Research Article

Assessment of Pesticide Residues in Peach (*Prunus persica L.*) from Swat and Peshawar, Khyber Pakhtunkhwa, Pakistan

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Abstract | Peaches, similar to many other fruits and vegetables, are treated with different pesticides at various stages of their growth and development, under conventional agricultural practices especially in developing countries. Pesticide residues in fruits and vegetables have achieved considerable attention due to the unempirical utilization of pesticides. Fruits and their products can serve as potential sources of toxic constituents such as pesticide residues, as they are often consumed unprocessed. This study was designed to assess the pesticide contamination level in different varieties of peach collected from the Swat and Peshawar districts of Khyber Pakhtunkhwa, Pakistan. The analytical method was optimized for a total of nine different pesticides (Atrazine, parathion-methyl, chlorpyrifos, captan, α-endosulfan, dieldrin, β-endosulfan, endosulfan sulfate, and α-cypermethrin) of various chemical classes, using Agilent's Intuvo-9000 GC-μECD system. A total of thirteen peach samples, ten from fruit orchards and three from fruit markets, belonging to five different varieties were collected and analysed. 38% of the samples were found to be non-compliant to EU-MRL for chlorpyrifos, 8% for atrazine and parathion-methyl, whereas no non-compliance was observed for FAO/codex maximum residue limits (MRLs). The designed method for the multi-class pesticide residues was standardized for peaches via GC-μECD. Based on the results of the present study, it is the need of time to conduct more research in this field; disseminate results, and develop stringent policies to screen the use of pesticides sustainably for the benefit of the environment and mankind.

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Introduction

Peach (*Prunus persica*) belongs to the 19th largest family of plant Rosaceae, subfamily Amydyloideae (Kant *et al*., 2018). Peaches are nutritionally and economically essential; one of the most popular fruits consumed worldwide (Zhao *et al*., 2015). Worldwide China is the top producer of peach with 15.8 million tons of production annually (FAOSTAT, 2020). Peaches are popular due to their desirable flavor and rich source of polyphenolic compounds, effective for the treatment of different diseases (Vizzotto *et al*., 2014).

Pakistan is ranked 25th in the production of peach worldwide with a total production of 73900 tons annually with an export share of 0.3% (Khan *et al*., 2008). Khyber Pakhtunkhwa contributes about 80% of the country's peach production with a total production of 56,800 tons annually (Agricultural Statistics of Pakistan, 2017-18). The popular peach varieties cultivated in Pakistan are Florida King (6-A), Early Grand, Shah Pasand, Golden Early, Shireen, 7, 8, and 9 numbers (Zeb and Khan, 2008). Pakistan exports peaches to Afghanistan, Kuwait, Hong Kong, Bahrain, Saudi Arabia, the UK, and UAE (Syed *et al*., 2014).

Because of intensification in agricultural activities, the latest technologies, and developments (industrialization), farmers have increased the use of pesticides by reducing the land size (Ecobichon, 2001). Agro-chemicals in various forms played an indispensable role to develop crop yields worldwide for the last few decades. By 2004, agrochemical use in Pakistan is increased by 2,159 times, which leads to a significant rise in imports over the last few years (Khan *et al*., 2010).

Plant protection mechanism products have significant roles to enrich food security by declining crop susceptibility to pathogens and plagues. However, these chemicals may have adverse environmental and health impacts; persistence in the soil makes it unusable for farming, bioaccumulation eradicates living creatures and sources of food, runoff and groundwater intrusion contaminate water, and produce nutrient pollution (US-EPA, 2005). Pesticide residues in fruits and vegetables have achieved considerable attention due to the unempirical utilization of pesticides (Pang *et al*., 2016). Educating the world to understand the agrochemical's hazardous effects will reduce the pesticide residues and ease the human health risk (Chen *et al*., 2011).

This study was designed with the aim of evaluating the multi-class pesticide residue in peach through GC-μECD. The method was applied in the determination of real samples collected from the Swat and Peshawar regions.

