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RESIDUAL ANALYSIS OF INSECTICIDES (LAMBDA-CYHALOTHRIN, SPINOSAD AND INDOXACARB) IN CABBAGEUSING HPLC-UVD

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ABSTRACT : The present investigations entitled "Development of IPM module for sustainable management of Diamondback moth (*Plutella xylostella* Linn.) and insecticide residues analysis in cabbage (*Brassica oleracea* Linn.)" was carried out during *Rabi*, 2018-19 and 2019-20 at White grub laboratory, Department of Entomology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.), India. Maximum residue of Spinosad was 1.25 and 1.17 (ppm) found in module-6 fallowed by 1.23 and 1.19 (ppm) in module-4 during both *Rabi,* 2018-19 and 2019-20. Maximum residue of indoxacarb was 1.12 and 0.95 (ppm) found in module -2 fallowed by 1.14 and 1.02 (ppm) in module-4 during both *Rabi,* 2018 and 2019. Maximum residue of Lambda-cyhalothrin was 0.13 and 0.11 (ppm) found in module-5 fallowed by 1.14 to 0.13 (ppm) in module-4 during both *Rabi,* 2018-19 and 2019-20.

Key words : Residual analysis, lambda-cyhalothrin, spinosad, indoxacarb, cabbage and HPLC-UVD.

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INTRODUCTION

Cabbage (*Brassica oleracea* Linn.) belongs to family Brassicaceae an important vegetable crop of Cyprus and Mediterranean origin. It is cultivated extensively in tropical and temperate regions of the world *viz*., China, Germany, India, Indonesia, Japan, Korea, Poland, Russia, Taiwan, Turkey, Ukraine, USA, Uzbekistan and several other countries with total area of 16.56 lakh hectares and output of 50.17 million tonnes. India's contribution is 3,862 lakh metric tonnes from an area of 2.2 lakh hectares with a productivity of 18.26 t/ha. The total area under cultivation of cabbage in India is 395 thousand hectares with an annual production to the tune of 8807 thousand tones with productivity of 18.3 metric tonnes (Anonymous, 2019).

Vegetables consume 14 per cent of the total pesticides used in India, in which, the share of different types of pesticides in Indian agriculture market shows that organophosphorus (50 per cent) ranked first followed by pyrethroids (19 per cent), organochlorines (18 per cent), carbamates (4 per cent) and biopesticides (1 per cent) (Dhaliwal and Singh, 2000). Pesticide application is a necessary step for management of insect-pests related problems and therefore, it is very important to assess their residues in vegetables. Many countries have established regular monitoring programs for quantitative determination of residues in food products (Reed, 1987) as pesticide residues above the maximum residues limits (MRL) at the time of harvest and subject to great concern both globally and nationally. Surveys carried out by institutions and spread throughout the country indicate that 50 to 70 per cent of vegetables are contaminated with insecticide residues (Karanth, 2002).

Pesticide residues in fruits and vegetables has affected exports in recent years and should be strictly monitored owing to the high concern about the toxic properties of residues. Maximum Residue Limit (MRL) often serve as safety limit that define the maximum expected level of a pesticide on a food commodity after its safe and authorized use. The limits are food specific and serve as monitoring tools. They serve both to prevent illegal and/or excessive use of a pesticide and act as an enforcement tool to ensure compliance with its registered label (Dureja *et al*, 2015).

MATERIALS AND METHODS

Working standard solution

The certified reference material (CRMs) of different insecticides used in the residue studies was procured from Dr. Ehrenstorfer Augusburg, Germany and used to prepare 1000 ppm stock solution (SS) of each insecticide. From each stock solution,respective working solutions (WS) of 100 ppm were prepared in a 10 ml volumetric flask by adding 1 ml of 1000 ppm solution to 9 ml acetone. The 50 ppm solution of respective insecticide was prepared by taking 5 ml of 100ppm (WS) and 5 ml of acetone. For GC determination, 1 ppm solution of each insecticide was prepared by taking 0.1 ml of 50 ppm solution and mixing in 4.9 ml of n-hexane.

