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Method Development and Validation for Determination of Indoxacarb Using LC-ESI-MS/MS and Its Dissipation Kinetics in Pigeonpea

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Abstract

A simple, sensitive and reproducible analytical method for quantitative determination of indoxacarb residues in pigeonpea green pod, pigeonpea dry grain and soil was developed and validated using LC-MS/MS. The developed methodology was linear having correlation coefficients (R^2) value of 0.999. The LOD and LOQ were 0.0015 and 0.005 μg g^{-1}, respectively. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique involved for extraction of indoxacarb residues with 1% ethyl acetate in acetonitrile provided acceptable recoveries in the range of 83.80–99.01% at 0.005, 0.025 and 0.050 μg g^{-1} spiking level with precision RSD of less than 1.20 %. Initial deposit on green pods following two application (15 days interval between application) of indoxacarb 14.5% SC at 60 and 120 g a.i.ha^{-1} was 0.583 and 1.342 μg g^{-1} and dissipated to 0.005 and 0.006 μg g^{-1} on 7 and 10 days after application, respectively. Half-life and safe waiting period (SWP) were ranged from 1.13 to 1.23 days and 3.23 to 5.03 days for the dose 60 and 120 g a.i.ha^{-1} in pigeonpea green pods, respectively. Hazard index (HI) value was more than 1 in green pods drawn on 5th and 7th day after application in recommended (60 g a.i.ha^{-1}) and double the recommended dose (120 g a.i.ha^{-1}), respectively.

Keywords Pigeonpea · Indoxacarb · Dissipation · LC-MS/MS · QuEChERS · Half-life

Introduction

Pigeonpea is commonly known as redgram, tur and arhar and an important drought resistance pulse crop cultivated mainly for its protein-enriched seeds in semi-arid and tropical region world (Shanower et al. 1999). It is an excellent source of protein (20–22 %), its green seeds are being used as vegetables and dried, dehulled split cotyledons (dal) are eaten along with rice and bread in India, while in Africa and Southern America, both whole dry and immature seeds are used as vegetable (Saxena et al. 2010). India produces more than 90% (4.83 million tonnes) of world's pigeonpea (Sameer Kumar et al. 2014). The diseases, insect pests and vector-borne diseases are the major bottlenecks in realizing higher yields in pigeonpea (Dubey and Sharma 2002; Shanower et al. 1999). Among them, the insect pests are the major factor contributed for 50–85% loss in grain yield, wherein the pod borer damages the crop to the tune of 20–57% and grain yield loss up to 28%; 20 to 60% in grain yield loss due to spotted pod borer, 70–80% of the total pod damage by pod fly and 25 to 40 % grain yield loss due to pod bug (Sahoo and Senapati 2000; Sharma et al. 2010). Farmers growing this crop are relying mainly on chemical pesticides because of quick action and high efficacy against insect pests infesting pods of pigeonpea. Chemical pesticides currently being used for management of pod borer and pod webber are indoxacarb and chlorantraniliprole (Sharma et al. 2010). Occurrence of residues of chemical pesticides needs to be monitored in view of quality food grain production and supply. Dissipation of chemical insecticide from crop ecosystem is useful in understanding the half-life and safe waiting periods for safe consumption of harvested grains.

Indoxacarb is a nonsystemic, reduced risk and synthetic organophosphate replacement insecticide that belongs to oxadiazine group used for managing lepidopteron larvae and sucking insects infesting many vegetables and commercial crops (United States Environmental Protection Agency

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(2000)). Two formulations, viz. indoxacarb 14.5% SC (suspension concentrate) and 15.8% EC (emulsifiable concentrate), were registered in India for managing lepidopteron larvae infesting cotton, cabbage, chillies, tomato, pigeonpea, rice and soybean. Similarly, they have been used in fruits, vegetables and cotton in other countries such as the USA (Allen et al. 1999; Liu and Sparks 1999) and Italy (Bassi et al. 2000). It mainly acts on the nervous system by blocking the voltage-dependent sodium channel in insect nerve cells resulting in paralysis and death of larvae (McCann et al. 2001). It has low side effect on non target insects and found non toxic to Trichogramma (Hewa-Kapuge et al. 2003). The consumption of food grains contaminated with indoxacarb leads to the acute onset of methemoglobinemia; a condition which reduces the effectiveness of red blood cells to exchange oxygen with organs can be noticed (Prasanna et al. 2008).

The method validation and dissipation kinetics of indoxacarb have been studied in edible crops such as apple using gas chromatography with electron capture and nitrogen phosphorus detector (Ewa Szpyrka et al. 2017), sugar beet leaf using HPLC with PDA detector and LOQ of 0.1 mg kg^{-1} (Sdeek Fayza and Taha 2018), cauliflower using GC-ECD with LOQ of 0.02 mg kg^{-1} (Ji-Yoon et al. 2013) and GC-MS with LOQ of 0.01 mg kg^{-1} (Takkar et al. 2011), cabbage using HPLC-PDA with LOQ of 0.01 mg kg^{-1} (Sun et al. 2012; Urvashi et al. 2012), Chinese cabbage using GC-ECD with LOD of 0.01 mg kg^{-1} (Lee et al. 2008), okra using GC-ECD with LOD of 0.02 mg kg^{-1} (Gupta et al. 2009) and brinjal with GC-ECD having LOQ of 0.01 mg kg^{-1} (Sinha et al. 2010). This review clearly indicated that the techniques used are less sensitive compared to LC-MS/MS (liquid chromatography tandem mass spectrometry). An attempt is made to develop a sensitive method for quantifying the indoxacarb residues involving modified QuEChERS extraction techniques (Anastassiades et al. 2003; Lehotay et al. 2005) for processing the pigeonpea green pod and dry grain samples. Similar studies were conducted using LC-MS/MS in pomegranate, apple and cauliflower with LOQ of 0.005, 0.044 and 0.01 mg kg^{-1}, respectively (Mohapatra et al. 2019; Tiryaki 2016; Prodhan et al. 2016). The present method is highly sensitive with reported LOD, and LOQ of 0.0015 and 0.005 μg g^{-1}, respectively, is the advantages over other technique in trace level analysis of indoxacarb in edible pigeonpea and other pulses.

