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Determination of Profenofos Residues using LC-MS/MS and Its Dissipation Kinetics in Pigeonpea Pods

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ABSTRACT

Background: Pigeonpea is a drought resistant legume crop, cultivated in semiarid tropical and subtropical regions of the world mainly for its protein enriched seeds. Insect pests are major biotic constraints implicating yield losses of staggering dimensions. Profenofos is being used for management of pigeonpea pod borer and studying the pattern of dissipation, residual occurrence and hazard index for consumption of pigeonpea grain contaminated with profenofos is very important.

Methods: A simple, sensitive and reproducible method for analysis of profenofos in pigeonpea green pods and dry grain was standardized and validated using liquid chromatography tandem mass spectrometry (LC-MS/MS) with electro spray ionization (ESI+). Modified QuEChERS methods with 1% ethyl acetate in acetonitrile involved in the extraction of profenofos residues from green pods and mature dry grains.

Result: The limit of detection and limit of quantification (LOD and LOQ) were 0.002 and 0.006 µg g^{-1}, respectively. The recovery ranges from 88.75 to 101.36% for the green pods and 88.34 to 98.77% for mature dry grains with relative standard deviation (RSD) was in the range of 0.99 to 4.05%. The field study was conducted to investigate the dissipation kinetics of profenofos in pigeonpea. Two applications of profenofos 50% EC at 500 and 1000 g a.i. ha^{-1} at 15 days intervals in pigeonpea at the time of pod formation recorded initial deposits of 20.28 and 41.64 µg g^{-1} in the green pod, respectively. At15 days after application, residues gradually dissipated to the level of 0.78 and 1.98 µg g^{-1} accounting to the loss of 96.15 and 95.58% at 500 and 1000 g a.i. ha^{-1}, respectively. The half-life values were 5.18 and 5.93 days. Hazard index (HI) was found less than 1 at 25th and 35th day after application at 500 and 1000 g a.i. ha^{-1}, respectively.

Key words: Dissipation, Hazard index, LC-MS/MS, Pigeonpea, Profenofos, QuEChERS.

INTRODUCTION

Pigeonpea is one of the major drought resistant pulse or grain legume cultivated in semiarid tropical and subtropical regions of the world mainly for its protein enriched seeds (both green and dry grains) and also as vegetable source (Shanower *et al.,* 1999; Nene *et al.,* 1990; Seetharamu *et al.,* 2020). Its mature green pods are used as a vegetable and de-hulled seed are used along with rice and bread. It is nutritionally rich vegetable containing good source of protein, vitamins (A, C and B complex) and minerals *viz.,* Ca, Fe, Zn, Cu (Saxena *et al.,* 2010). India accounts for 75% of total area with 65% production in the world and thus India being recognized as largest producer and consumer in the world (FAO Stat, 2011). The crop being predominantly drought hardy in nature and affected by an array of biotic and abiotic factors during its growth. Among the biotic factors, insect pests are major biotic constraints implicating yield losses of staggering dimensions. Insect pests *viz.,* pod boring larvae, pod sucking bugs and pod fly on pigeonpea are the major constraints contributed to poor productivity (Minja *et al.,* 2000). Among these insects, pod borer complex devastating the pigeonpea and is been the major yield limiting factor in production. The yield loss by this pest alone accounts for 20-57% and loss in grain yield up to 28% (Lateef and Reed, 1983; Sahoo and Senapati, 2000) and recently differential population of leafhopper had impact in reduction 10% of the grain yield (Rachappa *et al.,* 2016).

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The management of pod borer complex in pigeonpea is highly dependent on large number of chemical insecticides including recently approved new chemistry group molecules such as chlorantraniliprole (18.5% SC), flubendiamide (39.35% SC), indoxacarb (15.8% EC) and emamectin benzoate (5% SG) and proved quite effective insecticides against pod borer (Sharma *et al.,* 2018; Srinivasan and Sridhar, 2008; Babriya *et al.,* 2010; Bhede *et al.,* 2015). Profenofos is an organophosphorus insecticide, chemically called as O-4-bromo-2-chlorophenyl O-ethyl Spropyl phosphorothioate (IUPAC) and O-(4-bromo-2-chlorophenyl) O-ethyl Spropyl phosphorothioate (CAS). It is an extremely toxic and persistent chemical as per the toxicity classification.

