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DETERMINATION OF PESTICIDE RESIDUES FROM GRAPES PROCURED FROM DIFFERENT MARKETS USING THROUGH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Abstract

Food safety has a substantial aspect of food production for quality assurance as well as public health concern. Farmers using pesticides to fulfil public needs with limited resources and lands. In this limelight, as the pesticides have numeral benefits but cause severe health hazards. Grape samples collected from the different markets of ten cities of Punjab Province and subjected to HPLC for pesticide residues analysis with maximum residual limit (MRLs) assessment. The results showed that grape samples from Faisalabad, Multan and Sahiwal were contaminated with the residues of Chlorothalonil. Samples procured from Lahore and Hafizabad were sprayed with acetamiprid. Meanwhile, Lufenuron residues were determined from grape samples secured from markets of Okara, Lahore and Hafizabad. Samples collected from Okara, Multan and Sahiwal were found contaminated with residues of indoxacarb. Besides, residues of Beta-endosulfan were analyzed from grape samples of Faisalabad and Multan. Almost all samples were contaminated with pesticides residues and pesticides were not applied wisely as suggested by maximum residual limits (MRLs) and good agricultural practices (GAPs). Farmers are not much aware of the threats of pesticide residues on human health due to lack of education and extension work.

Key words: Grapes; Pesticide residue; HPLC; MRLs; Food safety; GAPs.

Introduction

Grapes (*Vitis vinifera*) are berry fruit and widely cultivated in the open and northern areas of Pakistan. Grapes are enriched in taste used as fresh fruit, and processed forms are seed extracts, jelly, jams, wine and resins. Grapes play a vital role in maintaining good health by averting various physiological illnesses like cancer, nausea, cholera, coronary heart disease, hypertension, liver disease, smallpox and constipation (Dohadwala & Vita, 2009; Mobeen *et al.*, 2021). Grapes have been grown all over the world due to their superior taste and health promising benefits along with handsome economic profit for the growers (This *et al*., 2006; Walayat *et al.*, 2021). Globally, in 2016 the total area covered by grapes cultivation was approximately 7.5 million hectares (Anon., 2017). Grapes have noteworthy importance among other fruits owing to their nutritious properties and feature to become the part of formulation in preparation of novel foodstuffs. All around the world, grapes production is approximately 74 million tons. Europe is adding his share of 41%, Asia 29% and America 21% respectively. Furthermore, around 45% of grapes used in the wine industry for the preparation of wines and 55% are preferred by people to consume whole. Grape vineyards have threats from diseases like downy mildew, powdery mildew and grey mold that impart their role in damaging the crops. So, pesticides used as an important source to control them (Flamini, 2003). Most destructive insects that attack grape yards are grape cane girdler, vine mealybug, grape berry moth and babesia botrana farmers used various pesticides to control them, but lack of education and awareness becomes the cause of extreme application which exceed their residual limit (Bakirci & Hişil, 2012).

Farmer's use of various pesticides is in attendance to improve their productivity and ultimately more profit from grapes per hectare (Sufyan *et al.*, 2021; Tariq *et al.*, 2021). Inappropriate application of pesticides like weedicides, fungicides, herbicides and insecticide can execute serious health outbreaks for the consumer (Noor *et al.*, 2021a, b). Contrary, the application of different pesticide left lethal effects on fruits and vegetables, eventually, threatening human health along with environmental hazards (Antle & Pingali, 1994). World population is increasing gradually it is noted that it has upsurges five times more in Pakistan in the last fifty years. Its approximately reach 32.5 million to 150.5 million from the day of freedom to 2004 with a 2% growth rate per annum. In tremendous increase of population in Pakistan, it is now assuming that it will spread to approximately 190 million (Azam & Shafique, 2017). However, now it becomes an alarming situation to

produce to such extend which will meet the horrible conditions. Farmers have limited resources, less productive lands and old strategies to fulfil these gap farmers are spraying pesticides extensively and applying other chemicals for more production from agriculture produce lands (Council, 1993).

Pakistan is one of the acquainted countries in the world which is best known for its agriculture production. Pakistan has infinite space among the wheat, cotton, sugarcane and maize cultivators among other countries due to its superiority production. Agricultural figures of Pakistan last few years showed that pesticides were broadly used to control various diseases of crops. Pesticides amplified from 580 million tons in 2000-2001 to 797 million tons in 2015-2016 million (Azam & Shafique, 2017). However, pesticides are sprayed for multiple benefits European Union also suggests their limits the methods for the determination of pesticide residue analysis with conventional ways are being advanced and improved (Tariq *et al.*, 2007). There are some major steps to ensure the detection are; extraction along purification of pesticide residues. High performance liquid chromatography is an advanced, reliable and cost-effective technique to determine the multiple pesticide residues from grapes (Debbab *et al.*, 2014).

