Surveillance and Dietary Risk Assessment of Endocrine Disrupting Pesticides in Brinjal and Cauliflowers

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Research Article

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Several pesticides (used in vegetable production) have recently been identified as potential Endocrine Disruptors. The current study aimed to determine the consumer exposure risk associated with eating contaminated vegetables. The European Union-citrate buffered QuEChERS extraction protocol, validated in accordance with the European Union guidelines, was used to monitor selected endocrine disrupting pesticides and their metabolites in brinjal (Solanum melongena L.) and cauliflower (Brassica oleracea) marketed in Rawalpindi/Islamabad, Pakistan. A total of 88 and 69 percent of brinjal (n=25) and cauliflower samples (n=26) were found contaminated among which 20 and 65 percent were exceeding the European Union maximum residue limits respectively. Both vegetables contained high levels of the androgen antagonist chlorpyrifos and the thyroid hormone inhibitor lambda-cyhalothrin. The acute health risk associated with dietary exposure to chlorpyrifos in brinjal and cauliflower was 1.88 and 22, respectively. The cumulative health index in cauliflower was found to be greater than one for both males and females (cumulative health index > 1). Thus, chlorpyrifos residues in brinjal and cauliflower pose both acute and chronic human health risks, whereas lambda-cyhalothrin residues in cauliflower samples only pose such an implication.

1 Introduction

The endocrine system (pituitary gland, pineal gland, pancreas, thyroid/hypothyroid, ovaries, and testes) is critical to overall health. Vegetables classified as “Protective Foods” by the Food and Agriculture Organization (FAO) of the United Nations protect the consumers from malfunctioning of the endocrine system (FAO, 2018; Gupta, et al., 2017; Yarmohammadi, et al., 2021). In order to meet the increasing demand for vegetables globally, farmers use chemical fertilizers and pesticides to increase per hectare yield. Imprudent use of registered pesticides without adhering to recommended good agricultural practices (GAP) and violating safe Pre-Harvest Intervals (PHIs) results in unwanted residues (EFSA, 2017), which may causes various acute and chronic health implications like memory loss, hormonal imbalance, birth defects, and cancer (Mnif, et al., 2011; Rawlings et al., 1998; Sugiyama et al., 2005) as well as can leads to export confiscation.

Several EDPs (organochlorine pesticides) were declared banned in Pakistan in 2001 after the country became a signatory to the Stockholm Convention (UNEP 2001; UNEP 2016). However, newer susceptible EDPs (several organophosphorus and pyrethroids) are still in use for vegetable production (Aamir et al., 2018). Furthermore, due to a lack of stringent policies, in addition to registered pesticides, the use of unregistered or banned pesticides, particularly suspected endocrine-disrupting pesticides (EDPs), for vegetable production was still reported (Aamir et al., 2018; Ali et al., 2019). Despite improvements in vegetable production due to the use of pesticides for pest control, the nutritional values of vegetables appear to be contradictory due to the detection of hazardous EDPs (Reiss, et al., 2012). Several studies have revealed alarming pesticide residue levels in vegetables from around the world (Ali et al., 2019; Hejji, et al., 2021). The surveillance of EDPs in vegetables lays the groundwork for determining their dietary risk (Chen et al. 2012).

Brinjal/Eggplant (Solanum melongena L.), a summer fruiting vegetable, and Cauliflower (Brassica oleracea), a winter flowering vegetable, are grown on an area of 8427 and 12620 hectares with a production of 84255 & 24623 tons respectively in Punjab province of Pakistan (AMIS, Pakistan, 2018). During vegetative growth of brinjal, the pest larvae perforate in shoots causing various diseases like root rot, soft rot, wilting, damping off, fruit rot, bacterial wilt, leaf rot, mosaic, collar rot (Ali, 2018) whereas cauliflower is attacked by black rot, blackleg, clubroot, black leaf spot, and downy mildew that adversely affect the productivity up to 31% and make the crop unfit for human consumption (Ali, 2018). Because both vegetables are susceptible to a variety of pests and diseases, they receive multiple applications in a short period of time (Panwar et al., 2013). Few studies have reported the residues of different pesticides in these vegetables (Chen et al., 2012; Ali et al., 2019). Consumption of these contaminated vegetables can be hazardous to one’s health (Chen et al., 2012), and dietary exposure assessments should be included in routine annual monitoring programs.

