



Persistence and Dissipation Pattern of Dimethoate 30 EC in / on Foxtail Amaranthus and Spinach

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A survey was conducted to know the insecticide usage pattern in leafy vegetables and found that dimethoate was the most frequently used insecticide. A field trial was conducted to know the dissipation pattern of dimethoate in foxtail amaranthus and spinach during October - November, 2017. Dimethoate 30 EC was sprayed twice as foliar treatment at 10 days interval @ 200 and 400 g a.i ha⁻¹. The samples were collected from 0, 1, 3, 5, 7, 10, 15 and 20 days after treatment (DAT), extracted by modified QuEChERS method and analyzed by LCMS. The mean initial deposit (1 h after spraying) of dimethoate at 200 and 400 g a.i ha⁻¹ were 7.25 µg g⁻¹ and 19.51 µg g⁻¹ for foxtail amaranthus and 18.37 µg g⁻¹ and 29.46 µg g⁻¹ for spinach, respectively. The residues reached below detectable level of < 0.05 µg g⁻¹ at 10 days after treatment in foxtail amaranthus and 15 and 20 DAT in spinach for both doses. Half-life of dimethoate residues were 0.85 and 0.86 days in foxtail amaranthus; 2.22 and 1.12 days in spinach at 200 and 400 g a.i ha⁻¹, respectively.

Key words: Foxtail amaranthus, Spinach, Dimethoate, QuEChERS, LCMS.

Foxtail amaranthus [*Amaranthus tristis* L.] and spinach [*Spinacia oleracea* L.] are green leafy vegetables which are grown and consumed all over the world. Amaranthus is rich in carbohydrate, protein, iron, potassium, zinc, sodium and vitamins like carotene, thiamine and riboflavin and several amino acids (Akubugwo *et al.*, 2007). Spinach is rich in vitamin C, A, B₁ and good source of iron and contains proteins and carbohydrates (Bagyalakshmi *et al.*, 2011). In green leafy vegetable cultivation, insect pests are considered as serious threat like in any other vegetable. More than twenty insect pests were reported on amaranthus.

Among the insect pests, amaranthus weevil and leaf webbers are of major importance causing heavy damage and yield loss while others are minor pests. Stem weevil *Hypolixus truncatulus* (Boheman), is a specific pest of amaranthus and widely distributed in India. The grub causes zig zag tunneling of stem resulting in longitudinal splitting of stem and drying (Butani and Jotwani, 1984; Tara *et al.*, 2009). Among the leaf webbers, *Hymenia recurvalis* (Fab.) is a destructive pest of regular occurrence and *Psara bipunctalis* (Fab.) is a sporadic pest. Cutworm *Agrotis segetis* (Hubner) also causes damage to foliage and yield loss in spinach. Spinach is damaged by *Lipaphis erysimi* (Kaltenbach), *Myzus persicae* (Sulzer) and *Hyadaphis indobrassicae* (Das). Marketability of spinach is affected by the mining of leaves by leaf miner *Liriomyza huidobrensis* (Blanchard) (Mou and Beiquan, 2008).

These insect pests are managed by insecticides to increase the production and marketability. Being short duration crop of 40 – 50 days, quick and effective

pest management is required. Most of the farmers growing green leafy vegetables resort to spraying of insecticides to minimize insect pests though none is recommended (CIBRC, 2016). Apart from the beneficial effect of insecticides in managing the pests they may also pose risk to the environment and health of humans by leaving residues on crop.

Insecticides namely, chlorpyrifos, λ-cyhalothrin, thiophanate methyl, metalaxyl and fluazifop-P-butyl) were widely used against major insect pests of leafy vegetables (amaranthus, spinach, and lettuce) under greenhouse condition in China (Fan *et al.*, 2013). Chlorpyrifos was reported to be extensively used on celery and lettuce under greenhouse condition to control insect infestation (Lu *et al.*, 2014). However insecticide dissipation studies on leafy vegetables are limited on leafy vegetables.

Therefore the present study was carried out to know the insecticide usage pattern on leafy vegetables in Tamil Nadu and to evaluate the dissipation pattern of commonly used insecticides on foxtail amaranthus and spinach.

Material and Methods

Survey was conducted on insecticide usage pattern in major leafy vegetables growing areas viz., Coimbatore, Nilgiris, Dindigul, Karur, Salem and Viluppuram of Tamil Nadu during September to November, 2016 by adopting a questionnaire. In each district, 25 farmers were selected and the information was collected regarding type of leafy vegetables grown, pest incidence and insecticide usage pattern.