It is a non systemic, broad-spectrum insecticide and acaricide with contact and stomach action and being extensively used for the control of lepidopteran group larvae, whitefly and mites on cotton, pigeonpea, chilli and vegetable crops (Sharma *et al.,* 2018; Yadav *et al.,* 2015). Further, profenofos efficacy on pod borer and pod fly infesting pigeonpea was in the range of 25 to 85% when used as ovicide and larvicide (Srivastava and Mohapatra, 2003; Chandrayudu *et al.,* 2006; Malathi, 2007). The information on the pattern of dissipation, residual occurrence and hazard index of profenofos in pigeonpea is not available. The repeated application may result in occurrence of residue in green pods and dry grains above the maximum residual limit. The codex maximum residue limits (MRLs) for profenofos in pigeonpea grains is 0.05 mg kg ^{1}, whereas for other commodities ranged from 0.01 to 2.00 mg kg ^{1} (FSSAI, 2018; Sonika and Rashmi, 2017). It was reported that, the initial deposits of profenofos was much higher in the tomato than the cypermethrin (Gupta *et al.,* 2010) indicate its high persistence nature. Several reviews have examined persistence and dissipation of profenofos and residue analysis in fresh and edible crops such as tea leaves (Pramanik *et al.,* 2005), okra (Paras *et al.,* 2005), green and cured cardamom (Renuka *et al.,* 2006), chillies (Reddy *et al.,* 2007), brinjal (Nigam *et al.,* 2009; Mukharjee *et al.,* 2012), tomato (Sahoo *et al.,* 2004; Romeh *et al.,* 2009; Gupta *et al.,* 2011).

The information related to the persistence of the profenofos in pigeonpea and other pulses is not available. Whereas profenofos residues in different crops was studied previously using GC-FID in tea leaves (Pramanik *et al.,* 2005), brinjal (Nigam *et al.,* 2009) and using GC-ECD in okra (Paras *et al.,* 2005), green and cured cardamom (Renuka *et al.,* 2006), brinjal (Mukharjee *et al.,* 2012) and tomato (Gupta *et al.,* 2011). In tomato, profenofos was quantified using GC-NPD (Sahoo *et al.,* 2004). Whereas the high-performance liquid chromatography (HPLC) was used for chilli (Reddy *et al.,* 2007) and tomato (Romeh *et al.,* 2009). Apart from these detectors, currently the chromatographic techniques coupled with mass spectrometry detector are the best choice for pesticide residue determination at trace levels (Lacina *et al.,* 2010). In this study, the method development and dissipation of profenofos in pigeonpea was attended using LC-ESI-MS/MS as it provides high sensitivity, selectivity and specificity over other methods. It was proved in case of indoxacarb residue analysis in pigeonpea green pod and dry grains using LC-MS/MS (Naik *et al.,* 2020). Therefore, a sensitive, effective and reproducible analytical method was developed involving modified QuEChERS technique. A supervised field trial was conducted to investigate the persistence and dissipation of profenofos in pigeonpea green, dry grains following the application of 500 and 1000 g a.i. ha^{-1} and calculated the safe waiting period, half life and hazard index to confirm the related risks on consumption of pigeonpea green pods.

MATERIALS AND METHODS

Chemical and reagents

Profenofos certified reference material (CRM) having purity of 99.0% was procured from Dr. Ehrenstorfer, Augsburg, Germany. Profenofos 50% EC was purchased from local authorized dealers at Raichur. LC-MS grade acetonitrile and methanol (≥ 99.9% purity) were procured from J.T. Baker (NJ, USA), anhydrous $MgSO_4$ (99.9% purity), sodium acetate (99.9% purity) were purchased from HiMedia, Bangalore; primary secondary amine (PSA) sorbent (AR grade, 40µm) was procured form Agilent Technologies, USA. NaCl (≥ 99.9% purity) from Merck Mumbai, India. Anhydrous disodium hydrogen citrate sesquihydrate and tri-sodium citrate dehydrate (99.00% purity) were procured from Sigma Aldrich, Germany. HPLC water collected through Milli-Q water purification system.

Preparation of standard solutions

Profenofos standard stock solution (1000 µg mL^{-1}) was prepared by weighing 10 mg (±0.1) of certified reference material in a calibrated volumetric flask (Class 'A'; 10 mL capacity) and volume made up with LC-MS grade methanol. An intermediate standard solution of 100 µg mL^{1} was prepared by drawing 1 mL of stock solution in 10 mL volumetric flask and volume made up using methanol. A working standard of 1 µg mL^{-1} was prepared in methanol and further the calibration standard solution ranging from 0.005 to 0.12 µg mL^{-1} were prepared. The higher residues recorded in samples outside the linear range were diluted, analyzed and calculated residues by adding dilution factor. The matrix match standards at the similar concentrations were prepared by using the control pigeonpea samples extract obtained through sample preparation.