Different vegetables have different phytochemicals which may possess physicochemical properties similar to targeted pesticides which results in false-positive results in chromatographic instruments (Besil et al., 2019). Moreover, these phytochemicals may contaminate the sensitive instruments and reduce the selectivity of the analytical method. Thus for robust and precise quantitation of multiple pesticides, the standard analytical method should be validated for each food commodity before analysis (Besil et al., 2019; SANTE, 2019).

Keeping in view malpractices in the production and marketing of vegetables, the current study was initiated to assess the levels of registered and unregistered/banned EDPs in market samples collected from Rawalpindi/Islamabad by using the verified standard method of the European Union Reference Laboratory for Fruits and Vegetable (EURL-FV-2010-M1), to check the compliance of market samples for the studied EDPs with maximum residue levels recommended by different regulatory agencies (FAO Codex Alimentarius Commission and European Union), evaluate the consumer health risk due to dietary exposure to these pesticides residues in brinjal and cauliflower. This study may provide baseline data to draft regulations related to the use of registered and illegal EDPs for vegetable production in Pakistan.

2 Material And Methods

2.1 Instruments, Chemicals and Reagents

Gas chromatograph equipped with micro-Electron Capture Detector (GC-μECD), HP-5 fused-silica capillary column and ChemStation® Software and gas chromatograph equipped with customized Pesticide Analyzer (GC-MSD), HP-5MS fused-silica capillary column and MassHunter Software®, NIST-MS library along with the Deconvolution DRS software and retention time locking (RTL) features (Agilent Technologies, USA) were used in this study. Other types of equipment used in this study were; laboratory food processor (Bliker® 5 Robot Coupe), vortex mixer (VELP, Scientifica: F202A0173), centrifuge (LX111HCS-LABDEX), Rotavapor (BUCHI Rota vapor: R-210), Nitrogen Air generator (CMC, Instruments GmbH Germany: NG UHP 5000X) and SMART2PURE-0.2µm water distillation unit (Thermo scientific, Sweden).
The extraction solvents and salts used in this study were HPLC grade acetonitrile, n-hexane, MgSO₄ anhydrous, and NaCl from DAEJUNG, Trisodium citrate dehydrate (Fisher Scientific UK), Disodium hydrogen citrate sesquihydrate (Sigma-Aldrich), and Primary Secondary Amine (PSA) (SUPELCO). The glassware used in this study was Pyrex class-A. Before use, they were sequentially washed with tap water, liquid detergent, NaOH solution, chromic mixture, distilled water, and then oven-dried to remove the other organic and inorganic pollutants. Certified analytical standards of bifenthrin, cypermethrin, deltamethrin, endosulfan sulfate, lindane, heptachlor, heptachlor exoepoxide A, heptachlor endoepoxide B, triadimenol, λ-cyhalothrin, chlorpyrifos, α-endosulfan, and β-endosulfan with purity > 95 percent were purchased from Sigma-Aldrich.

2.2 Criteria for selection of EDPs for study

The criteria for including a EDPs in the study was based on its registration with the Federal Department of Plant Protection (DPP), its common usage on brinjal/cauliflower (Ali, 2018), and their persistent banned EDPs (UNDP, 2001) which has previous long application history, reported in environmental matrices in past ten years (Ali et al., 2019), and may be alarming for the overall agro-exports from the country. The selected EDPs along with their physicochemical properties, impact on endocrine system and European Union-Maximum Residues Limit (EU-MRL) are given in the Supplementary Information (ESI) Table S-1.

2.3 Preparation of Solutions

Individual pesticide standards stock solutions were prepared in HPLC grade n-hexane and/or acetone. Different dilutions ranging from 0.1–20 µg/L were prepared as per the requirement of the respective pesticide/commodity MRL. Organically grown brinjal and cauliflower samples were obtained from the Horticulture Research Institute, NARC, Islamabad for the preparation of matrix blanks. Matrix matched calibration standards were prepared by spiking matrix blanks with appropriate concentrations of the standard mixture to obtain desired fortification level corresponding to 1×MRL, 2×MRL, and 4×MRL.

