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Persistence and dissipation kinetics of phorate in the soil of sugarcane ecosystem

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Abstract
An experiment was conducted to determine persistence and dissipation kinetics of phorate in sugarcane grown soil under South Gujarat condition. The experimental plots were subjected to application of phorate 10G (1.5 kg a.i./ha) at the time of planting and 60 days after planting of sugarcane. The soil samples were periodically collected at 0 (2 hrs.), 1, 3, 5, 10, 20, 30, 60 and 90 days after last application of phorate. Prior to analysis, acetonitrile based extraction and dispersive clean-up approach adopted to quantify the residues of phorate and its metabolites with LC-MS/MS from soil, sugarcane leaves and juice were verified on method performance verification parameters. The analytical method was accurate, sensitive and precise enough as per SANTE guideline. Residues of phorate and total phorate (phorate + metabolites *i.e* phorate sulfone and phorate sulfoxide) were observed upto 30 days after application and reached below quantification level at 60 days after application. Phorate and total phorate followed the second order dissipation kinetics in sugarcane grown soil. However, sugarcane leaves and juice contains residues of phorate and its metabolites BQL which are well within the safety limit of MRL.

Keywords: Dissipation, metabolites, persistence, phorate and sugarcane

Introduction
Sugarcane is the one of principal cash crop of India with the highest production of sugar after Brazil. Like other annual crops of economic importance, several factors are responsible for the low productivity of sugarcane in country. Insect-pests are among the important constraints accounting a significant loss in cane yield and reduction in sugar recovery resulted, a huge annual revenue loss each year. Sugarcane crop is also subjected to ravage by borer and white grub causing widespread damage to roots and underground stem. For control of these insect-pests, soil applied granular formulation of phorate is extensively used in sugarcane grown areas.

Phorate is one of the systemic insecticide that inhibits the cholinesterase enzymes involved in transmitting nerve impulses. Chemically phorate is an organophosphate, *O,O*-diethyl *S*-(ethylthio) meththyl phosphorodithioate. As a systemic, phorate is absorbed by the plant roots and moves to the different above ground parts, where it is concentrated in new leaves and fruits and kill sucking pests that consume sufficient plant sap containing chemical. Because of its persistence in soil it is also useful in controlling soil dwelling nematode [1].

On the one hand, the extensive use of pesticides for the control of pests enhanced the agricultural productivity many folds, but on the other hand, they are responsible for creating a severe threat to the environment and ecology with widespread pollution. Though the advantages of pesticides application is undisputed, but the residues of applied pesticides stay in the environment for variable periods of time, which may pose a serious threat to environmental quality and indeed can lead to acute and chronic effects on human life and biodiversity [2].

Recommendations for the use of agrochemicals for a crop cannot be made until its persistence and dissipation studies have been carried out in different components of environment. Information on degradation rate of pesticides also helps to assess and predict the environmental behaviour of the chemicals [3]. Little information is available on the persistence and dissipation of phorate and its metabolites especially under South Gujarat conditions. Therefore, by considering these facts, this experiment was conducted.

Materials and Methods

An experiment was conducted at Main Sugarcane Research Station farm of Navsari Agricultural University, Navsari, Gujarat. The farm is located at $20^0$92' N and $72^0$89' E at an altitude of about 10 m above MSL. According to agro-climatic condition, Navsari is located in South Gujarat heavy rainfall zone-I (Agro-ecological situation-III). The climate of this zone is typically tropical, characterized by humid and warm monsoon with heavy rains, quite cold winter and fairly hot summer. The average annual rainfall of the tract is about 1450 mm. The soil of experimental field was clay in texture having $pH_{2.5}$ 7.7, $EC_{2.5}$ 0.48 dS/m and organic carbon 0.68%.