Field experiment

A supervised field trial was conducted for studying the persistence and dissipation of profenofos in pigeonpea ecosystem at Entomological Experimental Plot, University of Agricultural Sciences, Raichur (Longitude: 77.3345°E and Latitude: 16.2043°N), India during the *kharif* 2018. The treatment plot size of 10 × 3.75 m^2 in a randomised block design (RBD) with 3 treatments and 8 replications. Pigeonpea (Variety: TS3R) was sown and managed as per the standard package of practice of UAS, Raichur, India. The crop was sprayed with 500 g.a.i. ha^{-1} (T1) as recommended dose as per the recommendation by the Central Insecticide Board and Registration Committee, India and 1000 g a.i. ha^{-1} as double the recommended dose (T2) and untreated control (T3). Profenofos 50% EC was applied twice using a high volume knapsack compression sprayer with spray volume of 500 L ha^{-1}. First spray was undertaken during flowering and pod initiation and subsequest application was made at 15 dyas interval. Temperature and relative humidity were recorded in the range of 17.60-32.00°C and 44.00 -55.00%, respectively, further, there was no rainfall during experiemental period.

Sampling

The pigeonpea green pod samples were collected randomly from each replicates treatment plots at regular interval on 0 (1 hr after spraying), 1, 3, 5, 7, 10, 15, 21, 25, 30 and 35 days after the second application and matured dry grains at harvest time *i.e.* 45 days after spraying. The collected samples were stored at -20°C until analysis.

Extraction

QuEChERS method and its modification described by Anastassiades *et al.* (2003) were followed with modification in extraction and cleanup of profenofos from pigeonpea green and dry grains. Five hundred grams pigeonpea green pod was grounded thoroughly using high volume homogenizer (Robo Coup). About 5 g of the ground sample was weighed and transferred into 50 mL centrifuge tube and 10 mL of distilled water was added, allowed to stand for 30 min. To this, 10 ml of 1% ethyl acetate in acetonitrile was added for better separation and efficient extraction of pesticides from the matrix and 6 g of anhydrous magnesium sulphate and 1.5 g sodium acetate was added. The sample mixture was then homogenized at 10,000-13,000 rpm for 3 min. The homogenized sample mixture was centrifuged at 5,000 rpm for 5 min. After centrifugation 7 mL supernatant was transferred into 15 mL centrifuge tube containing 350 mg primary secondary amine (PSA), 1.05 g anhydrous magnesium sulphate and 25 mg charcoal. The mixture was then vortexed for one minute followed by centrifugation at 12000 rpm for 5 min. Then transferred 3 mL extract into a test tube and evaporated the content using nitrogen flash evaporator at 35°C to dryness and reconstituted the residue with 1.5 mL of LC-MS grade methanol. Sonicated the mixture through ultrasonicator to dissolve residues completely then filtered content using 0.22 µ PTFE membrane filters in to LC vials.

LC-MS/MS parameters

The LC-MS/MS (Shimadzu®, LCMS 8040) assembled with 1200 series UHPLC, solvent degassing unit, a quaternary pump, an autosampler and a thermo stated column compartment system. Separation of the analyte was attained on a Shimpack XR ODS C_{18} column (150 × 2 mm i.d.) with 40°C column oven temperature. The mobile phase consisted of 0.0314 g ammonium formate (5 mM) + 2 mL MeOH + 10 µl formic acid (0.01%) made-up the volume with HPLC grade water to 100 mL as the component of mobile phase A and 0.0314 g ammonium formate (5 mM) + 10 µl formic acid (0.01%) and made-up the volume with 100% MeOH to 100 mL as component of mobile phase B was used at 0.4 mL/min flow rate. The profenofos was separated with the following gradient programme of 60% A and 40% B at start for 12 minutes followed by 100% B up to 20 minutes and then 60% A for 3 minutes. A full scan mass spectrum of profenofos with electro-spray ionization positive mode (ESI+) was documented to choose the most intense m/z value. Further, the parent ion $(M+H)^+$ was identified and selected as the precursor ion. The transitions of multiple reaction monitoring (MRM) along with acquisition parameter were optimized for the high abundance of selected ions with ESI positive mode. The MS source parameters were as follows; interface voltage of 4.5 kV, desolvation temperature of 250°C, heat block temperature of 400°C, desolvation gas (N2) of 2.9 L/min and drying gas at 2.9 L/min. Then collision with argon gas was done and different collision energies were optimized. LabSolution® LCMS Version 1.5 software was used for the system control, data acquisition and analysis.