Material and Methods

Samples collection and preparation for pesticide residues: Grapes were randomly collected from various cities of Punjab province.

Collection of samples: Samples for analysis were collected from various selected cities (Faisalabad, Okara, Lahore, Multan, Hafizabad, Sahiwal, Chiniot, Nankhana, Sheikupura and Pakpattan) and brought to Food Safety laboratory, National Institute of Food Science and Technology, University of Agriculture, Faisalabad in sealed polyethene bags. Afterwards, samples were stored.at -40^{o}C for further analyses.

Analysis of pesticides residues: Pesticides were analyzed by using HPLC UV-Visible (Perkin Elmer) detector method illustrated by (Jodeh *et al.*,) with slight modifications.

Extraction of residues: Pesticide residues extracted from the grapes samples by blended in a Blender so that homogenous slurry/paste is formed. Ethyl acetate was used as a solvent because of its efficient recovery. According to this method, 50 g of homogenized sample was taken in 250 mL Erlenmeyer flask. 20 g anhydrous sodium sulphate (HPLC grade) was added and mixed in homogenized grapes sample in a flask to prevent the clod formation. 10 mL saturated sodium chloride solution was added in the mixture. 75 mL ethyl acetate (HPLC grade) was added in the sample. The glass beads were added in the mixture to facilitate the extraction process. The mixture in the flask was shaken at a speed of 240 rpm on a horizontal mechanical shaker for the time of 1 h. The extract was collected in an inert plastic bottle. The sample extract was filtered using Whatman (No.4) filter paper. The filtered extract was stored at -40^{o}C before further analysis.

Purification of filtered extract: The glass wool was used to support the column, and that was located at the bottom of the column. The silica gel and charcoal were activated at 200^{o}C for 24 h before the filing of the column. The activated charcoal and silica gel were mixed at a ratio of 7:5 (w/w). A thin layer of anhydrous sodium sulphate was placed on glass wool. The activated mixture (12 g) of silica gel and charcoal was placed on sodium sulphate layer. The activated mixture was covered with a thin layer of anhydrous sodium sulphate and glass wool, respectively. The washing of the prepared column containing the adsorbents with acetone (HPLC grade) was done just before using the column. The flow rate through the column was also adjusted at the rate of 1 ml per minute before loading the target sample.

After column preparation, loading of sample extract was done, and the extract was eluted using 50 mL of acetone and hexane mixture (3:7 v/v). The cleaned-up elute received in 150 round bottom flasks. Elute was then concentrated in a rotary evaporator at 40°C up to 1-1.5 mL. The concentrated elute transferred to small vials of volume 1.5 by using glass suckers for this purpose. Elute in the vial was placed under a gentle stream of nitrogen until elute had completely dried.

Preparation of the mobile phase: The mobile phase of high-performance liquid chromatography was prepared with the amount of 80:20 (v/v) acetonitrile and water.

Filtration and Sonication of the mobile phase: The mobile phase of acetonitrile and water was placed to vacuum hood by filtering with filter paper with a pore size of 2 µm. After the mobile filtration phase was taken into the flask and sonicated for 10 min at 30^{o}C to remove the air bubbles.

Statistical Analysis

Significance of all parameters was analysed through by succeeding the principle and strategies of Montgomery (2008).

Results and Discussion

Outcomes of research showed that there were different pesticide residues like chlorothalonil, acetamiprid, lufenuron, indoxacarb and beta-endosulfan screened from the samples secured from different cities Faisalabad, Okara, Lahore, Multan, Hafizabad, Sahiwal, Chiniot, Nankhana, Sheikupura and Pakpattan of Punjab province.

Chlorothalonil: Chlorothalonil residues were detected from grape samples of Faisalabad S_1=0.964, S_2=1.684 and S_3=0.258 mg kg-1, Multan S_1=1.202, S_2=0.986 and S_3=1.654 mg kg^{-1}, Sahiwal S_1=1.754, S_2=1.431 and S_3=0.436 mg kg^{-1}, Nankana S_1=0.342, S_2=1.781 and S_3=0.594 mg kg^{-1} and samples from Pakpattan contains S_1=1.452, S_2=0.088 and S_3=2.023 mg kg^{-1}, respectively that shown in Fig. 1. Chlorothalonil residues were not detected from the samples of Okara, Lahore, Hafizabad, Chiniot and Sheikhupura. Chlorothalonil maximum residual limit is 3 mg kg^{-1} for grapes, and all the

contaminated sample of chlorothalonil did not show any residues which were above from its maximum residual limit (MRL) as per recommended by FAO. Hou *et al*., (2016) reported that the residues of chlorothalonil in cabbage. The authors demonstrated that chlorothalonil was present in cabbage at the detection rate of 0.05 mg kg^{-1}, which clearly showed results were below the maximum residual limit of pesticides conferring to European Union rules. Jongen *et al*., (1991) investigating that the chlorothalonil residues in carnation culture with high Performance liquid chromatography column C18, mobile phase methanol and water with ratio of 60:40. Although, Samples were extracted and cleaned with a foresail run through HPLC. Results were obtained with in a range of 0.5 µg L^{-1} at 325nm.

