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LC-ESI-MS/MS Method for Determination of Chlorantraniliprole Residue and its Dissipation Kinetics in Pigeonpea

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A simple and sensitive LC-MS/MS analytical method was developed and validated for determination of chlorantraniliprole residue in green pigeonpea pods, dry grains and soil. The limit of detection (LOD) and limit of quantification (LOQ) of the method were 0.002 and 0.06 µg g^{-1}, respectively. Samples were extracted with 1 per cent ethyl acetate in acetonitrile and cleaned up with primary secondary amine. Average recoveries were in the range of 91.91 to 109.84 per cent with the relative standard deviation of less than 12 per cent. The validated method was applied to analyse chlorantraniliprole residue in pigeonpea matrices. The chlorantraniliprole 18.5 per cent SC was applied in pigeonpea at 30 and 60g a.i. ha^{-1} at 10 d intervals. The initial deposits of chlorantraniliprole residue on green pigeonpea pods were 0.685 and 1.334 µg g^{-1} respectively at the recommended and double the recommended doses. The dissipation pattern followed first order kinetics with half-life of 0.48 to 1.47 d at 30 and 60 g a.$i.^{-1}$ ha, respectively. The safe waiting period (SWP) of 5 d was suggested for safe consumption of green pods at the recommended dose.

Key words: Chlorantraniliprole, LC-MS/MS, half life, safe waiting period, pigeonpea.

Pigeonpea is a major grain or seed legume and commonly called as redgram, tur, arhar, etc. About 75 per cent of world pigeonpea production is from India and annually, 2.0 to 2.5 million tonnes of grains are harvested (Sharma *et al*., 2010). Pigeonpea grains are important source of dietary protein and are consumed as green seeds and dried split seeds. The young and mature green seeds are consumed fresh as a vegetable (Saxena *et al.,* 2010). Insect pests are major production constraints in realizing higher yields (Dubey and Sharma, 2002; Sahoo and Senapati, 2000; Sharma *et al*., 2010). Pigeonpea is infested by more than 30 different species of lepidopteron insects which feed on pods and seeds (Shanower *et al*., 1999). Insecticide application is essential to overcome the incremental crop damage and grain yield loss in pigeonpea. Recently introduced newer insecticides *viz.,* indoxacarb 14.5 per cent SC, spinosad 45 per cent SC, emamectin benzoate 5 per cent SG, flubendiamide 480 per cent SC and chlorantraniliprole 20 per cent SC are commonly being used because of their quick action and high efficacy against lepidopteron larvae infesting pigeonpea (Sharma *et al*., 2010; Singh and Yadav, 2005).

Chlorantraniliprole (5-bromo-*N*-[4-chloro-2-methyl-6-(methylcarbamoyl) phenyl]-2-(3-chloropyridin-2-yl) pyrazole-3-carboxamide) is a new class of selective insecticide that belongs to anthranilic diamide group. It is highly effective on insect pests such as *Ostrinia nubilalis* and *Helicoverpa armigera* (Bassi *et al*., 2009). It activates the insect ryanodine receptors in insects and stimulates the release and depletion of intracellular calcium stores from the sarcoplasmic reticulum of muscle cells causing impaired muscle regulation (Cordova *et al*., 2006). The chlorantraniliprole 18.5 per cent SC has been

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recommended at 30g a.i ha^{-1} for the management of pod borer in India (CIB&RC) during flowering to pod maturity stage and various other formulations were registered for lepidopteron insect pests infesting crops *viz.,* rice, cabbage, cotton, sugarcane, tomato, chilli, brinjal, soybean, Bengal gram, black gram, bitter gourd and okra (http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides).

Reviews indicated that a method for the analysis of chlorantraniliprole residues in pigeonpea matrices has not been developed so far. However, the earlier reports revealed the method developed for other matrices such as fruits and vegetables using LC-MS/MS having the limit of detection (LOD) and limit of quantification (LOQ) of 0.8 and 1.6 µg kg^{-1} respectively; soil, rice straw, paddy water and brown rice having LOQ of 0.15 µg kg^{-1} and fruits and vegetables having LOQ of 0.10 µg kg^{-1} (Caboni *et al*., 2008; Pierluigi *et al*., 2008; Singh *et al*., 2012; Zhang *et al*., 2012) and indoxacarb in pigeonpea using LC-MS/MS (Naik *et al.,* 2020).

