg mL^{-1}. The LOQ of the method was 0.005 mg kg^{-1}, which was the lowest level, at which carbendazim could be measured with acceptable accuracy and precision in pomegranate whole fruit, aril, and

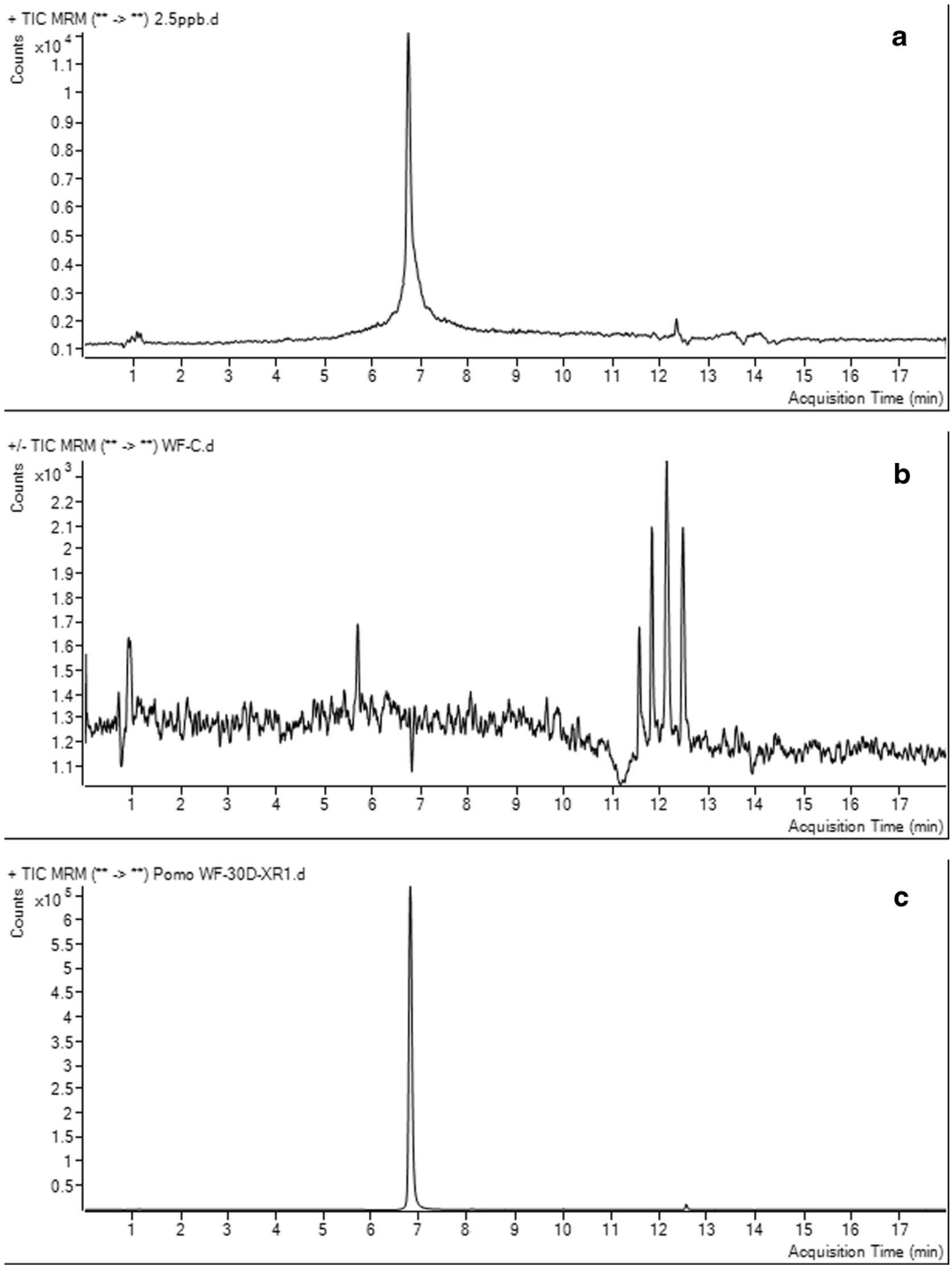

Fig. 1 Chromatogram of **a** carbendazim standard solution at 0.0025 μg mL^{-1}, **b** pomegranate control sample, and **c** pomegranate whole fruit sample on the 30th day at the standard dose

soil. Recoveries of carbendazim were within the acceptable range of 78.92–96.28 % (Table 2; SANCO 2013). The accuracy of the method was demonstrated by spiking carbendazim at 0.005–0.1 mg kg^{-1}, which gave satisfactory results. The precision of the method, expressed as the %RSD of repeated observations (n =