Materials and Methods

Samples collection, processing, and preparation

Ten samples (10) were collected from District Swat, while 03 samples were collected from Peshawar, Khyber Pakhtunkhwa, Pakistan, for pesticide residue analysis. The samples were brought to the laboratory and investigated properly, damaged samples were discarded, while the rest of the samples were cut manually in four pieces and the alternate slices were blended using Robot Coupe Blixer 5 plus. The samples were stored in transparent polythene plastic zipper bags, tagged, and kept frozen at -20 °C until further analysis.

Chemicals and reagents

Acetonitrile (HPLC) grade and n-Hexane (extra pure GC) grade were purchased from DaeJung (Korea). Acetone from Merck (Germany), anhydrous magnesium sulphate from Scharlau (Spain), acetic acid and anhydrous sodium acetate from Sigma-Aldrich (Germany), primary-secondary amine (PSA), carbon activated and glass wool from Supelco (USA), and sodium chloride was purchased from Fisher Scientific (USA). Pesticide standards were purchased from Dr. Ehrenstorfer Augsburg, (Germany) and Sigma Aldrich (Germany).

Preparation of standards stock solution

Individual stocks solution of pesticide standard (1000 μg/mL) was prepared in 25 mL volumetric flask (Certified class "A" glass), using n-hexane, and acetone as per best solubility and stability of the concerned pesticide. Working mixture (03 μg/mL) was prepared for spiking while the mixture was further diluted over the range 0.003, 0.006, 0.012, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 μg/mL. The stock solution, along with the working mixture and all dilutions, were prepared in n-hexane and kept at -20°C till further analysis.

Extraction procedures

The chopped, frozen samples were taken out and kept

open to attain room temperature before the extraction procedure. The method used in this study was slightly modified form reported by (Samad *et al.*, 2019) with modification in the clean-up phase to remove the additional colour pigment from the samples i.e. activated carbon was added during solid-phase extraction. For extraction 15±0.01 g of the blended homogenized sample was taken in a 50 mL screw-cap centrifuge tube. 15 mL of acidified acetonitrile (0.1% with acetic acid) was added to the tube and vortex for 02 minutes. 1.5 g sodium acetate (anhydrous), 1.0 g sodium chloride, and 6.0 g of magnesium sulphate (anhydrous) were added into samples followed by vortex (02 minutes) and centrifuged (05 minutes at 4000 rpm). For the clean-up phase, a solid-phase extraction-packed column was prepared by adding a small amount of glass wool followed by adding 300 mg of PSA, 450 mg $MgSO_{4\ (anhydrous)}$, and 200 mg of activated carbon. The supernatant (04 mL) was passed through the SPE (solid phase extraction) column and collected in a round bottom flask; evaporated to dryness through the rotary evaporator (Ika Werke Rv06 ML) and made up to 01 mL volume using n-hexane in a GC vial before instrument analysis.

Analysis of pesticide residues by GC–μECD

The Agilent Technologies Intuvo 9000 GC System was used for analysis, equipped with an auto-sampler (G4523A) and OpenLAB CDC Chem Station (Rev.C.01.08 [210]; online and offline software). The system was optimized by keeping the injector's temperature at 250°C under a constant flow (02 mL/min) of nitrogen gas as the carrier. The nitrogen gas was produced by a nitrogen generator (NG 2081) and for purification, Agilent Technologies gas clean purification system (Hydrocarbon filter- TUV 2400-B-104) was used. The temperature for the detector was kept at 300°C with a make-up flow of 60 mL/min . The column coupled to the μECD was an Agilent HP-5MS UI, temperature limits -60°C to 350°C (30m×0.320mm×0.25μm). The temperature for the oven was programmed as the initial temperature was 70°C (for 1 min); ramped to 150°C at the rate of 50°C/min, followed by 06°C/min rise until 200°C (for 5 min) which was further raised to 280°C with a rate 16°C/min resulting in a total run time of 20.933 min. The injection volume was kept at 01 μL, in splitless mode.

Method validation for peach analysis

The method accuracy, precision, and validation were evaluated to analyse the analyte of interest in peach samples. The experimental analyses were carried out in triplicates. The limit of quantification (LOQ) was kept to the lowest validated level (10 times of LOD), to calculate with recovery (70-120%), acceptable accuracy, and precision. For the said purpose, the guidelines were taken from (SANTE/12682/2019). The matrix-matched standards were prepared, to understand the behavioural changes in the concerned analytes.