A supervised field trial was conducted in farmer's field at Madhampatti village of Coimbatore during

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October - November, 2017. Foxtail amaranthus (c.v. - green) and spinach (c.v. - All green) were raised by following the recommended agronomic practices with a spacing of 15 x 10 cm. The commercial formulation of dimethoate 30 EC was sprayed at 200 g a.i ha⁻¹ (recommended dose) and 400 g a.i ha⁻¹ (double the recommended dose) and replicated thrice in 20 m² plot in Randomized Block Design (RBD). The first spraying was done at 10 days after sowing using power operated knapsack sprayer during morning hours and second spraying was done at 10 days after first spraying.

Collection and processing of samples for residue analysis

One kilogram leaf sample was randomly collected from each treated plot from 0 (one h after spraying), 1, 3, 5, 7, 10, 15, 20, 25 and 30 days after spraying. The collected samples were taken to the laboratory, homogenized and stored at -10°C for further analysis.

Extraction and cleanup

The samples were processed by adopting modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.*, 2003). A representative sample of 10 g was transferred into a 50 ml centrifuge tube and vortexed for one min after adding 20 ml of acetonitrile. About four grams of anhydrous Magnesium Sulphate (MgSO₄) and one gram of Sodium Chloride (NaCl) were subsequently added and again shaken well by vortexer, then centrifuged at 6000 rpm for 10 min.

Then, nine ml of supernatant was transferred to test tube containing four gram anhydrous sodium sulphate (Na₂SO₄) and hand shaken for one min. Six ml of supernatant aliquot was transferred into a 15 ml centrifuge tube containing 100 mg Primary Secondary Amine (PSA), 600 mg anhydrous MgSO₄ and 25 mg Graphitized Carbon Black (GCB), vortexed for one min and then centrifuged for 10 min at 3000 rpm. The upper extract (4 ml) was transferred into a turbovap tube and concentrated to near dryness under a gentle stream of nitrogen in a turbovap LV at 40°C for 30 min. The final volume was reconstituted to about one ml using ultra-pure water for analysis of dimethoate residues in LCMS.

Chemicals and reagents

The reference standard of dimethoate (99.5% purity) was purchased from M/s Sigma Aldrich, Bangalore, India. Acetonitrile (HPLC grade) and sodium chloride of analytical grade were purchased from M/s Merck (Mumbai, India). Anhydrous magnesium sulphate (analytical grade) was purchased from M/s HiMedia (Mumbai, India). Primary Secondary Amine (PSA) (Bondsil 40 µm) and Graphitized Carbon Black (GCB) were purchased from M/s Agilent technologies, USA.

Preparation of standard solutions

A standard stock solution of dimethoate (425.86 µg ml⁻¹) was prepared by dissolving 10.7 mg of analyte

in 25 ml HPLC grade acetonitrile in volumetric flasks. The flask was labeled and stored in refrigerator at -20°C. Intermediate stock solutions of 100 µg ml⁻¹ and 10 µg ml⁻¹ and working standard of 0.01 to 0.5 µg ml⁻¹ of analyte were prepared for further analysis

Instrumentation

The analysis of dimethoate residues in leaf samples of foxtail amaranthus and spinach was carried out using Shimadzu 2020 Liquid Chromatograph Mass Spectrometer (LCMS). The instrument parameters for estimation of dimethoate residues in foxtail amaranthus and spinach are given in Table 1.

Determination of LOD and LOQ

The limit of detection (LOD) was calculated by considering signal-to-noise ratio of three with reference to the background noise obtained from blank sample and the limit of quantification (LOQ) by considering a signal to noise ratio of ten.

Recovery study

The extraction efficiency of the modified QuEChERS method was determined by spiking the analytical standards of dimethoate at five different levels (0.01, 0.025, 0.05, 0.1 and 0.25 µg g⁻¹) and repeated three times to determine repeatability.

