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PERSISTENCE AND DEGRADATION BEHAVIOUR OF DIMETHOATE IN GRAPES

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ABSTRACT

Through a field experiment, the dissipation pattern of dimethoate in grapes was evaluated during December 2020 - February 2021. Dimethoate 30%EC was sprayed twice at ten-days interval at recommended (X) dose (445 g a.i ha^{-1}) and double the recommended (2x) dose (890 g a.i ha^{-1}). The samples were collected from 0 (2hr), 1, 3, 5, 7, 10, 15 and 20 days or till degradation to below detectable level (BDL) and at harvest time. The residues were extracted by the modified QuEChERS method and analyzed by LCMS. The method performance was satisfactory in terms of SANTE guidelines and with good linearity (r^2>0.99). The mean total dimethoate residue including omethoate was 1.047 and 2.168 mg kg^{-1} at x and 2x doses, respectively with half-lives of 5.47 and 5.59 days. The calculated Risk Quotient (RQ) at both x and 2x dose with Good Agricultural Practices (GAP) indicated that for dimethoate these are not safe for human health due to the intake of residue.

Key words: Grapes, dimethoate, omethoate, method validation, QuChERS, dissipation, risk assessment, risk quotient, acceptable daily intake, maximum residue level

Organophosphorus (OP) pesticides account for 34% of global insecticide consumption and are the most extensively used broad spectrum insecticides. There are around 100 different OP pesticides that are used to control pests in horticultural crops (Jaipieam et al., 2009). Among the OP pesticides, dimethoate [O, O-dimethyl S-(2-(methylamino)-2-oxoethyl) phosphorodithioate] is a broad spectrum systemic insecticide largely used to manage pests in fruits and vegetables (Zheng and Sun, 2014). Dimethoate is transformed to omethoate in crops due to the oxidation process, which is more harmful than dimethoate itself (EFSA, 2013).

In India, dimethoate is registered for control of aphids, mealybugs, hoppers and stem borer in various fruit crops viz., mango, banana, citrus, apple, fig and apricot but not registered for use in grapes (CIBRC, 2021). However, a survey conducted in major grape growing districts of Tamil Nadu revealed that 93.33% of farmers are using dimethoate as a plant protection input to mitigate the pest problem in grapes (Jayabal et al., 2020). Dimethoate dissipation has been widely investigated in various fruit crops viz., mango (Bhattacherjee and Dikshit, 2016), pomegranate (Utture et al., 2012) and guava (Khan et al., 2009; Devi et al., 2016). It is one of the most widely used insecticides in Indian viticulture and globally for pest management (Moyer and Neal, 2014; Patil et al., 2017; Preetha and Stanley, 2020).

In a desperate bid to save the crop, farmers often take intensive sprays of dimethoate at the berry initiation stage which is likely to leave toxic residues in/ on berries and may be unsafe to the consumers. The consumption of insecticide treated product become risky due to the residual persistence of the insecticide. So, it is necessary that insecticide should be active against pests while leaving only tolerable residue on food commodity. OPs are common pollutants that pose significant toxicological risks to soil, aquatic ecosystems, and human health (Ji et al., 2019). Therefore, it is important to understand how pesticide residues dissipate at various stages in order to ensure the supply of safe and fresh produce to the end users.

To our knowledge, there are no published reports in India on the dissipation of dimethoate and its metabolite omethoate in grapes. Therefore, a field study was conducted to monitor the degradation pattern of dimethoate in response to the above issue. Using Indian dietary data, the preharvest interval (PHI) and dietary risk assessment were calculated.

MATERIALS AND METHODS

For the estimation of dimethoate and omethoate residues, certified reference material (CRM) of dimethoate (99.5%), omethoate (96.8%), anhydrous sodium citrate dibasic sesquihydrate (≥ 99% purity) (Sigma Aldrich, Bangalore, India); HPLC grade of ethyl acetate (≥ 99.9%), n-hexane (≥ 99.9%) (Sisco Research Laboratories, Mumbai, India); LCMS grade methanol (> 99.9%) (Avantor Sciences, PA, USA); Anhydrous sodium chloride (≥ 99%), anhydrous tri-sodium citrate dihydrate (≥ 99%) (Merck, Mumbai, India); anhydrous magnesium sulphate (≥ 99%) (Himedia Laboratories, Mumbai, India); primary secondary amine (PSA, 40 µm) and graphitised carbon black (GCB) (Agilent Technologies, USA); and formic acid (≥ 99%) (Fisher Scientific Limited, Czech Republic) were obtained. The commercial formulation of dimethoate 30% EC was purchased locally from a pesticide dealer in Coimbatore, Tamil Nadu, India.