2.4 Sampling and sample processing

Twenty-five brinjal and twenty-six cauliflower samples (1Kg) were collected randomly from the different markets of Rawalpindi and Islamabad in triplicate as per European Union Commission's Directives: 2002/63/EC (EC, 2002). Relevant information for traceability of the samples i.e. the origin of the sample, quantity marketed, and possible plant protection measures, etc. was collected from the respective market sources (ESI-Table S-2). The collected samples were transported to the laboratory intact on the same day. The samples were cut into smaller pieces on a cutting board with a knife and then chopped to get a smaller particle size and homogenized in Stephen Chopper (Blixer®5 Plus robot coupe). A lab portion of approximately (100 g) was taken in zip mouth bags and stored at -20 °C in the freezer until further processing.

The extraction and cleanup of the samples were done according to the EU-citrate buffered QuEChERS (EURL-FV 2010-M1). Briefly, a homogenized 10g sample was taken in a 50 mL falcon tube; acetonitrile 10 mL, MgSO₄ anhydrous 4g, NaCl 1g, tri-sodium citrate dihydrate 1g, and sodium hydrogen citrate sesquihydrate 0.5g were added, vortexed for 2 minutes, and then centrifuged for 5 min at 4000 rpm. About 6 mL of the supernatant (upper acetonitrile layer of the extract) was taken in a 15 mL polyethylene centrifuge tube to which PSA 0.15 g and MgSO₄ anhydrous 0.9 g were added, shook vigorously for 1 min and then centrifuged at 6000 rpm for 2 min. One mL of the final acetonitrile extract was evaporated under a nitrogen stream and then reconstituted in 1 mL n-hexane in a 1.5 mL GC vial for gas chromatography.

2.5 Chromatographic Analysis

In the present study, GC-µECD and GC-MSD techniques were involved for the identification, quantification, and confirmation of the selected pesticides. The pesticides were identified on the basis of retention time and quantified on the basis of peak area. The analyses were performed on GC-µECD, HP-5 fused-silica capillary column, and ChemStation® Software (Agilent Technologies, USA). Analytical grade nitrogen (purity 99.99 percent) obtained from Nitrogen Air Generator (NG-UHP-5000 Ox) was used as carrier gas at a flow rate of 2.4 mL/min and 11.07 psi pressure. Samples (1µL) were injected in splitless injection mode with an inlet liner of a glass wool frit. The makeup gas (N₂) flow rate was 60 mL/min. The injector temperature was kept at 280°C. The initial oven temperature was 70°C (hold time 1 min), ramped to 150°C at 50°C /min (hold time 0 min), to 200°C at 6°C/min (hold time 0 min), and then to 280°C at 16°C /min (hold time 5 min). Thus the overall run time was 20.93 min.

The confirmation of positive sample was done on GC/MSD (Customized Pesticide Analyzer) equipped with HP-5MS fused-silica capillary column and Agilent MassHunter Software, NIST-MS library (version 17) along with the Deconvolution Reporting Software (DRS) software and retention time locking (RTL) features. Helium (purity 99.99 percent) was used as carrier gas at a flow rate of 2.0 mL/min. Samples were injected with a multimode inlet in splitless injection mode with an inlet splitless single tapered ultra-inert liner. Post-run time was set at 4 min with 25 mL/min flow. The MS was run in electron impact (EI) ionization mode with electron multiplier voltage set at 1217V. The temperatures of the MS interface, ion source, and the quad were kept at 280°C, 250°C, and 180°C, respectively. The rest of the GC thermal programming was the same as mentioned above for GC-µECD. Details of MS SIM parameters for the current analysis were tabulated in ESI Table S3.

2.6 Method Validation

The method was validated in terms of accuracy, precision, and limit of detection, the limit of quantification, lowest calibration level, linearity, and matrix effect to demonstrate its “fitness for the purpose” as per EU criteria (SANTE, 2019). Accuracy and precision of the analytical method were determined in terms of percent recovery and %RSD respectively at three different spiking levels equivalent to one, two, and four times of a given MRL. The LOD was calculated by multiplying the standard deviation of the recovered concentration of n-1 replicates (n = 6) at the lowest spiking level at which recovery and precision are satisfactory with the One-Tailed ‘t’ statistic. The limit of quantification is the concentration of pesticide that could be measured with precision and accuracy with EU-SANTE limits. It was calculated with equation-3.LCL is the least injected amount for which the S/N was greater than 10. The LCL was set lower than the LOQ of the method for the selected compounds. Linearity was evaluated by Procedural Standard Calibration (PSC). In the PSC method, the recovery of each spiked level was plotted against actual spiked concentration, and $R^2$ from the regression line demonstrated the linearity of the analytical method. Matrix Effect is the mutual effect of each co-eluting analyte after clean-up. It was calculated as percent of deviations of the chromatographic response of the
compound in matrix-matched standards (MMS) from its response in n-hexane by using equation-1. Positive matrix effect (ME) values indicate enhancements and negative values showed the suppressions of the analyte signals. The ME is classified into three categories as given below (Ferrer et al., 2011).