Results and Discussion

Survey

The major insecticides used by farmers growing leafy vegetables were dimethoate 30 EC (54.4 %), chlorpyrifos 40 EC (33.6 %), quinalphos 25 EC (32.8 %), chlorantraniliprole 18.5 SC (23.2 %), triazophos

Table 1. Instrument parameters of LCMS

Particulars	Dimethoate
Model	LCMS (Shimadzu, series 2020)
Column	Reverse phase - C18 (Agilent) column, 250 mm length x 4.6 mm id x 5 µ particle size in a column oven, at 40°C
Detector	Diode Array Detector (SPD-M20A)
Mobile phase	Ultra-pure water with 0.1 % formic acid and methanol with 0.1 % formic acid (60:40)
Flow rate	0.3 ml min ⁻¹
Mass ratio (m/z)	230
Ionization mode	Positive SIM (Single Ion Monitoring)
Injection volume	20 µl
Drying gas flow rate	15 L min ⁻¹
Nebulizer gas flow rate	1.5 L min ⁻¹
Desolvation line (DL) temperature	280°C
Heat block temperature	400°C
Run time	30 min.
Retention time of analytes	26.09 min.

40 EC (20.0 %) and imidacloprid 48 FS (18.4 %) and hence dimethoate was used for dissipation study. On

an average, 2.48 sprays were given during a crop growth period of 45 days and the harvest interval practiced by farmers was 2.15 days.

Table 2. Recovery percentage of dimethoate in foxtail amaranthus

Spiking level ($\mu\text{g g}^{-1}$)	Recovery %						Mean \pm SD	RSD (%)
	R1	R2	R3	R4	R5	R6		
0.05	106.90	108.08	105.79	105.79	107.40	106.34	106.72 \pm 0.92	0.86
0.1	100.73	105.09	102.72	100.65	105.82	103.38	103.07 \pm 2.15	2.09
0.25	97.98	107.78	108.51	98.13	108.45	109.84	105.11 \pm 5.51	5.24
0.5	110.61	110.86	112.61	111.02	112.15	113.34	111.76 \pm 1.10	0.98
1	101.96	110.73	110.57	101.79	111.10	112.13	108.05 \pm 4.81	4.45

SD – Standard Deviation, RSD – Relative Standard Deviation

Method validation

Linearity

The linearity of the calibration curve of dimethoate

was established using six different levels of concentration (0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 $\mu\text{g g}^{-1}$). The r^2 value was 0.9974 (Fig.1).

Table 3. Recovery percentage of dimethoate residues in spinach

Spiking level ($\mu\text{g g}^{-1}$)	Recovery %						Mean \pm SD	RSD (%)
	R1	R2	R3	R4	R5	R6		
0.05	90.22	112.46	119.06	99.62	113.37	111.23	107.66 \pm 10.65	9.89
0.1	91.03	98.82	101.95	91.88	101.94	104.87	98.41 \pm 5.73	5.82
0.25	106.34	119.61	123.56	103.97	119.28	112.17	114.15 \pm 7.92	6.93
0.5	83.70	106.44	105.32	80.25	100.80	105.85	98.06 \pm 11.90	12.26
1	65.29	75.96	82.61	60.92	70.95	73.83	71.59 \pm 7.74	10.81

SD – Standard Deviation, RSD – Relative Standard Deviation

Determination of LOD and LOQ

The LOD and LOQ values were found to be 0.015 and 0.05 $\mu\text{g g}^{-1}$ for dimethoate in LCMS.

Recovery

The mean per cent recoveries of dimethoate were

110.27, 109.99, 102.24, 101.13 and 95.74 in foxtail amaranthus and Relative Standard Deviation (RSD) percentage values were 1.83, 1.34, 3.54, 3.63 and 3.91 at spiking levels of 0.05, 0.1, 0.25, 0.5 and 1 $\mu\text{g g}^{-1}$, respectively.

Table 4. Persistence and dissipation of dimethoate 30 EC residues in/on foxtail amaranthus

Days after treatment	Dimethoate @ 200 g a.i ha ⁻¹ (X dose)					Dissipation percentage (%)	Dimethoate @ 400 g a.i ha ⁻¹ (2X dose)					Dissipation percentage (%)
	Residues ($\mu\text{g g}^{-1}$)						Residues ($\mu\text{g g}^{-1}$)					
	R1	R2	R3	Mean \pm SD	RSD (%)		R1	R2	R3	Mean \pm SD	RSD (%)	
Control	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-
0 (1 hour)	7.25	7.08	7.41	7.25 \pm 0.165	2.27	-	19.51	19.47	19.25	19.41 \pm 0.138	0.71	-
1	4.78	4.45	4.95	4.73 \pm 0.255	5.39	34.77	13.08	13.46	13.47	13.34 \pm 0.219	1.64	31.29
3	3.00	3.22	3.24	3.15 \pm 0.134	4.25	56.51	1.57	1.78	1.84	1.73 \pm 0.140	8.13	91.07
5	0.06	0.07	0.05	0.06 \pm 0.007	11.88	99.15	0.28	0.32	0.30	0.30 \pm 0.016	5.67	98.46
7	0.05	0.06	BDL	0.05 \pm 0.003	6.17	99.28	0.10	0.11	0.10	0.10 \pm 0.006	6.68	99.47
10	BDL	BDL	BDL	BDL	-	100.00	BDL	BDL	BDL	BDL	-	100.00

