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Dissipation Study of Thiophanate Methyl Residue in/on Grapes (*Vitis vinifera* L.) in India

Sudeb Mandal · Saktipada Das · Anjan Bhattacharyya

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Abstract A multi-location field trial was conducted in India during 2006–2008 to evaluate the dissipation pattern of thiophanate methyl (75% WP) in/on grapes at two application rates (500 and 1,000 g a.i. ha^{-1}). The quantitative analysis of the fungicide residues as carbendazim was performed using a UV/VIS spectrophotometer at the maximum absorption band of 281 nm. The average recovery was found 87% and the relative standard deviations (RSD) were below 3.8%. Following the first order kinetics the fungicide dissipates in grapes with a half-life ($t_{½}$) value of 4.74–6.52 days irrespective of locations and doses.

Keywords Thiophanate methyl residue · Dissipation · Grapes · UV/VIS Spectrophotometer

Thiophanate methyl [1, 2-bis-(3-methoxy carboxy-2-thioureidobenzene)] is a broad spectrum systemic fungicide which is widely used to control important fungal diseases of crops (Traina et al. 1998; Maranghi et al. 2003) which upon spraying on plant is quickly absorbed on its surface and easily transported to the various parts of the plant as methyl benzimidazol carbamate (MBC). MBC is actually responsible for the fungicidal activity of this chemical (Buchenauer et al. 1973a, b). Biochemical transformations in presence of plant enzyme or sunlight are responsible for the conversion of thiophanate methyl to MBC (Buchenauer et al. 1973a, b; Soeda et al. 1972) (Fig. 1). MBC is a chemically stable metabolite and relatively persistent fungicide, which only metabolised to a limited extent in plants and soil. Thiophanate methyl can be determined in the form of MBC after conversion (Muccio et al. 1995). The principle of the most cost effective analytical method used for supervised residue trials is that thiophanate-methyl and carbendazim are extracted and thiophanate-methyl is converted to carbendazim by refluxing under acidic conditions. Then the carbendazim is determined by UV/VIS spectrophotometer (Ono 1973). It is used for the protection of various crops including grapes, lettuce, citrus fruits, potatoes, and cereals.

Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production of around 69 million tons in 2006 (FAOSTAT 2007), contain large amounts of phytochemicals including anthocyanins and resveratrol, which offer health benefits (Pezzuto 2008), suffers yield losses due to fungal diseases. Because benzimidazole fungicides in food constitute a significant health risk (Banks and Soliman 1997; Urani et al. 1995) and grapes undergo a high consumption rate, the analysis of thiophanate methyl as well as carbendazim residues in grapes is of great importance. The dissipation of any compound depends on various factors, including plant species, chemical formulation, climatic conditions, physical phenomenon (mainly volatilization), application method and chemical degradation, in which sunlight plays a prominent role. Therefore, dissipation studies for a given crop in the specific conditions of each growing area are necessary. Residues of thiophanate methyl on tomatoes and cucumber under field and protected cultivations were reported by Abdel Megeed et al. (2000). Dutta et al. (1995) reported the

S. Mandal · S. Das
Department of Chemistry, University of Kalyani,
Kalyani 741235, West Bengal, India
e-mail: sudeb_ku@rediffmail.com

A. Bhattacharyya (✉)
Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, West Bengal, India
e-mail: anjan_84@rediffmail.com

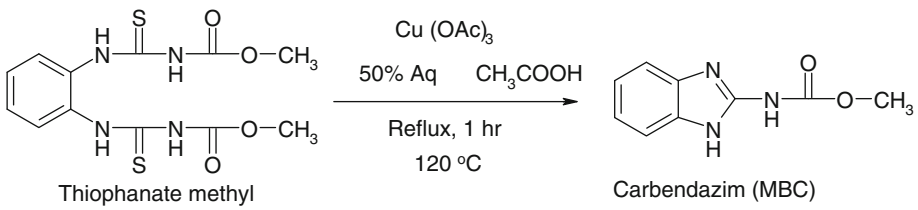

Fig. 1 Conversion of thiophanate methyl to carbendazim (MBC)

dissipation study of thiophanate methyl on mango at Mondouri Horticultural Research Station, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India. A literature survey reveals that no work has been published on the dissipation of thiophanate methyl residue on grapes in Indian condition. The objective of the present work was to study the dissipation and the fate of thiophanate methyl residue in/on grapes grown under the different agroclimatic conditions of India.