Results and Discussion

Validation of analytical method

The evaluation and validation of the analytical method were performed for a representative group of nine pesticides belonging to different chemical classes. Pesticide selection was not just based on those which are frequently applied in the fruit orchards but also on the ones previously reported and having a long application history in the study area (Table 1). The analytical method used in this study was slightly modified form of the one previously validated by Samad *et al.* (2019), the inter-laboratory reproducibility, as well as revalidation of the method, was performed and results are presented in Table 2.

Linearity and calibration range

The method's validation parameters (Table 2) were linearity, the limit of detection (LOD), the limit of quantification (LOQ), seven-point calibration/measurement range, percentage recoveries (i.e. intra and inter-day repeatability), and percentage matrix effects following the European Commission's guidelines (SANTE/12682/2019).

Limit of detection (LOD) and limit of quantitation (LOQ)

The analytical method's limits of detection and quantification (Table 2) were either lower or equal to the EU-MRL values for almost all other compounds except atrazine (Triazine) which has a higher LOD and consequently LOQ values both in solvent and MMS (matrix-matched standards) as it gives a lower response on GC-μECD. Although the LOD, as well as LOQ values for all the compounds, were considerably lower than those of the FAO/codex MRL values (Table 2).

Recovery and matrix effect

The method's accuracy in terms of percentage recoveries (70-120%) and precision in terms of relative standard deviation (RSD: ±20%) of the extraction

Table 1: *Details of the pesticide compounds; i.e. chemical and general class, EU and FAO/Codex MRLs (mg/kg) and n-octanol/water partition coefficients.*

S#	Compound name	Chemical class	General class	EU MRL (mg/kg)	FAO/Codex MRL (mg/kg)	log K_{ow} at ≤ 25°C, (pH 5-7)
1	Atrazine	Triazine	Herbicide	0.05	n.a	2.5
2	Parathion-methyl	Organophosphorus	Insecticide	0.01	0.3	3
3	Chlorpyrifos	Organophosphorus	Insecticide	0.01	0.5	$5.0*10^4$
4	Captan	N-trihalomethylthio	Fungicide	6	20	610
5	α-Endosulfan	Chlorinated cyclodiene	Insecticide, acaricide	0.05	n.a	4.74
6	Dieldrin	Organochlorine	Insecticide, acaricide	0.01	n.a	5.4
7	β-Endosulfan	Chlorinated cyclodiene	Insecticide, acaricide	0.05	n.a	4.79
8	Endosulfan sulfate	Organochlorine	Insecticide, acaricide	0.05	n.a	3.66
9	α-Cypermethrin	Pyrethroid	Insecticide	2	n.a	$0.87*10^7$

Table 2: *Analytical method's validation parameters for the pesticide compounds.*

S#	Compound name	GC-μECD RT (min)	R^2		LOD (mg/kg)		LOQ (mg/kg)		Matrix effect (%)	Calibration range (mg/kg)
			Solvent	Matrix	Solvent	Matrix	Solvent	Matrix		
1	Atrazine	9.113	0.9957	0.9775	0.0500	0.0500	0.1327	0.2654	-23	0.0500 - 0.7380
2	Parathion-methyl	11.036	0.9982	0.9638	0.0050	0.0070	0.0099	0.0197	-1	0.0030 - 0.3000
3	Chlorpyrifos	12.249	1.0000	0.9605	0.0030	0.0050	0.0050	0.0090	25	0.0030 - 1.2300
4	Captan	13.064	1.0000	0.9638	0.0050	0.0050	0.0090	0.0180	7	0.0050 - 0.6576
5	α-Endosulfan	13.579	0.9997	0.9628	0.0030	0.0050	0.0085	0.0171	15	0.0030 - 0.3000
6	Dieldrin	14.029	1.0000	0.9636	0.0030	0.0050	0.0073	0.0146	1.5	0.0030 - 0.3000
7	β-Endosulfan	14.531	0.9996	0.9822	0.0030	0.0050	0.0086	0.0171	-2	0.0030 - 0.3000
8	Endosulfan sulfate	15.118	0.9993	0.9759	0.0030	0.0050	0.0111	0.0223	0.6	0.0030 - 0.3000
9	α-Cypermethrin	18.369	0.9995	0.9968	0.0050	0.0050	0.0103	0.0207	15	0.0030 - 0.3000

RT: retention time (in minutes), R^2: correlation coefficient, LOD: limit of detection, LOQ: limit of quantification.