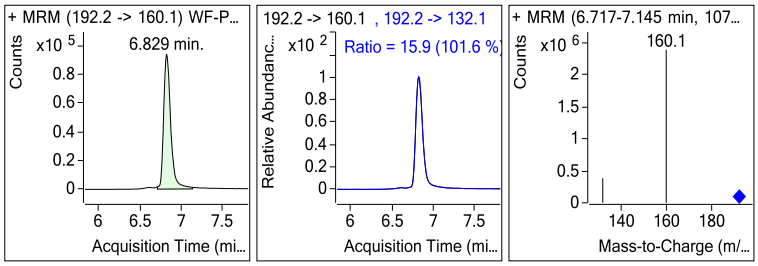

Fig. 2 Extracted ion chromatograms of carbendazim analysis by LC-MS/MS

6), was within 3.8–10.9 % (Table 2). The method was highly selective as determination of carbendazim could be carried out at a range of 0.005–0.1 mg kg^{-1} in pomegranate fruit and soil with acceptable accuracy and precision.

Carbendazim residues in/on pomegranate fruit

Average initial residue deposits of carbendazim in/on pomegranate whole fruit were 1.36 and 2.35 mg kg^{-1} from treatment at the standard and double doses of 500 and 1000 g a.i. ha^{-1}, respectively (Table 3). Dissipation of carbendazim on pomegranate whole fruit and aril is presented in Fig. 4. Carbendazim residue dissipation from pomegranate fruits was a very slow process. Within a day, about 11 % of the residues dissipated from the two treatments and within 5 days, 22 % residues had dissipated. From the tenth day, onward carbendazim residues from standard dose treatment dissipated faster compared with the double-dose treatment. Carbendazim residues on pomegranate dissipated by 53.5 and 44.2 % after 20 days and by 66.2 and 58.3 % after 30 days from treatments at standard and double-doses, respectively. At harvest (60 days), the residue level at the standard dose treatment reached the European Union maximum residue limit (MRL) of 0.1 mg kg^{-1} (Anonymous 2008). In the double-dose treatment, the residues exceeded the MRL even after 60 days. Carbendazim residues in the edible aril increased from 0 to 10 days and decreased thereafter. On day 0, the residue level of carbendazim in aril at the standard dose treatment was 0.01 mg kg^{-1}, which had increased to 0.11 mg kg^{-1} by day 10 and reached <LOQ by day 35. Similarly, in the double-dose treatment, the residue level in the aril on day 0 was 0.02 mg kg^{-1}, increased to 0.24 mg kg^{-1} by day 10, and reached <LOQ by day 40. At harvest (60 days), the edible aril was free from residues; however, carbendazim residues could be detected on the whole fruit.

Pomegranate fruit contains edible and non-edible portions. The edible arils of the pomegranate fruit are enclosed in a thick leathery rind, which is also known as the husk (Babu et al. 2014). As a result of the systemic nature of carbendazim, it can be absorbed by the fruits quickly. However, the movement to the edible aril may have been minimized by the thick outer layer of the fruit. The residues absorbed by the fruits would have remained beyond 60 days in the current study. Sharma