In this study, the analytical method developed using LC-MS/MS with positive ESI techniques is extremely sensitive with level of quantification of 0.06 µg g^{-1}.

Chlorantraniliprole residue and its dissipation kinetics have been studied under different crop ecosystems of corn grain, tomato, cowpea, cauliflower, brinjal, okra and sugarcane (Farag 2012; Fengshou *et al*., 2011; Kar *et al*., 2013; Neeraj *et al*., 2014; Vijayasree *et al*., 2013, 2015). However, no systematic research has been conducted on dissipation of chlorantraniliprole in pigeonpea under open field condition. A simple, sensitive, accurate, quick and reproducible LC-MS/MS method has been reported using modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method for extraction and clean up of pigeonpea green and dry grains (Anastassiades *et al*., 2003; Lehotay *et al*., 2005). The present study reports the dissipation kinetics in pigeonpea under open field condition and thereby provides an estimate of the application, harvesting interval required for the safe use of chlorantraniliprole in pigeonpea.

MATERIALS AND METHODS

Chemicals and reagents

Chlorantraniliprole certified reference material (CRM) of purity 98.40 per cent was procured from Dr. Ehrenstorfer, (Augsburg, Germany); LC-MS grade acetonitrile and methanol (purity ≥ 99.999 %), ammonium formate (≥ 96.00% purity) and formic acid (≥ 90.00% purity) were purchased from J. T. Baker (NJ, USA). Ethyl acetate (purity ≥ 99.999%), sodium chloride, disodium hydrogen citrate sesquihydrate and tri-sodium citrate dehydrate (≥ 99.00% purity) were procured from Merck (Mumbai, India). Diethylene glycol (purity > 99.00%), anhydrous magnesium sulphate, sodium acetate and sodium sulphate (99.00% purity) were procured from Himedia (Bangalore, India). Primary secondary amine (PSA) (particle size 40µm, irregular) was obtained from Agilent Technologies India Pvt. Ltd., (Bangalore, India). Chlorantraniliprole 18.5 per cent SC (Coragen, EI Dupont India Pvt. Ltd.) was purchased from local market, Raichur, India.

Preparation of standard solutions

Chlorantraniliprole standard stock solution (1000 µg mL^{-1}) was prepared by weighing accurately 10 ± 0.1mg and transferring into a 10mL clean, calibrated volumetric flask and dissolved in methanol and volume made to 10mL. Intermediate stock and working solutions of known concentrations i.e., 100 and 0.5 µg mL^{-1} were prepared from stock solution by serial dilution technique using methanol and subsequently for linearity study, concentrations ranging from 0.005 to 0.100 µg mL^{-1} were prepared. All the prepared standard solutions were stored at –20 °C. The matrix match standards at the same concentrations were prepared by using the control pigeonpea green pods, dry grains and soil and extracts obtained by sample preparation procedure.

Field experiment

The persistence and dissipation kenetics experiment was conducted at the Main Agriculture Researech Station, (UAS, Raichur, Karnataka, India; longitude:77.3345°E and latitude:16.2043°N). The field trial was laid out in a

randomised block design (RBD) with 3 treatments and 8 replications. The treatment plot size was 30 x 10 m. Pigeonpea (cv: TS-3R) was grown by adopting good agricultural practices reccommended by the University Agricultural Sciences, Raichur. Chlorantraniliprole 18.5 per cent SC was applied as foliar spray @ 30 (reccommended dose) and 60 g. a.i.ha^{-1} (double the recommended dose) along with an untreated control. Two foliar sprays were given at 15 d interval during pod formation to maturity. Chlorantraniliprole was applied using a high volume knapsack compression sprayer using spray fluid volume of 500 L ha^{-1}. There were no rains througout the experimentation, however, mean minimum and maximum temperature and relative humidity recorded were 17.6 and 32^{o}C and 44 and 55 per cent, respectively.

Sample collection and preparation

Pigeonpea green pods (500 g) were collected randomly from each plot in clean and inert plastic containers separately at 0 (1h after spraying), 1, 3, 5, 7, 10 and 15 d after the application of chlorantraniliprole. The samples were transported to the laboratory under dry ice condition and processed immediately for residue analysis. The fresh pods were homogenized using a high volume homogenizer (Robo coup). The soil (1 kg) samples were collected from each plot after 15 d of the last application following the standard sampling procedure. The samples from each plot were pooled, air dried, and then sieved through 2 mm sieve.