Chemicals and reagents: Certified reference standards of phorate, phorate sulfone and phorate sulfoxide having purity 95.6, 97.3 and 99.3%, respectively were supplied by Sigma-Aldrich, USA. Solvent like water (LCMS grade), acetonitrile (purity ≥99.9%) and anhydrous magnesium sulfate (purity ≥99.5%) were procured from Merck KGaA, Germany. Primary Secondary Amine (PSA) was from SUPELCO, Bellefonte, USA. Acetone, sodium chloride and anhydrous sodium sulfate were obtained from Fisher Scientific, UK.

Instrumentation: LC-MS/MS was used for quantification of phorate and its metabolites from soil, sugarcane juice and leaves matrix and its details are given below.

uHPLC	:	Thermo Scientific Dionex UltiMate-3000
MS/MS	:	Thermo Scientific Quantum Access Max
Column	:	C_{18} Column (100 mm X 2.1 mm i.d.1.9 µm)
Mobile phase	:	A- Water + 0.1% Formic acid B- Acetonitrile + 0.1% Formic acid
Column oven temp.	:	25 ^{0}C
Flow rate	:	0.3 mL/min.
Auto sampler	:	Dionex ultimate 3000 R
Injection volume	:	5 µL
Ion spray voltage	:	4500 V
Vaporizer temp.	:	350 ^{0}C
Capillary temp.	:	325 ^{0}C
Sheath gas pressure	:	48 arb
Aux gas pressure	:	18 arb

Standard solution: A traceable technical grade of phorate, and its metabolites *viz.* phorate sulfone and phorate sulfoxide standard was accurately weighed on Oahu's (maximum capacity 210 g and sensitivity 0.001 g) and transferred to 100 mL capacity volumetric flasks. The content was dissolved with acetonitrile and final volume was made up. From the primary standard, a suitable aliquot was diluted with acetonitrile in volumetric flask to prepare intermediate standard mixture of 10.0 µg/mL. The intermediate standard was further diluted with acetonitrile to obtain final working standard mixtures.

Method validation: The method performance verification studies were partially validated according to SANTE guidelines [4] in terms of linearity, detection limits, accuracy and precision for phorate, phorate sulfone and phorate sulfoxide. The linearity of a method is measured of range within which the measurements or observations recorded with any instrument are directly or by well-defined mathematical transformation, proportional to concentration of analyte in a given range. To work out the linearity, response (area) of the MS detector *vs*. concentration was plotted. Further, per cent residual was calculated to work-out the predictable strength of both variables of the calibration curve with the following formula.

% residual = [(Actual response – Anticipated response) / Actual response] X 100

The limit of detection **(**LOD) is the lowest concentration of analyte detects by an analytical instrument and limit of quantification (LOQ) is the lowest concentration that can be determined with acceptance accuracy and precision under the particular experimental condition with 90% or more confidence level. LOD and LOQ were determined on the basis of signal to noise ratio of 3 and 10, respectively.

LOD = 3 X Mean SD / Slope of regression equation
LOQ = 3.33 X LOD

In order to ensure quality assurance information such as accuracy or trueness and precision of the analytical method, the recovery study was carried out from soil, sugarcane leaves and juice before taking up analysis of test samples. As per guidelines of SANTE [4], per cent recovery and Relative Standard Deviation (RSD) is the indicator of trueness and precision of any analytical method employed for the quantitative estimation of insecticides. A representative sample was fortified with 5, 10 and 25 ng/g level of phorate and 10 and 25 ng/g level of phorate sulfone and phorate sulfoxide.

Application of phorate: The required quantity of phorate 10G (1.5 kg a.i./ha) was mixed thoroughly with dry sand of very fine texture and uniformly distribute in the gross plot at the time of planting and 60 days after planting of sugarcane.

Sampling: Periodic soil sampling were started at 60 days after planting or after last application of phorate from 0-15 cm soil depth with the help of soil auger and carried out at the Food Quality Testing Laboratory, Navsari Agricultural University, Navsari. The samples were processed on the same day for residue study. The soil samples was taken at 0 (2 hrs), 1, 3, 5, 10, 20, 30, 60 and 90 days after last application of phorate. Plant samples were taken at the time of harvest for terminal residue analysis.

