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DETECTION OF PESTICIDE RESIDUES IN THREE MAJOR MARKETED FRUITS IN PUNJAB, PAKISTAN

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Three major fruits, mango, guava and citrus, collected from the consumer markets of five cities viz. Bahawalpur, Multan, Faisalabad, Rawalpindi and Lahore of Punjab province were analyzed for pesticides residues. A total of 1200 fruit samples were collected consisting 400 samples of each fruit analyzed. Concentration of eight pesticides were quantified using QuECHERS sample preparation followed by analysis with gas chromatography-mass spectrometry (GC-MS) in fruit samples. Results revealed that more than 65% samples were contaminated with pesticide residues with violation rate of 38.28%. Among the total fruit samples analyzed, 66.25% mango samples were contaminated with more than 25% sample above MRL. Guava followed with 65 and 23.75% contamination and violation rate while citrus exhibited 64% sample contamination with more than 25% violation rate. Cypermethrin was determined in maximum (45%) samples while more than 24% samples were recorded above MRL with cypermethrin. This study provided important information on the current contamination levels of some commonly consumed fruits and pointed out an urgent need to mitigate some potentially persistent pesticides with excessive use.

Keywords: Food chain, fruits shipment, pest management, pesticide residues, residual limit.

INTRODUCTION

Despite enormous fruit production, export of fruits from Pakistan is greatly hammered by the confiscation of the shipments from European countries. Rejection of the shipments is attributed to food additives, microbiological contaminants, veterinary drug residues, heavy metals, foreign bodies, pesticide residues and allergens (Ahmad, 2009; Dawn, 2012).

Fruits are highly perishable commodity, so a variety of pesticides in large quantity has been applied on fruits that result in contamination of these fruits with toxic pesticide residues. Imidacloprid, acetameprid, cyhalothrin, thiamethoxam, chlorpyrifos and buprofezine are the key pesticides used for the management of these pests in fruits (Sarwar, 2006; Tariq *et al*., 2007; Batool *et al*., 2007). Mostly pyrethroid, organophosphate, neonicotinoid and carbamate pesticides are applied against these pests (Ashraf *et al*., 2014). Various chemicals such as chlorpyrifos, lambda cyhalothrin, profenophos, deltamethrin, bifenthrin (Gulzar *et al.,* 2015), neonicotinoids and carbamates are recommended for the management of these pests (Aslam *et al*., 2004). Pesticide residues are among the top few reasons for the rejection of food products from Europe and other developed countries (Ahmed *et al*., 2010).

A considerable percentage of applied pesticides do not reach the target rather enter the ecosystem and affect the non-target biota including human being (Ali *et al*., 2014). More than 25 million individuals are exposed to poisoning each year only in developing countries and most of them are farm workers (Jeyaratnam, 1990). Pesticides have been associated with various chronic health effects (Mahboob *et al*., 2011) such as mutagenicity, carcinogenicity (Sarabia *et al*., 2009), cytotoxicity (Wagner *et al*., 2005; Giordano *et al*., 2007), teratogenicity (Kang *et al*., 2004), genotoxicity (Cakir and Sarikaya, 2005), imunotoxicity (Yeh *et al*., 2005) and hormones disruption (Vos *et al*., 2000) in human and other organisms.

Contamination of the food products especially fruits and vegetables, is a major current issue worldwide especially in the developing countries such as Pakistan. Despite the severity of the residue problem in Pakistan, just a few individual regional studies have been conducted (Latif *et al*., 2011). Considerable proportion of the population is not familiar with the problem of pesticide residues and accumulation of residues in the food chain due of lack of knowledge. This study focused on five most populated cities of Punjab, Pakistan and three fruits with maximum production and consumption in the province.

MATERIALS AND METHODS

Simple and direct approach was applied for the quantification of pesticide residues in fruits samples at all steps from collection of samples, preparation and analytical analysis.