Method Validation

The different parameters such as linearity, matrix effect, limit of detection (LOD), limit of quantification (LOQ), Specificity, Trueness (bias), precision in terms of repeatability (RSD_r), precision in terms of reproducibility (RSD_{wR}) and robustness were validated following SANTAE/11813/2017 (European Commission, 2017). Calibration curve was drawn for profenofos by plotting the peak areas against their corresponding concentrations ranged between 0.002 and 0.12 µg mL^{-1}. The LOD was calculated by preparing different solutions with low concentration that is expected to produce a response that is 3 times baseline noise. LOQ in the same manner and selected as the concentration of pesticide that gives S/N ration of 10 and recovery of lowest spike level within the limit of 70-120% with RSD of ≤ 20%. Trueness of the developed method was evaluated by estimating the average recovery for each spike level tested. Recovery experiments were carried out at 3 fortification levels (0.006, 0.03 and 0.06 µg g^{-1}) by spiking blank sample with working standard solution. The fortified samples were extracted using the procedure described in the materials and methods. The method precision was ascertained with regards to the repeatability relative standard deviation (RSD_r) exactly similar extractions of blank samples spiked with profenofos at the same fortification levels (0.03 µg g^{-1}) and RSD with respect to reproducibility (RSD_{wR}) by attending the fortification and extraction at two different dates. The matrix effect was calculated by comparing the angular coefficients obtained by the curves in the solvent and in the matrix according to the following equation:

$$\text{Matrix effect (\%)} = \frac{(b_m - b_s)}{b_s} \times 100$$

Where

b_m and b_s are the angular coefficients of the curve in the matrix and in the solvent, respectively (Naik *et al.,* 2020).

The dissipation of profenofos residues in pigeonpea green pods was analyzed by using first-order dissipation kinetics equation *i.e.*

$$Ct = Coe^{-kt}$$

Where

Ct = Pesticide concentration (µg g^{-1}) at time t (d).

Co = Apparent initial concentration (µg g^{-1}).

k = Dissipation rate constant.

The half-life (t1/2) was determined as:

$$DT_{50} = \log 2/k$$

t1/2 is the insecticide half-life in green pigeonpea pods. Calculation of dissipation percentage, waiting period and half-life was done as per the following mathematical formulae given by Regupathy and Dhamu, (2001).

Dissipation percentage =

$$\frac{\text{Initial deposit (mg kg}^{-1}) - \text{Residues at given time (mg kg}^{-1})}{\text{Initial deposit (mg kg}^{-1})} \times 100$$

The waiting periods were calculated by the following mathematical formulae:

$$T_{tol}\ (\text{days}) = \frac{[a - \text{Log tol}]}{b}$$

Where

T_{tol} = Minimum time (days) required for the pesticide residue to reach below the tolerance limit.

a = Log of apparent initial deposits obtained in the regression equation (Y = a+bx).

tol = Tolerance limit (MRL).

b = Slope of the regression line.

Half-life (RL_{50}) was calculated mathematically,

$$t_{½} = \frac{e}{b} = \frac{0.301}{b}$$

Where

e = log 2 = 0.301.

b = Slope of the regression line.

Risk assessment

Profenofos is being used in pigeonpea during flowering to pod maturity and the risk associated with consumption of green pods from treated plots is an essential requirement to know the hazard level. Based on the average profenofos residual concentration (µg g^{-1}) quantified in different days samples drawn from treated plot and per capita pigeonpea consumption rate (kg day^{-1}), the estimated average daily intake (EADI) of profenofos was arrived. Per capita consumption of the green pod is not available. By considering recommended daily intake of 40 grams of dry pulses for a balanced diet of average men in India, the EADI was calculated. Hazard index (HI) was then calculated dividing the EADI (mg kg^{-1} day^{-1}) with acceptable daily intake (mg kg^{-1} day^{-1}) of profenofos. Acceptable daily intake (ADI) for profenofos was 0.01 mg kg^{-1} day^{-1}. The inference was made based on the HI values *i.e.*, if the calculated hazard index (HI) more than 1, then the green pod is not safe for human consumption (Darko and Akoto, 2008).

Maximum permissible intake (MPI) of 550 µg $person^{-1}$ day^{-1} was arrived by multiplying the ADI of profenofos 0.01 mg $kg^{-1}day^{-1}$with the average weight of 55 kg for the person. Theoretical maximum residues contribution (TMRC) values were arrived by multiplying the mean residues obtained in different day samples drawn in single and double doses with recommended pulses consumption (40 g $person^{-1}day^{-1}$). Inference was made by comparing the TMRC with MPI values. If the TMRC values are lower than MPI, then the dietary exposure to profenofos is within safety zone and no health hazard is expected (Mukherjee and Gopal, 2003).