Sampling

Extraction and clean-up

Cabbage heads (5kg) were homogenized with robot coupe blixer and homogenized

↓

15±0.1g sample was taken in 50 ml centrifuge tube

↓

Required quantity of standard (CRM) added to get desired fortification level

↓

30±0.1 ml acetonitrile was added to the tube

↓

The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher

↓

3±0.1g sodium chloride was added to tube and mixed by shaking gently

↓

Centrifuged for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube

↓

9±0.1 g anhydrous sodium sulphate was added to remove the moisture content

↓

8 ml of extract was taken in to 15 ml tube containing 0.4±0.01gPS Asorbent (for dispersive solid phased-SPE cleanup)and 1.2±0.01 gr anhydrous magnesium sulphate

↓

The sample tube was vertexed for 30 sec followed by centrifugation for 5 min at 2500-3000 rpm

↓

The extract of about 2ml was filtered by using PTFE filter and finally 1 ml filtered extract is taken for injaction in to the HPLC

Principle of QuEChERS technique

QuEChERS stands for quick, easy, cheap, effective, rugged and safe technique for residue determination. In this technique fruit samples were extracted with acetonitrile and sodium chloride was also added in the extract in order to reduce the amount of polar interferences. The extract was cleaned up with dispersive solid-phase by using anhydrous magnesium sulphate and primary secondary amine (PSA). Anhydrous magnesium sulphate was used to remove water from organic phase while PSA was utilized to remove sugars, fatty acids, organic acids, lipids and some pigments.

Determination of residues

The SHIMADZU HPLC 2010 was used for the determination of residues of both the groups of insecticides. In case of organophosphate insecticides (chlorpyriphos, monocrotophos and quinalphos), Flame Photometric Detector (FPD) maintained at 280^0 C was used, whereas for the residues of synthetic pyrethroids (lambda-cyhalothrin and cypermethrin), Electron Capture Detector (ECD) maintained at 300^0C was utilised.

RESULTS AND DISCUSSION

Determination of residue of Spinosad (ppm)

The method for determination of Spinosad was based on use of reverse-phase high performance liquid chromatography presented in Table 1 and chromatograms of Spinosad standred in acetonitrile and in peach matrix, as well as their overlapped spectra under aforementioned condition. Retention time of Spinosad A and Spinosad D under the set condition was found to be 4.15 and 4.34 minute. Evaluation of the chromatographic condition was carried out through the detector response linearity, the precision and accuracy of the method, the matrix effect as well as by determination of detection and quantification limit.

Under the operating mentioned earlier, Spinosad gave a distinct peak at retention time 4.15 and 4.34 minute. Spinosad was applicationed as foliar application on cabbage in module-4 and module-6. Result from table-4.27 revealed that the minimum residue of Spinosad was 1.25 (ppm) found in module -6 fallowed by 1.23 (ppm) in module -4 during *Rabi*, 2018.

Same trend was observed in year 2019-20 in which minimum residue of Spinosad 1.17 (ppm) was found in module-4 fallowed by 1.19 (ppm) in module-6.

Determination of residue of Indoxacarb (ppm)

The method for determination of indoxacarb is based on use of reverse-phase high performance liquid chromatography in Table 1 were presented