Material and Method

A field study was conducted in four different locations in India viz., (1) Grapes & Onion Research Station, M.P.K.V, Pimpalgaon, Baswant, Nasik (Variety-Thomson seedless) (2) Farmers name: Srinivasa Choudhary, Village: Chirala Mandal Kisara, District: Ranga Reddy, Hyderabad, Andhra Pradesh, (Variety-Thomson seedless), (3) Agricultural college and Research Institute, Department of Plant Pathology, Madurai, Tamilnadu, (Variety-Thomson seedless) and (4) Department of Horticulture, GKVK Campus, Bangalore, Karnataka, (Variety-Bangalore Blue) were taken into account and the experiment was conducted in randomized block design in three replicates (each replicate considered as five plant) during 2006–2008. The formulation of thiophanate methyl 75% WP was applied in the recommended dose i.e., 500 g a.i. ha^{-1} (T_1) and double the recommended dose i.e., 1,000 g a.i. ha^{-1} (T_2). For the dissipation study of thiophanate methyl in grapes, samples were collected periodically at different time interval 0 (2 h post application), 1, 3, 5, 7 and 15 days after the application. About 0.5 kg of the grape was harvested from each replicate of both the treatment and control plots and brought to the laboratory each time. The samples were transported from respective location in a thermocol box with dry ice packing and extracted immediately after receipt as far as practicable. Whenever not possible, the samples were stored in a cold room at −18°C for a minimum period of time.

An analytical standard of Carbendazim (purity 99.90%) was supplied by M/S Biostadt India Ltd, Mumbai, India. High purity solvent (chloroform), sodium hydroxide pellets and hydrochloric acid that were used in the extraction and clean up, were purchased from M/S Spectrochem, India. The other reagents viz., reagent grade anhydrous sodium sulphate, acetic acid and cupric acetate of M/S Qualigen, India were used. Glass distilled water was used in the entire study. pH meter was CL 46 type (Toshniwal Instruments, Pvt, Ltd, India). A high-speed commercial Remi automix blender with stainless steel container and rotary vacuum evaporator of Eyela (SB-1000, Japan) were used in this study.

The chopped grapes sample (50 g) was taken in 250 mL conical flask & then added 4 g of anhydrous sodium sulfate and blended thrice (3 min) with (100 + 50 + 50) mL of chloroform in remi automix blender. The content was then filtered and passed through anhydrous sodium sulphate. The solvent was evaporated to dryness in a rotary vacuum evaporator at 40°C. The residue was dissolved with 10 mL aqueous solution of 50% acetic acid and added 100 mg cupric acetate and a piece of pumic stone in a round bottom flask which was fitted with condenser and heated in oil bath at 120°C for 1 h. After cooling, 10 mL of 1(N) HCl was poured into the flask through the condenser and contents of the flask were transferred to a 100 mL separating funnel with additional 20 mL of 1(N) HCl. The acidified solution was washed thrice with 10 mL portion of chloroform. The chloroform layer was discarded and the acidified solution was transferred to a beaker and the pH was adjusted to 6.0–6.2 by adding 2 (N) NaOH solution using a pH meter. After neutralization, the solution was transferred to a 150 mL separatory funnel and then extracted with 30 mL of chloroform by shaking for 5 min. The chloroform extract was taken into a 100 mL separatory funnel and extracted with 20 mL of 1(N) HCl by shaking for 5 min.