RESULTS AND DISCUSSION

Optimization of method parameters in LC-MS/MS

The chromatographic conditions were optimized that the mobile phase with methanol provides better ionization. As described in the experimental section, the column and gradient program for 20 minutes produce the better separation and good peak shape. A full scan mass spectrum of profenofos was recorded to select the most abundant m/z ion (mass-to-charge). For the analyte, the protonated molecular ion $(M+H)^+$ of 374.95 was determined and chosen as the precursor ion. The multiple reaction monitoring (MRM) transitions and associated acquisition parameters were optimised for the maximum abundance of the fragmented ion under ESI positive mode condition by injecting 2 µl of 0.1µg mL^{-1} standard solution of profenofos into the tandem mass spectrometer. Then dissociation with argon was induced and different collision energies were tested to find the most abundant product ion. The optimized precursor m/z (374.95) and product ion transitions m/z 304.90 with CE of -14 was used for quantification and m/z of 346.95 and 128.15 with CE of -47, -21, respectively were used for confirmation of profenofos residue in samples (Fig 1c). The developed LC-MRM mode provides high sensitivity and selectivity requirements for analytical method used for the detection of profenofos at lower concentration from 0.002 mg kg^{-1} in the pigeonpea matrix. In this method, the profenofos was found to show a peak at a retention time of 13.177±0.1 min. The total and exacted ion chromatograms of profenofos standard are shown in Fig 1a and Fig 1b, respectively.

Method validation

Different known concentration of profenofos *viz.*, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.12 µg mL^{-1} were prepared in solvent and matrix match for linearity test and produced a linear relationship between detector response (y) and analyte concentration (x). The parameters obtained by the selected chromatographic conditions for profenofos calibration correspond to y = 11216x - 1333 and R^2 is 0.999 for solvent and y = 10205x + 8646 for R^2 is 0.999 for matrix linearity (Fig 2). The obtained LOD and LOQ of profenofos was 0.002 and 0.006 µg g^{-1}, respectively. The per cent recoveries of profenofos in pigeonpea green pod at 0.006, 0.03 and 0.06 µg g^{-1} were 100.74, 89.23 and 92.44 with RSD of 2.434, 1.292 and 4.349%, respectively. The precision in terms of repeatability was evaluated and recovery of 90.43% with RSD of 1.991% was recorded. Inter-day precision estimated at 0.03 µg g^{-1} resulted in recovery of 87.35 and 93.66% on first and second day, respectively with RSD of 4.927% (Table 1). Matrix effect was less than 9.90% indicated negative matrix effect in the pigeonpea which found below the acceptable limits (20%). The validation results were in complies with the SANTAE/11813/2017 (European Commission, 2017) (Fig 2).

Persistence and dissipation kinetics of profenofos

The average initial deposits of profenofos residues on pigeonpea green pod were 20.28 and 41.64 µg g^{-1} at the recommended dose (500 g a.i.ha^{-1}) and double the recommended dose (1000 g a.i.ha^{-1}) which dissipated to an extent of 74.80 and 70.43%, respectively (Table 2) on first