In pulses, more particularly in pigeonpea, the method for analysis of indoxacarb and its dissipation pattern under field condition has not been studied. Extensive use of indoxacarb may leave potential residues in green pods, which have toxicological implication (Prasanna et al. 2008). Realizing these importances, an analytical method was developed and validated in liquid chromatography tandem mass spectrometry (LC-MS/MS). Further, a supervised field trial was conducted to study the persistence and dissipation of indoxacarb in pigeonpea green pods and calculated the safe waiting period and risk associated with consumption of green pods.

Materials and Methods

Chemical and Reagents

Indoxacarb (98.4% purity) certified reference material was purchased from Dr. Ehrenstorfer (Augsburg, Germany), LC-MS grade acetonitrile and methanol (≥ 99.9% purity) were procured from J. T. Baker (NJ, USA), ethyl acetate (≥ 99.8% purity) was procured from Merck, Mumbai (India). Ammonium formate and formic acid (90% purity) were purchased from Empart, Hyderabad (India). Anhydrous magnesium sulphate, sodium acetate and sodium sulphate (> 99% purity) were procured from Himedia, Bangalore, India. Solid phase extraction sorbent, primary secondary amine (PSA) (AR grade, particle size of 40 μm), was obtained from Agilent Technologies India Pvt. Ltd., Bangalore, India. Sodium chloride (≥ 99% purity), disodium hydrogen citrate sesquihydrate and trisodium citrate dihydrate (≥ 99% purity) were procured from Merck, Mumbai (India). Diethylene glycol (> 99% purity) was purchased from Himedia, Bangalore, India. Indoxacarb 14.5% SC (Auvant®, E. I. DuPont India Pvt. Ltd) formulation was purchased from the local market. HPLC grade water was obtained with Milli-Q water system.

Apparatus

LC-MS/MS (SHIMADZU LC-MS 8040®), analytical balance (Sartorius®, model- BSA224S-CW), homogenizer (IKA®, model- T_{18}), centrifuge (Gyrozen®, model- 1736R), vortexer (REMI®), evaporator (Turbo Vap®), horizontal shaker (Rotek®), rotary vacuum evaporator (IKA, model-RV 10), centrifuge tube (Tarsons®) and Milli-Q water purification unit (Milli-Q®) were used in the experiment.

Preparation of Standard Solutions

Indoxacarb standard stock solution (1000 μg mL^{-1}) was prepared by weighing accurately 10.163 ± 0.1 mg and transferred into a 10 mL cleaned, calibrated volumetric flask and dissolved with 10 mL of methanol (LC-MS grade). Intermediate stock and working solution of known concentration, i.e. 100 and 0.5 μg mL^{-1}, were prepared from stock solution by serial dilution technique using methanol. For linearity study, the concentration ranging from 0.005 to 0.12 μg mL^{-1} was prepared from 0.5 μg mL^{-1} working standard solution. The concentration recorded outside the linear range was diluted, the samples were analysed, and residue was calculated adding dilution factor. 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 pod sample extract obtained through sample preparation procedure described below.

Field Experimentation

The persistence and dissipation kinetics experiment was conducted at entomological experimental block of University of Agricultural Sciences, Karnataka, India (longitude 77.3345° E and latitude 16.2043°N). The field trial has been laid out in a randomized block design (RBD) with 3 treatments and 8 replications. The treatment plot size was 30 × 16 m. Pigeonpea local cultivar, TS-3R, was cultivated by adopting good agricultural practices recommended by the University Agricultural Sciences, Raichur. Indoxacarb 14.5% SC was applied as foliar spray at 60 g.a.i.ha^{-1} (recommended dose) and 120 g. a.i.ha^{-1} (double the recommended dose) along with an untreated control. Two foliar sprays were given at 15 days interval during pod formation to maturity. Indoxacarb was applied using a high-volume knapsack compression sprayer using spray fluid volume of 500 L ha^{-1}. There were no rains throughout the experimentation; however, mean maximum and minimum temperature and relative humidity were 17.6 and 32 °C and 44 and 55%, respectively.

Sample Collection and Preparation

Pigeonpea green pods and dry grain (500 g) samples were collected randomly from each replicates which represents a whole treated and control plot in clean and inert plastic containers separately at a regular interval on 0 (1 hr after spraying), 1, 3, 5, 7, 10, 15, 21 and 25 days after the application of indoxacarb. The samples were transported to the laboratory under dry ice condition and processed immediately for residue analysis. The soil samples were collected from each treatment plot at harvest following the standard sampling procedure (Sharma et al. 2010). From each replicate plot, 1 kg soil sample was drawn from 15 cm depth using soil auger and collected in a clean container. The samples were pooled together and mixed thoroughly and processed at laboratory. Further, samples were air-dried and passed through 2 mm sieve, and about 20 g of sample in triplicate was weighed in 50 ml polypropylene tube for analysis.