Extraction and clean up

Soil: The QuEChERS method was adopted for insecticides residue analysis from soil [5]. A representative 10 g of fine ground soil sample was transferred in 50 mL capacity centrifuge tube, to which 20 mL of acetonitrile was added and shaken it vigorously for 1 minute. After this 4 g $MgSO_4$ and 1 g NaCl were added in the tube and vortex followed by centrifuged at 3500 rpm for 2 minute. After it, 10 mL of supernatant solution was transferred in the 15 mL capacity centrifuge tube containing 1.5 g $MgSO_4$ and 0.250 g PSA and again centrifuged it for 2 minute at 3500 rpm. 4 mL of aliquot was transferred in test tube and evaporated it to dryness with TurboVap at 40 °C. Finally 1 mL volume was make-up by using acetonitrile and filtered it in to the glass vial.

Sugarcane leaves: Modified QuEChERS method was adopted for insecticides residue analysis from sugarcane leaves samples. Sugarcane leaves sample (1-2 kg) was chopped and homogenized in high volume homogenizer. A 10

g of representative sample was transferred in 50 mL capacity polypropylene centrifuge tube, to which 20 mL of acetonitrile was added and homogenized the sample at 5000 rpm for 3 minute. After this 3 g NaCl was added and shake well for 2 minute followed by centrifuged for 3 minute at 3000 rpm to separate organic layer. 12 mL of organic layer was transferred into 50 mL capacity centrifuge tube and added about 10 g sodium sulphates and shake well for 1 minute again centrifuged at 3000 rpm. From this 6 mL organic layer was transferred to 15 mL capacity centrifuge tube containing 0.3 g PSA and 0.9 anhydrous $MgSO_4$ and centrifuged it for 5 minute at 3000 rpm. 2 mL of supernatant aliquot was transferred in test tube and evaporated it to dryness with TurboVap at 40 °C. Finally 2 mL volume was make-up by using acetonitrile and filtered it in to the glass vial.

Sugarcane juice: The modified QuEChERS method was adopted for insecticides residue analysis from sugarcane juice [6]. 10 mL of sugarcane juice sample was transferred in 50 mL capacity polypropylene centrifuge tube, to which 10 mL of acetonitrile was added and vortex for 1 minute. After this 4 g $MgSO_4$ + 1 g NaCl was added and mixed again for 1 minute followed by centrifuged it for 10 minute at 1200 rpm. From this 4 mL upper layer of aliquot was transferred in 15 mL capacity centrifuge tube having 600 mg $MgSO_4$ and 200 mg PSA and vortex for 30 second followed by centrifuged for 3 minute at 3500 rpm. 2 mL of supernatant aliquot was transferred in test tube and evaporated it to dryness with TurboVap at 40 °C. Finally 2 mL volume was make-up by using acetonitrile and filtered it in to the glass vial.

Dissipation kinetics: The dissipation kinetics of phorate in experimental soils was determined by using three dissipation kinetics models *viz*., zero, first and second order models [7]. The detail descriptions of models are given in Table 1.

Table 1: Dissipation kinetics models

Model	*k (Graphical)*	*k (Theoretical)*	*DT_{50}*
Zero order $C_t = C_0 - kt$	Slope of kinetic model	$k = \frac{C_0 - C_t}{t}$	$DT_{50} = \frac{C_0}{2k}$
First order $C_t = C_0 e^{-kt}$	Slope of kinetic model	$k = \frac{2.303}{t} \log \frac{C_0}{C_t}$	$DT_{50} = \frac{\ln 2}{k}$
Second order $\frac{1}{C_t} = \frac{1}{C_0} + kt$	Slope of kinetic model	$k = \frac{C_0 - C_t}{t(C_t X C_0)}$	$DT_{50} = \frac{1}{k C_0}$