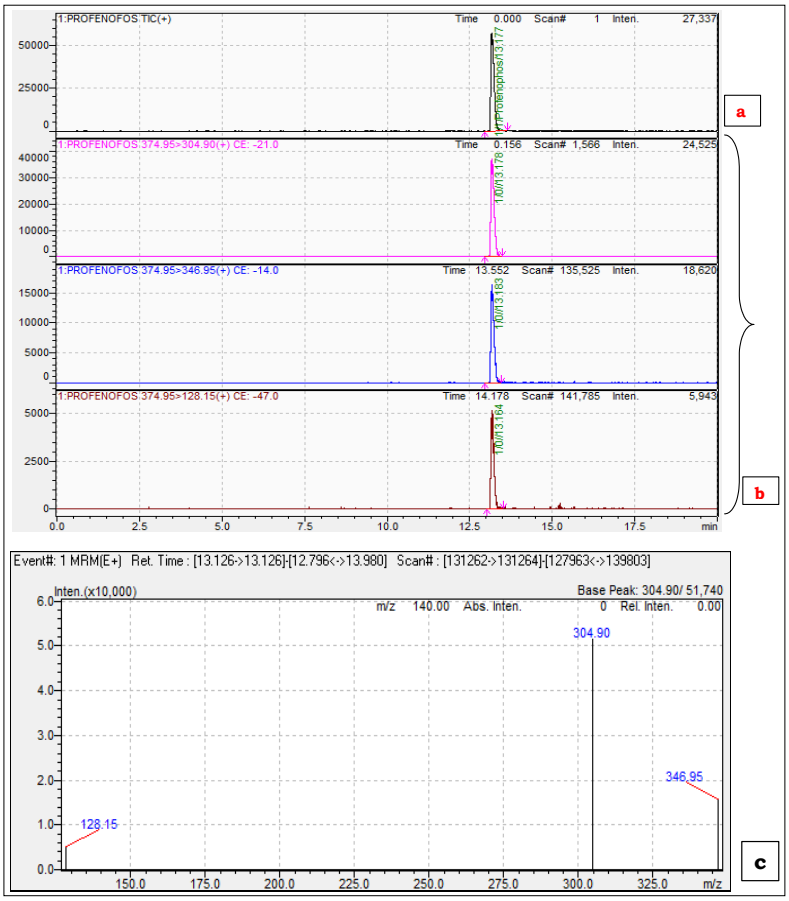

Fig 1: Profenofos total ion chromatogram (a), profenofos product ions extracted chromatogram (b), MRM transitions (c).

Table 1: Recovery, repeatability and reproducibility for profenofos in pigeonpea green pod at different spiking level by the proposed method (r=6); recovery in green pods and dry grains spiked at different LOQ levels.

Replications	% Recovery at different spiking levels			Repeatability at	Reproducibility and ruggedness	
	1 × LOQ	5 × LOQ	10 × LOQ	5 × LOQ	(% Recovery @ 5 LOQ) (0.03 µg g^{-1})	
	(0.006 µg g^{-1})	(0.03 µg g^{-1})	(0.06 µg g^{-1})	(0.03 µg g^{-1})	Day 1	Day 2
R1	101.10	88.15	86.45	88.56	83.89	95.41
R2	97.39	88.30	94.68	89.84	84.90	95.03
R3	101.89	91.30	95.49	93.20	89.17	95.92
R4	98.24	89.59	88.54	89.00	88.15	95.77
R5	103.84	88.78	93.20	90.00	89.42	89.90
R6	101.98	89.26	96.29	92.00	88.59	89.93
Mean	100.74	89.23	92.44	90.43	87.35	93.66
%RSD	2.434	1.292	4.349	1.991	2.697	3.115
						4.927
						(% RSD for inter-day comparsion).

Substrates	Spiked concentration (µg/g)	[a]Recovered concentration (µg/g)	[a]Recovery (%)	*RSD (%)
Pigeonpea green pods	0.06	0.0551	91.90	4.05
	0.03	0.026	88.75	0.99
	0.006	0.0061	101.36	1.49
Mature dry grains	0.06	0.0530	88.34	2.10
	0.03	0.0293	98.77	1.71
	0.006	0.0058	97.65	3.10

[a]Mean of six replicates; *Relative standard deviation.

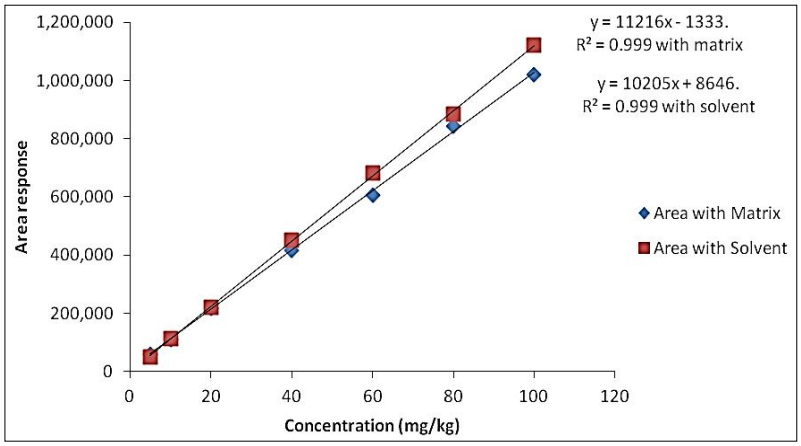

Fig 2: Calibration curve of profenofos from concentration range of 0.005 to 0.12 mg kg^{-1} in solvent and pigeonpea matrix.

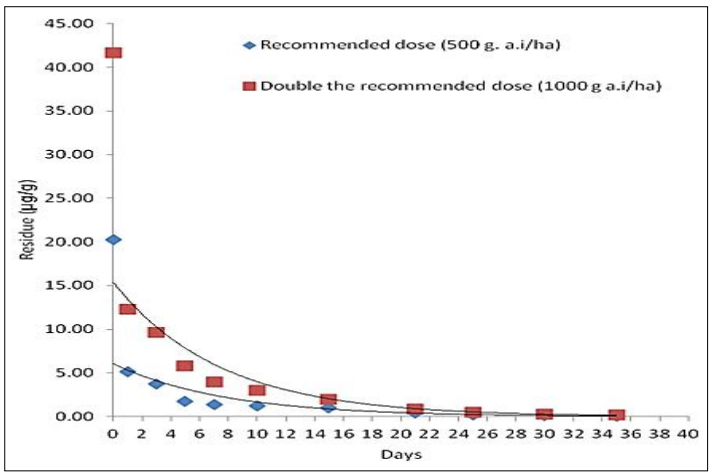

Fig 3: Dissipation curve for profenofos 50% EC sprayed at 500 and 1000 g. a.i ha^{-1} in pigeonpea.