Acetamiprid: Maximum residual limit of acetamiprid is 0.5 mg kg^{-1} for grapes. Acetamiprid residues were detected from the samples of Lahore S_1=0.257, S_2=0.097 and S_3=0.362, Hafizabad S_1=0.481, S_2=0.515 and S_3=0.085 mg kg^{-1}, Sheikhupura S_1=0.096, S_2=0.155 and S_3=0.276 and Pakpattan S_1=0.278, S_2=1.324 and S_3=0.052 that shown in Fig. 2. Meanwhile, acetamiprid residues were not detected from the Faisalabad, Okara, Multan, Sahiwal, Chiniot and Nankana. All the contaminated samples of acetamiprid residues did not show any limit of residue which exceeded its maximum residual limit. Martinez *et al*., (2002) evaluated the presence of acetamiprid residues in vegetables. Results disclosed that the substantial quantity of residues were found in the vegetables. Residues of acetamiprid were detected by using the HPLC with mobile phase of (80:20) acetonitrile and water, detection was made at 325 nm wavelength and the results that were obtained 0.1µg mL^{-1} and it was below the range of maximum residual level.

Lufenuron: Maximum residual limit (MRLs) of lufenuron pesticides is 1 mg kg^{-1} and its residues were detected from the samples secured from cities Okara S_1=0.736, S_2=0.138 and S_3=0.571 mg kg^{-1}, Lahore S_1=0.378 and S_3=0.167 mg kg^{-1}, Hafizabad S_1=0.569, S_2=0.632 and S_3=0.493 mg kg^{-1}, Chiniot S_1=1.536, S_2=0.842 and S_3=0.235 mg kg^{-1} and Sheikhupura S_1=0.836, S_2=0.318 and S_3=0.019 mg kg^{-1} in Fig. 3. Residues of lufenuron were not screened from the samples of Faisalabad, Multan, Sahiwal, Nankana and S_2 sample of Lahore. There was only one sample S_1=1.536 mg kg^{-1} from Chiniot city showed the residues above to its maximum residual limit. Likas and Tsiropoulos (2011) screened that the residues of lufenuron in grapes and wine making process through using high pressure liquid chromatography (HPLC) with UV detector. Lufenuron was sprayed on grapes in vineyard with different intervals of the day. After 42 days of treatment pesticide showed an important decline came in the rate of pesticide residues with a reduction of 0.011 mg kg^{-1} each day. Though, pre-harvest contamination did not incline from 0.27 mg kg^{-1}. When the grapes were managed to wine, there was no lufenuron residue observed.

Indoxacarb: Although, indoxacarb was another pesticide have a maximum residual limit of 2 mg kg^{-1} and its residues were perceived from the samples of Okara S_1=1.375, S_2=0.965, S_3=1.246 mg/kg, Multan S_1=1.748, S_2=1.059 and S_3=1.285 mg kg^{-1}, Sahiwal S_1=0.953, S_2=1.581 and S_3=1.83 mg kg^{-1} and Sheikhupura S_1=1.496, S_2=0.698 and S_3=1.294 mg kg^{-1} are presented in Fig. 4. Samples from Faisalabad, Lahore, Hafizabad, Chiniot, Nankana and Pakpattan did not show any residues of indoxacarb residues. All of the samples showed the amount of indoxacarb residues, but they were not above their maximum residual limit. Pujeri *et al*., 2016 conducted a study on brinjal and tomato to determine the indoxacarb residues in tomato and brinjal. All analyses were carried on high performance liquid chromatography, result exhibited that one of tomato samples contained 0.015 mg kg^{-1} of indoxacarb residues. On the other hand, Brinjal samples did not showed any amount indoxacarb residues. The level of indoxacarb remaining in eggplant and its dissipation rate was determined through high performance liquid chromatography. The outcomes showed that early screening of indoxacarb residues on eggplant was approximately 2.599 to 2.629 mg kg^{-1} with two different treatments. After 15^{th} to 20^{th} days, the residues of indoxacarb were detected to below limit less than 0.019 mg kg^{-1}.