Extraction and clean-up of pigeonpea green pods and dry grains

About 5 g of ground sample was weighed into 50 mL centrifuge tube and distilled water (10 mL) and 10 mL of 1 per cent ethyl acetate in acetonitrile were added followed by the addition of anhydrous magnesium sulphate (6 g) and sodium acetate (1.5 g). Homogenized the sample mixture using low volume homogenizer (Make-IKA®, model-T_{18}) at 13000 rpm for 3 min and centrifuged at 5000 rpm for 5 min. An aliquot of 7 mL of supernatant was transferred into a 15 mL centrifuge tube containing 0.350 g PSA (primary secondary amine) and 1.05 g anhydrous magnesium sulphate. The mixture was vortexed for one min and centrifuged at 12000 rpm for 5 min. The upper organic layer 3 mL was transferred into a test tube containing 300 µL of 10 per cent DEG (diethylene glycol) and evaporated the content using nitrogen flash evaporator at 35ºC. The residue was reconstituted with 1.5 mL of methanol and filtered using 0.22 µ PTFE nylon filter in LC vials for LC-MS/MS analysis.

Extraction and clean-up of soil sample

About 20 g of sieved soil sample was weighed in 250 mL conical flask, 12 mL distilled water was added and allowed to stand for 30 min. Then acetonitrile (20 mL) was added and shaken for 4 h in horizontal shaker at 250 rpm. The sample mixture was then transferred into 50 mL centrifuge tube and centrifuged for 3 min. at 5000 rpm. The whole supernatant aliquot was transferred in to 50 mL centrifuge tube containing 6g of magnesium sulphate, 1.5g of sodium chloride, 1.5g of trisodium citrate dehydrate and 750 mg of disodium hydrogen citrate sesquihydrate. The sample mixture was vortexed for 1 min and then the content was centrifuged at 5000 rpm for 5 min. Six mL supernatant was transferred in to 15 mL centrifuge tube containing 150 mg PSA and 900 mg magnesium sulphate ($MgSO_4$). Vortexed the mixture for 30 sec and centrifuged at 5000 rpm for 5 min. Finally, 2 mL of extract was transferred into a test tube containing 200 µL of 10 per cent DEG in methanol and evaporated to dryness using nitrogen flash evaporator at 35 ºC. Reconstituted the residue with 1.5 mL methanol (LC-MS grade). Sonicated the mixture in ultrasonicator to dissolve residues completely and then filtered the content using 0.22 µ PTFE nylon filter from test tube in LC vials.

LC-MS/MS parameters

The LC-MS/MS system equipped with Shimadzu 1200 series UHPLC and LCMS 8040 with triple quadrupole detector (TQD) was used. Lab Solution® LCMS Version 1.5 software was used for instrument control, data acquisition and processing. The C_{18} column (Shimpack XR, ODS III, 2 mm i.d x 150 mm; particle size of 1.9 µm; Shimadzu India Pvt. Ltd, Mumbai) was used for chromatographic separation. HPLC conditions for the analysis were as follows: the mobile phase A consisted of

0.0314 g ammonium formate (5mM) + 2mL MeOH + 10 µL formic acid (0.01%), made-up to volume with HPLC water to 100 mL and mobile phase B consisted of 0.0314 g ammonium formate (5mM) + 10µL formic acid (0.01%), made-up to volume with 100 per cent MeOH to 100 mL. The flow rate was 0.4 mL min^{-1}; injection volume was 2 µL and column oven temperature 40°C. The pesticide chlorantraniliprole was separated with the following gradient programme: 60 per cent A and 40 per cent B at beginning for 15 min followed by 100 per cent B upto 20 min and then 60 per cent A and 40 per cent B for 25 min. The MS/MS conditions were as follows: mass spectrometer was operated in electro spray ionization (ESI+) mode, interface current was 0.1 µÅ, heat block temperature was 400 °C, DL temperature was 250 °C, nitrogen gas (2.9 L min^{-1}) was used as nebulizer gas and drying gas (15 L min^{-1}), argon (230 kpa) was used as current ionization detector (CID) gas. The fragmentation, voltage current (V) and the collision energy (CEs) were optimized to achieve the highest sensitivity in separation.