Table 2: Persistence and dissipation of profenofos residues in pigeonpea green pods.

Days after treatment	Recommended dose (500 g a.i. ha^{-1})		Double the recommended dose (1000 g a.i.ha^{-1})	
	Residue (µg/g) [a] (Mean±SD)	Dissipation (%)	Residue (µg/g) [a] (Mean±SD)	Dissipation (%)
0 (1hr)	20.28±0.689	-	41.64±1.138	-
1	5.11±0.212	74.80	12.31±0.580	70.43
3	3.73±0.185	81.60	9.61±0.287	76.92
5	1.72±0.101	91.51	5.79±0.162	86.10
7	1.40±0.093	92.85	3.98±0.064	90.44
10	1.20±0.064	93.09	2.99±0.035	92.81
15	0.78±0.041	96.15	1.98±0.057	95.58
21	0.34±0.093	98.38	0.86±0.076	97.93
25	0.22±0.032	98.91	0.49±0.047	98.82
30	0.13±0.029	99.35	0.29±0.018	99.30
35	0.08±0.1310	99.60	0.18±0.15783	99.56
Mature dry pods (45 days)	ND	-	ND	-
Correlation coefficient	r = 0.951		r = 0.950	
Regression equation	y= 1.794-0.058 x		y=2.084-0.061 x	
t1/2 (days)	5.18		5.93	
K	-0.058		-0.061	
SWP (days)	53.37		55.50	

[a]Mean of eight replicates; ND, SD- Standard deviation; SWP: Safe waiting period.

day after the application. The residue gradually dissipated in 15th day samples contained 0.78 and 1.98 µg g^{-1} residues accounting to loss of more than 95.00% in both dosages as shown in Table 2 and Fig 3. In green pods, profenofos persisted up to 35 days and recorded residues of 0.08 and 0.18 µg g^{-1} in both the dosages, respectively and found below the quantification level after 35th days. Nigam *et al.* (2009) reported about 91.00% of residues of profenofos was dissipated initially and found below LOQ from brinjal at 15 days after application whereas in pigeonpea, it took more than 35 days to reach level below LOQ indicated the slower dissipation rate in pigeonpea. The dissipation is greatly varied with the ecosystem and conditions prevailed in them. In the present study, the persistence nature of profenofos, pigeonpea ecosystem and weather parameter operated during the time of experiment was contributed for quick degradation at the initial period and remained longer period in the field.

The kinetic equation, half-life values, correlation coefficient and safe waiting period of profenofos calculated from the experimental data are summarized in Table 2. The dissipation dynamics of profenofos in green pods followed a first-order rate equation which follows: y = 1.794-0.058 x (r = 0.951) and y = 2.084-0.061 x (r=0.950) with half-lives of 5.18 and 5.93 days at the recommended dose and double the recommended dose, respectively. In the tomato, it was persisted beyond 10 and 15 days with half-life period of 2.2 and 5.4 days for recommended and double recommended dose, respectively (Gupta *et al.,* 2011) which indicated less time required dissipating half of the concentration at the recommended dose than its double dose. Whereas, it was no much differ in the half life values recorded in case of pigeonpea ecosystem. The dissipation kinetics of profenofos in okra recorded the half life 1.35 days after the spray followed a biphasic dissipation pattern with faster dissipation in phase I (0-1 days) and manifesting slower rate of dissipation in phase II (1-15 days) as reported by Paras *et al.* (2005). Similar dissipation pattern is noticed in pigeonpea wherein profenofos dissipated quickly between 0-1 day in both the doses (500 and 1000 g a.i. ha^{-1}) and dissipated at the slower rate from 3 to 15th days after last application.

The waiting period of profenofos on pigeonpea was found to be 53.37 and 55.50 days at recommended and double recommended doses, respectively. Similarly, the waiting period of profenofos in different crops was recorded such as, 19 days in green chilli (Reddy *et al.,* 2007), 11.10 days in green and cured cardamom (Renuka *et al.,* 2006). It was evident that, the crop exudates and ecosystem character influenced more in chilli, green and cure cardamom for quick degradation of profenofos as compare to pigeonpea, as it persisted beyond 35 days and warrants for waiting up to 53.37 days. The profenofos residues persisted up to 30 days and dissipated below determination limit (0.006 µg g^{-1}) from green pods whereas, pigeonpea dry grain drawn at 45th day did not record any residues (Table 2 and Fig 3).