- Weak ME: (ME < 20%) assumed as no significant matrix effects. This is because the difference is within the range of allowable chromatographic shifts.
- Medium (ME 20–50%).
- Strong ME (> 50%).

The validated method was used for the analysis of pesticide residues in 25 brinjals and 26 cauliflower samples collected from selected markets of Rawalpindi/Islamabad. The matrix-matched calibration standards were analyzed in the same batch as the anonymous sample to check system suitability for the analysis.

### 2.7 Quality assurance and quality control

The quality of analytical work was ensured by including a reagent blank, one organic matrix blank, and quality control samples at least two spiking levels (High and lowest level of method validation) in each analytical batch. The reagent blanks were included to eliminate any chances of laboratory and glassware contamination. The matrix blank were included in each sequence to eliminate for false positive. To eliminate the chances of false positive due to carryover from previous injections, back-flush settings were ensured in instrumental post run setting.

### 2.8 Consumer Health Risk Assessment

The consumer dietary exposure to pesticide residues through intake of contaminated vegetables is estimated by using the secondary data on food consumption pattern, individual bodyweight, and pesticide residue levels (median mg/kg/BW). The exposure estimates are then compared with toxicological criteria such as Acute References Doses (ARfD) and Acceptable Daily Intake (ADI) (FAO, 2017a).

As per capita consumption of brinjal and cauliflower in the country was not available, therefore, the FAO Food Balance Sheets (FAO, 2017b) was used in this assessment process. The per capita consumption of brinjal and cauliflower is calculated from the quantity produced in a country plus quantity imported and minus the quantity exported divided by the number of individuals in a given population under consideration. The estimation of consumers' dietary health risk has calculated for two sub-groups; adult males (with an average body weight of 60 kg) and adult females (55 kg) in the age ranging from 19–60 years as Food and Agriculture Organization of the United Nations classification (FAO, 2018).

There are several empirical indices that are used in human health risk assessment studies. The dietary exposure is estimated in terms of estimated daily intake, which was calculated by using equation-1. (It was measured in mg/kg/BW). For acute risk assessments, these ESRI were divided with FAO/WHO established ESTI/arfD (Eq. 5) while for chronic FAO/WHO established ADI (Turner, 2018). The health quotient (HQ) indicates an unacceptable risk when it is higher than 100%. The accumulative risk indices can be calculated by the summation of health quotien (HQs) from the multiple pesticide residues of the same group by using equation-7.

\[
\text{EDI} = MC \times F.C / bW \quad \text{(Eq. 1)}
\]
\[
\text{ESTI} = HR \times Ws^* v^+ (Lp - Ws) \times HR / bW \quad \text{(Eq. 2)}
\]
\[
\text{aHQ} = \text{ESTI} / \text{aRfD} \times 100\% \quad \text{(Eq. 3)}
\]
\[
\text{cHQ} = \text{EDI} / \text{ADI} \times 100 \quad \text{(Eq. 4)}
\]
\[
HI = \sum (HQ)\]

Where as EDI = Estimated Daily Intake (mg/kg/BW); MC = Median concentration of residue in food commodity (mg/kg); F.C = Food Consumption data (Kg/person/day); bW = average body weight (Kg); ESTI = Estimated Short term intake (mg/kg/BW); HR highest residues of pesticide in food commodity (mg/kg) during surveillance study, Ws Weight of individual vegetable unit, variability factor for sample (for market samples it is 3), Lp large portion of population consuming the vegetables at 97.5% confidence interval, ADI = Acceptable Daily Intake; aRfD = Acute References Dose; aHQ = Acute health risk quotient; cHQ = Chronic health risk quotient; HI, health index cumulative. i index of the health quotients of multiple pesticides found in each contaminated sample.