Individual stock solutions of 400 mg/l dimethoate and omethoate were made in LCMS grade methanol by independently weighing 10.05 and 10.33 mg of the analytical standards into a calibrated glass A volumetric flask (25 ml). Dimethoate and omethoate secondary stock solutions (40 mg/ l) were made separately from the stock solution in a 25 ml container by transferring 2.5 ml of each. A working standard mixture of 10 mg/ l was prepared using secondary stock solution. The linearity and spiking standard solutions were obtained by serial dilution from the mixed standard solution in the range of 0.005 - 0.1 mg/ l. All standard solutions were maintained at -20°C in the deep freezer until use. Matrix matched standard solutions (0.01, 0.025, 0.05, 0.075, and 0.1 mg/ l) were prepared with immature and mature grape matrix.

Dissipation study was carried out in a farmer's field in Theni district of Tamil Nadu, India (9^o N, 76^o E and 375 masl) following good agricultural practices. The plot size was $50m^2$ area that had no application of dimethoate before taking up the study, with three replicated plots. The commercial product of dimethoate 30% EC was applied (Muscat Hamburg variety) at the berry initiation stage (45 days after flowering) at the recommended dose (445 g a.i ha^{-1}) and double the recommended dose (890 g a.i ha^{-1}). A high-volume sprayer with 500 l ha^{-1} of spray fluid, was used and two foliar sprays were given at an interval of 10 days for dissipation study. During the field trial, maximum and minimum temperatures were recorded as 28.16 and 19.16^o C, respectively and relative humidity was 78.16%. No rainfall was received.

The grape berries for residue analysis (0.5 kg) were randomly picked from vines from each replication at 0 (2hr), 1, 3, 5, 7, 10, 15, 20, 25, 30 and 35 days after second spraying and at the time of harvest. A high-volume blade homogeniser was used to homogenise samples using a Robot Coupe cutter mixer (Blixer 6 VVA, France) and stored at -20^oC for residue analysis,

A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique was adapted, validated and used for residue extraction (Anastassiades et al., 2003). 10 g of grape sample was added in a 50 ml centrifuge tube and vortexed for one min after adding 20 ml of ethyl acetate. After that, 1 g NaCl and 4 g anhydrous MgSO4 were added, vortexed and centrifuged completely for 10 min at 6000 rpm. Following centrifugation, a 6 ml aliquot of the supernatant was transferred to a 15 ml centrifuge tube containing 100 mg PSA, 600 mg anhydrous MgSO4, and 10 mg GCB and vortexed for one min before centrifugation at 3000 rpm for ten min. In a low volume concentrator at 40^oC, 2 ml of aliquot was evaporated to near dryness under a gentle stream of nitrogen gas. Finally, the residue was mixed in 1.0 ml methanol and filtered by using a 0.2 µm membrane syringe filter and transferred into 1.0 ml autosampler vials for analysis of residues by LC-MS.

The detection, estimation and confirmation of dimethoate and omethoate were done in Shimadzu 2020 series LCMS equipped with a reverse phase C18 (Eclipse plus- Agilent) column (250 mm length x 4.6 mm id, 5 µm particle size) at a column oven temperature of 40˚C. The mobile phase used for the separation of target compounds were methanol and ultra-pure water with 0.05 % formic acid in the ratio of 50:50. The LCMS pump was run in binary mode at a pressure of 48 kgf/ cm^2 to discharge the mobile phase at a constant isocratic flow rate of 0.5 ml/ min. The chromatograms were analysed using Shimadzu lab solutions software (5.6) and further calculations were based on obtained peak areas in the chromatograms. Samples were ionized using positive electron spray ionization (ESI+) mode and 0.1 μA^o of interface current, 350^o C of heat block temperature, 250^oC as desolvation line (DL) temperature, nebulizer gas (N_2-99.99%) flow of 1.5 l/ min, drying gas of 12 l/min and scan speed of 15000 sec were employed.

The method used to determine the residues of dimethoate and omethoate in grape matrices was validated as per SANTE guidelines (SANTE, 2019) and evaluated with parameters viz., linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, precision, repeatability and matrix effect. The Horwitz ratio (HorRat) is a measurement tool that reflects the acceptability of analytical processes in terms of interlaboratory precision (reproducibility). It is the ratio of the observed relative standard deviation among laboratories (RSD), to the corresponding predicted relative standard deviation (PRSD), calculated using the Horwitz equation PRSD = $2C^{(-0.15)}$, where C is the concentration found or added, expressed as a mass fraction and PRSD was calculated for all the fortified concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1ppm (Horwitz and Albert, 2006). The matrix effect (ME) was assessed using the formula mentioned by (Dong et al., 2018).

