Research Article

Determination of Pesticide Residue in Brinjal Sample Using HPTLC and Developing a Cost-Effective Method Alternative to HPLC

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Two analytical techniques HPLC (high performance liquid chromatography) and HPTLC (high performance thin layer chromatography) were validated to reveal the quality and quantity of pesticide residues (organophosphorus, organochlorine, and pyrethroids) in brinjal samples collected from a local market of Faisalabad. The HPTLC methods showed linear behavior for standard samples and residue was in the range of 1–130 ng. The organochlorine (α-endosulfan) contaminates the samples at 4, 5, 9, and 10 weeks, and detected quantity was less than MRL (minimum residue level) of the FAO (Food and Agriculture Organization of United Nations), i.e., 0.5 mg·kg⁻¹. The organophosphorus pesticide (chlorpyriphos, methamidophos, monocrotophos, dichlorvos, carbosulfan, profenophos, and dimethoate) residue contaminated the samples and violated the MRL limit. Pyrethroids (deltamethrin, β-cyhalothrin, and cypermethrin) were present at appreciable levels, in samples of 1, 3, 4, 6, 8, and 9 weeks. The concentration of β-cyhalothrin (0.25 mg·kg⁻¹) and cypermethrin (0.205 mg·kg⁻¹) was significantly higher than that of all detected pesticides. The carbosulfan and deltamethrin contaminated all 10-week samples. The HPLC analysis of samples was carried out to confirm the efficiency of HPTLC as cost-effective method. The concentration of α-endosulfan, chlorpyriphos, dimethoate, monocrotophos, profenophos, deltamethrin, and cypermethrin in brinjal samples through the HPTLC method showed similar residual concentration with HPLC analysis.

1. Introduction

Pesticides are used extensively at growth stage of vegetable/crops, to provide protection from insect pest problems [1]. From past few decades, it is observed that the pesticide used for agriculture is one of the leading weapons for crops protection [2]. For a target pest, the pesticides are classified as insecticides, herbicides, fungicides, rodenticides, and nematicides [3]. The insecticides are further divided into organophosphate, organochlorine (most used by grower), carbamates, pyrethroids, and neonicotinoids; there is a wide and extensive usage of endosulfan (colorless and most controversial insecticides) in the world [4]. According to Enviro-news Forum-1999, due to extensive sprays of pesticides, approximately one million deaths per year occur [5]. The monitoring and proper investigation of pesticide residues in fruits and vegetables have great importance to consumer health protection, and the development or usage of robust and economical analytical methods is of great interest [6]. After extraction, the various types of validation...
Organochlorine pesticides showed its toxicity to numerous plants and also affect the human life by taking part in food chain as contaminants. Dioxin is produced when organic matter is burnt in the presence of numerous insecticides and chlorin such as DDT. It is a true fact DDT is widely used as to control insect in 20th century [2]. Pesticides cause very serious health effects on human health, mostly the people engaged in agriculture sector. The acute poisoning of pesticides causes health effects like seizures, rashes, and gastrointestinal illness, and due to chronic effects of pesticides, the genotoxic potential is a key factor for long-term health effects such as cancer. The neurological disorders are reported due to pesticide contamination including headache, dizziness, fatigue, weakness, nausea, chest tightness, difficulty in breathing, insomnia, and confusion [9]. The endosulfan contamination also affects the human organs very badly; some study on rats showed kidney problems and reduced sperm quality in blood [10]. Carbamate and organophosphorus insecticides are still recognized as important pesticides not only for being confined to preventing damage due to insects but also for control of insect-borne diseases. For human health, the minimum risk is an approximation of the regular human contact to the toxic substances that is likely to be without considerable risk of adverse, noncancer health possessions over a quantified duration of exposure of 0.002 mg·kg⁻¹ per day according to FAO-2006 [11]. According to European Union 2014, total pesticide marketed sales in the European Union-28 calculated were almost 400000 tons [12]. The extensive use of carbamates contaminates not only foods but also the aqueous environment by leaching from soil, and due to their high solubility, they create threats for human and aquatic life [13].

The brinjal (Solanum melongena L.) belongs to the family of Solanaceae and is a widely consumed vegetable and most native in Asia region specially in India and Pakistan. Brinjal (Solanum melongena L.) is easily available throughout the year but its peak season starts from August to September [1]. Brinjal is grown on wide scale in China, India, Pakistan, and Japan [14]. Total production of brinjal fruit, about 4.2 million tons, was recorded according to FAO-2012 [15]; the total cultivated area of brinjal in Pakistan is 8325 ha with 82999 tons of annual production. Punjab is the agricultural backbone of Pakistan, the total cultivated area of brinjal is about 4452 hectares having annual production of 54159 tons, and it is a remarkable contribution in vegetable production system [16].

