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RESEARCH ARTICLE

Simple detection method for paraquat dichloride in various matrices of cotton and sugarcane using liquid chromatography mass spectrometry

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ABSTRACT

Field trials were conducted to determine residues of paraquat dichloride in cotton and sugarcane at harvest time. Paraquat dichloride was sprayed on weeds at two to three leaves stage at doses of 480 and 960 g/ha. Samples collected at 87 and 102 days after herbicide application in cotton and 199 and 341 days after herbicide application in sugarcane were subjected to residue analysis by employing modified QuEChERS method. Recovery studies were conducted to determine the accuracy of method by spiking each matrix with a known concentration of paraquat dichloride. A satisfactory recovery rate of 70 to 120% and RSD < 20% were obtained in all the matrices. In harvest time samples of cotton (87 and 102 DAT) and sugarcane (199 and 341 DAT) matrices analysed, paraquat dichloride residues were less than the limit of quantification (0.05 mg/kg).

Keywords: Detection method, Herbicide residue**,** Paraquat dichloride, Cotton, Sugarcane

INTRODUCTION

Paraquat dichloride (1,10-dimethyl-4,40-bipyridinium dichloride), a contact herbicide is commonly used in agricultural fields to control broad-leaved and grassy weeds. It exerts a strong herbicidal effect on plants during photosynthesis by interfering with electron transport system by preventing NADP from being reduced to NADPH that resulting in the production of reactive oxygen species (ROS), which in turn reacts with unsaturated lipids found in cell membranes there by destroying plant organelles resulting in cell death.

Due to its crystalline structure, hygroscopicity, odour lessness and low vapour pressure, the chemical composition confers significant properties, such as ease of handling, high solubility in water, high binding capacity to soil, and stability in soil environment with a half-life of 1000 days (Vencill 2002). Apart from being used as herbicide in coffee, beans, soy, and citrus fields, it is also used as a desiccant on potatoes before harvest (Macbean 2012).

Concern about the residues left over by the widespread use of paraquat dichloride in agriculture have increased over the years due to its high toxicity to humans, farm and household pet animals, and particularly to aquatic animal species. Hence its usage is prohibited in several countries such as Sweden, Denmark, Austria, China, and Finland (Tingting 2015).

To determine paraquat dichloride residues in food crops, analytical methods such as capillary electrophoresis (Wigfield *et al.* 1993), gas chromatography with solid-phase extraction (SPE) (Almeida and Yonamine 2007), liquid chromatography (Ruan *et al.* 2014) and enzyme-linked immunosorbent assay (ELISA) (Garcia 2014) have been used. To increase the sensitivity of detection of paraquat dichloride residues in food and water samples, methods based on mass spectrometry, such as gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), are critical (Vince 1998). However, when these methodologies are used, the presence of high buffer and ion-pairing concentrations compete with the analyte during ionisation, thereby reducing the sensitivity. Additionally, buffers containing high salt concentration also clog the analyte spray unit of MS equipments, necessitating frequent and thorough cleaning of the spray unit (Tingting 2015). To overcome these practical challenges associated with sensitive detection of paraquat residues, we have developed a method using a low salt concentration buffer and tested the sensitivity of the detection of paraquat residues in cotton and sugarcane.

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MATERIALS AND METHODS

Trials were conducted during two seasons in cotton and sugarcane to determine the residues of paraquat dichloride at harvest time in Kondaiyampalayam (10.9791° N, 76.8112° E) village in cotton and Panaimarathur (11.0046° N, 76.9298° E) village, Coimbatore district in sugarcane. The details of soil physiochemical properties and micronutrient status of the above region were collected from the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, India. The mean soil pH, 7.33 slightly acidic to alkaline nature; electrical conductivity, non - saline; mean Soil Organic Carbon, 5.4 g/kg (medium); available N, medium to high; available P, medium to high (47 kg/ha); available K, medium to high (352 kg/ha); Zn, 0.42 – 9.45; Fe, 5.62 – 30; Mn, 1.42 – 17; Cu, 0.59 – 9.76.

For this study, fields growing *Bollgard II* cotton variety and *Mandiya* sugarcane variety with recommended agronomic practices were selected. The paraquat dichloride 480 and 960 g/ha was applied twice with 15 days intervals in the interrow space, at 30 days after sowing (DAS)/planting (DAP) of cotton and sugarcane, or on weeds at 2 to 3 leaf stage. Throughout the study period, an untreated control area in the fields was marked and maintained without spraying the herbicide. Each treatment was replicated three times, in a 45 m^2 plot per replication. The herbicide was applied using 500 L/ha with a knapsack sprayer equipped with a flood jet nozzle with a hood to avoid drift of herbicide spray on to the main crop. Two season samples of cotton (87 and 102 DAT) and sugarcane (199 and 341 DAT) were collected during harvest.