Method validation

The method was validated as per SANTE/11813/2017 guidelines for linearity, sensitivity (LOD and LOQ), matrix effect (ME), recovery, precision (repeatability) and ruggedness (European Commission, 2017). The linearity study was conducted by preparing different standard concentrations ranging from 0.001 to 0.10 µg mL^{-1}. The LOD and LOQ were determined by the signal-to-noise ratio (S/N) of 3 and 10, respectively by obtaining the response on injecting lowest concentration. Accuracy was evaluated by fortifying the pigeonpea green pods, dry grains and soil sample at concentration level of 0.006, 0.030 and 0.060 µg g^{-1}. The spiked samples were equilibrated and processed as per the methodology described. The method repeatability was ascertained with regards to the relative standard deviation (RSD_r) of the six replicates, exactly similar extractions of blank pigeonpea samples spiked with chlorantraniliprole at the 0.030 µg g^{-1} fortification level in pigeonpea green pod matrix.

Reproducibility test was done by spiking the chlorantraniliprole in pigeonpea sample at 0.030 µg g^{-1} levels at two different dates with six replications and calculated the per cent RSD. The ruggedness of the method was evaluated by spiking the sample at 5 times of LOQ and performing analysis by changing the analysts. Per cent matrix effect was calculated comparing the peak area of matrix match with solvent standard. The matrix effect caused by the green pod extract was calculated by comparing the angular coefficients obtained by the curves in the solvent and in the matrix according to the following equation:

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

where, b_m and b_s are the angular coefficients of the curve in the matrix and in the solvent, respectively (European Commission, 2017; Naik *et al.,* 2020).

Statistical analysis

The dissipation of chlorantraniliprole residues in green pigeonpea pods was analyzed by using first-order dissipation kinetics equation i.e. $Ct = Coe^{-kt}$, where Ct is the pesticide concentration (µg g^{-1}) at time t (d), Co is the apparent initial concentration (µg g^{-1}), k is the dissipation rate constant. The half-life ($t_{1/2}$) was determined as DT_{50} = log2/k; $t_{1/2}$ is the insecticide half-life. Safe waiting period (pre-harvest interval, PHI) for green pigeonpea pods was calculated by the following formula:

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

where, T_{tol} = minimum time (d) 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 of the insecticide (MRL); b = Slope of the regression line (Regupathy and Dhamu, 1990).

Half-life (RL 50)

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

(e = log 2=0.301).

RESULTS AND DISCUSSION

Optimization of LC-MS/MS parameters

The UHPLC parameters such as, pump flow rate 0.4 $mLmin^{-1}$, column oven temperature of 40 ^{o}C, rinse dip time 5 sec., heat block temperature of 400 ºC, DL (disolvation temperature) temperature of 250ºC provided a good resolution for chlorantraniliprole and was maintained. The mobile phase used in this study provided adequate resolution and separation of chlorantraniliprole from pigeonpea matrix. The chlorantraniliprole estimation was optimized in the positive electro spray ionization (ESI+) mode. Its multiple reaction monitoring (MRM) was conducted. For chlorantraniliprole, the protonated molecular ion $(M+H)^{+}$ of 483.75 was determined and chosen as the precursor ion. Then dissociation with argon was induced, and different collision energies were tested to find the most abundant product ion. The most intense product ion transition (m/z) of 452.95 with CE of -17 (collision energy) was used for quantitation, while the daughter ion (m/z) of 285.90 with CE of -16 and 177.05 with CE of -50 was employed for confirmation. The chlorantraniliprole eluted at 8.26 ± 0.1 min under optimized conditions (Fig. 1). The optimized parameters are presented in Table 1.

Method validation

A very good linearity was obtained from 0.006 to 0.1 µg mL^{-1} with the correlation coefficients greater than 0.999 for both solvent and matrix match standard solutions. (Fig. 2a and 2b). The limit, of detection (LOD) and quantification (LOQ) were 0.002 and 0.006 µg g^{-1}, respectively. The average recoveries of 109.84, 105.57 and 91.92 per cent at fortified concentrations of 0.006, 0.03 and 0.06 µg g^{-1} were recorded in pigeonpea green pods with relative per cent deviation (RSD) ranging from 5.18 to 11.27 per cent. The per cent recoveries of 103.53, 108.63 and 104.83 were recorded in dry grains with RSD ranging from 2.83 to 3.84 percent. The per cent recovery of 84.32, 90.00 and 77.53 were rerecorded in soil samples at 0.06, 0.03 and 0.006 µg g^{-1} of fortification levels, respectively with RSD range of 1.56 to 3.77 (Table 3 and Fig. 3). Recoveries recorded were within the acceptance range of 70 to 120 per cent and relative per cent deviation found below 15 per cent showed high accuracy of the developed method. The precision was evaluated and found recovery percentage of 96.79 with RSD of 8.581 (Table 2). The reproducibility with respect to changed date of analysis gave an acceptable per cent recovery of 107.86 to 101.59 with RSD of 4.236 per cent (Table 2) and this complies with the SANTE/11813/2017 guidelines (European Commission, 2017).