The quantitative analysis of thiophanate methyl residues as MBC was performed with a Varian CARY 50 UV/VIS spectrophotometer which is controlled by Cary WinUV software at the maximum absorption band at 281 nm. The limit of detection (LOD) and limit of quantification (LOQ) were found 0.1 and 0.3 μg/g respectively. A calibration curve (Fig. 2) was constructed by plotting five concentrations (0.1–2.5 μg/g) of standard MBC versus absorption. Determining the concentration in μg/g of MBC in samples from calibration curve, then it was calculated the concentration in μg/g of thiophanate methyl residues according to the following equation:

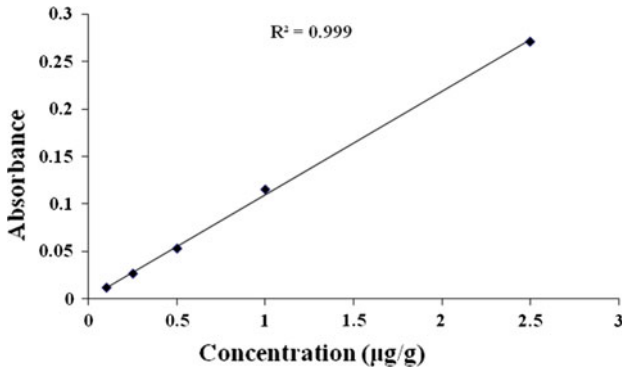

Fig. 2 Calibration of carbendazim (MBC) at 0.1–2.5 µg/g level

$$\text{Concentration of thiophanate methyl } (\mu g/g) = \frac{\text{Concentration of MBC}(\mu g/g)}{\text{g/mL sample}} \times 1.79$$

[Where 1.79 is the conversion factor for MBC to thiophanate methyl]

$$\text{Correction factor} = \frac{\text{Molecular weight of thiophanate methyl}}{\text{Molecular weight of MBC}} = 1.79$$

The efficiency of extraction and clean up method was checked by recovery study. Control samples were fortified at the level of 0.3, 1 and 2 µg/g with analytical standard of MBC (purity 99.90%). The average recovery of MBC was found to be 87.0% for grape samples and the relative standard deviations (RSD) were below 3.8%.

Results and Discussion

The residue data in the present study, at different days interval, dissipation percentage, regression equation and half life values in grapes samples in four different locations have been presented in Tables 1, 2, 3, 4. A straight line was found when a log of residue was plotted against time and values of coefficient of determination (R^2) in all four locations varies from 0.900 to 0.999, thereby establishing that first order reaction kinetics was involved in the dissipation process. The initial deposits (2 h after spraying) of thiophanate methyl in grapes in Nasik were found 1.81 µg/g (T_1) and 3.18 µg/g (T_2) and the half-life values ($t_{½}$) were found to be 4.93 days (T_1) and 6.52 days (T_2). The initial deposits of thiophanate methyl in grapes in Hyderabad were found 1.85–3.12 µg/g irrespective of doses and the half-life values ($t_{½}$) were found to be 5.91 days (T_1) and 6.38 days (T_2). The lowest initial deposits of thiophanate methyl in grapes were found in Madurai samples which ranges 1.79–3.00 µg/g and the half-life values ($t_{½}$) were found sample 4.74 and 5.67 days corresponding to the T_1 and T_2 respectively. The highest initial deposits of thiophanate methyl in grapes were found to ranges 2.06–3.54 µg/g and the half-life values ($t_{½}$) were found to be 5.15 and 5.81 days corresponding to the T_1 and T_2 respectively in Bangalore samples. More than 50% of the initial deposit was dissipated within 7 day irrespective of any locations and doses. The dissipation patterns as well as half life values of the present study are in well agreement with the earlier studies conducted in tomato, cucumber and mango (Abdel Megeed et al. 2000; Dutta et al. 1995).