### 2.9 Statistical analysis

Descriptive statistical analyses were carried out by using statistical software (SPSS 16.0)

### 3 Results

#### 3.1 Validation of the analytical method

A good extraction technique for pesticide residues from a matrix involves the selection of proper solvent, sorbent, salts, and other experimental conditions. The EURL-FV method (EURL-FV (2010-M1) based on QuECHERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) was used for the extraction of pesticide residues from brinjal and cauliflower samples. However, beforehand it is imperative to demonstrate that the method being used is fit for its intended purpose. Therefore, the method performance characteristics including accuracy, precision, limit of detection (LOD), the limit of quantification (LOQ), linearity and matrix effect were assessed as per European Union Guidelines (SANTE, 2019).
The perilous effect of chlorpyrifos on human health and the environment, the European Union legislated to ban the use of chlorpyrifos in 2020 (EFSA, 2020).

This study reports on the levels of pesticide residues in brinjal (25) and cauliflower (26) samples collected randomly from the different markets of Rawalpindi and Islamabad. The frequency & range of contamination and their compliance with EU-MRLs are summarized in Table 2. Briefly, 15 brinjal and 15 cauliflower samples were found contaminated with varying levels of pesticide residues. Out of 13 compounds tested only five pesticides viz. chlorpyrifos, heptachlor endoxide B, endosulfan sulfate, α-cyhalothrin and α-cypermethrin were detected. The most culprit was found to be chlorpyrifos detected in 58% cauliflower samples in the range of 0.16–182.5 mg/kg and in 20% brinjal samples in the range 0.24–5.78 mg/kg; all exceeding the EU-MRLs. The confirmatory analysis of positive samples detected on GCµECD was carried out on GC/MS in both total ion current (TIC) and selected ion monitoring (SIM) modes. The matching inRetention Times (RT), NIST library, and extracted ion chromatogram confirm the presence of chlorpyrifos in the samples.

Cyhalothrin was detected in 88% brinjal (n = 25) and 8% (n = 26) cauliflower samples. None of the samples in brinjal violated the safety limits while 8% of cauliflower samples were contaminated with residues above EU-MRL. Rest of the compounds viz. Heptachlor endoxide B, endosulfan sulfate, and α-cypermethrin detected in brinjal and/or cauliflower samples were within permissible limits (Fig. 1). However, their isomers like α-endosulfan, and β-endosulfan were not found in any sample. The most common combination of two pesticides in either vegetable was chlorpyrifos/α-cyhalothrin and endosulfan sulphate/λ-cyhalothrin. The frequency of occurrence of three pesticides in brinjal and cauliflower samples were 4% and 5% respectively.

3.3 Dietary human risk assessment

The consumers’ dietary exposure and associated health risks due to pesticide residues in brinjal and cauliflower samples were evaluated in terms of estimated daily intake (EDI) and health quotient (HQ) (Table.3).

Acute risk assessment

For short-term risk assessment, ESTI values for highest conc. of selected pesticide residues were much less than the ARFD values hence aHi less than 1 except for chlorpyrifos. The hazard index of brinjal and cauliflower contaminated with chlorpyrifos were 1.78, 21 for males and 1.98, 23 for females respectively. Cauliflower samples contaminated with λ-cyhalothrin also showed aHi of 18 for males and 20 for females (Table.3-a).

Chronic risk assessment for individual pesticide

In the long-term risk assessment, the health risk quotient (HQs) for all selected pesticides was negligible except chlorpyrifos. HI, for chlorpyrifos was 1.87 for males and 2.07 for females for brinjal while 15 for males and 16 for females for cauliflower. Cyhalothrin-λ present in cauliflower samples also showed risk in terms of HI of 4.6 for males and 5.1 for females (Table.3-b). The cumulative risk (cHI) for the tested pesticides was 0.41 and 0.85% lower than unity.

4 Discussion

Pesticide residues analysis involves sensitive instrumental techniques and proper skills in handling the whole experimental procedure. A small negligence can give rise to gross errors in the results. Therefore, it should be demonstrated that the method being used is fit for the intended purpose.

In the present study, EU-citrate buffered QuEChERS method has been validated for the extraction of selected organochlorine, organophosphate, and synthetic pyrethroids in brinjal and cauliflower. The method showed linearity \(\left( R^2 > 0.99 \right) \) in the working range \(\left( 1 \times \text{MRL}, 2 \times \text{MRL, and } 4 \times \text{MRL} \right) \) and the residual concentration was within the limit of ± 20 percent as per SANTE Guidelines (2019). The method accuracy was within the acceptable limits. The sensitivity of the method was sufficient enough to be used for routine pesticide residue monitoring studies. The matrix effect was in the range of -11.4 to 54.7 percent indicating sufficient cleaning efficiency of the procedure. Quality control (QC) checks at the limit of quantification (LOQ) were run with real samples in each sequence.