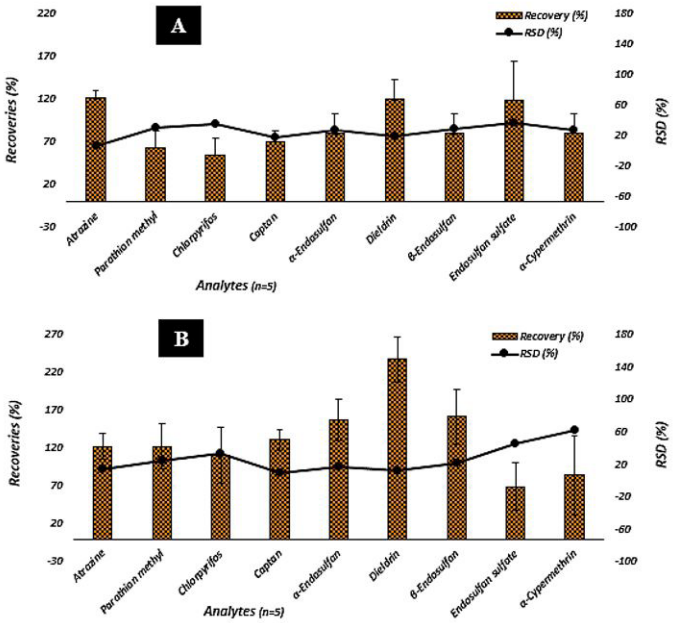

Figure 1: *Intra-day (A) and inter-day (B) repeatability for the pesticides in the peach matrix (n=5).*

method was evaluated at a spiking concentration ranging from 0.27-0.7 mg/L. The intra-day and inter-day repeatability studies were performed for the pesticides in the peach matrix, every batch comprised of five spiked samples, one matrix blank, and one reagent blank. The recoveries were within the range for all other analytes but were slightly higher for α-endosulfan, β-endosulfan, and dieldrin, whereas, the RSD was higher for chlorpyrifos, endosulfan sulfate, and α-cypermethrin, in the inter-day repeatability studies (shown in Figure 1). The matrix effect (±20%) was within the range for almost all the studied compounds with the highest (i.e. 25%) for chlorpyrifos and lowest (-23%) for atrazine (Table 2).

Real samples analysis

The validated method was applied for the determination of the pesticide residues in 13 peach samples collected from peach orchards, District Swat, and fruit markets, Peshawar city, Khyber Pakhtunkhwa. The samples were comprised of different varieties such as; golden (31%), early grand (23%), NJC-84 (15%), Maria Delizia (15%), NJC (08%), and Indian blood (08%). All the varieties were locally grown. The early grand is cultivated and distributed more throughout

Table 3: *Real samples results (n=13).*

S#	Compound name	Contaminated samples (%)	Detection range (mg/kg)	EU MRL Non-compliance	FAO/Codex MRL non-compliance
1	Atrazine	8	0.0839	1	n.a
2	Parathion-methyl	8	0.0179	1	0
3	Chlorpyrifos	38	0.0113 - 0.1247	5	0
4	Captan	8	0.0144	0	n.a
5	α-Endosulfan	31	0.0064 - 0.0275	0	n.a
6	Dieldrin	8	0.0063	0	n.a
7	β-Endosulfan	8	0.0083	0	n.a
8	Endosulfan sulfate	15	0.0060 - 0.0080	0	n.a
9	α-Cypermethrin	31	0.0115 - 0.3783	0	n.a

the country. The results showed maximum contamination (35%) in the samples belonging to the early grand variety, followed by golden (30%), NJC-85 (15%), NJC-84, and Maria Delizia (10% each). Indian blood (08%) was found to be free of pesticide contamination. Based on the mode of action of pesticides, the insecticides/acaricides are applied more in the peach orchards as compared to other types of pesticides, whereas, the detection rate of organophosphorus pesticides (OPs) was higher followed by organochlorines (OCs) and pyrethroid groups (Figure 2).