Extraction of Indoxacarb from Pigeonpea Green and Mature Pods

QuEChERS method and its modification described by Anastassiades et al. (2003) and Lehotay et al. (2005) were adopted for extraction and cleanup of indoxacarb from pigeonpea green pods and dry grain. Whole laboratory samples (500 g) was grounded thoroughly using high-volume homogenizer (Robot Coup). About 5 g of ground sample was weighed in analytical balance and transferred into 50 mL centrifuge tube, and distilled water (10 mL) was added and, further, allowed to stand for 30 min. After 30 min., 10 mL of 1% ethyl acetate in acetonitrile (for better resolution of peak of the analyte) was added followed by the addition of anhydrous magnesium sulphate (6 g) and sodium acetate (1.5 g). Homogenize the sample mixture using low-volume homogenizer at 10000–13000 rpm for 3 min and centrifuged at 5000 rpm for 5 min. After time lapsed, 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, and then vortex the sample mixture for 1 minute, and contents were centrifuged at 12,000 rpm for 5 min. Transfer 3 mL of each extractant into a test tube containing 300 μL of 10% DEG (diethylene glycol), and evaporate the content using nitrogen flash evaporator at 35 °C to dryness. Later reconstitute the residue with 1.5 mL of methanol (MeOH), and sonicate the mixture using ultrasonicator to dissolve residues completely. Then sample content was filtered using 0.22 μ PTFE nylon filter for subsequent analysis in LC-MS/MS.

Extraction of Indoxacarb from Soil Sample

About 20 g of sieved soil sample was weighed in 250 mL conical flask, and then 12 mL distilled water was added and allowed to stand for 30 min. Then acetonitrile (20 mL) was added and shaked for 4 hours 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. Then, aliquot supernatant (20 mL) was transferred in to 50 mL centrifuge tube containing 6 g of anhydrous magnesium sulphate, 1.5 g of sodium chloride, 1.5 g of trisodium citrate dehydrate and 750 mg of disodium hydrogen citrate sesquihydrate. Further, sample mixture was vortexed for 1 min. and then content was centrifuged at 5000 rpm for 5 min. Then, 6 mL supernatant was transferred in to 15 mL centrifuged tube containing 150 mg of PSA and 900 mg anhydrous magnesium sulphate. Further, vortex the mixture for 30 sec. and centrifuge at 5000 rpm for 5 min. Finally, 2 mL of extractant was transferred into a test tube containing 200 μL of 10% DEG (diethylene glycol) in methanol, and evaporate the content near to dryness using nitrogen flash evaporator at 35 °C to dryness. Later reconstitute the residue with 1.5 mL methanol (MeOH). Sonicate the mixture in ultrasonicator to dissolve residues completely; then filter the content using 0.22 μ PTFE nylon filter from test tube in LC vials for LC-MS/MS analysis.

Instrumentation (LC-MS/MS)

A LC-MS/MS system equipped with Shimadzu 1200 series UHPLC and LCMS 8040 with triple quadrupole detector (TQD) was used. LabSolution® Version 1.5 software was used to instrument control, data acquisition and processing. 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 solvent systems used were (A): 0.0314g ammonium formate (5mM) + 2mL MeOH + 10 μl formic acid (0.01%) made-up the volume with HPLC water to 100 mL and (B) 0.0314 g ammonium formate (5mM) + 10μl formic acid (0.01%) made-up the volume with 100 % MeOH to 100mL with flow rate of 0.4mL/min. The flow rate was 0.4 mL min^{-1}, injection volume was 2 μL, and column temperature was 40 °C. The pesticide indoxacarb was separated with the following gradient programme: 40% B and 60% A at the beginning for 13 minutes followed by 100% B up to 1 minute and then 40% A for 1 minute. A full-scan mass spectrum of indoxacarb 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+. The MS source parameters used were as follows: interface voltage of 4.5 kV, desolvation temperature of 250 °C, heat block temperature of 400 °C, desolvation gas (N_2) of 2.9 L min^{-1} and drying gas at 2.9 L min^{-1}. Then collision with argon gas (230 kpa) was done and different collision energies were optimized. The transitions optimized for indoxacarb MS/MS analysis are presented in Table 1.

Method Validation

In the extraction method, clean up to remove co-extractives with PSA and anhydrous $MgSO_4$, and identify and quantify indoxacarb from pigeonpea (green and mature pods), and soil samples were optimized and validated according to the SANTE/11813/2017 (European Commission 2017) by ascertaining the different parameters: linearity, matrix effect, limit of detection (LOD), limit of quantification (LOQ), specificity, trueness (bias), precision in terms of repeatability (RSDr-intraday) and precision in terms of reproducibility (RSDwR-interday).

Linearity of the method was assessed in both solvent and matrix matched by injecting the standard concentrations at 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, 100.0 and 120.0 μg L^{-1} and calculated the coefficient of variation. The matrix effect was calculated by comparing the angular coefficients obtained by the calibration 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 coefficient of the curve in the matrix and in the solvent, respectively (European Commission 2017; Santiago da Silva et al. 2012).