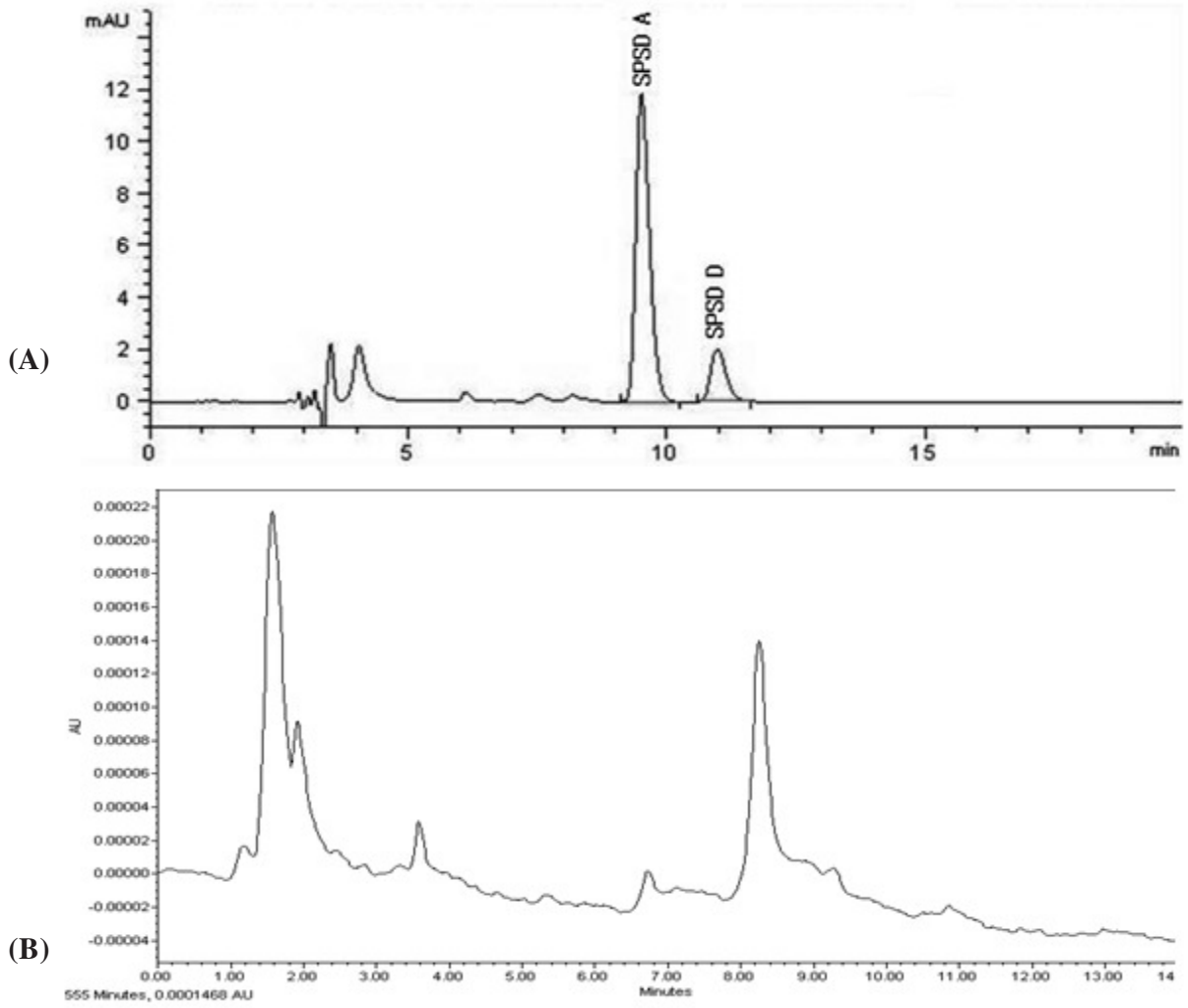

Plate 1 : HPLC chromatograms of **(A)** Spinosad Standard, 0.25 mg/kg. **(B)** HPLC chromatograms of Spinosad residues.

chromatograms of indoxacarb standred in acetonitrile and in peach matrix, as well as their overlapped spectra under aforementioned condition. The retention time of indoxacarb under the set condition was found to be 4.22 minute. Evaluation of the chromatographic condition was carried out through the detector response linearity, the precision and accuracy of the method, the matrix effect as well as by determination of detection and quantification limit.

Under the operating mentioned earlier, indoxacarb gave a distinct peak at retention time indoxacarb was application as foliar application on cabbage in module-2 and module-4. Result from Table 1 revealed that the minimum residue of indoxacarb was 1.12 (ppm) found in module-2 fallowed by 1.14 (ppm) in module-4 during *Rabi*, 2018-19.

Different trend was observed in year 2019-20 in which minimum residue of indoxacarb 0.95 (ppm) was found in module-2 fallowed by 1.02 (ppm) in module-4.

Determination of residue of Lambda-cyhalothrin (ppm)

The method for determination of Lambda-cyhalothrin was based on use of reverse-phase high performance liquid chromatography. Table 1 was presented chromatograms of Lambda-cyhalothrin standred in acetonitrile and in peach matrix, as well as their overlapped spectra under aforementioned condition. the retention time of Lambda-cyhalothrin under the set condition was found to be 3.499 minute. Evaluation of the chromatographic condition was carried out through the detector response linearity, the precision and accuracy of the method, the matrix effect as well as by determination of detection and quantification limit.