Dissipation kinetics of chlorantraniliprole

The initial deposits of chlorantraniliprole on pigeonpea green pods were found to be 0.685 and 1.334 µg g^{-1} at the recommended and the double doses, respectively (Table 4). These deposits dissipated to 0.015 and 0.043 µg g^{-1} at 3^{rd} d of application, the dissipation being more than 96 per cent of the initial deposit. The residues reached below its LOQ of 0.006 µg g^{-1} on 7^{th} d. Within 3 d of application, the concentration of chlorantraniliprole reduced drastically because of its sensitivity to light and other weather parameters existing in pigeonpea ecosystem during experimentation. The dissipation or degradation of pesticide residues in crops depends on various factors including environmental condition, formulation, method of application, plant species, dosage and interval between applications (Cabras *et al.,* 1989).

Pigeonpea dry grains after 25 d and the soil samples drawn at 15 d after application did not reveal the presence of chlorantraniliprole residue (Table 4).

Results of the present findings showed a close similarity to the dissipation of chlorantraniliprole in sugarcane field soil at application rate of 100 and 200 g a.i ha^{-1} where the average initial deposits were found to be 0.88 and 1.59 µg g^{-1} respectively, the deposit being 1.9 times lower in single dose when compared to the double dose (Neeraj *et al.,* 2014). An initial deposit of 2.308 µg g^{-1} was observed following application of chlorantraniliprole at the recommended dosage in tomato fruits (Malhat *et al*., 2012).

The residue data on chlorantraniliprole in pigeonpea indicated that there was initially rapid loss of residues

