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Persistence and Dissipation of Dichlorvos and Profenofos on Mulberry Leaves

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Persistence and dissipation properties of dichlorvos and profenofos in/on mulberry leaves were studied. Dichlorvos (76 WSC) and profenophos (50 EC) were sprayed at recommended dose (2 mL/L) and double the dose (4 mL/L) and leaf samples were drawn at different intervals in triplicate and residues derived from the QuEChERS method were quantified on gas chromatography-electron capture detector (GC-ECD). The initial deposits of dichlorvos and profenofos were between 1.18 to 2.79 and 2.19 to 4.72 µg g^{-1} in the recommended and double the dose, respectively. Dichlorvos dissipated to 0.03 (98.8%) and 0.031 µg g^{-1}(97.38%) in recommended and double the doses, respectively three days after spray. Residues of profenofos dissipated to the extent of 95.8% and 94.30%, respectively, at recommended and double doses in 15 days. Dissipation pattern followed first order kinetics for both the insecticides with half life periods of 0.52 and 4.29 days for dichlorvos and profenophos, respectively. Present studies suggest that the use of dichlorvos and profenofos at the recommended doses does not pose any hazard to silkworm, if waiting periods of 2.89 and 32 days respectively,were observed before harvesting mulberry leaves to feed silkworms.

Key words: Dichlorvos, profenofos, residues, GC-ECD, mulberry, QuEChERS

India is the second largest producer of silk in the world next to China. Mulberry (*Morus* spp.) is an important crop because silk production depends on the quality of the leaves, but this crop is attacked by many insects which require frequent use of insecticides. The use of profenofos, imidacloprid, acephate, dimethoate and dichlorvos may achieve a measure of control of mealybug in mulberry plant (Mahalingam *et al.,* 2010), but residual toxicity in the treated plants results in reduced palatability of the leaves to silkworm larvae, reduction in larval growth and silk production.

Therefore, it is essential to know the extent to which the pesticide residue remains on mulberry leaves after application. Recently, quick, easy, cheap, effective, rugged and safe (QuEChERS) sample preparation approach (Anastassiades *et al.,* 2003) has been used around the world in many studies for pesticide residue analysis in different matrix samples (Lehotay *et al.,* 2005 ; Aysal *et al.,*2007; Nguyen *et al.,*2008; Paramasivam and Banerjee, 2011). The method has gained popularity among pesticide residue scientists due to its inherent advantages such as speed, low cost and wide applicability. So far no research has been conducted on persistence and dissipation of dichlorvos and profenofos on mulberry leaves under Indian climatic conditions. In order to provide scientific basis for setting up a safe plucking time of leaves of mulberry and to determine the residues and degradation dynamics of dichlorvos and profenofos on mulberry leaves, the current study was carried out using QuEChERS method.

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Materials and Methods

Analytical reference standards of dichlorvos (99.1% purity) and profenophos (99.6% purity) were purchased from Sigma -Aldrich and commercial formulation of dichlorvos (76 WSC) and profenofos (50 EC) were purchased from the authorized dealers in Coimbatore, Tamil Nadu. All the other chemicals and solvents were from M/s. E Merck, Mumbai, (India) and were of analytical grade and the Primary secondary amine (PSA, 40µm, Bondesil) was purchased from M/s. Varian (India).

Stock solutions were prepared by dissolving appropriate quantities of dichlorvos and profenofos in HPLC grade hexane. Fortification levels and calibration solutions for GC analysis were prepared by diluting the stock solution using hexane to concentrations 0.01 to 1 µg mL^{-1}. Fortification and calibration standard solutions were stored at -4°C.

The experimental trial was conducted in a randomized block design (RBD) on standing mulberry plant at the experimental farm of Tamil Nadu Agricultural University, Coimbatore. The commercial formulations of dichlorvos and profenofos at the recommended doses (2 ml/L) and

double the doses (4ml/L) were sprayed onto mulberry plants using a knapsack sprayer. No other pesticide sprays were given. Two treatments and one untreated plots in triplicate was laid out. For every treatment 10 plants were treated per replication. Random samples of approximately 500 g were collected at each sampling date, at 0 (2 h), 1, 3 and 5 days for dichlorvos and for profenofos 0 (2h), 1, 3, 5, 7, 10, 15, 22 and 30 days after application of the insecticide. Collected leaf samples were chopped immediately, homogenized and stored at - 4°C until analysis. Dichlorvos and profenofos residues were determined by the analytical protocol described below, and their log values over times (days after application) were subjected to weighted linear regression. The half-life (time in days required to reduce the residues to half of initial deposits) was calculated from regression equation.