The study was designed to analyze the level of pesticide residual concentration in brinjal samples collected from a local market of Faisalabad, to introduce and develop simple and cost-effective method HPTLC for pesticide residue analysis as an alternative to HPLC, and also to develop good comparison with designed MRL (minimum residue level) of Codex Alimentarius Commission and Thai Agriculture Standard.

2. Materials and Methods

2.1. Collection and Preparation of Brinjal Samples. Fresh brinjal samples were collected in triplicate, 1–10 samples, with one-week interval from local sale market (Jhang Bazar Faisalabad, Sabzi Mandi Ghulam Muhammad Abbab, Model Bazar Main Jhang Road) of Faisalabad city and treated as laboratory samples. The samples were washed with tap water to remove dirt and debris and were homogenized using blender (Braunmultimix MX 32, Germany) at high speed with distilled water. The samples were cut into small pieces after air-drying.

2.2. Reagent Preparation Used for HPTLC Methods. 0.5 g o-tolidine was mixed in 10 mL acetic acid, and 2.0 g of KI (potassium iodide) was dissolved in 10 mL of distilled water. In a volumetric flask (500 mL), both solutions were mixed and diluted up to the mark with distilled water. Then reagent solution was stored in refrigerator.

The horse blood 200 mL was collected from DVM Clinical Laboratory, University of Agriculture, Faisalabad, and brought to Pesticide Chemistry Laboratory, NIAB Faisalabad. The clotted blood was fragmented with glass rod and moved into the centrifugal tubes (100 mL) and centrifuged at 4000 rpm for 30 minutes. In 10 mL portions, the serum was collected in glass vial and kept in freezer. Further the 10 mL serum will be diluted with 7 mL of tris buffer solution. DCPIP solution, 250 mg (2,6 dichlorophenol-indophenol Na salt), was added in a 500 mL glass stopped volumetric flask and dissolved in distilled water, and then the volume was made up to the mark. The solution was mixed thoroughly and stored in refrigerator. 0.05 molar tris-buffer solution was prepared: a 3.04 g of tris (hydroxymethyl) amino methane was weighed then put into a 500 mL flask and diluted up to mark with distilled water. After preparation, it was stored in refrigerator. Substrate solution was prepared: the 60 mg of acetylthiocholine iodide was put into a 50 mL flask, dissolved in distilled water, and made up the volume to the mark. The solution was stored in refrigerator at 40°C.

Borax buffer solution was prepared: the sodium borate (Merck; Germany) solution was prepared by dissolving a 9.5 g of it in 500 mL distilled water; after this, 350 mL of this borax buffer solution was mixed with 150 mL of 0.1 N HCl for borax buffer solution and then stored in refrigerator. DCPIP solution was prepared: 200 g of 2,6-dichlorophenol-indophenol Na-salt (Merck, Germany) was dissolved in 500 mL borax buffer solution and then stored in refrigerator.

Detecting reagent was prepared: a 10 mL wheat extract was mixed with 10 mL of DCPIP solution and then added dropwise until the color of this mixture becomes bluish-green. This solution was enough for four plates of size 20 × 20 cm.
Fungi spore (Aspergillus niger) multiplication was prepared: different fungi strains, from NIAB and NIBGE, were collected and multiplied at Pesticide Chemistry Laboratory, NIAB, Faisalabad, using potato culture media. Reagent was collected and multiplied at Pesticide Chemistry Laboratory, prepared: different fungi strains, from NIAB and NIBGE, were mixed. The mixture was kept warm (40°C) and the suspension was filtered through 2-layer gauze.

Preparation of fungi culture media was as follows: from the local market, the fresh potato was collected and sanitized with Dettol about 10–12 minutes. The potatoes were washed with distilled water, peeled off, and ground in high-speed blender (Multimix Braun, Germany). A 50 g of ground potato was boiled along with distilled water 250 mL in a 500 mL flask for 45 minutes. Concentrate was filtered through one-layer gauze cotton cloth. Glucose (5 g) and agar-agar (5 g) were mixed in filtrate and autoclaved at standard temperature 121°C and pressure 15 lbs. The autoclaved growth media were cooled down to 45°C, poured into 10 sterilized Petri plates, and incubated for 48 hours at 37°C to remove any infectivity. Non-contaminated Petri dishes were chosen and cultured with Aspergillus niger spores. The Petri dishes were placed into the incubator for fungus multiplication and then these were utilized for the determination of fungicide residues in samples.