The LOD was calculated by preparing different solutions with low concentration that is expected to produce a response that is 3 times baseline noise. The LOQ was selected based on 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 or bias of the developed method was evaluated by estimating the average recovery for each spiked level tested. Recovery experiments were carried out at 3 fortification levels by spiking the pigeonpea green pods, dry grains and soil at the concentration of 0.005, 0.025 and 0.05 μg g^{-1} with the analytical standard solution of indoxacarb. Spiking was done such that the fortification level is at LOQ level, 5 times LOQ and 10 times LOQ with six replications for each level. The spiked samples were then kept at room temperature of 25 °C for 2 hours to attain sample stability. The fortified samples were analysed by following the previously described sample extraction procedure in the material and methods. The extracted aliquots were were injected to LC-MS/MS and the area under the peaks of the know amount of analytes in the spiked matrix prior to extraction and with sample extract spiked near the chromatographic analyses (matrix matched standards) was compared to calculate recovery percentage. The method precision was ascertained with regard to the repeatability relative standard deviation (RSDr) of the six replicates exactly similar extractions of blank pigeonpea samples spiked with pesticide at the same fortification levels (0.005, 0.025 and 0.05 μg g^{-1}) and RSD with respect to reproducibility (RSDwR) by doing the fortification at two different dates with six replications.

Data Analysis

The dissipation of indoxacarb residues in green pigeonpea pods was analysed 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}) and k is the dissipation rate constant. The half-life ($t_{1/2}$) was determined as $DT_{50} = \log 2/k$. $T_{1/2}$ is the

Table 1 ESI-MS/MS mass spectrometer parameters for indoxacarb

Name of the pesticide	Retention time (min)	Precursor ion (m/z)	Product ions (m/z)	Collision energy (V)
Indoxacarb	11.79 ± 0.1 min	527.95	293.00 (quantifier)	−17
			218.15 (confirmation)	−22
			149.80 (confirmation)	−36

insecticide half-life in green pigeonpea pods. The waiting periods or pre-harvest interval were calculated by the following formula

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

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 of the insecticide (MRL)
b Slope of the regression line (Regupathy and Dhamu 2001)

Half-life ($T_{1/2}$) was mathematically calculated

$$T_{1/2} = \frac{e}{b} = \frac{0.301}{b} (e = \log 2 = 0.301).$$

Risk Assessment

Hazard index (HI) was calculated by arriving the estimated average daily intake (EADI) from highest residual indoxacarb concentration (mg kg^{-1}) obtained on different day samples drawn from treatment plots and multiplied with per capita food consumption rate (kg day^{-1}) for pigeonpea. The per capita consumption of pulses as 40 g per day (Indian Council of Medical Research recommended daily intake of 40 g of pulses for a balanced diet of an average sedentary man in Indian context) (Gopalan et al. 1989) as quantity of green pod seed consumption is not available with published documents. As per the procedure given by the World Health Organization (WHO, Geneva), the estimated hazard indices were calculated by dividing the estimated average daily intake (EADI) (mg kg^{-1} day^{-1}) by their corresponding values of acceptable daily intake (ADI). If the hazard index (HI) is more than 1, the food is considered as not safe for human consumption (Darko and Akoto 2008).

Results and Discussion

Optimized Conditions for 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 programme for 15 minutes produces better separation and good peak shape. A full-scan mass spectrum of indoxacarb was recorded to select the most abundant m/z ion (mass-to-charge). For the analyte, the protonated molecular ion (M + H)+ of 527.95 m/z was determined and chosen as the precursor ion. The multiple reaction monitoring (MRM) transitions and associated acquisition parameters were optimized for the maximum abundance of the fragmented ion under ESI-positive mode condition by injecting 2 μL of 0.1 μg mL^{-1} standard solution of indoxacarb 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 (527.95) and product ion transitions m/z 293.00 with CE of −17 was used for quantification, and m/z of 218.15 and 149.80 with CE of −22 and −36, respectively, was used for confirmation of indoxacarb residue in samples (Table 1 and Fig. 1b). The developed LC-MRM (multiple reaction monitoring) mode provides high sensitivity and selectivity requirements for analytical method used for the detection of indoxacarb at lower concentration from 0.0015 μg g^{-1} in the pigeonpea matrix. Under the developed method, indoxacarb was found to elute at a retention time of 11.79 ± 0.1 min. The total and exacted ion chromatograms of indoxacarb standard are shown in Fig. 1a and Figure 1c, respectively.

Method Validation

The standardized method in the present study was validated according to SANTE/11813/2017. The pigeonpea green pod, dry grain and soil sample collected from control experimental plot was used as matrix during method validation. After the matrix extraction, it was injected to LC-MS/MS and found free from indoxacarb (Fig. 3a). The sensitivity or linearity of indoxacarb with pure solvent as well as calibrations with matrix matched standard showed that the developed methodology was linear having R^2 value of 0.999 (Table 2 and Fig. 2). The matrix effect was found –14.151 (Table 2). The detection and quantification limits for the indoxacarb were 0.0015 and 0.005 μg g^{-1}, respectively (Table 2), which were very less compared to maximum residue levels (MRLs) established by European Union and Export Inspection Council of India (European Commission 2017). The trueness was evaluated in three matrices, viz. pigeonpea green pod, dry grains and soil at spiking levels of 0.005, 0.025 and 0.05 μg g^{-1}, and the mean recovery was found to be 83.80 to 99.01% with relative standard deviation values of < 15% (Table 3 and Fig. 3b). In accordance with EU guidelines for method validation, the recovery values should be within the range of 70–120% at all levels of spiking. The precision was evaluated and recovery percentage of 93–114 was found with RSD of 3 to 9% for each spike level, i.e. 0.005, 0.025 and 0.050 μg g^{-1} (Table 3). The reproducibility with respect to changed date of analysis gave the acceptable recovery of 89–107% with % RSD of 4 to 10 (Table 3).