A total of 25 brinjal samples and 26 cauliflower samples were analyzed among which 22 and 18 samples (88, 69 percent) were contaminated and 20 percent were exceeding MRLs set by EU. Chlorpyrifos was the most frequently detected pesticide found in both brinjal and cauliflower. It was detected in brinjal samples from Dera Ismail Khan, Mansehra, and Mandi-bahauddin and cauliflower samples originated from Swabi, Mansehra, Huzro, Sawat, Haripur, and Multan. Chlorpyrifos is an organophosphorus insecticide classified as WHO category II (Turner, 2018) that is widely used for the treatment of pests (leaf hopper, fruit borer) affecting brinjal and cauliflower production (Omparakash, 2014). The results were consistent with other studies reporting chlorpyrifos in brinjal samples from district Gujranwala (Iqbal et al., 2009, Latif, et al., 2011). It has been recognized as endocrine disruptor chemicals (UNEP, 2016). Due to the perilous effect of chlorpyrifos on human health and the environment, the European Union legislated to ban the use of chlorpyrifos in 2020 (EFSA, 2020).
Another interesting finding of the study is that endosulfan has not been detected in any sample but the presence of one of its isomer endosulfan sulfate in 24 percent brinjal and 3.8% of cauliflower samples indicates its translocation/uptake from drift or previous applications from the soil. These samples were supplied in the Islamabad market from Mandi bahauddin, Bhakkar, Gorakhpur, Faisalabad, Sargodha, Khushab, and Narowal. Endosulfan is an organochlorine compound and its use has recently been prohibited in Pakistan as it has been enlisted as Persistent Organic Pollutants (POPs) under Stockholm Convention on POPs in 2017. Since most of the organochlorine pesticides (OCPs) are banned and organophosphate (OPs) are highly toxic and hazardous compounds hence pyrethroids are among the most extensively used pesticides in the world for vegetables. Among the four pyrethroids viz. bifenthrin, cypermethrin, \( \lambda \)-cyhalothrin, and deltamethrin were investigated, only the residues of \( \lambda \)-cyhalothrin were detected in both brinjal and cauliflower samples. \( \lambda \)-cyhalothrin is recommended and widely used for the treatment of bugs and fruit borer which affects brinjal production severely in Pakistan (Ali, 2018). The contaminated samples were originated from Multan, Faisalabad, Mandi bahauddin, Layyah, Bhakkar, Mardan, Sahiwal, Attock, Sargodha, DI khan, and Mansehra. The residues of different PYs in vegetables have also been reported in Pakistan and other countries like Bangladesh (Amjad et al., 2019; Hossain et al., 2013, Ali et al., 2019). In the present study although the concentration of \( \lambda \)-cyhalothrin in brinjal samples was below EU-MRL. Its higher detection frequency may be of great concern as dietary intake from other sources might pose serious accumulative implications on human health. Long-term exposure to a low dose of pyrethroid residues could lead to chronic diseases e.g nervous and immune system impairment, cardiovascular, and genetic disorders (Zhao et al., 2014). This compound is more toxic than the majority of the active ingredients approved in the European Union (EU 2017).

The causes of the occurrence of multiple residues in brinjal and cauliflower might be because different compounds are used individually or as a mixture. Another cause might also be spray drift from neighboring plots (Lozowicka, 2015). Endosulfan is a highly persistent pesticide and may travel long distances in the air. The researcher has reported residues of endosulfan in crops approximately 121 miles from the sprayed agricultural site (Bradford et al., 2010). Moreover, this pesticide may persist in soil for several years before degradation. The concentration of pesticide mixture applied to the crop before harvesting above the recommended level may be the reason for the high level of chlorpyrifos in cauliflower samples.