ND - Not Detected, BDL – Below Detectable Level (< 0.05 $\mu\text{g g}^{-1}$)

Similarly, the mean per cent recoveries in spinach at the same spiking levels were 109.02, 108.23, 99.78, 95.56 and 88.99 with RSD percentage of 1.39, 0.85, 3.15, 2.67 and 2.93, respectively.

The recovery percentage was in the acceptable range of 70 to 120 with RSD less than 20 (Table 2 and 3).

Persistence and dissipation of dimethoate residues in/on foxtail amaranthus and spinach

Persistence and dissipation pattern of dimethoate under field condition was studied in foxtail amaranthus and spinach. In foxtail amaranthus, the mean initial deposit (1 h after spraying) of dimethoate was found to be 7.25 $\mu\text{g g}^{-1}$ when sprayed at 200 g a.i ha⁻¹ (X

dose) and 19.41 $\mu\text{g g}^{-1}$ at 400 g a.i ha^{-1} (2X dose) (Table 4). In X dose, more than 90 per cent residues were lost on 5 DAT while it was 3 DAT in 2X dose

showing higher dissipation rate. However, in both doses the residues reached BDL ($< 0.05 \mu\text{g g}^{-1}$) on 10 DAT (Fig. 2).

Table 5. Persistence and dissipation of dimethoate 30 EC residues in/on spinach

Days after treatment	Dimethoate @ 200 g a.i ha^{-1} (X dose)					Dissipation percentage (%)	Dimethoate @ 400 g a.i ha^{-1} (2 X dose)					Dissipation percentage (%)
	Residues ($\mu\text{g g}^{-1}$)						Residues ($\mu\text{g g}^{-1}$)					
	R ₁	R ₂	R ₃	Mean \pm SD	RSD (%)		R ₁	R ₂	R ₃	Mean \pm SD	RSD (%)	
Control	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-
0 (1 hour)	16.52	18.74	19.82	18.37 \pm 1.371	7.46	-	28.54	30.60	29.22	29.46 \pm 0.859	2.91	-
1	10.43	11.28	11.81	11.18 \pm 0.568	5.08	39.11	18.65	18.62	21.11	19.47 \pm 1.167	5.99	33.91
3	8.85	8.62	9.46	8.98 \pm 3.947	3.94	51.10	10.98	11.40	11.27	11.22 \pm 0.173	1.54	61.91
5	2.67	2.76	2.73	2.76 \pm 2.445	2.44	84.99	3.25	2.99	3.17	3.14 \pm 0.106	3.38	89.33
7	1.75	1.87	1.90	1.85 \pm 3.714	3.71	89.95	2.88	3.07	3.05	3.01 \pm 0.082	2.75	89.79
10	0.83	0.82	0.88	0.85 \pm 1.926	1.92	95.37	1.14	1.40	1.45	1.33 \pm 0.141	10.62	95.48
15	BDL	BDL	BDL	BDL	-	100	0.83	0.82	0.88	0.85 \pm 0.029	3.44	97.11
20	BDL	BDL	BDL	BDL	-	-	BDL	BDL	BDL	BDL	-	100

ND - Not Detected, BDL – Below Detectable Level ($< 0.05 \mu\text{g g}^{-1}$)

The half-life value was less than a day (0.85 and 0.86) in both treatments. The dimethoate residues dissipated following first order kinetics with r^2 value of 0.8795 and 0.9821 at X and 2X dose. Since, Maximum Residual Limit (MRL) for dimethoate in foxtail amaranthus is not available under Food Safety

and Standard Authority of India (FSSAI), Codex Alimentarius Commission (CAC) MRL for cabbage ($0.05 \mu\text{g g}^{-1}$) was used to work out the safe waiting period. The calculated safe waiting periods were 3.99 and 4.73 days for X and 2X doses, respectively.