2.3. Extraction of Pesticide Residues. From the well-homogenized sample, 50 g of each sample was weighed correctly into Erlenmeyer flasks (500 mL) with Quick fit glass stopper and 75 mL of ethyl acetate of (analytical grade), 2.5 g sodium chloride of (Analytical grade) Merck, and 10 g sodium sulphate (anhydrous) were added in each flask and stoppered, with shaking the flask at moderate speed for one hour in a shaker (GFL Shaker, Germany). The organic layer of ethyl acetate was separated and filtered using Whatman filter paper number 1. The chlorophyll and other color materials were present in extracted solvent and removed by using clean up column (GPC) of activated charcoal and sodium sulphate (anhydrous, Merck). When sample passed through columns, the content was evaporated up to dryness with the help of rotary evaporator (Buchi 011, Switzerland), and then the analytical grade acetone 1 mL was added into the vials and analyzed with analytical techniques [17].

2.4. HPTLC Detection Method. The protocols for pesticide residue analysis continuously improved with new conventional technology. Due to low cost, wide range of application and simplicity HPTLC gained popularity for all classes of pesticides including herbicides, fungicides, and insecticides as reliable method [18].

2.4.1. *o*-Tolidine + Potassium Iodide (*o*-TKI). The *o*-tolidine + potassium iodide (*o*-TKI) detection method was used for the determination of any pesticide. The factory-made silica gel coated plates (0.25 mm thickness, 20 × 20 cm, Merck 1.05721) were used. The plates were freshly activated in oven at 105°C for 30 minutes and appropriate volume of all samples was spotted according to the written plan starting from left corner of the plate using microliter syringe (Hamilton) at 1.5 cm space for spots. The plates were developed in the developing tank already saturated with ethyl acetate (analytical grade) solvent. The time and temperature were noted and plates were developed up to the mark 12.0 cm from the origin. After elution, the plates were removed, dried in fume hood, and placed in another glass tank saturated with Cl vapors (8 g KMnO₄ + 10 mL Conc. HCl) for 45 seconds. To evaporate the excess chlorine, plates were removed from the tank and put in the fume hood for some time, 45 min. Then plates were sprayed with reagent solution using Desaga sprayer gun. After a few seconds, blue spots appeared on a white background. The positions of the spots were marked and the distance of the spots and area of spots were recorded. The \( R_f \) and MDQ of each pesticide were calculated [19].

2.4.2. Enzyme Inhibition Method with Horse Blood Serum and Acetylthiocholine Iodide Substrate (Eacl). The method is designed for those pesticides, which show enzyme inhibition properties, especially for phosphoric and thio-phosphoric acid esters and organophosphorus and carbamate pesticide residues. Plant extraction usually does not interfere in the detection process. The factory-made silica gel 60, 0.25 mm (Merck, 1.05721) plates were incubated at 105°C for 30 minutes before use. For work the standard solutions of different pesticides were prepared in acetone (analytical grade, Merck, Aldrich, Sigma). The plates were spotted according to the designed plan with microliter syringe. The plates were developed in presaturated developing tank and eluted to the mark (12 cm). The plates were air-dried in fume hood and treated with Br vapors for 15 minutes in a presaturated tank (0.5 mL bromine was pipette out into the beaker). The plates were removed and kept for 45 minutes in fume hood to remove excess bromine. After this, the plates were sprayed properly with freshly made enzyme solution (10 mL horse serum + 10 mL distilled water). Then, plates were incubated in glass tank presaturated with vapors at 300°C about 30 minutes. After incubation, the plates were removed from the tank and extra water was evaporated with hair dryer. The plates were again sprayed with substrate solution and incubated at 37°C for 15 minutes in the similar water vapor saturated tank. The plates were again removed back from the tank, air dried for 5–7 minutes under air stream, and sprayed with reagent solution. The blue spots/dots against white background appeared within few seconds, and the spots were marked immediately because they disappeared in five minutes. The eluted distance of solvent, spots, and spot diameter was measured and calculations were made for \( R_f \) and MDQ [19].
2.4.3. Photosynthesis Inhibition Method. This method is specifically used for determination of herbicide residue, which inhibits the photosynthesis function. Extraction of chlorophyll suspension was as follows: for the active detection of herbicides in vegetable extract, the chlorophyll of rice, wheat, and spinach leaves was used. Wheat leaves extract is used for this work. Fresh wheat leaves 30 g cut and ground with pestle and mortar were homogenized completely and then 3 mL glycerin and 15 mL distilled water is added. The suspension is poured through thin cloth into a flask to obtain chlorophyll from leaves. Flask covered with aluminum foil and stored in refrigerator. The ready-made silica gel 60 plates were activated at 105°C for 30 minutes. Then plate was removed from oven, fixed in spotting rack, and spotted with sample extracts (20 μL) and authentic standards (isoproturon, metoxuron, atrazine approximately IOP). After this, the plates are activated in a tank using ethyl acetate. The plates were dried using fume hood, sprayed with reagent solution, and put under light (60 W bulb) for about five minutes for extreme visibility of spots. The spot disappeared after 10 minutes, so the spot area was measured quickly. The bluish color against the greenish background appeared [19].