From a consumer health perspective, it is important to compare exposure estimates to established toxicological criteria such as EDI, ESTI, which are the realistic estimation of pesticide residues exposure (Lehmann et al., 2017; Li et al., 2018). All the aHIs for selected pesticides were less than unity except chlorpyrifos, which meant there was a negligible short-term or acute risk except chlorpyrifos. Chlorpyrifos presented an HQ close/exceeding the unity. Chlorpyrifos may induce hematological malignancies, behavioral and developmental anomalies, oxidative stress, genotoxicity, and immunotoxicity (ur Rahman et al., 2021). Ventura et al., (2016) reported chlorpyrifos is one of risk factor for breast cancer. It proliferate the human breast cancer cells by enhancing progesterone receptor (PgR), decreased serum the progesterone, estradiol, and luteinizing hormone levels (Ventura et al., 2016). In study conducted on Thai pesticide sprayer, Kongtip, et al., 2021 concluded its acute exposures adversely affect the hypothalamic–pituitary–thyroid (HPT) axis, which result in disturbance of human thyroid hormone levels (Kongtip, et al., 2021). Other pesticides (\( \lambda \)-cyhalothrin, endosulfan sulfate) were found in concentrations yielding smaller HQ values. From the public health point of view, the observed levels of pesticide residues do not pose a serious health risk to the consumers, but the presence of pesticide residues in food items even at the lowest possible level should be a matter of concern as the low level does not remain low forever.

The difference in potential health risks in gender-based two sub-population may be to the fact that everyone does not necessarily consume both vegetables each day and in similar proportions. For this reason, a deterministic approach or average portions might underestimate or overestimate an individual's dietary intake. To overcome this shortage, the assumption was made that only 68 percent of the Pakistan population consuming these vegetables. This assumption is supported by the fact that the global diet is generally poor and monotonous (Savy et al., 2003). In absence of extreme intake values, these average estimates yielded no acute or chronic risk. A food frequency questionnaire (FFQ) could be used in further studies to validate the estimation of propensity-to-consume items and refined the presented assessment in the future.

Exposure to pesticide mixtures in cumulative risk assessment (HI) was associated with a larger intake of pesticides with the same mode of action thus resulting in a higher hazard for the consumer. As chlorpyrifos and \( \lambda \)-cyhalothrin do not share the same mode of action thus they are not considered in the same cumulative assessment group (CAG). Under these conditions, \( \sum \) HIs which correspond to the sum of HIs from the same CAG will not yield a value significantly different from the HIs of these pesticides. Joint use of several formulation/trademarks alone or in combination with a single vegetable is the probable reason for multiple pesticide detections. Moreover, illiteracy, lack of awareness, and poor labeling quality (foreign language, etc.) and counterfeiting could have led to misinterpretations.

**Conclusion**

The pesticide is a two-edged sword. It is used to control pests and diseases but if not used as per Good Agriculture Practices (GAP) they can cause pesticide residue issues and human health implications. Vegetables supplied to the residents of Rawalpindi/Islamabad contain EDPs residues about regulatory limits. The health quotient (HQ) for Androgen antagonist chlorpyrifos was exceeding unity indicating potential human health risk. However, residues of other compounds do not pose any serious health issues. It was observed that these health risk indices associated with dietary exposure to pesticide residues in brinjal and cauliflower might be underestimated due to the scarcity of secondary data on food consumption and the empirical form of the model. Furthermore, pesticide residues in food items even at a safe level should be a matter of concern as low levels do not remain low forever.

**Recommendation:**

The developed method can confidently be used in routine pesticide residue monitoring studies. If the use of pesticides is inevitable, then the principles of Good Agriculture Practices (GAP) should be observed.
Declarations

Ethics approval and consent to participate:
This article does not contain any studies with human participants or animals.

Consent for publication:
Not applicable

Conflicts of Interest:
The authors declare no conflict of interest.

Availability of data and material:
All the data used within this manuscript are available online.

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Conceptualization: Karam Ahad, Method optimization and validation: Farwa Talat, Hina Aslam, Nazia Rafique, Data analysis & interpretation: Karam Ahad, Nazia Rafique, Hina Aslam, Manuscript writing: Karam Ahad, Nazia Rafique, Farwa Talat. Funding acquisition: Karam Ahad

References

17. FDA-Import Alerts. [Cited 2020/ 05/11], Available from: https://www.accessdata.fda.gov/cms_ja/industry_25.html.


### Tables

#### Table 1

Method performance characteristics for determination of pesticide residues in brinjal and cauliflower