Table 6. Rate of residue decay and half-life of dimethoate 30 EC in foxtail amaranthus and spinach

Crop	Rate of application (g. a.i./ha)	Regression equation	Half life ($t_{1/2}$) (days)	Modified r^2
Foxtail amaranthus	200	$Y = -0.8149x + 2.3986$	0.85	0.66
	400	$Y = -0.8074x + 3.1194$	0.86	0.88
Spinach	200	$Y = -0.3129x + 2.8591$	2.22	0.96
	400	$Y = 0.0633x + 0.1714$	1.12	0.97

In spinach, the samples collected from plots treated with dimethoate @ 200 and 400 g a.i ha^{-1} , after one h of spraying showed mean initial deposits of 18.37 and 29.46 $\mu\text{g g}^{-1}$, respectively (Table 5). At

X dose, the dimethoate residues dissipated to more than 90 per cent on 10 DAT. At 2X dose, 95 per cent of dimethoate residues were lost within 10 DAT (Fig. 3).

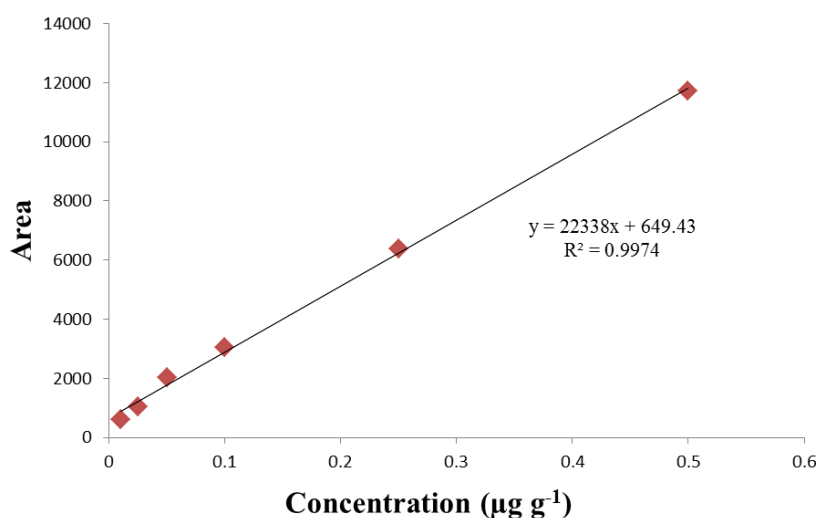


Figure 1. Linearity calibration of dimethoate in LCMS

(Linearity of dimethoate was calibrated in LCMS using six different concentrations from 0.01 to 0.5 $\mu\text{g g}^{-1}$ with R^2 value of 0.9974)

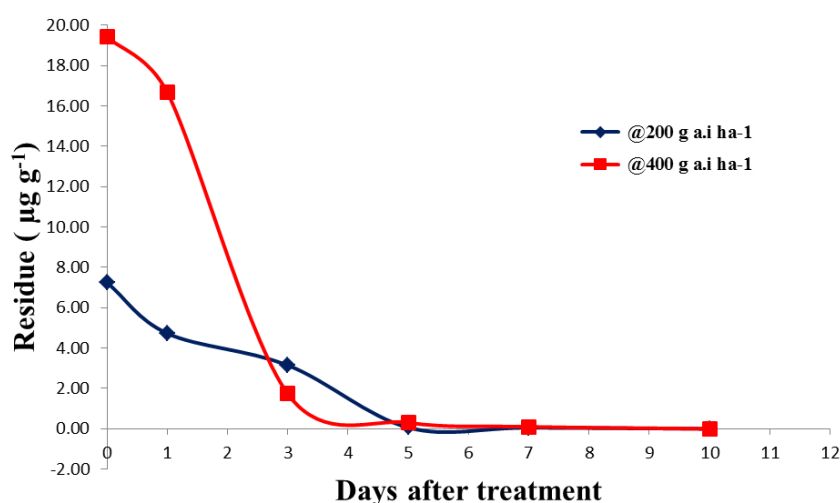


Figure 2. Degradation pattern of dimethoate residues in foxtail amaranthus
(The persistence of dimethoate residue was similar for both the treatments, which reach below detectable level on 10 DAT)

The residues reached BDL on 15 DAT in X dose and 20 DAT in 2X dose. The half-life of dimethoate residues in spinach were 2.22 and 1.12 days at X and 2X doses. (Table 6). The dimethoate residues dissipated following first order kinetics for X dose and 1.5th order kinetics for 2X dose with r^2 value of 0.9728

and 0.9759 at X and 2X dose, respectively. Since, there is no FSSAI MRL for dimethoate in spinach, the safe waiting period was calculated using CAC MRL for cabbage ($0.05 \mu\text{g g}^{-1}$). The calculated values were 7.02 and 8.35 days for X and 2X dose, respectively.