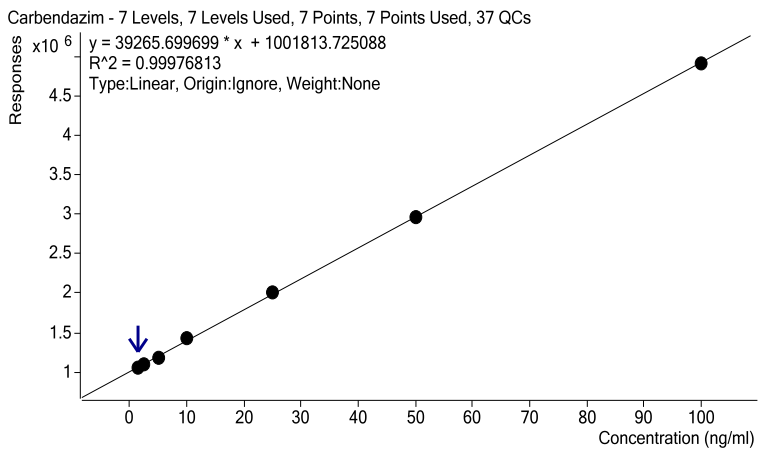

Fig. 3 Standard curve of carbendazim

Table 2 Recovery of carbendazim from pomegranate whole fruit, aril and soil at various spiked levels

Spiked concentration (mg kg^{-1})	Average recovery (%) ± SD[a]					
	Pomegranate whole fruit		Pomegranate aril		Soil	
	Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)
0.005	78.92 ± 8.6	10.9	84.70 ± 6.3	7.4	82.64 ± 7.4	8.9
0.010	83.60 ± 6.5	7.8	88.36 ± 5.8	6.5	86.78 ± 7.0	8.0
0.025	87.55 ± 5.4	6.1	90.67 ± 5.3	5.8	87.53 ± 6.5	7.4
0.050	90.86 ± 5.0	5.5	92.76 ± 4.6	4.9	91.95 ± 5.1	5.5
0.100	94.34 ± 4.8	5.1	96.28 ± 3.7	3.8	93.66 ± 3.6	3.8

[a] Average of six replicate analyses ± standard deviation

and Bharat (1994) showed that carbendazim persisted on apple fruit up to 35 days and on apple leaves up to 45 days. Movement of carbendazim to the inner part of apple has been reported by Leroux et al. (1975).

Pesticides disappeared rapidly from the surface of plants, but the absorbed residues were more persistent (Moody et al. 1977). Reduction of pesticides on fruits can be attributed to the dilution effect caused by fruit growth (Cabras et al. 1995; Juraske et al. 2012). Pomegranate fruits grow slowly, and the time taken from fruit set to maturity is about 120–130 days. Therefore, residue dissipation due to growth dilution would be lower in

Table 3 Residue level of carbendazim on pomegranate

Days after treatment	Untreated control	Residues of carbendazim recovered (mg kg^{-1}) ± SD[a]			
		Treatment at 500 g a.i. ha^{-1}		Treatment at 1000 g a.i. ha^{-1}	
		Whole fruit	Aril	Whole fruit	Aril
0	ND	1.36 ± 0.150	0.01 ± 0.001	2.35 ± 0.025	0.02 ± 0.001
1	ND	1.20 ± 0.098 (11.7)	0.07 ± 0.008	2.10 ± 0.202 (10.6)	0.10 ± 0.011
5	ND	1.06 ± 0.076 (22.0)	0.06 ± 0.004	1.83 ± 0.166 (22.1)	0.09 ± 0.008
10	ND	0.84 ± 0.080 (38.2)	0.11 ± 0.003	1.70 ± 0.180 (27.7)	0.24 ± 0.017
15	ND	0.76 ± 0.053 (44.1)	0.06 ± 0.005	1.52 ± 0.134 (35.3)	0.11 ± 0.008
20	ND	0.63 ± 0.053 (53.6)	0.05 ± 0.004	1.31 ± 0.121 (44.2)	0.09 ± 0.007
25	ND	0.54 ± 0.028 (60.3)	0.05 ± 0.004	1.16 ± 0.150 (50.5)	0.08 ± 0.008
30	ND	0.46±0.033 (66.2)	0.04 ± 0.003	0.98 ± 0.092 (58.3)	0.06 ± 0.004
35	ND	0.38 ± 0.021 (72.0)	<LOQ	0.82 ± 0.078 (65.1)	0.03 ± 0.003
40	ND	0.32 ± 0.016 (76.5)	<LOQ	0.74 ± 0.060 (68.5)	<LOQ
50	ND	0.18 ± 0.015 (86.7)	<LOQ	0.53 ± 0.032 (77.4)	<LOQ
60	ND	0.1 ± 0.011 (92.6)	<LOQ	0.32 ± 0.026 (86.4)	<LOQ
Field soil before spray	ND	0.02 ± 0.011		0.06 ± 0.013	
Field soil on 0 day	ND	0.37 ± 0.032		0.60 ± 0.044	
Field soil on 15 days	ND	0.27 ± 0.014		0.48 ± 0.025	
Field soil on 30 days	ND	0.05 ± 0.001		0.15 ± 0.016	
Field soil on 60 days	ND	<LOQ		<LOQ	