2.4.4. Fungi Spore (Aspergillus niger) Inhibition Method (FAN). This method is used for the determination of those pesticides, which have the properties to inhibit the growth of *Aspergillus niger*. This method is selectively used to detect fungicides. Plant extracts usually do not interfere in the detection process. Factory-made silica gel 60 plates were initiated at 105°C for 30 minutes. Working standard solutions of different pesticides were prepared in acetone. The plates were spotted according to the written plan with microliter syringe (Hamilton). The plates were developed in presaturated developing tank and eluted to the mark (12 cm). The plates were air dried in fume hood and sprayed with fungi suspension solution thoroughly. The plates were kept in the tank presaturated with vapors using deionized water. After 48 hours, the plates were observed and spots were found and marked. The eluted distance of solvents, spots, and spot diameter was measured and calculations were made for $R_f$ and MDQ [19].

2.5. High Performance Liquid Chromatography Analysis. Vegetable samples were analyzed by HPLC method, described by Ohlin 1986 and Dekok and Himestra in 1992. Methanol was used to redissolve the vegetable extracts. The next step was to run the samples through the HPLC. Gradient and Isocratic System with a reverse phase C-18 column 25 × 4.6 mm (I.d) was used to perform analysis. The UV/visible detector having a wavelength of 214 nm was used. During the experiment, the oven temperature ranged 30 to 50°C. The equipment was in position, to attain a maximum pressure of 210 kg/cm² and range was 1.40 AUFS (absorption unit full scale). The method was used for the detection of organophosphorus and pyrethroids residues using isocratic mode in reverse phase system. The following conditions were used for the analysis of brinjal samples for organophosphorus, organochlorine, and pyrethroids. Liquid chromatography (Shimadzu LC 10 A) was done using C-18, 250 × 4.6 mm, 5 μm column with flow rate of 1.5 mL/min at 30°C column temperature. The acetonitrile/water was used as a mobile phase with injection loop of 20 μL using Class LC-10 software at 214 nm.

2.6. Statistical Analysis. For the validation of four methods (see Table 1), the values are in the terms of means and SD and the linear response was observed for pesticides using Microsoft Excel 2016. The results of pesticide residual concentration are shown in Table 2. Each value is a mean of ≥3 samples from each week interval and 1 to 10 weeks sampling intervals were used. The means comparisons were set by applying ANOVA (general linear model). Tukey Simultaneous 95% CI comparisons were performed by Minitab-17. The means that do not share letter are significantly different and results are also described in the form of graphs (conc. vs weeks) with S.E (standard error) using Microsoft Excel 2016.

3. Results and Discussion

3.1. Validation of HPTLC. Numbers of techniques and their versions are used to analyze the pesticide residue, but HPTLC is considered as efficient version of chromatography to analyze almost all classes of pesticides due its mode of detection simplicity and cost-effective application [20]. The HPTLC is a versatile version of TLC having advancement to prevent the possible result variation due to human error [3]. Before residual analysis, the method used for analysis was validated to evaluate the sensitivity and linearity. Four methods were used for residue analysis. Types of organochlorine, organophosphorus, and pyrethroids pesticides were used as indicator compounds and results showed good spot visibility. Calculated values for $R_f$, SD, MDQ, and CV are listed (see Table 1). The linear response was noticed between average spot diameter and maximum detected quantity of pesticides (Figure 1). The graphs show linear behavior of indicator compounds and it is concluded that the behavior of pesticide standard was linear (see Figure 1). Statistics were applied on data and regression analysis was carried out. The value of $R^2$ was in the range of 0.9615–0.9458 according to results of [21].

3.1.1. Reproducibility of $R_f$ Values. The result values were mean of three replicates. From Table 1, it is illustrated that $R_f$ with standard deviation, $RR_f$, is coefficient of variation for different described four methods. For ethyl acetate system, the value of $R_f$ ranges from 0.05 ± 0.006 to 0.60 ± 0.01 (Table 1). The value of $CV$ for all pesticides is less than 10, except methomyl, and results are in agreement with the already reported study [8]. The value of $RR_f$ is varied from 0.07 to 1 (Table 1) and $RR_f$ of atrazine, fenamidone, thiophanate-methyl, malathion, linuron, and thiabendazole were in promising agreement with $RR_f$ value of reported data [8]. The $R_f$ value (0.05–0.66) achieved in Table 1 is within range of already reported results [19]. $RR_f$ values calculated for
marker compounds, atrazine, and captan show good agreement with literature [19]. For o-TKI and photosynthesis inhibition method, the RR values of pesticides are in good agreement with literature and the marker compound atrazine was reported with $R_f (0.62)$ and $RR_f (1)$ [22]; also of atrazine, oxamyl, diuron, linuron fenarimol, captan, and parathion-methyl was in effectively close agreement with markup and selected compounds of reported data [19]. The four used methods for pesticide evaluation/analysis were in good agreement for detection of organophosphorus, organochlorine, and pyrethroids because the various compounds show very close $R_f$ trend. The $R_f$ values of atrazine, linuron, methomyl, thiabendazole, malathion, thiophanatemethyl, fenarimol α-endosulfan, and chlorpyriphos were almost related to already reported results so it is validated that the TLC method was promising technique for detection of pesticides [8, 22].