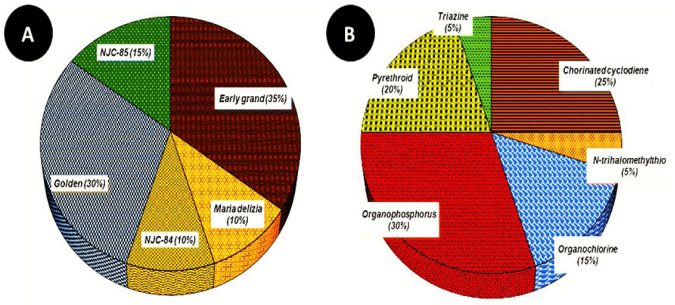

Figure 2: *Contamination trend: (A) among the analysed varieties. (B) based on the chemical classes of the studies compounds.*

The results (Table 3) showed maximum contamination of 38% (5 samples) for chlorpyrifos, which is very commonly used on fruits and vegetables for the control of flies, aphids, leaf miners, and other insect species, 31% (4 samples) for α-cypermethrin and α-endosulfan each, 15% (2 samples) with endosulfan sulfate, and 08% (only 1 sample) was found contaminated with captan (fungicide), atrazine (herbicide), and parathion-methyl (insecticide). Traces of dieldrin and β-endosulfan were also found in one sample. The compounds investigated are mostly used on peach orchards in many parts of the world as recommended crop protection products. Researchers from different parts of the world have reported residues of the above-mentioned pesticides in peach samples (Zioris *et al.*, 2009). Zioris *et al.* (2009) reported 44.2% contamination of the analysed peach samples with chlorpyrifos at an average concentration of 0.036 mg/kg and 22.1% contamination with captan at an average concentration of 0.09 mg/kg, in Northern Greece. Traces of endosulfan (0.004 mg/kg) and cypermethrin (0.031 mg/kg) were also reported in peach samples in Nawabshah, Sindh, Pakistan (Anwar *et al.*, 2011). Chlorpyrifos with 0.924 mg/kg and cypermethrin with 1.63mg/kg was also reported in peach samples in Bahawalpur, Punjab, Pakistan (Ata *et al.*, 2013). Peach samples analysed in Valencia (Spain) were also found contaminated with chlorpyrifos with an average concentration of 0.09 mg/kg (Berrada *et al.*, 2010). In the present study, 38.5% (i.e. 05 samples) were found to be non-compliance to EU-MRLs for chlorpyrifos and 8% (01 sample each) non-compliance was observed for atrazine and parathion-methyl, whereas no non-compliance was observed to FAO/codex MRLs.

Conclusions and Recommendations

The designed method for the multi-class pesticide residues was standardized for peaches via GC-μECD. The results of the present research revealed the presence of different pesticides including atrazine, parathion-methyl, chlorpyrifos, captan, α-endosulfan, dieldrin, β-endosulfan, endosulfan sulfate, and α-cypermethrin in real samples. These agrochemicals were applied at different stages of harvesting peaches. The proper mechanism must be followed in order to bring these residues concentrations within the limits of MRLs and minimize the hazardous effects on human health. For quality control, it is the need

of time to conduct more research in this field; disseminate results, and develop stringent policies to screen the use of pesticides sustainably for the benefit of the environment and mankind.

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Novelty Statement

Fewer work had been done on multi-pesticide residues analysis in different varieties of peaches.

Author's Contribution

All authors contributed to this study, authors 1 and 2 contributed equally.
Farida Anjum and Bilal Jan: Conceptualization.
Farida Anjum, Bilal Jan and Aasma Bibi: Methodology.
Farida Anjum, Bilal Jan, Syed Roohul Hussain, Abdul Ahad and Azeem ud Deen: Formal analysis.
Bilal Jan and Farida Anjum: Writing original draft preparation.
Farida Anjum, Bilal Jan and Aasma Bibi: Writing review and editing.
Farida Anjum and Zia Ullah: Resources
Farida Anjum, Zia Ullah and Farrakh Mehboob: Supervision.

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All the co-authors/participants of this article have no objection to the correspondence and are willing to publish the article in the Pakistan Journal of Agriculture Research.

Conflict of interest
The authors have declared no conflict of interest.

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