Figures in the parenthesis are the percent dissipation of residues

ND not detected, *LOQ* limit of quantification

[a] Average of three replicate analyses ± SD

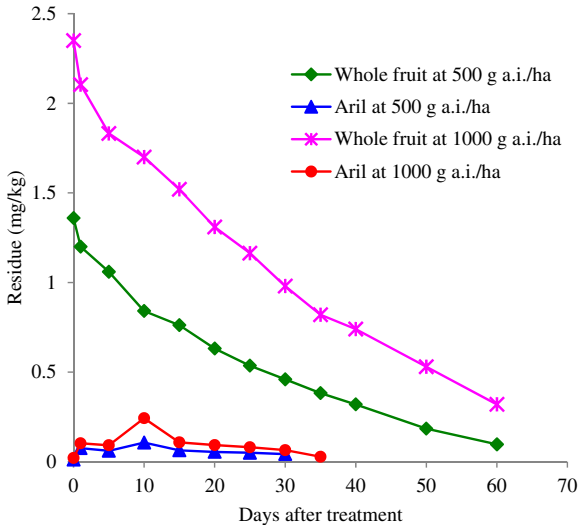

Fig. 4 Dissipation of carbendazim in pomegranate whole fruit and aril

pomegranate than in many other fruits. Long persistence of cypermethrin on pomegranate has also been reported in previous work (Mohapatra 2014).

Carbendazim residues in soil

Carbendazim residues from treatments at the standard and double doses of 500 and 1000 g a.i. ha^{-1} were 0.02 and 0.06 mg kg^{-1}, respectively, just before the second application (Table 3). The residue levels were 0.37 and 0.60 mg kg^{-1} after the second application. Carbendazim residues degraded in soil and reached 0.27 and 0.48 mg kg^{-1} after 15 days and 0.05 and 0.15 mg kg^{-1} after 30 days. However, at harvest (60 days) the residue level in soil was <LOQ. The adsorption of carbendazim was inversely correlated to the pH of the soil (Paszko 2012). As the pH of the experimental field soil was 6.6, adsorption of carbendazim may be reduced and it is more likely to be leached after rainfall. Carbendazim is known to persist for a long time in soil but degrades faster with high moisture level and soil microorganisms (Gupta and Sharma 1989). The total cumulative rainfall of 384 mm (during the study) could have accelerated degradation of carbendazim in soil in the current study.

Half-life and PHI

The degradation kinetics of carbendazim was studied by fitting the data into a first-order rate equation. The half-life of degradation (DT_{50}) of carbendazim was defined as the time required for the amount of the fungicide to fall to half of its initial concentration. Carbendazim residues on pomegranate whole fruits degraded at the half-lives of 17.3 and 22.8 days from treatments at the standard and double doses of 500 and 1000 g a.i. ha^{-1} (Table 4). The half-life of carbendazim on mushroom was about 1 day and brinjal, 5 days (Dubey et al. 1994; Mousa et al. 2004). These DT_{50} values are much lower than that of pomegranate. The higher DT_{50} values of pomegranate can be attributed to its slow-growing nature, where residue dilution due to fruit growth is reduced. In a previous study, we found that the half-life of cypermethrin on pomegranate was 11.5 days when applied at 500 g a.i. ha^{-1} (Mohapatra 2014). Carbendazim half-life determined from a post-harvest treatment to mango was within 17.3–21.6 days (Devarajan et al. 2000).