Thus, it may be concluded that the dissipation of thiophanate methyl in four fields conformed to first order kinetics in respect of any locations and doses. The initial deposit of thiophanate methyl ranges from 1.79 to 3.54 µg/g and more than 50% of the residue was degraded in Grape samples within 7 days irrespective of any location and treatment. The initial deposits were found to be less than its Indian maximum residue limit (5 µg/g for apple)

Table 1 Persistence of thiophanate methyl in grapes of Nasik

Days after application	Residue in µg/g [M* ± SD] (% of dissipation)	
	T_1 (500 g a.i. ha^{-1})	T_2 (1,000 g a.i. ha^{-1})
0	1.81 ± 0.05 (–)	3.18 ± 0.06 (–)
1	1.77 ± 0.02 (2.03)	2.86 ± 0.08 (10.22)
3	1.43 ± 0.03 (20.81)	2.33 ± 0.12 (26.81)
5	0.98 ± 0.04 (45.86)	1.91 ± 0.06 (39.94)
7	0.71 ± 0.02 (60.59)	1.47 ± 0.12 (53.77)
15	BDL (–)	BDL (–)
Regression equation	$Y = 3.2973 - 0.061X$	$Y = 3.5017 - 0.0462X$
Half life ($t_{½}$)	4.93 days	6.52 days

BDL below detectable limit, *M** mean of three replicate

Table 2 Persistence of thiophanate methyl in grapes of Hyderabad

Days after application	Residue in µg/g [M* ± SD] (% of dissipation)	
	T_1 (500 g a.i. ha^{-1})	T_2 (1,000 g a.i. ha^{-1})
0	1.85 ± 0.65 (–)	3.12 ± 1.10 (–)
1	1.67 ± 0.24 (9.91)	2.66 ± 0.59 (14.85)
3	1.48 ± 0.54 (19.82)	2.37 ± 0.22 (23.93)
5	1.06 ± 1.39 (42.88)	1.87 ± 1.11 (40.06)
7	0.81 ± 2.19 (56.04)	1.38 ± 1.99 (55.88)
15	BDL (–)	BDL (–)
Regression equation	$Y = 3.282 - 0.050X$	$Y = 3.489 - 0.047X$
Half life ($t_{½}$)	5.91 days	6.38 days

BDL below detectable limit, *M** mean of three replicate

Table 3 Persistence of thiophanate methyl in grapes of Madurai

Days after application	Residue in μg/g [M* ± SD] (% of dissipation)	
	T_1 (500 g a.i. ha^{-1})	T_2 (1,000 g a.i. ha^{-1})
0	1.79 ± 0.63 (–)	3.00 ± 1.06 (–)
1	1.59 ± 0.21 (11.03)	2.56 ± 0.55 (14.83)
3	1.18 ± 0.65 (33.94)	2.34 ± 0.23 (22.17)
5	0.87 ± 1.46 (51.40)	1.89 ± 1.10 (37.08)
7	0.65 ± 2.25 (63.55)	1.18 ± 2.06 (60.75)
15	BDL (–)	BDL (–)
Regression equation	$Y = 3.2573 - 0.0635X$	$Y = 3.492 - 0.0531X$
Half life ($t_{½}$)	4.74 days	5.67 days

BDL below detectable limit, *M** mean of three replicate

Table 4 Persistence of thiophanate methyl in grapes of Bangalore

Days after application	Residue in μg/g [M* ± SD] (% of dissipation)	
	T_1 (500 g a.i. ha^{-1})	T_2 (1,000 g a.i. ha^{-1})
0	2.06 ± 0.73 (–)	3.54 ± 1.25 (–)
1	1.89 ± 0.31 (8.41)	2.89 ± 0.63 (18.64)
3	1.57 ± 0.51 (23.79)	2.46 ± 0.20 (28.25)
5	1.16 ± 1.36 (43.69)	1.84 ± 1.12 (52.26)
7	0.80 ± 2.19 (61.17)	1.53 ± 1.94 (56.21)
15	BDL (–)	BDL (–)
Regression equation	$Y = 3.3373 - 0.0585X$	$Y = 3.5335 - 0.0518X$
Half life ($t_{½}$)	5.15 days	5.81 days

BDL below detectable limit, *M** mean of three replicate

value fixed as by Ministry of Health & Family Welfare, Govt. of India (Anonymous 2004). Interestingly thiophanate methyl residue was not detected in all the samples collected at 15th day after application irrespective of any location.

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