Under the operating mentioned earlier, Lambda-cyhalothrin gave a distinct peak at retention time Lambda-cyhalothrin was applicationed as foliar application on cabbage in module-4 and module-5. Result from Table 1 revealed that the minimum residue of Lambda-cyhalothrin was 0.13 (ppm) found in module -5 fallowed by 1.14 (ppm)

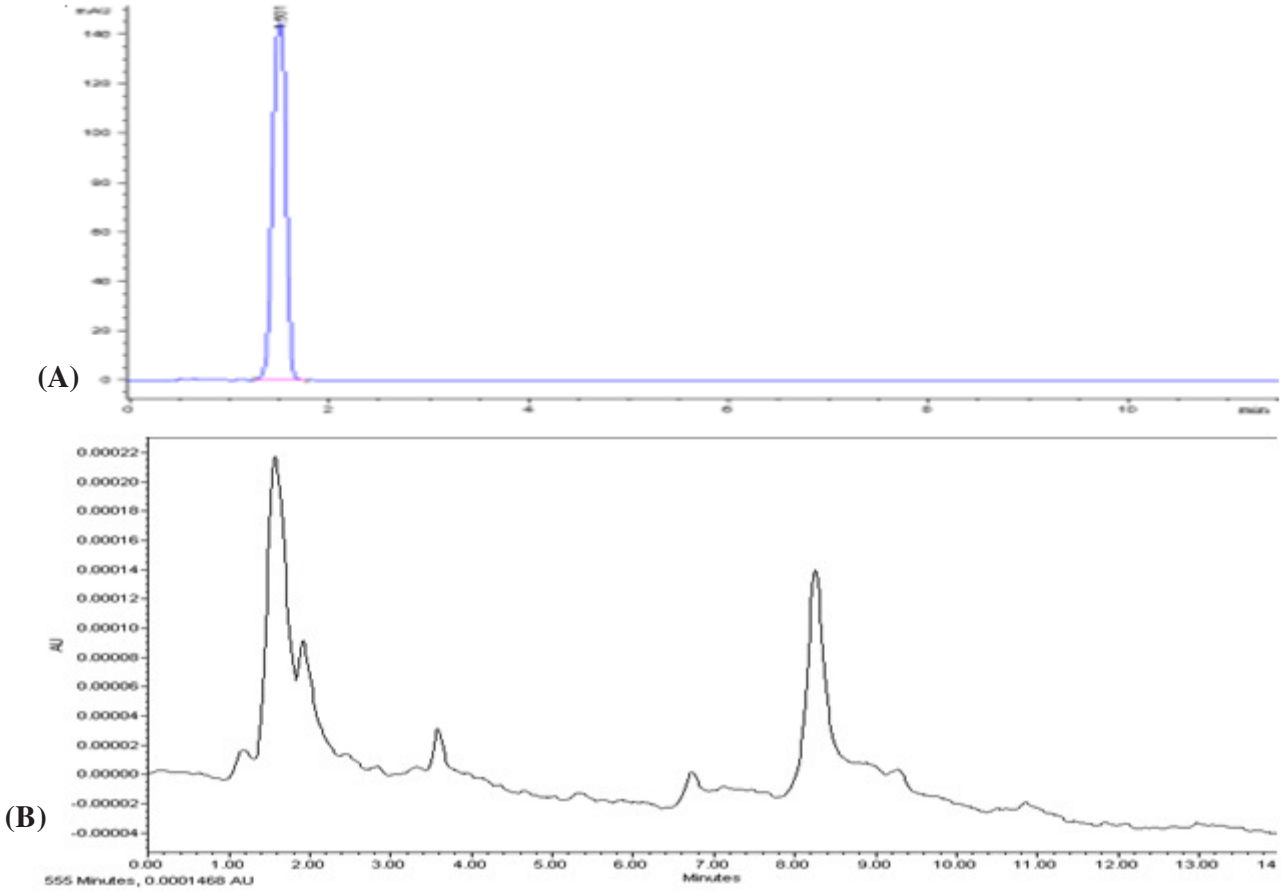

Plate 2 : HPLC chromatograms of **(A)** Indoxacarb Standard, 0.25 mg/kg. **(B)** HPLC chromatograms of Indoxacarb residues.

in module-4 during *Rabi*, 2018-19. Same trend was observed during *Rabi*, 2019-20 in which minimum residue of Lambda-cyhalothrin 0.11 (ppm) was found in module-5 fallowed by 0.13 (ppm) in module-4.