Sample: Samples of three fruits were collected from fruit markets of five cities viz. Bahawalpur, Multan, Faisalabad, Rawalpindi and Lahore of Punjab, Pakistan for the determination of pesticide residues. A total of 10 samples of each fruit were collected from 8 shops/retailers from 5 markets of a city making 400 samples of each fruit and 1200 total samples. Fruit samples of citrus, guava and mango were transported to pesticide residue and IPM laboratory, department of entomology, University of Agriculture, Faisalabad following the procedure of Cook (2002). Each fruit sample was weighted as much as 1000g and stored in 4°C until subjected to homogenization, only edible parts of the fruits were subjected to residual analysis (Chowdhury *et al*., 2013). Samples were analyzed in Central High Tech. laboratory, University of Agriculture, Faisalabad.

***Chemicals and reagents*:** All solvents and reagents were dissolved as required for the sample extraction protocol and preparation of mobile phases. Anhydrous magnesium sulphate ($MgSO_4$), Acetonitrile (MeCN), primary secondary amines (PSA), anhydrous sodium acetate (NaAc) and insecticide reference standards were purchased from SIGMA-ALDRICH Pvt. Ltd®. Pesticides, currently in use or being recommended by the authorities for the management of fruit pests lambda cyhalothrin, cypermethrin, indoxacarb, imidacloprid, pyriproxifin, acetameprid, buprofezine, chlorpyrifos (Qin *et al*., 2015) were targeted for detection and quantification in fruits. The purity of all pesticide standards and other chemicals were not less than 98%.

***Extraction and clean up*:** In total, 1 g of each sample (edible pulp and peel mixed) was weighed by electric balance and was added with 4 ml of acetonitrile. Sample was placed in homogenization vial after addition of acetonitrile and homogenization was performed at 5634×g for 20 seconds followed by 90 seconds of cool down time. Two such cycles were performed for homogenization. Additionally 6 ml of ACN was added after getting the vial out of homogenizer and sample was placed in 15 ml vial/tube.

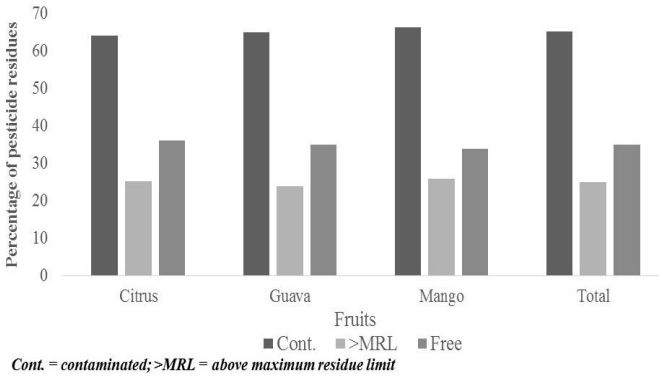
For extraction and clean up, Agilent technologies method of QUeCHERS (AOAC) Zhao (2007) was followed according to which 100 µl of internal standard was added in accordance with the target pesticides followed by an addition of 6 g of $MgSO_4$ and 1.05 g NaOac in the homogenized sample in the 15 ml vial and shaking it up with hand or vortex for a minute to mix both the solid and liquid ingredients completely. The vial with mixture was centrifuged at 4695×g for 5 minutes. About 1.05 ml of supernate was taken and placed in the vial containing 2 ml of dispersive SPE (primary and secondary amides and $MgSO_4$). The mixture was shaken by hand and centrifuged for 5 minutes at 10285×g. A supernatant appeared after shaking was taken from the vial without any solid particles in it and poured into a lid vial which was left in centrifuge for overnight. After the sample was dried overnight, 100 µl acetonitrile was added and vortexed for re-suspension. Sample was placed in centrifuge for 1 minute to separate any possible solids and was then transferred into LC vials for analysis (Anastassiades *et al*., 2003; Martı´nez-del-Rı´o *et al*., 2013; Rejczak and Tuzimski, 2015).