The amount of dimethoate residue (log value) over time (days after application) was subjected to weighted linear regression to calculate half-life and safe waiting period values of dimethoate (Hoskins, 1961; Handa et al., 1999). Half-life of dimethoate was determined as $T_{1/2}$ = 0.693/k. The maximum residue limit (MRL) for dimethoate in grapes was 0.01 mg/kg as per the European pesticide (EU) database, 2020. The safe waiting period of dimethoate was calculated using the formula mentioned as PHI = [log (A)-log (MRL)]/K

EDI was divided by the acceptable daily intake (ADI) to arrive the risk quotient (RQ), which was used to quantify the long-term risk of intakes in comparison to pesticide toxicological data (Dong et al., 2018). ADI value of dimethoate is 0.002 mg/ kg/ bw/ day (EFSA, 2013). The average body weight of an Indian (male- 65kg and female- 55kg) adult (NIN, 2020) and recommended total fruit consumption is 150 g/ day (NIN, 2020). If the RQ is <1, the risk of long-term human dietary consumption of dimethoate is acceptable; if the RQ is >1, the risk is unacceptable.

RESULTS AND DISCUSSION

Instrument conditions were optimised using single quadrupole LC-MS to identify, confirm, and quantify dimethoate and its metabolite omethoate in grapes. By setting standard chromatographic conditions, dimethoate and omethoate were separated and eluted at retention times of 8.47 and 5.31 min, respectively (Fig. 1). Quantification of dimethoate and omethoate was carried out using selected ion monitoring (SIM) mode and sensitivity was increased by using positive (+) SIM mode with target m/z at 230 and 214 for dimethoate and omethoate, respectively.

The recovery %, RSD, and linearity of the analytical

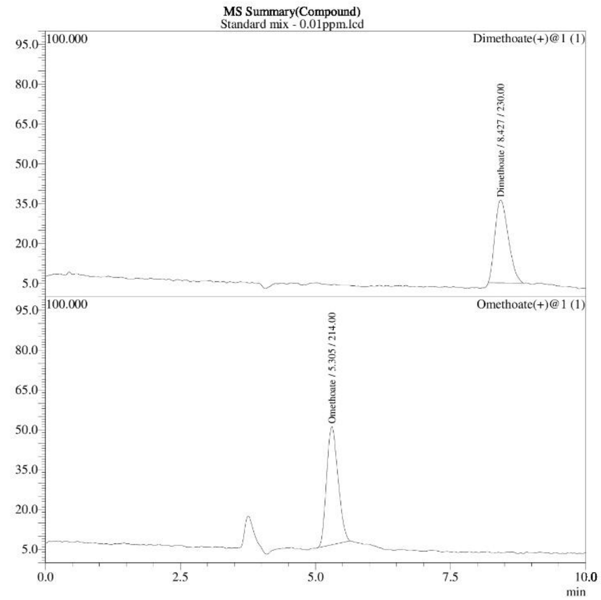

Fig. 1. Standard chromatogram of dimethoate + omethoate (0.01ppm)

method used to estimate the residues of dimethoate and omethoate were calculated and validated. In both immature and mature grape matrices, the method's linearity was determined to be between 0.005 to 0.1 mg/l. For both solvent and matrix-matched calibration standards, the correlation coefficient of dimethoate and omethoate was >0.99 (Table 1). The LOD (at a signal-to-noise ratio greater than 3) and LOQ (at a signal-to-noise ratio greater than 10) were determined as 0.005 and 0.01 mg/kg, respectively. The proposed LOQ (0.01 mg/ kg) of the method was in agreement with the MRL (0.01 mg/ kg) value fixed by EU pesticide database. Dimethoate and omethoate recovery in grapes was between 70 and 120% confirming to SANTE (2019) guidelines. RSD (<20%), matrix effect (<20%), horwitz ratio (0.5-2.0) were in acceptable range validating the analytical method (Table 1).

Residues of dimethoate reached BDL on 30 and 35 days after spraying at recommended (X) and double the recommended dose (2X), respectively (Table 2). Omethoate residues reached BDL 7 days after spraying. At harvest time samples, neither dimethoate nor omethoate was detected (45 days after spraying). Total residue (dimethoate + omethoate) was used to determine the half-life values and confirmed as 5.47 and 5.59 days at x and 2x doses, respectively. Reports on the dissipation of dimethoate in fruit crops like mango, guava, citrus, pomegranate are documented and available but could not get for grapes. Pappas et al. (2003) reported dissipation as 16.7 and 30.1 days

Table 1. Recovery, linearity parameters and matrix effect of dimethoate and omethoate in grape matrices

Pesticides	Calibration (matrix)	Calibration range (mg/ l)	Regression equation	Correlation coefficient (R^2)	Matrix effect (%)
Dimethoate	Solvent	0.005-0.1	y = 2E+07x + 39129	0.999	-
	Immature grapes	0.005-0.1	y = 2E+07x + 15789	0.9981	4.09
	Mature grapes	0.005-0.1	y = 2E+07x - 42273	0.9962	1.68
Omethoate	Solvent	0.005-0.1	y = 2E+07x + 36193	0.9993	-
	Immature grapes	0.005-0.1	y = 3E+07x + 13261	0.9972	3.56
	Mature grapes	0.005-0.1	y = 2E+07x - 4107.6	0.9985	6.79