The pre-harvest intervals (PHIs) of carbendazim on pomegranate fruits calculated as per Hoskins (1961) were 65.4 and 103.4 from treatments at the standard and double doses of 500 and 1000 g a.i. ha^{-1} (Table 4). "The PHI is the time interval between the last application of the pesticide and harvest of the crop and should be long enough to allow the pesticide residues in the harvested crop to reduce to the MRL" Mohapatra (2015). The PHI is dependent on the nature of the pesticide, growth dilution factors and various environmental factors (Gupta and Sharma 1989; Sadlo 2001). The persistent nature of carbendazim, coupled with the slow growing nature of pomegranate fruit and low MRL value (0.1 mg kg^{-1}), is likely to cause the high PHI values. The PHI of cypermethrin on pomegranate was 50–73 days (Mohapatra 2014), substantially lower than that of carbendazim. The systemic nature of

Table 4 Rate of residue decay and safety constants of carbendazim residues on pomegranate

Rate of application (g a.i. ha^{-1})	Regression equation	Regression coefficient	Half-life ($t_{½}$) (days)	PHI (days)
500	$Y = 3.13666 - 0.01739X$	−0.99	17.3	65.4
1000	$Y = 3.36709 - 0.01323X$	−0.99	22.8	103.4

carbendazim coupled with its low water solubility (http://www.inchem.org/documents/jmpr/jmpmono/v073 pr11.htm) may be the reason for its longer persistence on pomegranate compared to other pesticides.

Conclusions

Validation of the QuEChERS method, in conjunction with LC-MS/MS, gave satisfactory results for analysis of carbendazim residues in pomegranate and soil. The limit of quantification (0.005 mg kg^{-1}) was substantially below the European Union MRL of 0.1 mg kg^{-1} (Anonymous 2008). Carbendazim recovery from pomegranate fruits and soil was satisfactory (within 70–120 %) and the precision, expressed as %RSD, was within 3.8–10.9 %. Carbendazim residues were detected on pomegranate whole fruits at harvest (60 days) but at the standard dose treatment the residue level reached the MRL by that time. Carbendazim residues were detected up to 30 days after application in the edible aril (standard dose treatment). However, excluding the tenth day, the residues in the aril were always less than the MRL. The field soil contained carbendazim residues, but at harvest these values were <LOQ. Carbendazim is effective against several diseases of pomegranate crop; however, as it is persistent in nature and requires a long PHI, it should be applied before fruit set.

Acknowledgments The author thanks the Director of the Indian Institute of Horticultural Research, Bangalore, India for providing the facilities to carry out the work for this study.

References

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *Journal of Association of Official Analytical Chemists, 86*, 412–431.

Anonymous. (2007). *Project report on export promotion of pomegranate from India by Agricultural Finance Corporation*. New: Delhi.

Anonymous. (2008). MRLs of concern for the active substance carbendazim. European Food Safety standard Authority. *EFSA Scientific Report, 158*, 1–31.

Babu, K. D., Singh, N. V., Chandra, R., Sharma, J., Malty, A., & Sarkar, P. C. (2014). Effect of surface coating with lac based formulations on postharvest quality of pomegranate (*Punka grantum* L.) fruits. *International Journal of Tropical Agriculture, 32*, 835–839.

Bakirci, G. T., Acay, D. B. Y., Bakirci, F., & Otles, S. (2014). Pesticide residues in fruits and vegetables from the Aegean region, Turkey. *Food Chemistry, 160*, 379–392.

Bhatt, H. R., & Jadeja, K. B. (2010). Mango stem end rot management with carbendazim. *Indian Phytopathology, 63*(1), 103–105.

Cabras, P., Melis, M., Cabitza, F., Cubeddu, M., & Spanedda, L. (1995). Persistence of pirimicarb in peaches and nectarines. *Journal of Agricultural and Food Chemistry, 43*, 2279–2282.

Devarajan, L., Chinniah, C., Jayakumar, R., & Mani, S. (2000). Evaluation of carbendazim residues from post-harvest treatment of ripening mango fruits. *Madras Agricultural Journal, 87*, 672–673.

Dubey, J. K., Sharma, V. P., Patyal, S. K., & Nath, A. (1994). Persistence of carbendazim on white button mushroom, *Agaricus bisporus. Mushroom Research, 3*, 81–82.

Gupta, V. K., & Sharma, S. D. (1989). Degradation of carbendazim in soil by microorganisms. *Indian Journal of Agricultural Sciences, 5*, 21–24.