An aliquot of homogenized mulberry leaf sample (5 g) was placed in a 50 mL screw capped polypropylene centrifuge tube and 20 mL of acetonitrile was added. The samples were shaken for 2 min. Then 2 g anhydrous magnesium sulfate and sodium chloride (0.5 g) were added to the tube and contents were shaken vigorously to prevent agglomeration of anhydrous magnesium sulfate. Samples were vortexed for 2 min and centrifuged at 10,000 rpm for 10 min. The supernatant (6 mL) aliquot was transferred to a small centrifuge tube containing with 25 mg primary secondary amine sorbent and 150 mg anhydrous magnesium sulfate and shaken for 30 seconds. Later, the tube was centrifuged at 5000 rpm for 5 min to separate solids from solution. The supernatant extract (4 mL) was concentrated by purging with gentle stream of nitrogen using Turbovap LV (Caliper Life Sciences, Russelsheim, Germany) set at 40 °C and 15 psi and final volume made up with hexane for GC analysis.

Analysis of residues was carried out using a gas chromatography (GC) with the following operating parameters: Shimadzu-GC-2010 with electron capture detector. A GC-2010 (Shimadzu, Japan), equipped with ECD with ^{63}Ni radio isotope 370 MBQ (10mci) as source of detector. Capillary column (J&W, scientific, USA), DB-5, 30m X 0.25 mm id X 0.25 µm film thickness was used. Carrier gas, Nitrogen; 2.0 ml/min (constant flow mode), Injector temperature 250°C and Detector temperature 300°C. The GC column oven was initially set at a temperature of 160°C for 1 min, increased at the rate of 3°C / min to 200°C, held for 2 min and finally increased at the rate of 4°C / min to 220°C held for 2 min. Split injection (1:10 ratio) was done at a volume of 1 µl by Shimadzu AOC 20i auto injector and AOC 20s auto sampler. With these operating parameters the retention time of the dichlorvos and profenofos was 11.79 and 17.61 min respectively.

Recovery studies were carried out in order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up steps employed for the present study. The recovery rate was determined by spiking appropriate standard solutions of dichlorvos and profenofos into untreated mulberry leaf samples (5 g homogenized sample) at three different concentrations (0.01, 0.05 and 0.1 µg g^{-1}). The spiked samples were then equilibrated for 30 min and residues were determined by above said method. Replicated (n = 3) samples were injected and the recovery values were calculated for each.

Results and Discussion

Quantification was carried out using an external standard. The recovery values of dichlorvos and profenofos (Table 1) were in the range of 89.42 to 93.65 and 91.65 to 96.65 %, respectively. The precision of the method was checked by calculating the RSD values which showed an acceptable range (<5 %). As the recovery percentage was more than 85 %, the method can be adopted for residue and dissipation studies of dichlorvos and profenofos in mulberry leaf samples. The sensitivity of the method was expressed in terms of the attained limit of detection (LOD), which was evaluated as three times the signal-to-noise ratio, 0.003 µg g^{-1}. The limit of quantification (LOQ) of the method with a signal-to-noise rate from untreated samples equal to 10 was calculated as 0.01ug g^{-1}.

Table 1. Fortified recoveries of dichlorvos and profenofos in mulberry leaves

Fortified concentration (µg g^{-1})	Recovery (%) ± SD			
	Dichlorvos		Profenofos	
	Mean ± SD	RSD (%)	Mean ± SD	RSD(%)
0.01	90.23 ± 2.78	3.08	92.05 ± 2.86	3.11
0.05	89.42 ± 1.21	1.35	91.65 ± 1.74	1.90
0.10	93.65 ± 4.32	4.61	96.65 ± 1.63	1.68