Extraction and clean-up was performed using the QuECHERS AOAC method and kits were purchased from Agilent technologies with the part numbers for citrus (5982-5755+5982-5058), guava (5982-5755+5982-5058) and mango (5982-5755+5982-5058) where the first number is the part number for extraction kit while the latter is kit number for clean-up kit.

***GC analysis*:** The samples were analyzed using GC-MS with the parameters as: Injector Temperature: was 220°C, Column used was 25 methyl silicon, I.D. 0.53 mm, 2.0 µm film thickness, Injection Volume was 1 µl split less, GC Detector was Electron Capture Detector (ECD- Ni 63). Temperature was 300°C, Carrier gas N2 (30-32 ml/ml), Oven Temperature: 60°C (0.5 min), flow rate 30°C/ min to 180°C (0 min), 4°C/min to 80°C and Injection method: Solvent flush technique (Auto-sample injection).

RESULTS

Quantification of pesticide residues showed 65.08% samples were contaminated, while 38.28% among the contaminated samples showed residues above the safe limits as specified by Codex Alimentarius Commission or the European Union (Fig. 1). Considering the fruits individually for pesticide residues, mango was found with the highest number 265 of contaminated samples (66.25%) with 103 (25.75%) exceeded maximum residue limits. Guava fruit was ranked 2nd in this study based on the number of contaminated samples 260 (65%) with different pesticide residues while the 95 (23.75%) among those contaminated samples exceeded residual limits. About 256 (64%) samples of citrus were found contaminated with variety of pesticide residues while 101 (25.25%) of were above safe residual limits (Fig. 1).

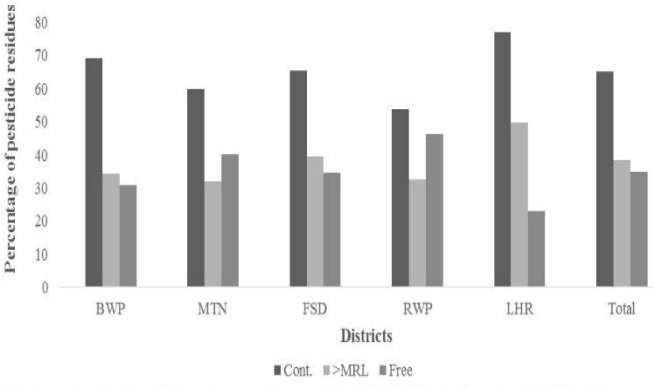


Cont. = contaminated; >MRL = above maximum residue limit

Figure 1. Percentage of pesticide residues in each fruit.

The analysis of contaminated samples on the basis of cities revealed that about 77.08% fruit samples collected from Lahore were contaminated among which 49.72% were above

MRL. Proportion of contaminated samples and violation rate from Faisalabad was 65.41 and 39.49%. Similarly 60 and 31.94% from Multan markets. A total of 32.55% were above MRL among 53.75% contaminated samples collected from Rawalpindi, while proportion from Bahawalpur was 69.16 and 34.33%, respectively (Fig. 2). The proportion of the sample contaminated with cypermethrin was 45% with violation rate of 24.16% followed by imidacloprid with 44.76% contaminated and 25% samples above MRL. Similarly chlorpyrifos was quantified in 44.58% samples with violation rate of 22.91% followed by acetameprid (43.75 and 22.91%), lambda cyhalothrin (43.75 and 23.33%). Buprofezin determined in 37.08% with violation rate of 4.16% followed by pyriproxifin (33.33 and 8.33%) and indoxacarb (31.25 and 0.41%) contaminated and violation rate, respectively.



Cont. = contaminated; >MRL = above maximum residue limit; BWP = Bahawalpur; MTN = Multan; FSD = Faisalabad; RWP = Rawalpindi; LHR = Lahore

Figure 2. Percentage of pesticide residues in each district.