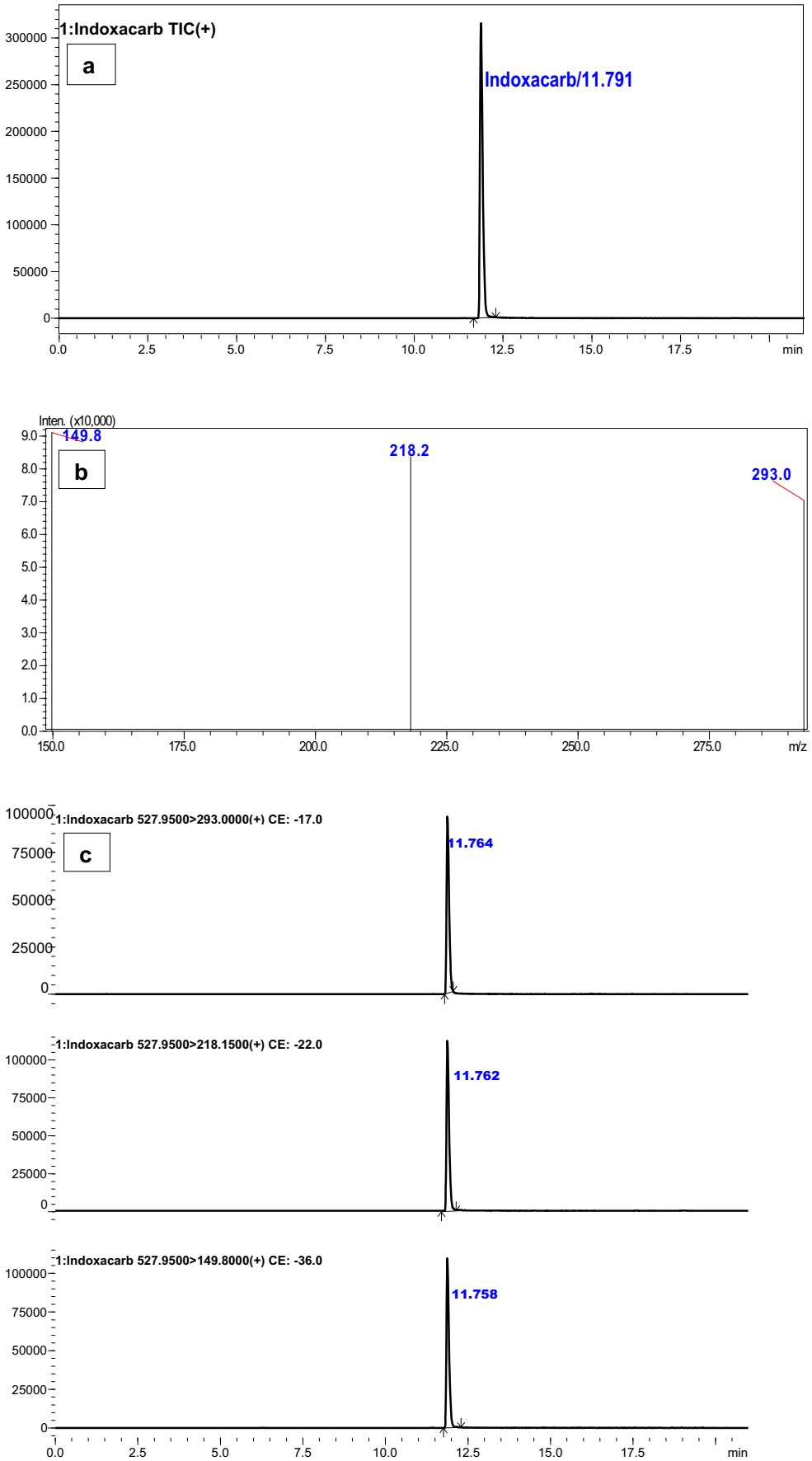

Fig. 1 **a** Indoxacarb total ion chromatogram. **b** MRM transitions. **c** Extracted chromatogram

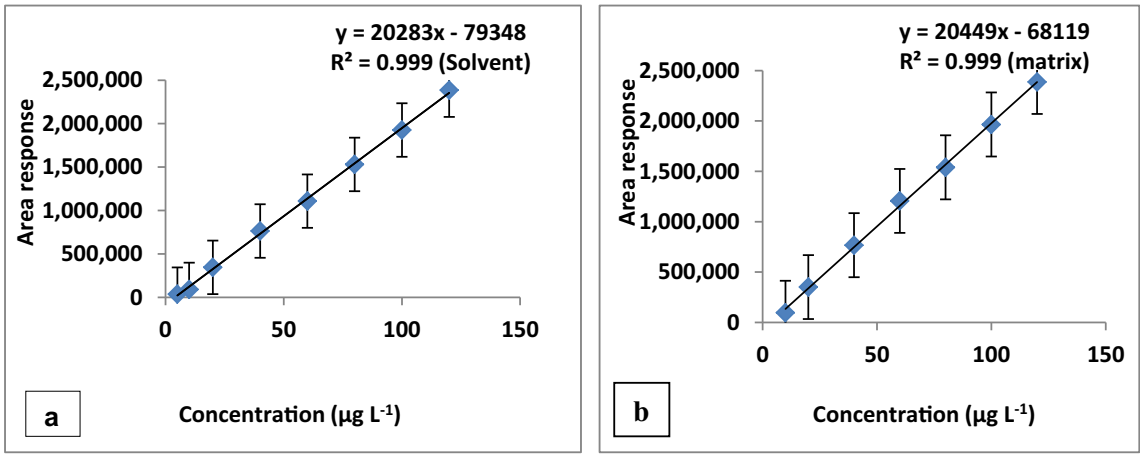

Fig. 2 **a** Calibration curve for the indoxacarb in methanol solvent. **b** pigeonpea matrix