3.1.2. MDQ for Different Detection Methods. The MDQ is a minimum amount of analytical standard and expressed in ng, and under average chromatographic condition it provides clear visible spot when spotted on plates [19]. The MDQ values are determined using markup compounds of individual method. The markup compounds are atrazine, fenarimol, linuron, and oxamyl (see Table 1) for present study and these compounds were also confirmed in literature as markup compounds in different methods [19]. The MDQ values were varying among different compounds as well methods. The atrazine is a markup compound for both photosynthesis inhibition and o-TKI method and similar findings were reported in literature [22]. For pesticide detection, the range of MDQ of 1–100 is required [19]; the MDQ value mentioned in Table 1 is within this range and provides good result reproducibility. The MDQ value of (Table 1) pesticides also shows confident results with reported results [23]. The o-tolidine+potassium iodide (o-TKI) method was noted as suitable for organophosphorus and carbamate residues and in good agreement with literature [8, 24].

3.2. Pesticide Residues in Brinjal Samples. The extract of ten samples collected at one-week interval was spotted on silica-gel plates along with reference standards purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The plates were developed in the presence of solvent and different spray used for spot visibility (see Figure 2). Pesticide residues were extracted with the use of ethylacetate as extraction media and multiresidue methodology was used for analysis of pesticide. The HPTLC recently gained popularity due to sensitivity and effectiveness. On the basis of average spot diameter, the concentration (mg·kg$^{-1}$) of pesticide was calculated. After one-week interval, the concentrations varied from sample to sample. All the pesticides residues were detected in the range of 0.08–0.67 $R_f$ value with (MDQ) range (0.5–100 ng) (see Table 2). For pesticide detection, the range of MDQ 1–100 is required [19], and the MDQ value mentioned in Table 2 is within this range and has satisfying results.

### Table 1: $R_f$ and MDQ values of four TLC validation methods.

| Pesticide                          | $R_f$  | $RR_f$ | MDQ (ng) | Coefficient of variation |
|-----------------------------------|--------|--------|----------|--------------------------|
| o-tolidine + potassium iodide (o-TKI) method |
| Oxamyl                            | 0.2 ± 0.001 | 0.32   | 90       | 0.52                     |
| Diuron                            | 0.37 ± 0.018 | 0.59   | 30       | 4.89                     |
| Atrazine                          | 0.62 ± 0.018 | 1      | 20       | 2.90                     |
| Dimethoate                        | 0.27 ± 0.002 | 0.43   | 100      | 0.73                     |
| Imidacloprid                      | 0.23 ± 0.002 | 0.37   | 130      | 0.88                     |
| Fungi spore method, FAN            |
| Captan                            | 0.63 ± 0.002 | 1      | 20       | 0.32                     |
| Fenarimol                         | 0.51 ± 0.004 | 0.80   | 1        | 0.20                     |
| Prochloraz                        | 0.37 ± 0.001 | 0.60   | 20       | 0.27                     |
| Imazolil                          | 0.15 ± 0.001 | 0.23   | 5        | 0.68                     |
| Thiophanate-e-methyl              | 0.58 ± 0.002 | 0.93   | 50       | 0.33                     |
| Horse blood serum method, Eacl     |
| Oxamyl                            | 0.18 ± 0.002 | 0.28   | 1.2      | 1.10                     |
| Parathion-methyl                  | 0.58 ± 0.010 | 0.90   | 9        | 1.72                     |
| Methomyl                          | 0.05 ± 0.006 | 0.07   | 75       | 12.00                    |
| Methidathion                      | 0.63 ± 0.001 | 0.98   | 100      | 0.16                     |
| Phosphamidon                      | 0.23 ± 0.001 | 0.35   | 50       | 0.44                     |
| Malathion                         | 0.64 ± 0.002 | 1      | 100      | 0.31                     |
| Dichlorovos                       | 0.50 ± 0.002 | 0.78   | 2.0      | 0.40                     |
| Photosynthesis inhibition method, Hill reaction |
| Atrazine                          | 0.66 ± 0.01 | 1      | 2        | 1.51                     |
| Linuron                           | 0.62 ± 0.007 | 0.93   | 2        | 1.12                     |
| Chlortoluron                      | 0.44 ± 0.006 | 0.66   | 3.5      | 1.36                     |
| Isoproturon                       | 0.41 ± 0.008 | 0.62   | 3        | 1.95                     |
| Thiabendazole                     | 0.40 ± 0.02 | 0.60   | 25       | 5.00                     |