Reagents used

Paraquat dichloride standard reference material was provided by M/s. Syngenta India Limited. Solvents such as acetonitrile (Lichrosolv and Chromosolv), ethyl-acetate, methanol, hexane, dichloromethane (M/s Merck Bangalore, India) (M/s. Fishers chemical Ltd., Chennai, India), acetone (M/s. Molychem, Mumbai, India) were purchased. Salts such as anhydrous sodium chloride, sodium sulfate AR grade (M/s Merck Bangalore, India), anhydrous Magnesium sulphate (M/s. Himedia Laboratory, Mumbai), sorbents such as Primary Secondary Amine (PSA, 40 ìm, Bondesil), Graphitized Carbon Black (GCB) (M/s. Agilent, USA) were also procured. The ultra-pure type I (18.2 MÙ) water was prepared using Merck (Direct - Q® 3) water purifier and filtered through 0.45 µm membrane filter paper using a millipore solvent filtration unit. The 0.45 and 0.20 µm membrane filter paper (Ultipor, M/s. Pal life Science, Mumbai) and LCMS grade formic acid (M/s. Sigma Aldrich, Bangalore) were also used in this study.

Standard preparation

A stock solution of 400 mg/kg of paraquat dichloride was prepared by dissolving 10 mg of Certified Reference Material (CRM) in a final volume of 25 ml of methanol in a clean Class A volumetric flask and stored at -20^{0}C for subsequent preparation of intermediate and working standards.

Instrument parameters

Chromatographic separations were performed using a Shimadzu Liquid Chromatography-Mass Spectrometry (LCMS-2020) system equipped with an electrospray ionization (ESI) interface. A concentration of 1.0 mg/kg paraquat dichloride was infused directly into the LCMS without using a column to determine the mass of the compound and to tune the conditions under which paraquat dichloride can be detected. Separation of paraquat dichloride was carried out in positive ionization mode (ESI+) at 185 m/z. The mobile phase ratio of water with 20 mM ammonium formate + 0.2% formic acid at 60% (solvent A): acetonitrile at 40% (solvent B) was used in a low-pressure gradient method using Agilent 5 TC-C18 (2) 250 x 4.6 mm column. The optimized instrument parameters were oven temperature 40^{0}C; interface temperature 350^{0}C; DL temperature 250^{0}C; heat block temperature 400^{0}C; nebulizing gas flow rate 1.5 L/min.; and dry gas flow rate 15 L/min.; flow rate 0.8 ml/min; injection volume 20 µl.

Validation

The linearity curve was established by injecting standard solution at concentrations of 0.05, 0.1, 0.25, 0.5, and 1 mg/kg in six replicates. The relative standard deviation (RSD) and coefficient of determination (R^2) was calculated using the analyte's mean response. The Limits of Detection (LOD) and Limits of Quantification (LOQ) were determined using three and ten levels of signal and noise intensity, respectively. The accuracy of the extraction method was determined using analyte concentrations of 0.05, 0.25, and 0.5 mg/kg. After spiking, the samples were mixed in a vortex and allowed for 30 minutes to equilibrate. The samples were then extracted using the procedure outlined below. To ensure the developed method's accuracy and precision, the percentage of analyte recovery and RSD were calculated with the appropriate matrix match standards.

Sampling

For sampling, two kilograms of cotton were collected on 87 DAT during season I and 102 DAT during season II from each treatment. A 200 g subsample of lint was ginned from each treatment to separate them from seeds. Another subsample of 500 g was taken for delinting seeds with 50 ml of concentrated H_2SO_4. Acid-treated seeds were continuously mixed with a wooden stick and then washed three to four times with water to remove residual acid. Two hundred grams of seeds were crushed in a mixer grinder and cotton oil was extracted in a Soxhlet apparatus using acetone as a solvent. To separate the oil and solvent, the mixture was evaporated at 30°C in a rotary vacuum. After separating oil from the seed, the seed cake was collected from the Soxhlet apparatus and subjected to residue analysis.

For sugarcane sampling, two kilograms of cane and 500 g of leaf from randomly selected plants in each treatment were collected on 199 DAT during season I and 341 DAT during season II. A 300 g subsample was taken from each cane sample and was finely chopped for analysing the residues in cane. The remaining canes were crushed and cane juice was extracted using a cane juice extractor. Leaf samples were cut and blended using blade homogenizer.