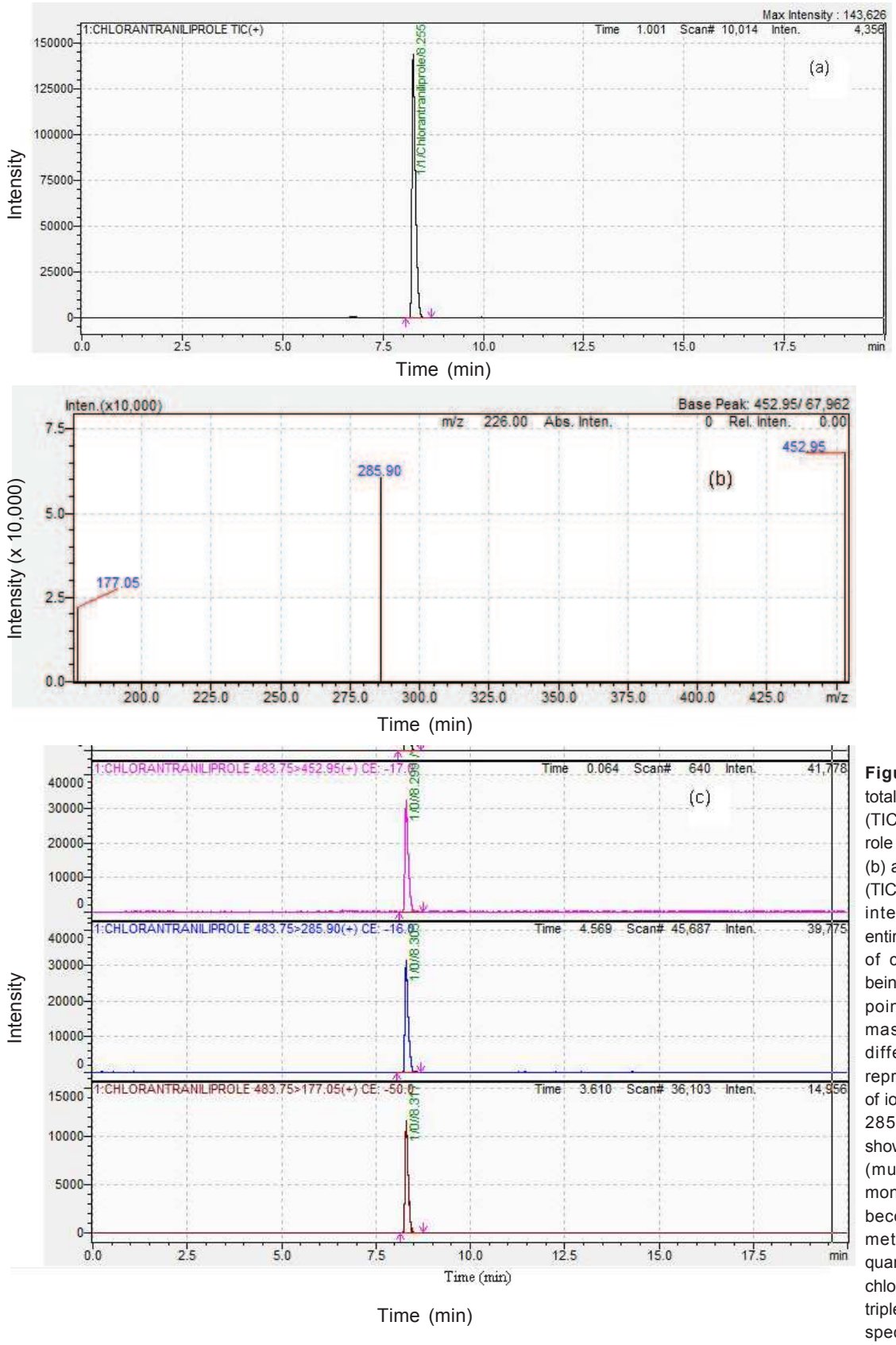

Figure 1. LC-MS/MS total ion chromatograms (TIC) for chlorantraniliprole (a) mass spectrum (b) and MRM mode (c) (TIC represents summed intensity across the entire range of masses of chlorantraniliprole being detected at every point in the analysis; mass spectrum with different m/z values represents distribution of ions such as 177.05, 285.90 and 452.95 shown by LC-MS; MRM (multiple reaction monitoring) mode has become the preferred method for the quantitative analysis of chlorantraniliprole using triple quadrupole mass spectrometry)

Table 1. ESI-MS/MS (+) mass spectrometer parameters for chlorantraniliprole

Pesticide	RT (min)	Precursor ion (m/z)	Quantifier ion (m/z)	Collision energy (CE)	Qualifier ion (m/z)	Collision energy
Chlorantraniliprole	8.26 ± 0.1	483.75	452.95	-17	285.90	-16
					177.05	-50

RT-retention time

Table 2. Method validation for the determination of chlorantraniliprole in pigeonpea green pod

Replication	Recovery (%) at different spiking levels (µg g^{-1})			Precision (% recovery at	Reproducibility (% recovery at 0.030 µg g^{-1})	
	0.006	0.030	0.060	0.030 µg g^{-1})	Day 1	Day 2
R1	113.41	87.16	93.32	91.59	107.95	101.45
R2	98.66	108.83	96.38	89.87	111.13	105.98
R3	99.47	95.11	97.71	95.33	114.25	114.18
R4	115.48	117.64	86.19	97.37	113.04	102.24
R5	119.05	114.71	87.00	93.74	103.60	98.28
R6	112.97	109.99	90.91	112.86	97.23	87.42
Mean	109.84	105.57	91.92	96.79	107.86	101.59
% RSD	7.850	11.269	5.182	8.581		4.236

RSD=Relative standard deviation

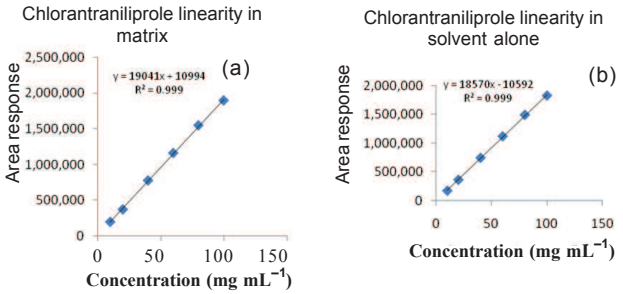

Figure 2. Calibration curve of chlorantraniliprole for the concentration range of 0.006 to 0.1 mg L^{-1} in pigeonpea blank matrix (a) and solvent (b)