Table 3: Theoretical maximum residue contribution (TMRC) and estimated average daily intake (EADI) and hazard index (HI) of profenofos residues in pigeonpea green pods.

Days after application	MPI (µg. person^{-1} day^{-1})	Recommended dose (500 g.a.i ha^{-1})		Double the recommended dose (1000 g.a.i ha^{-1})		ADI (mg kg^{-1} day^{-1})	Recommended dose (500 g a.i ha^{-1})		Double the recommended dose (1000 g a.i ha^{-1})	
		Mean residues in green pods (µg/g)	TMRC (µg person^{-1} day^{-1})	Mean residues in green pods (µg/g)	TMRC (µg person^{-1} day^{-1})		EADI (mg kg^{-1} day^{-1})	Hazard Index (HI)	EADI (mg kg^{-1} day^{-1})	Hazard index (HI)
0 (1hr)	550	20.28	811.20	41.64	1665.60	0.01	0.8112	81.12	1.666	166.56
1	550	5.11	204.40	12.31	492.40	0.01	0.2044	20.44	0.492	49.24
3	550	3.73	149.20	9.61	384.40	0.01	0.1492	14.92	0.384	38.44
5	550	1.72	68.80	5.79	231.60	0.01	0.0688	6.88	0.232	23.16
7	550	1.40	56.00	3.98	159.20	0.01	0.0560	5.60	0.159	15.92
10	550	1.20	48.00	2.99	119.60	0.01	0.0480	4.80	0.120	11.96
15	550	1.04	41.60	1.98	79.20	0.01	0.0416	4.16	0.079	7.92
21	550	0.44	17.60	0.86	34.40	0.01	0.0176	1.76	0.034	3.44
25	550	0.22	8.80	0.49	19.60	0.01	0.0088	0.88	0.020	1.96
30	550	0.13	5.20	0.29	11.60	0.01	0.0052	0.52	0.012	1.16
35	550	0.08	0.32	0.18	7.20	0.01	0.0032	0.32	0.0072	0.72

MPI- Maximum permissible intake, ADI= Acceptable daily intake, EADI- Expected average daily intake.

Risk assessment

The maximum residue limit (MRL) of profenofos on pulses has been prescribed as 0.05 mg kg^{-1} (FSSAI, 2017). The statistical analysis revealed that the residues of profenofos on pigeonpea dissipated below the MRL after 35 days in both doses (500 and 1000 g a.i. ha^{-1}). The TMRC values were observed as 811.20 and 1665.60 µg $person^{-1}day^{-1}$, respectively for initial deposits shown in Table 3, were found above the permissible intake and not safe for the consumption. Further, theoretical maximum residual concentration values were less than maximum permissible intake at 1st day of sampling at 500 and 1000 g a.i. ha^{-1} doses, respectively found safe for consumption green pods as vegetable.

In an another approach, the calculated hazard index based on the mean residual concentration obtained in the respective treated dose reflected more than 1 up to 21 and 35th day samples drawn from 500 and 1000 g a.i. ha^{-1}, treated plots respectively and it was advised to the consumer that not to use green pigeonpea pod whenever the profenofos is being used as a plant protection chemical (as ovicide and larvicide) during flower initiation to pod maturity for management of pod borer in pigeonpea ecosystem. In the present study, the hazard index (HI) value was found less than one on 25th and 35th day in both the treated doses indicating safely for consumption and no risk for the consumers (Table 3).

CONCLUSION

A simple, sensitive and reproducible method was standardized and validated in pigeonpea matrices using LC-MS/MS with QuEChERS extraction procedure, whereas the methods published so for depicted the use of GC-ECD, FPD, NPD, HPLC-UV for quantifying trace level profenofos from edible crops. The persistence of profenofos was up to 35 days and unnoticed after 45 days indicated the longer persistent period in pigeonpea ecosystem. Profenofos is being commonly used for management of lepidopteron larvae and its leftover residues may have significant toxicological effects on exposing living biota in the crop ecosystem. The safe waiting period of 53.37 days was observed on use of profenofos in pigeonpea at the recommended dose (500 g a.i. ha^{-1}). Hazard index was more than 1 up to 30th day's samples and found high risk upon consumption of green pod during the period and can be used pods as vegetable after 35th day after application.

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Conflict of interest

Author and co-authors declares no conflicts of interest.

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