Persistence and Dissipation of Indoxacarb

Sampling of green pods at application and on subsequent days, followed by QuEChERS extraction and analysis using LC-MS/MS (liquid chromatography tandem mass spectrometry), was attended for quantification of residues. The persistence of indoxacarb applied at recommended (60 g a.i ha^{-1}) and double the recommended dose (120 g a.i ha^{-1}) on pigeonpea green pods is given in Table 4. The initial deposits were found to be 0.583 and 1.342 μg g^{-1} at recommended dose and double the recommended dose, respectively. The residues recorded higher the concentration in indoxacarb applied at 120 g a.i ha^{-1}. There are several factors, viz. type of pesticide and its formulation, concentration of active ingredient and carrier and substrate characteristics, and weather parameters such as relative humidity, temperature, precipitation and wind movement affects the initial residual deposition of a pesticide. Besides these, plant type, shape of plant parts and growth of plant parts (slow or fast) also influence the persistence of residues (Ebeling 1963). The indoxacarb residues of 0.343 μg g^{-1} and 0.806 μg g^{-1} at recommended and double the recommended dose in the green pod samples analysed after a day of application accounted for total loss of 41.16 and 39.94%, respectively. A day after application, the loss of residues was near to the 50 % of its initial concentration was observed. The residue started decreasing from a day after treatment imposition and gradually dissipated to 0.005 μg g^{-1} on the 7th day accounting to loss of 99.31% and became non-detectable on subsequent analysis (Fig. 4). In the case of double the recommended dose, the residue gradually dissipated to 0.006 μg g^{-1} at the 10th day accounting to loss of more than 99.55% and completely absent from the pigeonpea green pod samples drawn after 15 days application (Table 4). The indoxacarb residues in dry grain and soil samples drawn at 25 days after application showed below detectable limit.

Growth rate of plants influences more in the case of initial deposition of chemical pesticides. On vegetation (plant leaves and fruits), indoxacarb degrades rapidly. It takes 2–34 days and average of 18 days for disappearance of 50% of its concentration from leaves and fruits of the vegetable crops depending upon the application rate (Brugger 1997). On pigeonpea green pod, recommended dose that recorded lower residue compared to its higher application rate justified quick dissipation from the vegetation. Several studies revealed the different pattern of degradation and initial deposit depends on growth rate of crops. Indoxacarb persisted for 2.5 months in apple ecosystem, and the initial residue levels were declined gradually and reached the level of 0.01 μg g^{-1} (Ewa Szpyrka et al. 2017). The application of indoxacarb at 100 and 200 g.a.i ha^{-1} in cauliflower recorded 0.29 and 0.58 μg g^{-1} of residues (Yoon et al. 2013).

Table 2 The linear regression equations, correlation coefficients (R^2) and matrix effect for indoxacarb in pigeonpea

Parameter	Indoxacarb	
	Solvent standard	Matrix match standard
Linear regression equation	y = 20,283x−79,348	y = 20,449x−68,119
Coefficient of determination (R^2)	0.999	0.999
Limit of detection (μg g^{-1})	0.0015	
Limit of quantification (μg g^{-1})	0.005	
Matrix effect (%)	−14.151	