Gurbuz, N., Arik, O., & Baykal, A. (2014). Screening of pesticide residues in fresh vegetables and fruits by LC-MS/MS and GC-MS/MS. *Asian Journal of Chemistry, 26*, 6975–6981.

Harzallah, A., Hammami, M., Kepczynska, M. A., Hislop, D. C., Arch, J. R. S., Cawthorne, M. A., & Zaibi, M. S. (2016). Comparison of potential preventive effects of pomegranate flower, peel and seed oil on insulin resistance and inflammation in high-fat and high-sucrose diet-induced obesity mice model. *Archives of Physiology and Biochemistry, 122*, 75–87.

Hoskins, W. M. (1961). Mathematical treatment of the rate of loss of pesticide residues. *FAO Plant Protection Bulletin, 9*, 163–168.

Indra, N. (2013). Prevalence of sapota leaf spot disease (*Phaeopleospora indica*) and its management with fungicides in Tamil Nadu. *Journal of Mycology and Plant Pathology, 43*(4), 419–422.

Jamadar, M. M., Sataraddi, A. R., Patil, P. V., Jawadagi, R. S., Patil, D. R., Sheikh, M. K., Mokashi, A. N., & Rokhade, A. K. (2011). Status of pomegranate diseases of northern Karnataka in India. *Acta Horticulturae, 890*, 501–507.

Janaki, R. S., Priya, C., & Chinnusamy, C. (2013). Field dissipation of oxyfluorfen in onion and its dynamics in soil under Indian tropical conditions. *Journal of Environmental Science and Health Part B, 48*, 941–947.

Jatav, N. K., Rana, R. S., Verma, J. R., & Verma, S. K. B. (2014). Chemical control of dry bubble disease induced by *Verticillium fungicola* [Preuss] Hassebr on white button mushroom, *Agaricus bisporous*. *African Journal of Microbiology Research, 8*, 2202–2207.

Juraske, R., Fantke, P., Ramírez, A. C. R., & González, A. (2012). Pesticide residue dynamics in passion fruits: comparing field trial and modelling results. *Chemosphere, 89*, 850–855.

Khosla, K., Bhardwaj, S. S., & Kumar, J. (2008). Field evaluation of fungicides against fruit spot and rot diseases of pomegranate. *Haryana Journal of Horticultural Sciences, 37*, 22–24.

Kjindal, K., Sharma, R. C., & Gupta, V. K. (1989). Chemical control of bacterial leaf spot and fruit gummosis caused by *Xanthomonas campestris* pv. *pruni* in almond (*Prunus*

dulcis). *Indian Journal of Agricultural Sciences, 59*(11), 754–755.

Kumar, J., Watpade, S., Pramanick, K. K., Shukla, A. K., & Kumar, J. (2015). Evaluation of some fungicides for control of smoky blight canker in apple (*Malus* × *domestica*). *International Journal of Tropical Agriculture, 33*(2), 1549–1551.

Leroux, P., Casanova, M., & Dachaud, R. (1975). A study of the penetration and persistence of benomyl and carbendazim in apples treated after harvest. *Phytiatrie-Phytopharmacie, 24*, 49–56.

Mohapatra, S. (2014). Residue dynamics of chlorpyrifos and cypermethrin in/on pomegranate (*Punica granatum* L.) fruits and soil. *International Journal of Environmental Analytical Chemistry, 94*(14-15), 1394–1406.

Mohapatra, S. (2015). Dynamics of difenoconazole and propiconazole residues on pomegranate over 2 years under field conditions. *Environmental Science and Pollution Research, 23*, 5795–5806.

Moody, R. P., Prasad, R., Greenhalgh, R., Weinberger, P., Roberts, J. R., Greenhalgh, R., & Marshall, W. K. (1977). *Translocation of ring labelled 14C-fenitrothion in conifers* (pp. 217–231). National Research Council Canada: Report.

Mousa, M., Sagar, V. R., Gajbhiye, V. T., & Kumar, R. (2004). Pesticides persistence in/on fresh and dehydrated brinjal. *Journal of Food Science and Technology (Mysore), 41*, 429–431.