In terms of pesticides residue concentrations quantified in citrus, cypermethrin was quantified with highest concentration (2.5987 mg kg^{-1}) followed by chlorpyrifos (1.7058 mg kg^{-1}), imidacloprid (1.7055 mg kg^{-1}). Concentration of acetameprid was 0.8245 mg kg^{-1} followed by lambda cyhalothrin (0.5238 mg kg^{-1}), pyriproxyfin (0.7129 mg kg^{-1}), buprofezin (0.0632 mg kg^{-1}) and indoxacarb (0.0625 mg kg^{-1}) (Table 1). Cypermethrin was quantified with highest residue concentration (0.8416 mg kg^{-1}) followed by imidacloprid (0.8038 mg kg^{-1}), pyriproxifin (0.6247 mg kg^{-1}), lambda cyhalothrin (0.3122 mg kg^{-1}). Concentration of buprofezin was 0.2141 mg kg^{-1} followed by chlorpyrifos (0.0837 mg kg^{-1}), acetameprid (0.0810 mg kg^{-1}) and indoxacarb (0.0141 mg kg^{-1}) in all the guava samples analyzed (Table 2). Similarly pesticide residue concentration determined in mango, imidacloprid was quantified with highest concentration (0.7972 mg kg^{-1}) followed by buprofezin (0.5931 mg kg^{-1}), pyriproxifin (0.0982 mg kg^{-1}). Concentration of cypermethrin quantified was 0.0896 mg kg^{-1} followed by chlorpyrifos (0.0818 mg kg^{-1}), acetameprid (0.0721 mg kg^{-1}), lambda cyhalothrin (0.0606 mg kg^{-1}) and indoxacarb (0.0197 mg kg^{-1}) (Table 3).

Method of laboratory fortified blanks (LFB) was used to calculate LOD and LOQ. LOD calculated for the samples from Bahawalpur ranged from 0.0013 to 0.0089 mg kg^{-1}, 0.0018 to 0.0076 mg kg^{-1} for Multan. LOD for Faisalabad ranged 0.0016 to 0.0085 mg kg^{-1}, 0.0011-0.0088 mg kg^{-1} for Rawalpindi and 0.0003 to 0.0089 mg kg^{-1} for Lahore. LOQ value for pesticide residues in fruit samples collected from Bahawalpur ranged from 0.0015 to 0.0096 mg kg^{-1} and 0.0026 to 0.0087 mg kg^{-1} from Multan. LOQ for the samples from Faisalabad ranged from 0.0027 to 0.0091 mg kg^{-1}, 0.0031-0.0091 mg kg^{-1} for Rawalpindi and 0.0011 to 0.0092 mg kg^{-1} for Lahore. Recoveries for the samples ranged from 89.44±0.54 to 96.67±0.27 ng ml^{-1}at three concentration levels (0.05, 0.1 and 0.5 ng ml^{-1}).

Table 1. Pesticide residue concentrations quantified in citrus samples collected from different cities.

District	Pesticides (Min.-Max.) mg kg^{-1}								R^2
	Lambda Cyhalothrin	Cypermethrin	Indoxacarb	Imidacloprid	Pyriproxyfin	Acetameprid	Buprofezin	Chlorpyrifos	
Bahawalpur	0.0162-0.4612	0.0282-1.8253	0.0063-0.0098	0.0271-1.5761	0.0239-0.7129	0.0053-0.5207	0.0043-0.0341	0.0412-1.3230	0.9948
Multan	0.0258-0.4285	0.0035-2.5453	0.0033-0.0120	0.0051-1.2442	0.0095-0.0997	0.0072-0.8245	0.0053-0.0632	0.0845-1.6214	0.9785
Faisalabad	0.0051-0.2732	0.0029-1.5251	0.0128-0.0625	0.0075-1.4287	0.0143-0.0323	0.0160-0.5598	0.0043-0.0341	0.0464-1.7058	0.9619
Rawalpindi	0.0546-0.4613	0.0205-2.5987	0.0042-0.0093	0.0057-1.6171	0.0307-0.0776	0.0259-0.8101	0.0052-0.0358	0.0528-1.4207	0.9957
Lahore	0.0038-0.5238	0.0069-1.8292	0.0063-0.0170	0.0035-1.7055	0.0078-0.0857	0.0824-0.6335	0.0046-0.0423	0.0052-1.1192	0.9981
MRLs	0.20	1.00	0.02	1.00	0.50	0.50	0.01	1.00	

Table 2. Pesticide residue concentrations quantified in guava samples collected from different cities.