For harvest time soil sampling, the surface litter in the sampling site was removed and soil samples were collected at depth ranging from 0 to 15 cm. One kilogram sample from each replication was taken and thoroughly mixed with a conical trier. The samples were immediately transported to the laboratory for residue analysis. Soil samples were dried, powdered, and then quartered to get a sub sample of 250 g. Soil samples were sieved and stored in a polythene bag until analysis.

Extraction and cleanup

A representative sample of 5 g of cotton lint was taken for each treatment and soaked in 200 ml of acetonitrile for 24 hours. To remove excess moisture, the acetonitrile extract was filtered through Whatman filter paper No. 1 containing 10 g sodium sulphate and concentrated to near dryness using a rotary vacuum evaporator. The final residue was reconstituted in 1 ml methanol containing 0.2% formic acid for LC-MS analysis.

Representative samples of seed (5 g), cake (5 g), sugarcane leaves (5 g), and soil (10 g) were weighed in a 50 ml centrifuge tube and vortexed for 1 minute with 5 ml distilled water and 20 ml acetonitrile. Five-grams of chopped cane sample was added to a 250 ml conical flask containing 20 ml of ethyl acetate and extracted using a mechanical shaker at 250 rpm for 1 hour. The extract was filtered through a funnel with a cotton plug and the filtrate was transferred to a 50 ml centrifuge tube. A 10 ml representative sample of cane juice was added to a 50 ml centrifuge tube containing 10 ml of ethyl acetate and vortexed for 1 minute.

For all matrices, following clean-up steps were followed. Four grams anhydrous magnesium sulphate and one gram of sodium chloride were added to 50 ml centrifuge tubes, vortexed for one minute, and then centrifuged at 6,000 rpm for ten minutes. Nine millilitres of supernatant were transferred to a glass test tube containing 4 grams of anhydrous Na_2SO_4 and shaken for one minute, 6 ml of supernatant was transferred to a 15 ml centrifuge tube containing 100 mg PSA, 10 mg GCB, and 600 mg anhydrous $MgSO_4$. The mixture was vigorously shaken by hand for 1 minute and then centrifuged at 3000 rpm for 10 minutes. Four millilitres of supernatant were transferred to a turbovap tube and evaporated to near dryness; the residue was dissolved in 1 ml methanol containing 0.2% formic acid and used for subsequent LC-MS analysis.

Five grams of the oil was taken in a 125 ml separating funnel, 50 ml hexane was added, and the mixture was partitioned using acetonitrile saturated with hexane (3x50 ml) and vigorously shaken for one minute. Once the layers separated, acetonitrile layer was drained carefully into a 1 L separator funnel. Brine solution (600 ml) was added and partitioned twice using 150 ml (2 x 75 ml) of dichloromethane filtered through anhydrous sodium sulphate and treated for 2 hours at room temperature with 500 mg GCB. The clear extract was concentrated to near dryness using Whatman filter paper No. 1., 20 ml of acetonitrile was added to the dried residues and concentrated to dryness using a rotary vacuum at 30°C. The procedure was repeated twice to completely remove all traces of dichloromethane, and the final residue was dissolved in 1 ml methanol containing 0.2% formic acid and used for LC-MS analysis. In order to eliminate the effect of matrix on residue determination, all samples were compared with the matrix match standard.

RESULT AND DISCUSSION

Linearity, LOD and LOQ

The standard solutions prepared linearly in methanol and acetonitrile solvents resulted in an unacceptable coefficient of determination (R^2).

However, standard solutions prepared using methanol containing 0.2% formic acid resulted in enhanced paraquat dichloride ionization in LCMS as well as a high degree of linearity and R^2 value of 0.99 as coefficient of determination. The LOD and the LOQ were estimated at 0.01 and 0.05 mg/kg, respectively (**Figure 1**).

Recovery

Recovery studies on paraquat dichloride were performed to ascertain the accuracy of our method described in this article. The herbicide recovery was determined in a wide range of cotton matrices (lint, seed, seed cake, oil, and soil) and sugarcane matrices (leaf, juice, cane and soil) (**Table 1** and **2**). The mean recovery rate of paraquat dichloride in various cotton and sugarcane matrices ranged between 74.42 and 111.24%.

Degradation of paraquat dichloride in cotton and sugarcane

The present study's findings indicated that paraquat dichloride residues in cotton and sugarcane matrices at harvest were less than the limit of quantification (0.05 mg/kg). The method's accuracy was estimated in terms of the recovery experiment by following the modified QuEChERS method. In the present study, all the matrices showed a satisfactory recovery and RSD percentage (SANTE 2019). In combination with ammonium format, formic acid enhanced the ionization of the analyte. The lowest concentration that produced a response three times that of the noise peak was used as the LOD (0.01 mg/kg). The LOQ (0.05 mg/kg) is estimated to be 3.3 times the LOD.