Nargund, V. B., Jayalakshmi, K., Benagi, V. I., Byadgi, A. S., Patil, R. V., Melgarejo, P., & Valero, D. (2012). Status and management of anthracnose of pomegranate in Karnataka State of India. *Options Mediterraneennes. Serie A Seminaires Mediterraneens, 103*, 117–120.

Navale, M., Suryawanshi, K. T., & Waskar, D. P. (2010). Response of *Bhagwa* variety of pomegranate to post-harvest treatments for extending the shelf life under different storage conditions. *Annals of Plant Physiology, 24*(1), 77–81.

Paszko, T. (2012). Effect of pH on the adsorption of carbendazim in Polish mineral soils. *Science of the Total Environment, 435*, 222–229.

Patel, D. S. (2009). Chemical management of fruit spot of pomegranate caused by *Colletotrichum gloeosporioides* Penz. and Sacc. *Indian Phytopathology, 62*(2), 252–253.

Petrucci, R. H. (2007). *General chemistry: principles and modern applications* (9th ed.). New Jersey: Pearson Education Inc.

Rinaldi, M., Caligiani, A., Borgese, R., Palla, G., Barbanti, D., & Massini, R. (2013). The effect of fruit processing and enzymatic treatments on pomegranate juice composition, antioxidant activity and polyphenols content. *Food Science and Technology, 53*, 355–359.

Sadlo, S. (2001). Disappearance of dichlofluanid, chlorothalonil, vinclozolin and carbendazim on greenhouse tomatoes. *Journal of Plant Protection Research, 41*, 5–11.

SANCO (2013). Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANCO/12571/2013.

Sarkale, V. M., Sanghavi, K. U., Dhemre, J. K., & Masalkar, S. D. (2003). Effect of post-harvest treatments on shelf-life and quality of pomegranate in cold storage and ambient conditions. *Journal of Food Science and Technology (Mysore), 40*(1), 67–69.

Sharma, S. K., & Bharat, N. K. (1994). Persistence of carbendazim on apple fruit and leaves. *Plant Disease Research, 9*, 204–206.

Sharma, K. K., Mohapatra, S., Ahuja, A. K., Deepa, M., Sharma, D., Jagdish, G. K., Rashmi, N., Battu, R. S., Sharma, S. K., Singh, B., Parihar, N. S., Sharma, B. N., Kale, V. D., Nakat, R. V., Walnuj, A. R., Singh, G., Kuldeep, K., Ravivanshi, Devi, S., & Noniwal, R. (2014). Safety evaluation of flubendiamide and its metabolites on cabbage and persistence in soil in different agroclimatic zones of India. *Environmental Monitoring and Assessment, 186*, 3633–3639.

Siddamallaiah, L., & Mohapatra, S. (2016). Residue level and dissipation pattern of spiromesifen in cabbage and soil from 2-year field study. *Environmental Monitoring and Assessment, 188*, 155.

Singh, J., & Majumdar, V. L. (2002). Fungicidal management of *Alternaria* rot in pomegranate. *Journal of Mycology and Plant Pathology, 32*(1), 134.

Tomlin, C. (1994). *The pesticide manual* (10th ed., pp. 149–150). U.K.: British Crop Protection Council and Royal Society of Chemistry.

Utture, S. C., Banerjee, K., Dasgupta, S., Pati, S. H., Jadhav, M. R., Wagh, S. S., Kolekar, S. S., Anuse, M. A., & Adsule, P. G. (2011). Dissipation and distribution behavior of azoxystrobin, carbendazim, and difenoconazole in pomegranate fruits. *Journal of Agriculture and Food Chemistry, 59*, 7866–7873.

Wang, Y., Li, S. H., Zhang, F. F., Lu, Y. F., Yang, B. C., Zhang, F., & Liang, X. M. (2016). Study of matrix effects for liquid chromatography-electrospray ionization tandem mass spectrometric analysis of 4 aminoglycosides residues in milk. *Journal of Chromatography A, 1437*, 8–14.

Yarita, T., Aoyagi, Y., & Otake, T. (2015). Evaluation of the impact of matrix effect on quantification of pesticides in foods by gas chromatography-mass spectrometry using isotope-labeled internal standards. *Journal of Chromatography A, 1396*, 109–116.