District	Pesticides (Min.-Max.) mg kg^{-1}								R^2
	Lambda Cyhalothrin	Cypermethrin	Indoxacarb	Imidacloprid	Pyriproxyfin	Acetameprid	Buprofezin	Chlorpyrifos	
Bahawalpur	0.0105-0.2750	0.0284-0.8239	0.0038-0.0141	0.0490-0.7098	0.0517-0.5837	0.0059-0.0723	0.0139-0.2141	0.0074-0.0837	0.9921
Multan	0.0099-0.3080	0.0057-0.7625	0.0035-0.0082	0.0428-0.8038	0.0084-0.6247	0.0073-0.0538	0.0056-0.1920	0.0055-0.0584	0.9832
Faisalabad	0.0104-0.3122	0.0546-0.7922	0.0033-0.0138	0.0042-0.6218	0.0534-0.6206	0.0053-0.0810	0.0410-0.1593	0.0068-0.0660	0.9584
Rawalpindi	0.0184-0.2733	0.0223-0.8324	0.0060-0.0091	0.0233-0.5165	0.0741-0.5430	0.0063-0.0733	0.0151-0.1216	0.0053-0.0625	0.9358
Lahore	0.0538-0.3208	0.0495-0.8416	0.0048-0.0098	0.0228-0.5240	0.0637-0.5825	0.0070-0.0526	0.0550-0.1614	0.0045-0.0603	0.9975
MRLs	0.02	0.05	0.02	0.05	0.10	0.01	0.30	0.05	

Table 3. Pesticide residue concentrations quantified in mango samples collected from different cities.

District	Pesticides (Min.-Max.) mg kg^{-1}								R^2
	Lambda Cyhalothrin	Cypermethrin	Indoxacarb	Imidacloprid	Pyriproxyfin	Acetameprid	Buprofezin	Chlorpyrifos	
Bahawalpur	0.0077-0.0473	0.0250-0.0896	0.0062-0.0164	0.0063-0.7901	0.0055-0.0672	0.0043-0.0386	0.0413-0.3463	0.0052-0.0702	0.9859
Multan	0.0042-0.0530	0.0248-0.0832	0.0038-0.0088	0.0084-0.6895	0.0061-0.0440	0.0041-0.0562	0.0063-0.5931	0.0081-0.0694	0.9684
Faisalabad	0.0069-0.0540	0.0287-0.0808	0.0078-0.0197	0.0075-0.6077	0.0149-0.0608	0.0057-0.0470	0.0057-0.0537	0.0023-0.0757	0.9895
Rawalpindi	0.0035-0.0606	0.0397-0.0827	0.0086-0.0169	0.0042-0.7106	0.0166-0.0560	0.0035-0.0721	0.0054-0.0971	0.0139-0.0759	0.9781
Lahore	0.0052-0.0606	0.0290-0.0705	0.0066-0.0178	0.0070-0.7972	0.0185-0.0982	0.0052-0.0399	0.0034-0.0551	0.0061-0.0818	0.9932
MRLs	0.20	0.70	0.02	0.20	0.50	0.50	0.01	1.00	

DISCUSSION

Several attempts have been made for the determination of pesticide residues in different food commodities in China (Nakata *et al*., 2002; Chen *et al*., 2011), Bangladesh (Chowdhury *et al*., 2013; Hossain *et al*., 2013), Africa (Darko and Akoto, 2008; Farag *et al*., 2011), South America (Hjorth *et al*., 2011), Turkey (Bakirci *et al*., 2014), Europe (Fantke *et al*., 2012; Szpyrka *et al*., 2015), South Asia (Ali *et al*., 2014), India (Abhilash and Singh, 2009; Sharma *et al*., 2010) and Pakistan (Parveen *et al*., 2011; Eqani *et al*., 2012; Akhtar *et al*., 2014).