The analysis of paraquat dichloride in various matrices was found to be complicated across all matrices with inconsistent recovery percentages and higher RSD. The problem could be rectified with the addition of 0.2% formic acid in methanol used finally to reconstitute the residues after evaporation and accuracy and precision were well within the acceptable limit (SANTE 2019). As a result, the developed method is deemed adequate for determining paraquat dichloride residues in cotton and sugarcane matrices.

The present study showed that paraquat dichloride residues were at less than the quantification limit of 0.05 mg/kg in cotton (lint, seed, seed cake, oil) and sugarcane (leaf, juice, cane) samples collected at harvest in both seasons. (**Figure 2**).

Paraquat dichloride is a contact herbicide that has not been shown to transfer to plant parts. Typically, paraquat dichloride disrupts the chloroplast's electron transport system (PS I). This inhibits oxygen and carbon dioxide fixation, forming the superoxide anion, which then reacts with the two hydrogen molecules to form hydrogen peroxide. Hydrogen peroxide decompose into free radicals in the presence of sunlight, and these free radicals cause cell death. Thus, once exposed to paraquat dichloride with sufficient sunlight, the plant will wilt or die. No

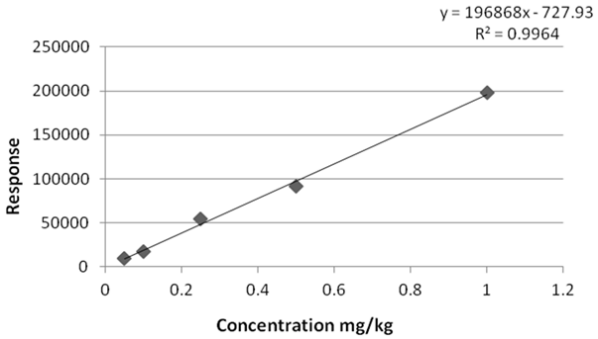

Figure 1. Mass spectrum and linearity curve of paraquat dichloride in LCMS

Table 1. Percent recovery of paraquat dichloride in cotton lint, seed, and oil

Fortification	Cotton lint		Cotton seed		Cotton oil		Cotton cake		Soil	
	Mean % recovery	RSD (%)	Mean % recovery	RSD (%)	Mean % recovery	RSD (%)	Mean % recovery	RSD (%)	Mean % recovery	RSD (%)
0.05 mg/kg	102.18	8.13	95.09	4.21	94.43	11.40	103.19	3.18	112.24	5.72
0.25 mg/kg	101.14	8.34	109.44	2.92	80.51	10.58	104.02	0.72	98.92	15.39
0.50 mg/kg	110.74	3.47	97.91	4.64	90.93	4.93	74.42	5.53	79.04	7.40

Table 2. Percent recovery of paraquat dichloride in sugarcane leaves, cane, juice and soil

Fortification	Leaves		Cane		Juice		Soil	
	Mean% recovery	RSD (%)	Mean% recovery	RSD (%)	Mean% recovery	RSD (%)	Mean% recovery	RSD (%)
0.05 mg/kg	91.87	2.77	102.68	5.52	104.54	9.51	92.88	9.82
0.25 mg/kg	90.04	10.62	103.07	6.19	95.13	7.67	83.18	5.47
0.50 mg/kg	89.31	8.41	85.40	1.94	110.28	3.57	82.10	8.38