Values are mean of three replicates. SD: standard deviation. MDQ: maximum detected quantity. C.V: coefficient of variation.
Table 2: Pesticide residue found in brinjal samples by different HPTLC methods.

| Pesticide       | Sample number 1 (mg·kg⁻¹) | Sample number 2 (mg·kg⁻¹) | Sample number 3 (mg·kg⁻¹) | Sample number 4 (mg·kg⁻¹) | Sample number 5 (mg·kg⁻¹) | Sample number 6 (mg·kg⁻¹) | Sample number 7 (mg·kg⁻¹) | Sample number 8 (mg·kg⁻¹) | Sample number 9 (mg·kg⁻¹) | Sample number 10 (mg·kg⁻¹) | Rf | MDQ (ng) |
|-----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----|---------|
| α-Endosulfan    | ND                        | 0.075E                    | ND                        | 0.105D                    | 0.22A                     | ND                        | 0.075E                    | ND                        | 0.155C                    | 0.2B                      | 0.67 | 50      |
| Chlorpyriphos   | 0.055G                    | ND                        | 0.075F                    | 0.085E                    | 0.105C                    | 0.095D                    | ND                        | 0.099CD                   | 0.135B                    | 0.145A                    | 0.669 | 0.5     |
| Methamidophos   | 0.025E                    | 0.035E                    | 0.065D                    | ND                        | 0.035E                    | 0.15A                     | 0.06D                     | 0.075C                    | ND                        | 0.085b                    | 0.11 | 10      |
| Monocrotophos   | 0.135C                    | 0.165A                    | ND                        | 0.095F                    | 0.12D                     | 0.105E                    | 0.15B                     | ND                        | 0.09F                     | 0.125D                    | 0.08 | 88      |
| Dichlorvos      | ND                        | 0.025D                    | 0.022D                    | 0.035C                    | ND                        | 0.035C                    | 0.062A                    | 0.055B                    | ND                        | 0.504                     | 10   |         |
| Profenophos     | 0.078C                    | 0.062E                    | ND                        | 0.058E                    | 0.098A                    | 0.068D                    | 0.098A                    | ND                        | 0.048F                    | 0.088B                    | 0.34 | 20      |
| Dimethoate      | 0.125A                    | D                         | 0.075D                    | 0.105C                    | 0.125A                    | 0.105C                    | ND                        | 0.065E                    | 0.055F                    | 0.12B                      | 0.64 | 25      |
| Carbosulfan     | 0.1B                      | 0.062C                    | 0.09B                     | 0.05C                     | 0.115A                    | 0.114B                    | 0.124A                    | 0.095B                    | 0.055C                    | 0.125A                    | 0.676 | 15      |
| Deltamethrin    | 0.095C                    | 0.09D                     | 0.06J                     | 0.12B                     | 0.085E                    | 0.08F                     | 0.125A                    | 0.065H                    | 0.1C                      | 0.075G                    | 0.226 | 50      |
| β-Cyhalothrin   | 0.125E                    | 0.15B                     | 0.25A                     | 0.115C                    | ND                        | 0.12C                     | 0.16B                     | 0.15B                     | 0.125C                    | ND                        | 0.55 | 100     |
| Cypermethrin    | 0.125F                    | ND                        | 0.2B                      | 0.135E                    | 0.145D                    | 0.135E                    | ND                        | 0.205A                    | 0.145D                    | 0.165C                    | 0.673 | 100     |

*Values are mean of 3 samples from each interval and 1–10 are sampling intervals. ND: not detected. Capital letters as subscript on values means separation.
Organochlorine pesticide residue was detected in brinjal samples. The α-endosulfan was found in higher concentration in samples of 4, 5, 9, and 10 weeks intervals with Rf value of 0.67 and MDQ 50 ng. But there is no endosulfan in samples 1, 3, 6, and 8 (Figure 3). Various types of organochlorine (α- and β-endosulfan) were banned in many developing countries due to its highly toxicity, bioconcentration, and high persistence. Its contamination in our food was unknown but its markup evidence of carcinogenicity and genotoxicity was found[24,25].