Here, k is dissipation rate constant, C_t is concentration at t time, C_0 is initial concentration and DT_{50} is 50% dissipation time

Results and Discussion

Method validation parameters: The calibration curve of phorate, phorate sulfone and phorate sulfoxide showed linear response in the concentration range of 1.0 to 250 ng/mL with coefficient of determination 0.982, 0.974 and 0.982, respectively. The average per cent residual of phorate and its metabolites over different concentration were found to be in the range of 1.55-13.64% (Table 2).The LOD values obtained for phorate, phorate sulfone and phorate sulfoxide was 0.962, 4.627 and 2.191 ng/g, respectively. The corresponding LOQ values of method was 3.21, 15.42 and 7.30 ng/g for phorate, phorate sulfone and phorate sulfoxide, respectively.

Table 2: Linearity parameters of phorate and its metabolites on LC-MS/MS

Compound	Linearity range (ng/mL)	Regression equation	% residual
Phorate	1.0 - 250	y=1745x+ 7563; R^2=0.982	1.55-11.47
Phoratesulfone	1.0 - 250	y=42.71x+ 253.4; R^2=0.974	3.15-13.64
Phoratesulfoxide	1.0 - 250	y=243.7x+ 987.9; R^2=0.982	3.87-11.44

Accuracy and precision was determined with the help of per cent recovery and per cent RSD (Relative Standard Deviation). The average recovery and RSD of phorate, phorate sulfone and phorate sulfoxide from soil, sugarcane juice and leaves are given in Table 3.

QuEChERS based extraction procedure and measurement on LC-MS/MS instrument for analysis of the residues of phorate, phorate sulfone and phorate sulfoxide had per cent residual <20%, recovery range between 70-120%, RSD <20% and LOQ of phorate <MRL (50 ng/g in sugarcane) which fulfilled the criteria of method validation as prescribed in guidelines of SANTE [4]. Several other scientists had also employed QuEChERS based extraction techniques and found that this analytical approach offered a potential alternative technique for extraction of organophosphate from soil [8] and from sugarcane juice [9].

Table 3: Accuracy and precision of extraction method for phorate and its metabolites

Compound	Level (ng/g)	Soil		Sugarcane juice		Sugarcane leaves	
		Mean Recovery (%)±SD	% RSD	Mean Recovery (%)±SD	% RSD	Mean Recovery (%)±SD	% RSD
Phorate	5	85.56±5.75	6.72	98.94±8.03	8.12	92.80±6.62	7.13
	10	85.14±5.70	6.69	98.91±5.14	5.20	97.44±6.04	6.20
	25	87.75±10.20	11.62	99.20±5.39	5.43	93.81±6.42	6.85
Phorate sulfone	10	91.86±11.80	12.85	96.18±10.13	10.53	91.47±5.41	5.91
	25	91.49±8.71	9.52	89.45±9.51	10.63	95.45±11.03	11.56
Phorate sulfoxide	10	86.62±5.11	5.90	96.93±2.74	2.83	97.86±7.57	7.73
	25	87.16±10.75	12.33	98.76±4.47	4.53	91.88±4.14	4.50

Persistence and dissipation study: The dissipation rates of phorate and total phorate were faster at the beginning, which slows down with the passage of time (Table 4). This indicated a non-linear pattern of degradation and implies that simple first order kinetics might not be adequate to explain the behaviour of phorate and total phorate in soil. Hence, the kinetics of the residues data of phorate and total phorate was evaluated under three different kinetic models. Among the kinetic models, first order and second order models were best fit for phorate and total phorate residues, judging from the significant of the coefficient of determination *i.e.* 0.810 and 0.788, respectively (Table 5). Further, for more accuracy, per cent variation in graphical and theoretical half-life was calculated which shows that second order kinetics had less per cent variation as compared to first order kinetics (Table 5). Therefore, it is recommended that half life of the phorate (3.00 days) and total phorate (2.60 days) be assessed from the second order kinetics rather than zero order and first order kinetic model in sugarcane grown soil under South Gujarat conditions.