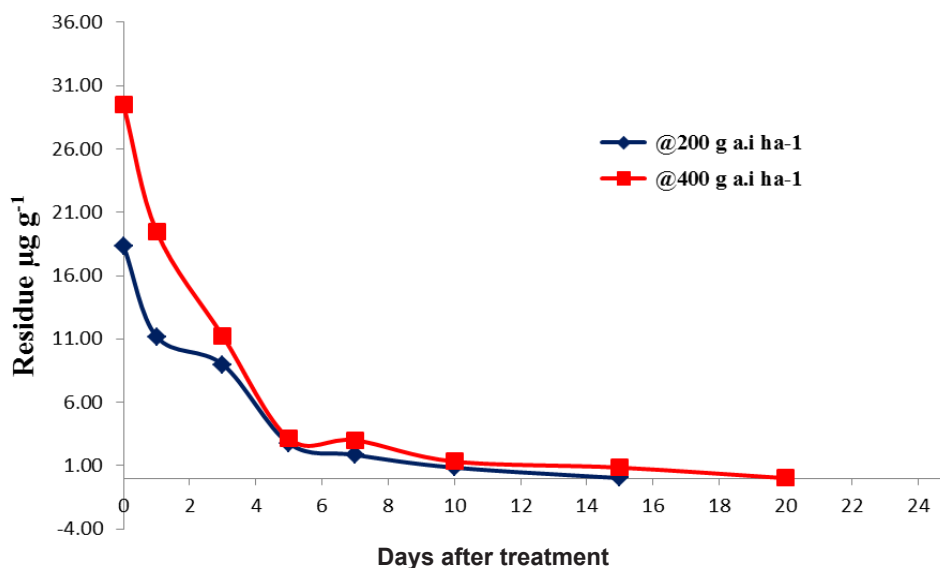


Fig. 3. Dissipation pattern of dimethoate residues in spinach

(The persistence of dimethoate residues was longer in higher dose [$400 \text{ g. a.i ha}^{-1}$] than recommended dose [$200 \text{ g. a.i ha}^{-1}$])

Similarly, in Chinese kale - a leafy vegetable, dimethoate dissipation rate was faster with 75 per cent loss of dimethoate residues on 7 DAT and more than 86.49 per cent loss on 10 DAT with half-life of 3.8 days (Chen *et al.*, 2017). In contrast, Hou *et al.* (2017) reported that more than 90 per cent of dimethoate residues were lost on 14 DAT with mean initial deposit of $15.25 \mu\text{g g}^{-1}$ in spinach.

The higher initial deposit of dimethoate in spinach than foxtail amaranthus can be attributed to the

possibility of varied level spray solution received due to differed leaf surface. The leaf surface is larger in spinach when compared to narrow leaf surface in foxtail amaranthus. Longer half-life of dimethoate in broccoli head when compared to leaf was attributed to larger surface area of broccoli head (Antonious *et al.*, 2007). The surface area plays an important role in deciding the initial deposit of residues and the dissipation in leafy vegetables (Lu *et al.*, 2014). In tea, initial deposit of dimethoate dissipated to 5.57

$\mu\text{g g}^{-1}$ with 83.8 per cent reduction on 3 days and reached BDL on 14 DAT (Pan *et al.*, 2015). Divakara *et al.* (2016) also reported that the persistence of chlorothalonil residue was longer in polyhouse condition than in field in spinach, which was due to the effect of UV spectrum on residues which caused maximum degradation of pesticides in field condition. The initial deposit reported was 111.98 and 110.23mg kg^{-1} @ 2 g/ L foliar spray under field and polyhouse condition, respectively. Nearly 80 per cent of residues were lost on 5 days after treatment with half-life of 2.03 and 2.12 days in field and polyhouse condition.

Conclusion

A method was validated for determination of dimethoate residues in foxtail amaranthus and spinach and the suitability of QuEChERS method was revealed by the acceptable range of recovery percentage (70 –120) and RSD (< 20). The persistence of dimethoate residues was found to be longer in spinach than foxtail amaranthus. The safe waiting period calculated for dimethoate were 3.99 and 4.73 days in foxtail amaranthus and 7.02 and 8.35 days in spinach for X and 2X dose, respectively.

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