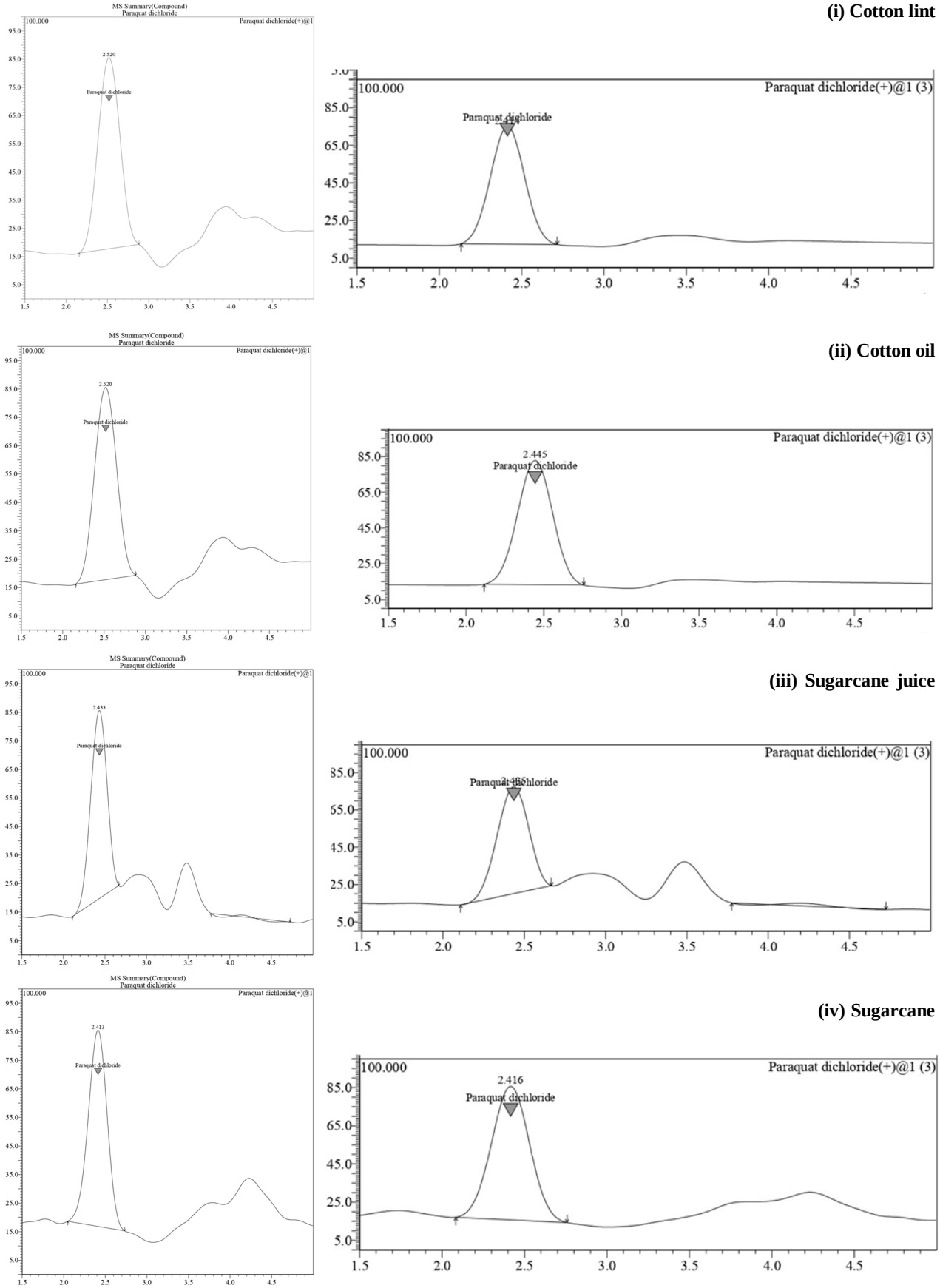

Figure 2. Chromatogram of matrix match standard (left) and recovery at LOQ level (right) for selected matrices in cotton and sugarcane by LCMS

such drying of crop plants was observed during the study period. Additionally, paraquat dichloride is highly soluble in water, it is typically trapped in soil or clay particles and degraded by microbial fauna (Alexander 1999 and Srinivasan 2004). No residues were reported in samples collected at 100 days after the application of paraquat dichloride 24% SL at a dose of 2 and 4 kg/ha in tea (Janaki and Chinnusamy 2016).

Cotton fiber is used to make natural textiles, cotton seed is used to make edible oil, and cotton meal is used to feed livestock. As a result, it is critical to maintain high-quality fiber, nutritional value and devoid of contaminants. Similarly, sugarcane juice is consumed fresh, and only very few studies were reported on pesticide residue in sugarcane juice. As a result, it is critical to investigate the fate of herbicides and their residue levels in these cropping ecosystems.

Thus, the study confirms the possibility of eliminating residues in plant and soil with an adequate gap between the last herbicide application and harvest. However, care should be taken to ensure that sound agricultural practices are followed to avoid residue deposition. Additionally, because of its high solubility and toxicity, indiscriminate use of paraquat dichloride may result in bioaccumulation in plants and animals, particularly in aquatic systems. The tolerance limits are established by CODEX Alimentarius and FSSAI for cotton seed (2 mg/kg) and cottonseed oil (0.05 mg/kg) by FSSAI. No MRL is available for paraquat dichloride in sugarcane. To ensure food safety, the MRL for paraquat dichloride need to be established for additional agricultural crops.

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