followed by slower rate of dissipation later. Dissipation of 83.33 per cent of chlorantraniliprole on brinjal is reported and the residues reached below the detectable limit on 10th d after spray (Vijayasree *et al*., 2015). In another study, the initial residues of 0.55 µg g^{-1} observed in cowpea dissipated rapidly to 0.02 µg g^{-1} in 7 d and reached below detectable limit on 10th d after spray following the application of chlorantraniliprole 18.5 per cent SC (Vijayasree *et al*., 2013). On berseem, chlorantraniliprole initial deposit was found to be 0.47 µg g^{-1} following application of 11.6 g a.i. ha^{-1}. It degraded by 40 per cent after a d and subsequently by more than 70 per cent on 3rd d and the residues reached below detection limit of 0.01 µg g^{-1} at 5 d (Mandal *et al*., 2014). On tomato crop a deposit of 2.308 µg g^{-1} on

Table 3. Recovery and relative standard deviation of chlorantraniliprole spiked in pigeonpea green pods, dry grains and soil at different spiking levels

Substrate	Spiked concentration (µg g^{-1})	Recovered concentration (µg g^{-1})	[a]Recovery (%)	*RSD (%)
Green pod	0.060	0.055	91.92	5.18
	0.030	0.032	105.57	11.27
	0.006	0.0066	109.84	7.85
Dry grains	0.060	0.062	103.53	3.84
	0.030	0.033	108.63	3.76
	0.006	0.006	104.83	2.83
Soil	0.060	0.051	84.32	2.22
	0.030	0.027	90.00	3.77
	0.006	0.005	77.50	1.56

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

application of chlorantraniliprole 20 per cent SC @ 60 mL per feddan (1 feddan = 4200 m^2) reached below determination limit of 0.03 mg kg^{-1} at 21 d after application (Malhat *et al*., 2012). Application of chlorantraniliprole on cauliflower curds revealed mean initial deposits of 0.18 and 0.29 µg g^{-1} at 9.25 and 18.50 g a.i. ha^{-1}, respectively and the residues following 3rd application of chlorantraniliprole reached below the level of determination (0.10 µg g^{-1}) in 3 and 5 d respectively (Kar *et al*., 2013).

Half life and waiting period

The kinetic equation, half-life and correlation coefficient (r) following chlorantraniliprole residue dissipation are reported in Table 4. A plot of log residues versus time provided a linear relationship. The dissipation pattern followed first-order kinetics with a half-life of 0.47-1.47 d. Earlier studies have reported the half-life of chlorantraniliprole in cauliflower as 1.36 and 1.25 d (Kar *et al.,* 2013), 3.30 d on tomato (Malhat *et al.,* 2012) and 3.50 d on rice straw (Zhang *et al.,* 2012).

In the present investigation the persistence of chlorantraniliprole was studied to generate data for fixing MRL. Furthermore, the limit of quantification and the safe waiting period have been suggested. The waiting periods of chlorantraniliprole on pigeonpea have been found to be 5.01 and 7.00 d at the recommended and the double doses, respectively. These values are close to the observations on dissipation of chlorantraniliprole on tomato where pre-harvest interval period of 8 d was reported (Farag, 2012).

CONCLUSION

A simple and sensitive LC-ESI-MS/MS analytical method has been standardized for the analysis of chlorantraniliprole in pigeonpea green pods, dry grains and soil. A faster degradation of chlorantraniliprole occurred in pigeonpea green pods and followed first order kinetics with half lives of 0.48 to 1.47 d when applied at 30 and 60 g.a.i ha^{-1}. A safe waiting period of 5 d at the recommended dose has been suggested for safe consumption of green pods.

Table 4. Chlorantraniliprole residues in pigeonpea ecosystem at different days after treatment

Days after treatment	Recommended dose (30 g. a.i. ha^{-1})		Double the recommended dose (60 g. a. i. ha^{-1})	
	Mean residues ± SD (µg g^{-1})	Dissipation (%)	Mean residues ± SD (µg g^{-1})	Dissipation (%)
0 (1 h)	0.685±0.271	-	1.334± 0.653	-
1	0.388±0.037	43.35	0.832± 0.230	37.63
3	0.015±0.005	97.81	0.043±0.003	96.77
5	0.008 ± 0.001	98.83	0.020± 0.002	98.50
7	BDL	-	BDL	-
15 (Soil)	ND	-	ND	-
25 (Dry grains)	ND	-	ND	-
Correlation Coefficient (r)	0.999		0.998	
Regression equation	y= 1.208-0.625x		y= 0.6321-0.2044x	
$t_{1/2}$ (d)	0.48		1.47	
SWP (d)	5.01		7.20	