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Typical Method Accuracy (%)</th>
<th>Matrix Effect (%)</th>
<th>LOD*(mg/kg)</th>
<th>LOQ**(mg/kg)</th>
<th>LCL*** (mg/kg)</th>
<th>EU-MRLs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brinjal</td>
<td>Cauliflower</td>
<td>Brinjal</td>
<td>Cauliflower</td>
<td>Brinjal</td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Lindane</td>
<td>96 ± 14.9</td>
<td>87.2 ± 18</td>
<td>-11.4</td>
<td>0.006</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>101.7 ± 13.4</td>
<td>90.4 ± 10.8</td>
<td>-0.6</td>
<td>0.008</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>82.3 ± 20.7</td>
<td>86 ± 20.8</td>
<td>-19.8</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Heptachlor endoepoxide</td>
<td>89.6 ± 19.2</td>
<td>84.3 ± 19.2</td>
<td>-14.7</td>
<td>0.007</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Heptachlor exoepoxide</td>
<td>91.7 ± 13.2</td>
<td>76.5 ± 7.2</td>
<td>-19.5</td>
<td>0.003</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Triadimenol</td>
<td>90 ± 19.7</td>
<td>88.7 ± 17.4</td>
<td>-6</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Endosulfan-α</td>
<td>103 ± 17.2</td>
<td>78.4 ± 8.7</td>
<td>-16.2</td>
<td>0.001</td>
<td>0.009</td>
<td>0.05</td>
</tr>
<tr>
<td>Endosulfan-β</td>
<td>77.8 ± 7.4</td>
<td>83.6 ± 13</td>
<td>-20.2</td>
<td>0.005</td>
<td>0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>112.9 ± 20.2</td>
<td>79.9 ± 17.2</td>
<td>-38</td>
<td>0.005</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>113.9 ± 8.6</td>
<td>79.6 ± 11.7</td>
<td>-22.3</td>
<td>0.08</td>
<td>0.05</td>
<td>0.3</td>
</tr>
<tr>
<td>Cyhalothrin-λ</td>
<td>98.7 ± 11</td>
<td>101.6 ± 15.8</td>
<td>54.7</td>
<td>0.04</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Cypermethrin-α</td>
<td>95.8 ± 12</td>
<td>84.4 ± 4.6</td>
<td>-33.1</td>
<td>0.02</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>79.4 ± 9</td>
<td>85.3 ± 7.6</td>
<td>-23.2</td>
<td>0.08</td>
<td>0.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*LOD Lowest calibration, **LOQ Limit of detection, ***LCL Limit of quantification

#### Table 2

Status of pesticide residue and MRLs compliance in cauliflower and Brinjal

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Contaminated samples</th>
<th>Samples above MRL</th>
<th>Range (mg/kg)</th>
<th>Compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brinjal</td>
<td>Cauliflower</td>
<td>Brinjal</td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>5</td>
<td>15</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Heptachlor endoepoxide</td>
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<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
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<tr>
<td>Endosulfan sulphate</td>
<td>6</td>
<td>2</td>
<td>n.d</td>
<td>n.d</td>
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<td>Cyhalothrin-λ</td>
<td>22</td>
<td>2</td>
<td>n.d</td>
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<td>Cypermethrin-α</td>
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<td>n.d</td>
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</tbody>
</table>

#### Table 3-a

Acute health risk associated with consumption of pesticide residues contaminated brinjal and cauliflower
### Table 3-b

Chronic health risk associated with consumption of pesticide residues contaminated brinjal and cauliflower

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Pakiastani male population</th>
<th>Pakiastani female population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARfD (mg/kg)</td>
<td>Male ESTI mg/kg/bw</td>
</tr>
<tr>
<td>Brinjal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.005</td>
<td>8.93E-05</td>
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<tr>
<td>Σ Heptachlor</td>
<td>n.d</td>
<td>8.49E-09</td>
</tr>
<tr>
<td>Σ Endosulfan</td>
<td>0.02</td>
<td>4.65E-06</td>
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<tr>
<td>Cyhalothrin-λ</td>
<td>0.005</td>
<td>2.55E-06</td>
</tr>
<tr>
<td>Cauliflower</td>
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<tr>
<td>Chlorpyrifos</td>
<td>0.005</td>
<td>0.001</td>
</tr>
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<td>Σ Endosulfan</td>
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<tr>
<td>Cyhalothrin-λ</td>
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</tr>
</tbody>
</table>

Figures
Figure 1

Frequency of pesticide residues in cauliflower and brinjal samples

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplentaryInformationBJCFV4NR.docx
- GraphicalAbstract.png