The present results are in conformity with Lina *et al* (2010), who analyzed residues were via a HPCL-UVD, and the lambda-cyhalothrin residue was analyzed via a GC-ìECD. The versatility of this method was evidenced by its excellent linearity (>0.9998 to 1) at broad concentration ranges. The average recoveries evaluated from the untreated sample spiked with two different fortification levels ranged from 72.45 to 113.90 per cent, and the repeatability (as a relative standard deviation) resulted from triplicate recovery tests was in a range from 0.80 to 11.75 per cent. The residues of all insecticides determined from treated pomegranate samples and their LOD levels (lunfenuron, 0.01; lambda-cyhalothrin, 0.005; thiamethoxam, 0.01; clothianidin, 0.02 mg/kg) were much lower than their MRLs (0.5 mg/kg). The present results are in accordance with Choudhury *et al* (2013), who recorded the contamination by SP group *viz.* fenpropathrin ranged from 0.001 to 0.003ìg g^{-1}, ë-cyhalothrin ranged from 0.001 to 0.003ìg g^{-1}, á-cypermethrin ranged from 0.001 to 0.002 ìg g^{-1} and deltamethrin ranged from 0.001 to 0.003ìg g^{-1}. The highest contaminated sample is brinjal followed by cauliflower, cabbage and tomato. Among the OC compound the major contaminants were â-HCH followed by ä-HCH, á-HCH, P,P'-DDE and P,P'-DDT. Similar observation are also recorded by Hammad (2015), who observed that the residues of insecticides were detected and measured using high performance liquid chromatography (HPLC). The results revealed that, all the samples contained lambda cyhalothrin and/or imidacloprid insecticides residues. The LODs (limit of detection) for the imidacloprid and lambda cyhalothrin were found to range between 0.3275 and 0.02818, and the LOQs (limit of quantification) ranged between 0.1087 and 0.09338 mg kg-, respectively. Present finding are agreed with Sanja *et al* (2018), who done determination of indoxacarb residues, the QuEChERS method coupled with high-performance liquid chromatographic (HPLC) analysis were carried out. The HPLC Agilent 1100 system with diode array detection and Zorbax Eclipse XDB-C18 column (50 mm × 4.6 mm, 1.8 ìm) were used. The mobile phase was water and acetonitrile (25:75). The flow rate was maintained at 1.0 ml/min in isocratic mode and the injection volume was 20 ìl. Chromatograms were