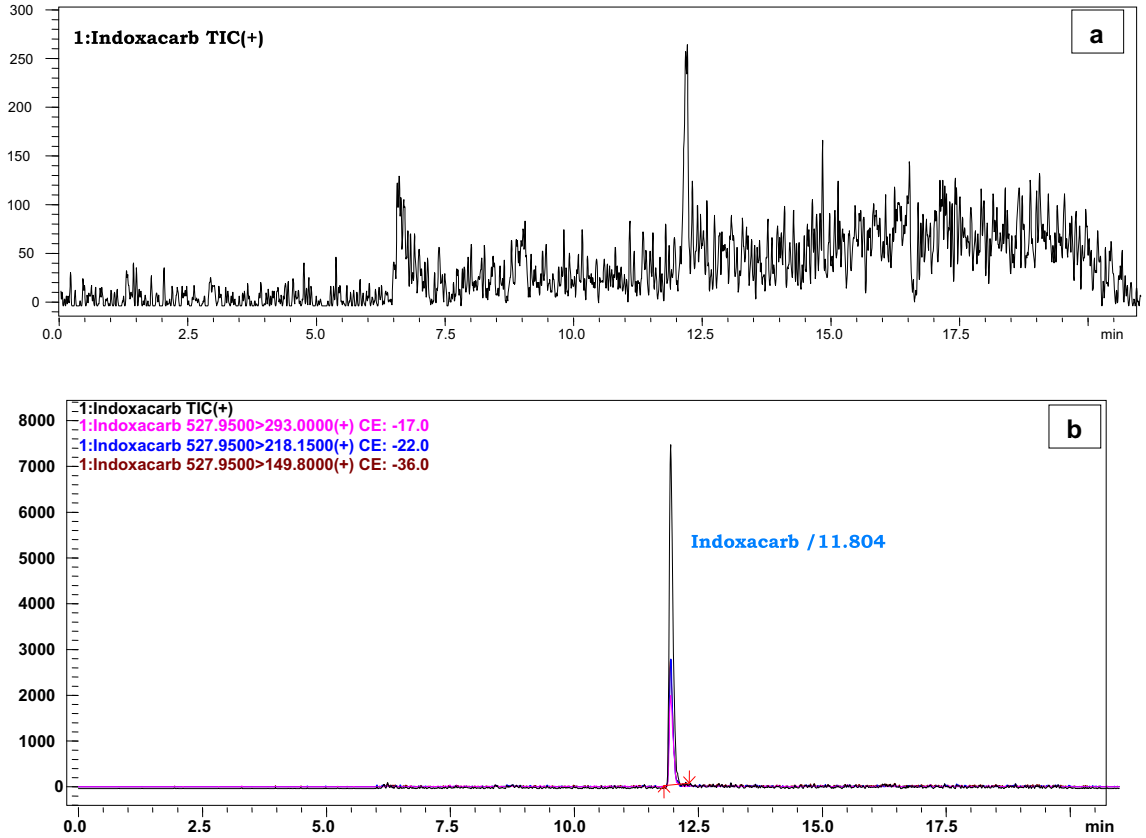

Fig. 3 **a** LC-MS/MS chromatogram of pigeonpea blank matrix (a). **b** recovery chromatogram of indoxacarb at 0.005 μg g^{-1} level of fortification

The initial concentration of 0.18 and 0.39 μg g^{-1} was recorded in cabbage when indoxacarb 14.8 % SC applied at 52.2 and 104.4 g a.i. ha^{-1}, respectively (Urvashi et al. 2012). The residues of 0.27, 0.39 and 0.58 μg g^{-1} were recorded on sugar beet leaf upon application of indoxacarb 15% EC at 150.75, 301.50 and 452.25 mL/feddan (1 feddan is equal to 1.03 acres), respectively (Sdeek Fayza and Taha, 2018), whereas in the case of cauliflower, 0.23 and 0.45 μg g^{-1} of residues were recorded following the application of indoxacarb at 52.2 and 104.4 g. a.i. ha^{-1}, respectively, and dissipated below its LOQ of 0.01 μg g^{-1} after 7 days (Takkar et al. 2011). These studies depicted the fast dissipation and less mean residues even at higher dose because

Table 3 The recoveries of indoxacarb in pigeonpea green pods, pigeonpea dry grains, and soil

Fortification level (μg g^{-1})	Recovery ± RSD % (n = 6)		
	Pigeonpea green pods	Pigeonpea dry grains	Soil
Trueness			
0.005	83.80 ± 0.53	88.30 ± 0.31	94.82 ± 0.21
0.025	89.30 ± 1.20	84.32 ± 0.63	97.34 ± 0.38
0.05	96.23 ± 0.64	99.01 ± 0.18	85.98 ± 0.29
Intraday precision (repeatability-RSDr)			
0.005	94.62 ± 4.66	92.72 ± 4.16	100.02 ± 2.95
0.025	113.51 ± 6.65	96.55 ± 3.84	98.28 ± 8.53
0.05	93.52 ± 4.78	93.62 ± 5.23	96.99 ± 3.83
Interday precision (reproducibility-RSDwr)			
0.005	89.78 ± 8.14	94.24 ± 4.16	98.24 ± 9.24
0.025	106.67 ± 5.27	89.24 ± 10.13	92.35 ± 8.94
0.05	101.36 ± 6.24	92.35 ± 9.34	94.83 ± 7.86

RSD Relative standard deviation, *n* Number of replications

Table 4 Persistence of indoxacarb residues in pigeonpea green pods, dry grains, and soil

Days after treatment	Recommended dose (60 g. a.i ha^{-1})		Double the recommended dose (120 g. a.iha^{-1})	
	Residue (μg g^{-1})[a] (Mean ± SD)	Dissipation (%)	Residue (μg g^{-1})[a] (Mean ± SD)	Dissipation (%)
0 (1 hr)	0.583 ± 0.007	-	1.342 ± 0.013	-
1	0.343 ± 0.016	41.16	0.806 ± 0.007	39.94
3	0.112 ± 0.003	80.78	0.351 ± 0.002	73.84
5	0.085 ± 0.013	85.42	0.201 ± 0.002	85.02
7	0.005 ± 0.001	99.31	0.016 ± 0.001	98.80
10	BDL	-	0.006 ± 0.001	99.55
15	BDL	-	BDL	-
Dry grains (25 days)	BDL	-	BDL	-
Soil (25 days)	BDL	-	BDL	-
	Correlation Coefficient (R^2) = 0.8987 Kinetic equation y = $0.7004e^{-0.611x}$ $T_{1/2}$ = 1.13 days K = −0.2760 SWP = 3.23 days		Correlation coefficient (R^2) = 0.99551 Kinetic equation, y = $0.16068e^{-0.564x}$ $T_{1/2}$ = 1.23 days K = −0.2815 SWP = 5.03 days	

[a] Mean of eight replicates; *BDL* below detection limit (< 0.005 μg g^{-1}), *SD* standard deviation, *SWP* safe waiting period

of fast growing and higher growth dilution in vegetables. It was proved in the case of Chinese cabbages because of its higher growth rate and dilution compared to cauliflower; rapid degradation pesticide concentrations were observed (Yoon et al. 2013). The dissipation rate in pigeonpea was slow because its slow growth, long durability and less photo degradation are also a reason for higher initial mean residues in dose applied at 120 g.a.i ha^{-1} pigeonpea.