Overall results revealed that more than 65% samples were contaminated with violation rate of 38.28%. These contamination levels are in accordance with Parveen *et al*. (2011) where 40% samples violated the limits. Similar results were reported by Masud and Hassan (1995) where more than 40% samples were found contaminated with pesticide residues. In another study pesticide residues were detected in mango fruits from Multan with more than 60% sample contamination and violation rate of more than 25% (Hassan *et al*., 2002). Difference in the results may be attributed to the target pesticides which were relatively degradable new chemistry insecticides in present study with shorter shelf life. Comparatively different results were reported by Tahir *et al*. (2009) but the target of their study was apple and citrus and sampling area was different markets of Lahore. A total of 40% samples were found contaminated with residues and a considerable percentage of the samples were found violating the maximum residue limits for each fruit. Unlikely, the results of present research exhibited that percentage of the contaminated samples was 68.5%. The findings of Zia *et al*. (2009), who reported 60% fruit samples contaminated with cypermethrin, are also partially in agreement with the findings of current study.

In a study guava and citrus showed 100% contamination but no violation was reported (Anwar *et al*., 2011) these results are partially different from the results of current study. Present study exhibited 65% and 64% contamination in guava and citrus, respectively. Differences in the results may attributed to the different environmental factors acting on the fate of pesticides in the sampling area. Samples were collected from Nawabshah district of Sindh in case of Anwar, *et al*., (2011) while in present study it was different cities of Punjab. Fate of the pesticides in the environment is dependent on different factors which are dissolution (water solubility), heat degradation, metabolic/microbial activity, photo degradation and volatilization (Amvrazi, 2011). Results of a study conducted in some parts of the lower parts of Punjab (Lodhran, Bahawalpur) and Nawabshah (Sindh) showed more than 60% contamination of the samples by Anwar (2008). Samples fruits analyzed in present study were same as in the study of Anwar (2008) but the sampling area was different. These variations depends on the target food item(s), especially when a single fruit or sample is analyzed by two different individuals. Chemicals and instruments used for the process may also cause variations even in the same lot of samples (Fantke and Juraske, 2013). In such monitoring studies, the variability highly dependent on sources of the sample such as either collected from a city where samples usually come from different ecological zones or from the farm (Syed *et al*., 2014). Quantification of different concentration levels of pesticide residues highly depends on different factors. Most important of these factors are lack of awareness in the farmers (literacy rates) and timing which include post-harvest interval (PHI) which determines the duration of the environmental forces to act on the chemicals for the degradation (Cengiz *et al*., 2006).

Comparing the results with other studies, present study exhibited that level of contamination in fruits is higher (65%) in comparison to India where contamination was 40% (Charan *et al*., 2010). A study from Saudi Arabia reported more than 55% contamination (Osman *et al*., 2010) in contrast to the present study, results may vary on the target fruits and sample source. Similar contamination levels (51.30% samples) from Bangladesh with different rates of violations (38.89% samples) were reported (Chowdhury *et al*., 2013) for fruits different from present study. A total of 15% samples violated the safe limits from Poland (Stachniuk *et al*., 2017) in comparison to 38.28% in present study. Therefore, injudicious use with high dose rates by the fruit growers due to lack of education may be one of the reasons of residue contamination in large number of samples (Zhou and Jin, 2009).

***Conclusion*:** Pesticide residues were determined for three

fruits from Bahawalpur, Multan, Faisalabad, Rawalpindi and Lahore. Levels of pesticide residue concentrations quantified for different pesticides are alarming in fruits so is the violation rate posing a great threat to human health. Organophosphates and pyrethroid were quantified in maximum number of samples, so the use of these insecticides must be checked. A more comprehensive monitoring study should be conducted in Pakistan for all food commodities to assess the situation.

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