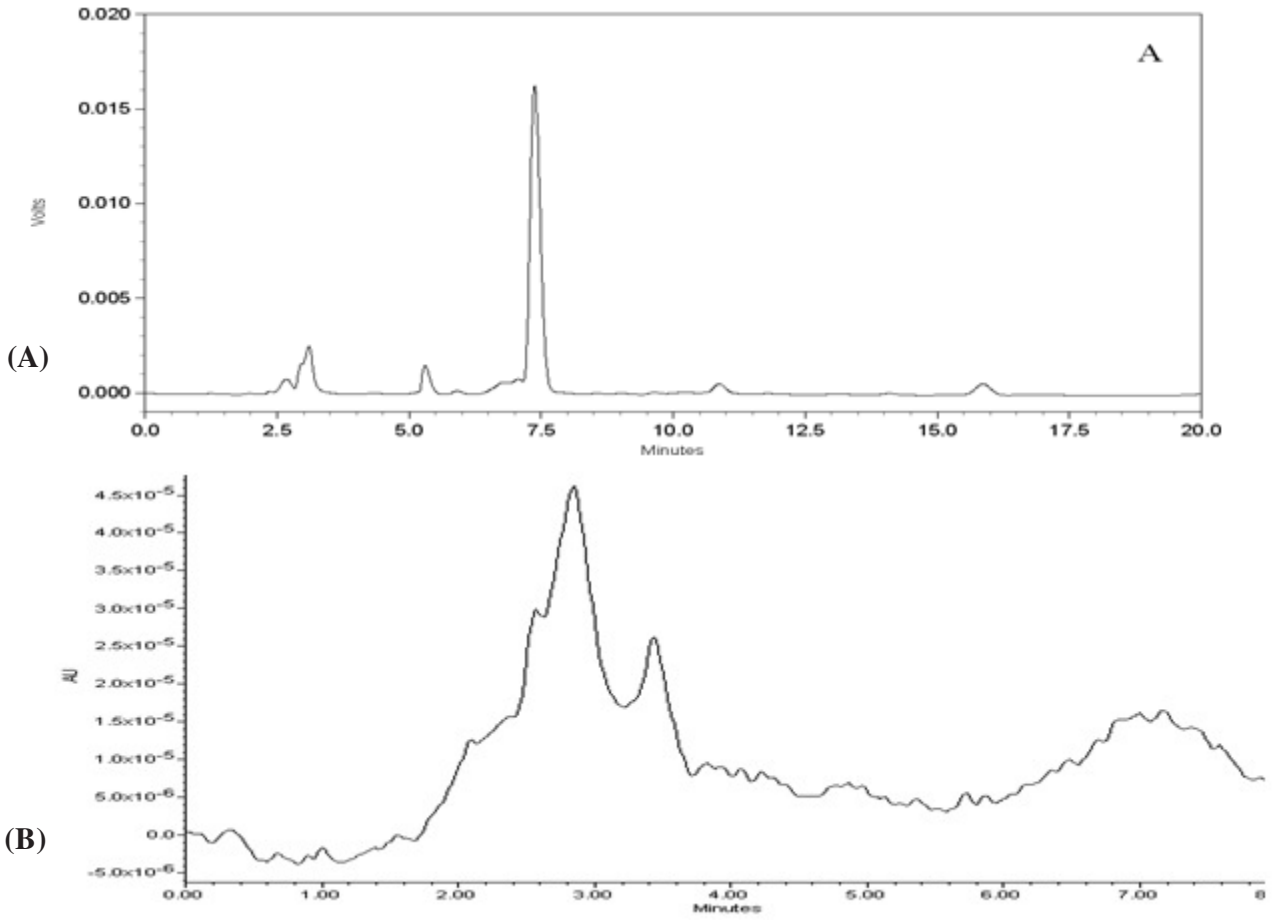

Plate 3 : HPLC chromatograms of **(A)** Lambda-cyhalothrin Standard, 0.25 mg/kg. **(B)** HPLC chromatograms of Lambda-cyhalothrin residues.

Table 1 : Residues of insecticide (ppm) in different modules on one day after application.

Module	2018-19			2019-20		
	Spinosad	Indoxacarb	Lambda-cyhalothrin	Spinosad	Indoxacarb	Lambda-cyhalothrin
M1	-	-	-	-	-	-
M2	-	1.12	-	-	0.95	-
M3	-	-	-	-	-	-
M4	1.23	1.14	0.14	1.17	1.02	0.13
M5	-	-	0.13	-	-	0.11
M6	1.25	-	-	1.19	-	-
M7	-	-	-	-	-	-

extracted at 310 nm. Under these conditions retention time of indoxacarb was 1.501 min. Similar observation are also found by Kim *et al* (2011), who reported that the spinosad was determined using HPLC with UV detector at 250 nm. Correlation coefficient (r2) for standard curve of spinosad A and D at standard concentration of 0.1-5.0 mg/kg were 0.999, respectively. Limit of quantitation (LOQ) of HPLC analysis was 0.005 mg/kg while limit of detection (LOD) was 0.001 mg/kg. Recovery experiments were conducted on five representative agricultural products to validate the analytical method. The recovery of proposed methods ranged from 74.9% to 104.0% and relative standard deviations wereless than 10%. Spinosad residues were investigated in 16 commodities collected from 22 provinces. In this study, residues on all samples were not detected.

Conflict of interest

The authors declare that they have no conflict of interest.

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