Recovery of dimethoate and omethoate in grape matrix at different spiking levels (n=7)

Matrix	Spiked concentration (mg/ kg)	Dimethoate Recovery (%)± SD	Dimethoate RSD (%)	Dimethoate HorRat	Omethoate Recovery (%) ± SD	Omethoate RSD (%)	Omethoate HorRat
Immature grape berries	0.010	98.79± 2.49	2.52	0.08	96.59± 2.28	2.36	0.07
	0.025	93.55± 1.92	2.06	0.07	92.16± 2.71	2.94	0.11
	0.050	102.31± 1.64	1.60	0.06	103.05± 2.34	2.27	0.09
	0.075	97.92± 2.01	2.05	0.09	95.06± 1.80	1.89	0.08
	0.100	95.44± 2.90	3.04	0.14	95.61± 2.20	2.30	0.10
Mature grape berries	0.010	95.68± 1.72	1.80	0.06	85.28± 3.09	3.62	0.11
	0.025	90.10± 1.50	1.66	0.06	98.58± 1.48	1.51	0.05
	0.050	85.04± 1.68	1.98	0.08	93.54± 1.96	1.02	0.04
	0.075	92.08± 2.61	2.83	0.12	97.82± 2.88	2.94	0.13
	0.100	95.28± 3.17	3.33	0.15	94.19± 1.91	2.02	0.09

SD- standard deviation; RSD- relative standard deviation, HorRat- Horwitz ratio

Table 2. Residues, dissipation and dietary risk assessment of dimethoate in immature grape berries at 445 g a.i ha^{-1} (X) and 890 g a.i ha^{-1} (2X)

x dose						2x dose					
Days after treatment	Dime-thoate residues* (mg/ kg)	Ome-thoate residues* (mg/ kg)	Total residues (mg/ kg)	Dietary risk assessment Risk quotient (RQ) Adult male (65 kg)	Adult female (55 kg)	Days after treat-ment	Dime-thoate residues* (mg/ kg)	Ome-thoater residues* (mg/ kg)	Total residues (mg/ kg)	Dietary risk assessment Risk quotient (RQ) Adult male (65 kg)	Adult female (55 kg)
0 (2hrs)	1.012	0.035	1.047	1.20	1.43	0 (2hrs)	2.107	0.061	2.168	2.50	2.96
1	0.607	0.026	0.633	0.73	0.86	1	1.098	0.051	1.149	1.32	1.56
3	0.527	0.022	0.549	0.63	0.75	3	0.802	0.035	0.837	0.96	1.14
5	0.385	0.011	0.396	0.46	0.54	5	0.632	0.028	0.660	0.76	0.90
7	0.272	BDL	0.272	0.31	0.37	7	0.567	0.014	0.581	0.67	0.80
10	0.162	BDL	0.162	0.19	0.22	10	0.337	BDL	0.337	0.39	0.46
15	0.099	BDL	0.099	0.11	0.13	15	0.295	BDL	0.295	0.34	0.40
20	0.078	BDL	0.078	0.09	0.11	20	0.205	BDL	0.205	0.24	0.28
25	0.044	BDL	0.044	0.05	0.06	25	0.091	BDL	0.091	0.10	0.12
30	0.014	BDL	0.014	0.01	0.01	30	0.051	BDL	0.051	0.06	0.07
35	BDL	BDL	BDL	-	-	35	0.010	BDL	0.010	0.01	0.01
Kinetic equation	y = 0.055x + 2.8862					y = 0.0538x + 3.1818					
R^2 value	0.974					0.947					
Half-life	5.47 days					5.59 days					
PHI	36.72 days					43.42 days					

BDL-Below Detectable Level (<0.01 mg/ kg); *Mean of three replicates, RQ- Risk Quotient in lemons and mandarins, respectively, 5.75 - 7 days in pomegranate (Utture et al., 2012), two days in mango (Bhattacharya and Diskhit, 2016) and 2.8 - 8.15 days in guava (Khan et al., 2009; Devi et al., 2016). The variation in the degradation pattern of dimethoate in different crops is due to the association of pesticide chemistry, plant architecture and environmental conditions in that particular crop ecosystem.

Risk assessment is the process of identifying potential threats and related hazards to life and health due to long term human exposure to chemicals found in food. The implication of total dimethoate residues including omethoate in grapes was evaluated by calculating risk quotient. The RQ value was >1 at both doses on the day of application and first day after spraying which indicated that grapes harvested on particular sampling days were not safe according to the calculated risk quotient value (RQ>1) under Tamil Nadu agroclimatic conditions.

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