The residue of dichlorvos was estimated in mulberry leaves over a period of 5 days. Initial residues of dichlorvos in mulberry leaves from treatments at recommended and double the recommended doses were 1.18 and 2.79 µg g^{-1}, respectively (Table 2). Dichlorvos residues persisted upto 3 days irrespective of the dose. Faster dissipation of insecticide residues was noted from day one after application, with a reduction value of 93-97 % from both the treatments. The residues reached below determination level (0.01 µg g^{-1}) on the 5th day at standard and double doses. Dichlorvos dissipation followed first- order dissipation kinetics (Table 2). The calculated half-life was 0.512 days. The pre-harvest interval calculated (based on the LOQ value of 0.01 µg g^{-1}) for recommended dose of dichlorvos was 2.89 days.

The dissipation studies of profenofos on mulberry leaf showed that profenofos residues

Table 2. Residues of dichlorvos in mulberry leaf sample

Days after Treatment	Residues recovered (µg g^{-1})							
	2 mL L^{-1}				4 mL L^{-1}			
	R1	R2	R3	Mean	R1	R2	R3	Mean
0	1.17	1.21	1.18	1.18 (-)	2.8	2.78	2.79	2.790 (-)
1	0.035	0.037	0.034	0.036 (97.00)	0.064	0.078	0.091	0.078 (93.45)
3	0.013	0.014	0.013	0.013 (98.87)	0.028	0.031	0.034	0.031 (97.67)
5	BDL	BDL	BDL	-	BDL	BDL	BDL	-
Regression equation	y =2.699 - 0.587x				y = 3.058 - 0.586x			
$T_{(1/2)}$ days	0.512				0.513			

Figures in parenthesis are dissipation percentage values, BDL-below detection limit (0.01 ìg g^{-1})

persisted for 30 days on the leaves at recommended and double dose of application (Table 3). The initial deposits of 2.19 and 4.72 µg g^{-1} immediately after application in/on mulberry leaves at recommended and double the dose resulted in 38.13 % and 37.92 % loss within 3 days, respectively. On the 5th day, loss of 53.7 % and 56.0 % was observed in the profenophos residues in recommended and double the dose, respectively. The residues gradually declined thereafter and persisted upto 30 days with dissipation of 99.04 % and 98.85 % recommended and double dose, respectively. Profenofos

Table 3. Residues of profenofos in mulberry leaf sample

Days after Treatment	Residues recovered (µg g^{-1})							
	2 mL L^{-1}				4 mL L^{-1}			
	R1	R2	R3	Mean	R1	R2	R3	Mean
0	2.170	2.210	2.200	2.193 (-)	4.680	4.780	4.700	4.720 (-)
1	1.600	1.580	1.650	1.610 (26.585)	3.280	3.210	3.310	3.267 (30.79)
3	1.350	1.400	1.320	1.357 (38.136)	2.980	2.890	2.920	2.930 (37.92)
5	1.020	0.980	1.040	1.013 (53.792)	2.040	2.090	2.100	2.077 (56.00)
7	0.350	0.390	0.350	0.363 (83.432)	0.940	0.930	0.980	0.950 (79.87)
10	0.290	0.270	0.270	0.277 (87.384)	0.680	0.610	0.680	0.657 (86.09)
15	0.090	0.089	0.091	0.090 (95.896)	0.280	0.270	0.250	0.267 (94.35)
22	0.045	0.051	0.041	0.046 (97.918)	0.080	0.078	0.090	0.083 (98.25)
30	0.020	0.024	0.019	0.021 (99.042)	0.052	0.050	0.060	0.054 (98.86)
Regression equation	y =3.238 - 0.070x				y = 3.579- 0.068x			
$T_{(1/2)}$ days	4. 30				4.42			

Figures in parenthesis are dissipation percentage values, BDL-below detection limit (0.01 ìg g^{-1})

dissipation followed first-order dissipation kinetics. The calculated half-life was 4.30 and 4.42 days from treatments at recommended and double doses, respectively. The pre -harvest interval calculated (based on the LOQ value of 0.01 µg g^{-1}) for recommended dose of profenophos was 32 days. The present studies suggest that the use of dichlorvos and profenofos at the recommended doses does not pose any hazards to silkworm if safe waiting periods of 2.89 and 32 days respectively, are observed before harvesting mulberry leaves for feeding silkworm.

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