The one part of added insecticides are immediately available in soil solution phase, degraded rapidly, leaving the other part adsorbed on the organic and clay fraction of soil, degraded slowly due to slow release in soil solution [10]. This might be the reason for fast degradation of phorate at initial phase as compared to later phase. In the present investigation, it was observed that metabolites of phorate *i.e.* phorate sulfoxide and phorate sulfone were present in the 0 days (2 hrs) samples which indicated that conversion of phorate into its metabolites are very fast. The solubility of phorate in water is low (50 mg/L) at 20^0C [11] but under alkaline (basic) conditions phorate is unstable, resulted a very small half life. One more interesting thing was also observed that about half of total residue of total phorate was contributed by phorate sulfoxide from 0 days to 30 days after application. Phorate rapidly oxidized into its sulfoxide metabolite in the sandy clay loam soil [12]. Phorate sulfoxide alone accounted for more than 20% of the total residues within 2 hours post application and it was more than 50% on the fifth day after treatment irrespective of the doses applied. Phorate persist in laterite and alluvial soils up to 45 and 60 days, respectively [13].

Residues in sugarcane: Residue of phorate and its metabolites in sugarcane juice and leaves were found below quantification level (BQL) at the time of harvest (Table 4). This might be due to availability of phorate for absorption by sugarcane plants was only up to 30 days after application of phorate and after 60 days it was not available for the plant.

Table 4: Persistence and dissipation behaviour of phorate and its metabolites in soil

Days after application	Phorate		Metabolites (ng/g)		*Total phorate	
	Residue (ng/g)	Dissipation (%)	Phorate sulfone	Phorate sulfoxide	Residue (ng/g)	Dissipation (%)
0 (2 hrs. after)	346.26	-	92.44	367.94	806.64	-
1	178.48	48.46	47.97	180.39	406.84	49.56
3	119.29	65.55	29.23	116.60	265.12	67.13
5	98.72	71.49	25.53	103.96	228.20	71.71
10	83.76	75.81	20.35	85.02	189.13	76.55
20	81.58	76.44	21.05	85.97	188.60	76.62
30	25.68	92.58	BQL	26.39	52.07	93.55
60	**BQL	-	BQL	BQL	BQL	-
90	BQL	-	BQL	BQL	BQL	-
At harvest						
Sugarcane juice	BQL	-	BQL	BQL	BQL	-
Sugarcane leaves	BQL	-	BQL	BQL	BQL	-

*Total phorate = Phorate + Metabolites; **BQL is <LOQ

Table 5: Kinetic parameters to estimate the models and half lives of insecticide in soil

Insecticide	Model	R^2	*k* (per day)		DT_{50} (days)		*% variation in DT_{50} over graphical
			Graphical	Theoretical	Graphical	Theoretical	
Phorate	Zero order	0.492	6.5373	10.6861	26.48	16.20	38.82
	First order	0.798	0.0636	0.0867	10.90	7.99	26.70
	Second order	0.810	0.0096	0.0012	3.00	2.40	20.00
Total phorate	Zero order	0.485	15.2245	25.1526	26.49	16.03	39.49
	First order	0.798	0.0667	0.0914	10.40	7.59	27.02
	Second order	0.788	0.0005	0.0006	2.60	2.07	20.38

* % variation in DT_{50} = (Graphical DT_{50} – Theoretical DT_{50})/ Graphical DT_{50}

Conclusion

The result of the study showed that modified QuEChERS method adopted for extraction and quantification on LC-MS/MS is accurate, precise and sensitive enough for estimation of phorate and its metabolites from soil, sugarcane juice and leaves. Phorate and total phorate followed second order dissipation kinetics. Sugarcane leaves and juice contains residues of phorate and its metabolites below quantification level at the time of harvest which is well within the safety limits of MRL.

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