The pesticide residues, chlorpyriphos, methamidophos, monocrotophos, dichlorvos, carbosulfan, and dimethoate, belong to class of organophosphorus determined with OT+KI and horse blood methods. The carbosulfan contaminates all samples with Rf values (0.68) and MDQ (15 ng). The maximum residue was found in sample as shown in Table 2. Samples 5, 6, 7, and 10 were significantly different and higher than others. Thai Agriculture Standard set the MRL for carbosulfan as 0.03 mg·kg\(^{-1}\) (TAS 9002-2013). All samples 1–10 violated the limit [23]. The sample of the second week revealed a higher concentration of monocrotophos (0.165 mg·kg\(^{-1}\)) than all the other samples and types of organophosphorus and the mean were significantly different than other means. Chlorpyriphos and dichlorvos are well detected using HPTLC with Rf (0.66 and 0.5) using ethyl acetate extraction method (Table 2). Rf (0.05) in literature were reported with effective decrease in residual concentration with 3-day interval [18]. According to Thai Agriculture Standard, the MRL of dichlorvos is 0.2 mg·kg\(^{-1}\) and no sample violates the set limit. Literature shows that the mean concentration of chlorpyriphos was 0.17 mg·kg\(^{-1}\) in some animals trials showed that the long-term exposure and swelling of endosulfan damaged kidney [10].

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Faisalabad region [26]. Organophosphates residual concentration showed good detection range in mg kg\(^{-1}\) with reported work of TLC combined with GC/HPLC. (Di-methoate contaminates samples 1–10 with 0.05 to 1.25 mg·kg\(^{-1}\) but was less detected than reported work [19].

The maximum concentration of chlorpyriphos (0.145 mg·kg\(^{-1}\)) is much better obtained as compared to literature but the monocrotophos was less detected [27]. The mean value from ten samples of monocrotophos was 0.0794 mg·kg\(^{-1}\) which is less than MRL of 0.5 mg·kg\(^{-1}\). But the mean value of profenophos (0.059 mg·kg\(^{-1}\)) was close to the MRL of 0.05 mg·kg\(^{-1}\). The MRL for methamidophos was 0.03 mg·kg\(^{-1}\) according to FAO 2016 and samples numbers 3, 7, 8, and 10 violate the set limit (Figure 4).

Pyrethroids pesticides (deltamethrin, cypermethrin, and β-cyhalothrin) were found in brinjal samples. The means of 1–10 samples with one-week interval were highly statistically significant for deltamethrin (Table 3). The MRL of deltamethrin is 0.01 mg·kg\(^{-1}\) for cattle and sheep for kidney organ and was set to found in different tissue (FAO/WHO CX/MRL 2-2018) and was 0.1 mg·kg\(^{-1}\) for vegetables. Samples 1, 2, 3, 5, 6, 8, and 10 showed less concentration than set limit. The MRL (0.03 mg·kg\(^{-1}\)) was set for cypermethrin by Thai Agricultural Standard (TAS 9002-2013). All the samples exceeded this limit except samples numbers 2 and 7 [23]. The cypermethrin MDQ (100 ng) was detected in higher concentration than other ones. It was evident that samples 1, 3, 4, 6, 8, and 9 showed all three types of pyrethroids and the deltamethrin was found in all samples of 1 to 10 with minimum detected quantity (MDQ = 50 ng). The means of β-cyhalothrin is significantly different (sample number 3 was more significantly different) and β-cyhalothrin was found with maximum concentration of 0.25 mg·kg\(^{-1}\) in sample

**Figure 2:** Spot visibility of four used HPTLC methods. (a) Spot visibility of pesticides in the OT + KI method. (b) Spot visibility of pesticides in the fungi spore inhibition method. (c) Spot visibility of pesticides in the horse blood serum method. (d) Spot visibility of pesticides in the photosynthesis inhibition (hill reaction) method.

**Figure 3:** Concentration of α-endosulfan pesticide residue in brinjal.

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number 3 (see Figure 5). The MDQ values of deltamethrin, cypermethrin, and dichlorvos showed good detection values with reported data [19].

Overall, from all the detected pesticide residues, a very low MDQ value was noted for chlorpyriphos (0.5 ng) with $R_f$ value of 0.67 and the highest value of MDQ for cypermethrin and $\beta$-Cyhalothrin is 100 ng. But the concentration of cypermethrin (0.20 mg·kg$^{-1}$) was significantly higher and also the concentration of $\beta$-cyhalothrin (0.25 mg·kg$^{-1}$) is found to be higher (see Table 2). The residue belonging to pyrethroid (cypermethrin and deltamethrin) contaminates all samples (Table 2). From all the detected pesticide residues (Table 2), cypermethrin showed higher value of $R_f$ and MDQ (0.67 and 100 ng) compared to other pesticide residues. The detected concentrations of cypermethrin in all samples except samples 2 and 7 violate the MRL for eggplant (0.03 mg·Kg$^{-1}$) according to Thai Agriculture Standards [23]. All the samples were tested for fungicides and herbicides but there was no such evident found for these types of pesticides. All the pesticide residues were detected from samples numbers 1 to 10. From all detected pesticides, a few samples were showing less quantity of residue than recommended MRL but its continuous use and the contamination could cause a very serious problem in humans' life.