SD=Standard deviation; ND=Not detected; BDL=Below detectable limit; $t_{1/2}$=Half life; SWP=Suggested waiting period

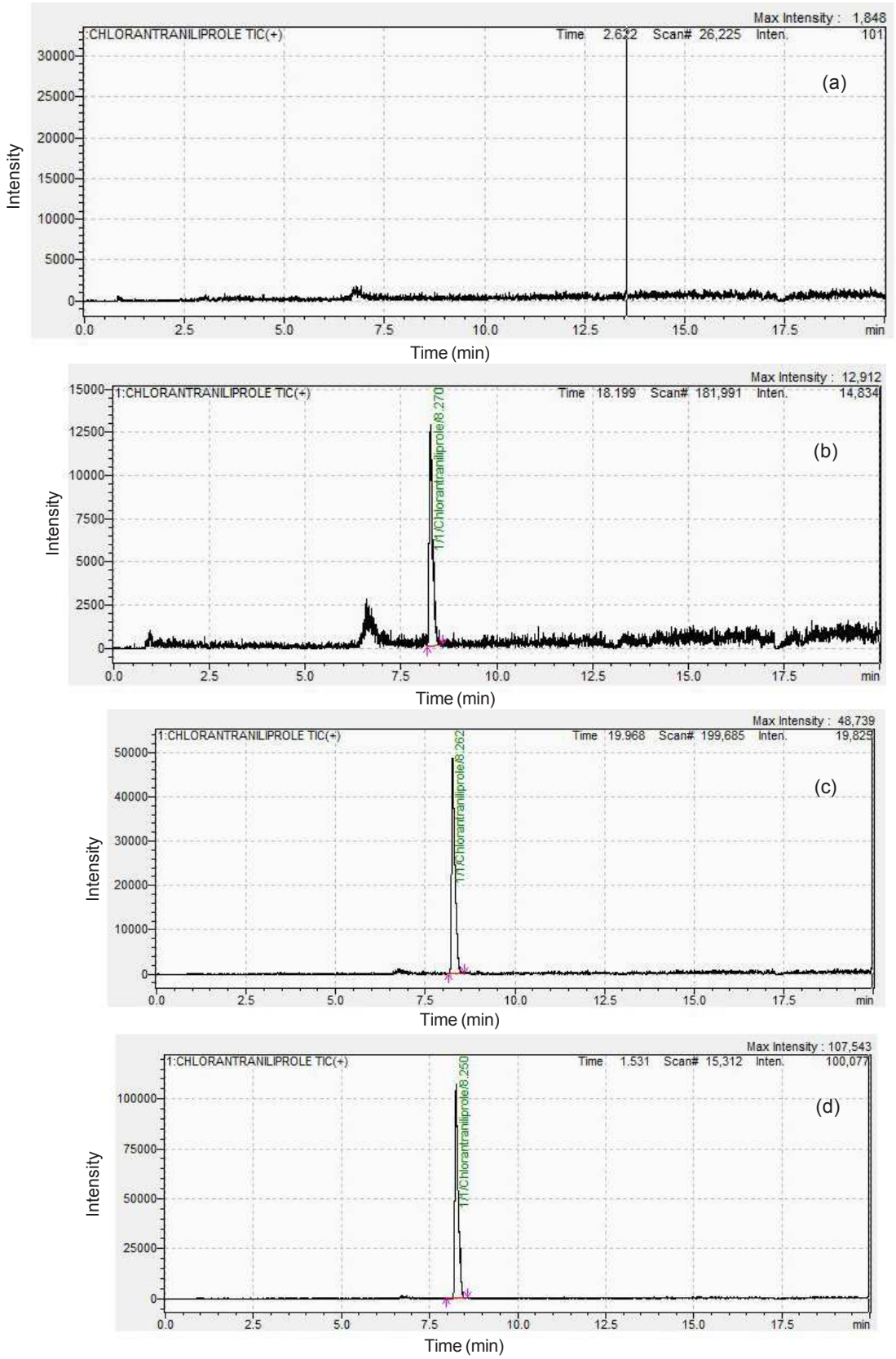

Figure 3. LC-ESI-MS/MS chromatogram of pigeonpea green pod blank (a), recovery 0.006 µg g^{-1} (b), 0.03 µg g^{-1} (c) and 0.06 µgg^{-1} (d)

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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