The dissipation of indoxacarb residue in green pods followed a first-order reaction kinetics in both doses with the half-life of 1.13–1.23 days. The similar findings were recorded in several other crops such as; in cabbage the half-life period of 1.12 and 1.31 days at single and double the dose, respectively (Takkar et al. 2011); in okra, 1.6 and 2.3 days when indoxacarb applied at 70 and 140 g a.i. ha^{-1}, respectively and residues were dissipated below the determination limit of 0.01 μg g^{-1} (Gupta et al. 2009); in cabbage, half life values of 2.88 and 1.92 days (Urvasi et al. 2012); 2.8 and 4.6 days (Sun et al. 2012) and in apple, the half life values were 20 and 24 days, respectively applied at 0.2 kg/ha (Ewa Szpyrka et al. 2017) was due to its slow dissipation in apple ecosystem. The correlation coefficient (R^2) of 0.8987 and 0.99551 for two different doses, respectively, indicated that the dissipation data conforms to first-order reaction kinetics, and degradation rate constant (k) was in the range of −0.2760 to 0.2815 day^{1}, and safe waiting periods (SWP) or pre-harvest interval (PHI) were 3.23 and 5.03 for

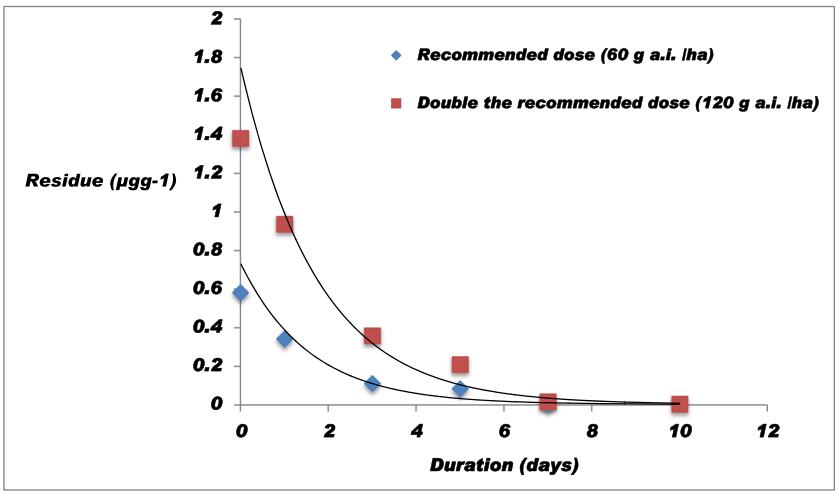

Fig. 4 Dissipation curve of indoxacarb applied at 60 g a.i ha^{-1} and 120 g.a.i ha^{-1} in pigeonpea green pod

Table 5 Hazard index values of indoxacarb residues in pigeonpea green pods

Days after application	Highest residue (μg g^{-1}) at 60 g. a.i ha^{-1})	ADI (mg kg^{-1} day^{-1})	EADI (mg kg^{-1} day^{-1})	Hazard index (HI)	Highest residue (μg g^{-1}) at 120 g. a. i.ha^{-1})	EADI (mg kg^{-1} day^{-1})	Hazard index (HI)
0 (1 hr)	0.597	0.01	0.060	5.97	1.383	0.138	13.83
1	0.366	0.01	0.037	3.66	0.938	0.094	9.38
3	0.117	0.01	0.012	1.17	0.360	0.036	3.60
5	0.085	0.01	0.009	0.85	0.211	0.021	2.11
7	0.005	0.01	0.001	0.05	0.018	0.002	0.18
10	BDL	0.01	-	-	0.006	0.001	0.06

BDL below determination limit (< 0.005 μg g^{-1}); *ADI* acceptable daily intake; *EADI* estimated average daily intake

the dose 60 and 120 g a.i ha^{-1}, respectively, and consumption pigeonpea green pod at 3.22 days after application of indoxacarb at recommended dose (60 g a.i ha^{-1}) was safe.

Risk Assessment

The maximum residue limit (MRL) of indoxacarb on pulses has been prescribed as 0.1 μg g^{-1} (FSSAI 2018). The residues of indoxacarb from pigeonpea green pod dissipated below the MRL on 5 and 10 days after application at 60 and 120 g a.i. ha^{-1}, respectively. MRL of indoxacarb for pigeonpea green pods under Indian condition is not available to make risk assessment on consumption green pods. The calculated hazard index based on the mean maximum concentration obtained in the respective treated dose reflected more than 1 up to 3 and 5 days after treatment. Hazard index value was found less than one on subsequent days (5th and 7th day, respectively, for 60 and 120 g a.i. ha^{-1}, respectively), and samples in both the treated doses indicated safe for consumption and health of consumers (Table 5). Present study supported by the findings of Brugger (1997) that, the low application rate and calculated dietary exposure to non-target organisms based on the residues obtained treated samples are is expected to be very low.

Conclusion

Highly sensitive, reproducible and cost-effective analytical method for quantification of indoxacarb residues in pigeonpea green pod, dry grains and soil was standardized and validated. The residues of indoxacarb dissipated below its LOQ of 0.005 μg g^{-1} after 7 and 10 days with half-life periods of 1.13 and 1.23 days, respectively, at recommended and double the recommended dosages. Hazard index values were less than 1 for those concentration dissipated less than LOQ in both the doses indicating safe for consumption. Hence the use of indoxacarb 60 g a.i.ha^{-1} (registered doses in India under CIB&RC) is safe in pigeonpea, and residues in pigeonpea green pod and dry grains and its products are unlikely to pose any undue hazard to the consumers, and PHI is observed as 3.23 days at application of indoxacarb 14.5% SC at 60 g.a.i ha^{-1} in pigeonpea.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict(s) of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Statement of Informed Consent Informed consent is not applicable in this study.

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