3.3. HPTLC and HPLC Analysis. The method was utilized for analysis of pyrethroids and organophosphorus. The chromatogram of standard pesticides and sample was analyzed by HPLC reverse phase in gradient program (see Figures 6 and 7).

No such difference was showed for analysis of pesticide residual concentration in brinjal samples with RP-HPLC as compared to HPTLC method. The organochlorine pesticide endosulfan showed almost similar concentration in all samples of HPTLC method to residual concentration of HPLC method except concentration of sample number 5. The higher concentration of 5th week in HPTLC may be obtained due to extensive sprays than other week samples.
For organophosphorus pesticide analysis, the concentration of chlorpyriphos (0.14 mg·kg\(^{-1}\)) of sample number 10, dimethoate (0.12 mg·kg\(^{-1}\)) of weeks 1, 5, and 10, and profenophos (0.98 mg·kg\(^{-1}\)) of weeks 5, 6, and 10 in HPTLC method showed good agreement with residual concentration of chlorpyriphos (0.14 mg·kg\(^{-1}\)) dimethoate (0.13 mg·kg\(^{-1}\)) and profenophos (0.11 mg·kg\(^{-1}\)) obtained from HPLC method (Tables 2 and 3). The obtained concentration of chlorpyriphos (0.14 mg·kg\(^{-1}\)) showed better detection compared to already reported work of Harshit et al. in HPLC and UV-spectrophotometric method [28]. In Table 3, it was clearly evident that concentration of monocrotophos in HPLC 0.17 mg·kg\(^{-1}\) was similarly detected in 2\(^{nd}\) week sample, 0.16 mg·kg\(^{-1}\), by HPTLC method. For pyrethroid pesticides, the residual concentration of deltamethrin (0.09 mg·kg\(^{-1}\)) and cypermethrin (0.12 mg·kg\(^{-1}\)) through HPLC show similar detection value with samples of HPTLC method, while the other pesticides such as methamidophos, carbosulfan, and \(\beta\)-Cyhalothrin are less detected in HPLC and showed better concentration in HPTLC residue. The high-performance liquid chromatography method was utilized for analysis of organophosphorus residue in variety of samples like fruits, vegetables, soil, and water [29]. No doubt the HPLC technique has extensive and precious application in analysis of food and is considered as more efficient analytical technique compared to TLC and HPTLC. The HPLC is more sensitive than HPTLC, but the pesticide residual concentration detected in HPTLC method showed
very close agreement with residual concentration using HPLC. Some of the pesticides in various weeks showed higher concentration in HPTLC; this might be due to extensive use of sprays or effect of solvent on extraction or sensitivity of HPLC for detected pesticides but it does not compromise the sensitivity of HPLC method. So, the findings of the present study by comparing the obtained residual concentration of insecticides from both methods HPLC and HPTLC confirm the good sensitivity of HPTLC and provide satisfactory results for HPTLC as cost-effective method for residual analysis of pesticides as an alternative to HPLC.

4. Conclusion

Pesticide residual contamination in food creates health problems worldwide due to its extensive toxicity and ambiguity. The brinjal samples collected from local market of Faisalabad were used for the pesticide residue detection. The four different high-performance thin layer chromatography methods were used for the detection of pesticide residue and validation of HPTLC method was performed to check the linear behavior between different pesticide standards. Analysis of brinjal samples (1 to 10 sample with one-week interval) shows that three classes of pesticides were detected: organophosphorus, organochlorine, and pyrethroids. The higher concentration of organochlorine (α-endosulfan) was present in 4, 5, 9, and 10 samples. The organophosphorus pesticides (chlorpyriphos, methamidophos, monocrotophos, dichlorvos, carbosulfan, profenophos, and dimethoate) contaminate the samples and violate the set MRL limit of FAO and TAS. The deltamethrin and carbosulfan contaminate the all samples of 1−10 weeks. The β-cyhalothrin (0.25 mg·kg⁻¹) and cypermethrin (0.205 mg·kg⁻¹) were found in higher concentration than all the other detected pesticides. The HPLC were performed to confirm the results of HPTLC by using reference standards. The residual concentration of α-endosulfan, chlorpyriphos, dimethoate, monocrotophos, profenophos, deltamethrin, and cypermethrin showed similar results with HPLC analysis. The finding suggested that the HPTLC as cost-effective method was effectively used as an alternative to HPLC for detection of insecticides residues.

Data Availability

Data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publishing of this work.

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