Beta-endosulfan: Beta-endosulfan have residual limit of 0.05 mg kg^{-1} and its residues were identified from the samples of Faisalabad S_1=0.013, S_2=0.039 and S_3=0.016 mg kg^{-1}, Multan S_1=0.072, S_2=0.0009, S_3=0.055, Chiniot S_1=0.018, S_2=0.021 and S_3=0.034 mg kg^{-1}, Nankana S_1=0.065, S_2=0.015 and S_3=0.041 and Pakpattan S_1=0.511, S_2=0.038 and S_3=0.267 mg kg^{-1} S_1 and S_3 samples were highly above from its maximum residual limit of 0.05 that shown in Fig. 5. Remaining samples of Okara, Lahore, Sahiwal, Hafizabad and Sheikhupura did not reveal any amount of beta-endosulfan residues. There were various samples from different cities showed the amount of beta-endosulfan residues which were above to their maximum residual limit like samples from Multan S_1=0.022 and S_3=0.005 mg kg^{-1} NankhanaS_1=0.015 mg kg^{-1} and Pakpattan showed the amount above the limit was S_1=0.46 mg kg^{-1} and S_3=0.21 mg kg^{-1} respectively. Paranthaman *et al*., (2012) worked on endosulfan that was sprayed by farmers on banana. Pesticide residues of the banana sample were extracted and detected by using high performance liquid chromatography. The results did not show any amount of endosulfan residues above its maximum residual limit. Another Research was carried by Anwar *et al*., (2011) on endosulfan residues in different fruits like orange, apple, grapes, peach and banana. Analysis showed that the amount of endosulfan residues in apple and peach was 0.774 and 0.004 mg/kg. Meanwhile, Banana, grapes and orange did not showed peak of endosulfan residues Hadjmohammadi *et al*., (2013) worked on high performance liquid chromatography to screen the endosulfan residues from rice. The results show that the amount of endosulfan residues in rice was 2.269 ppm and the recovery rate was 71.70%. Residues of endosulfan were also screened from the rice, which was purchased from market and the level of residues in that sample was approximately 0.04 mg kg^{-1} (This *et al*., 2006).

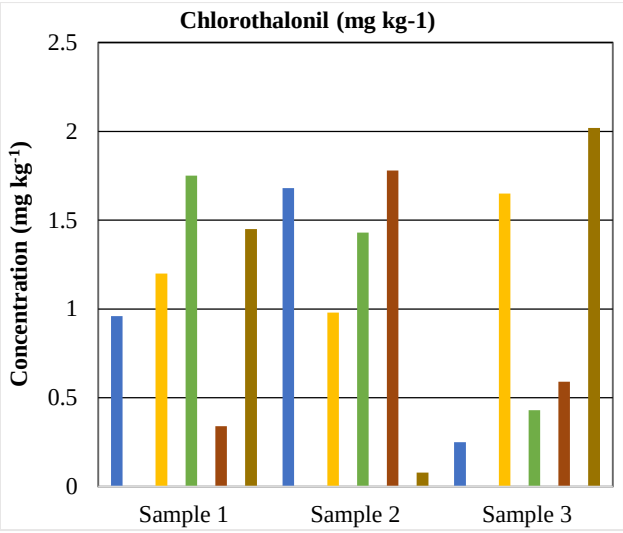

Fig. 1. Concentration of chlorothalonil mg kg^{-1} in grapes samples of different cities.

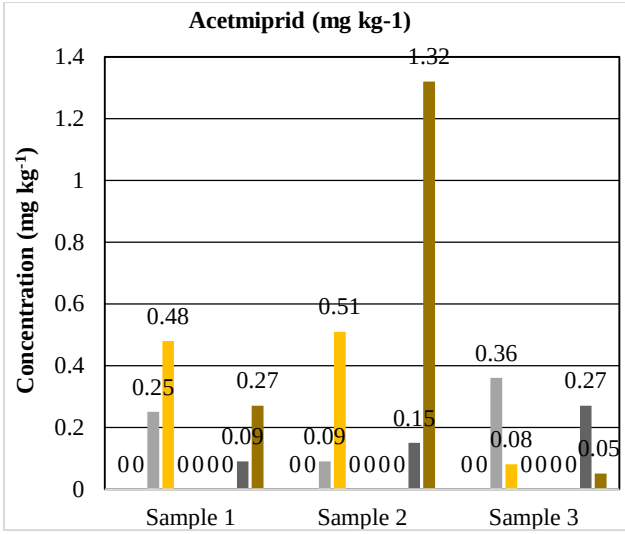

Fig. 2. Concentration of acetamiprid mg kg^{-1}in grape samples of different cities.

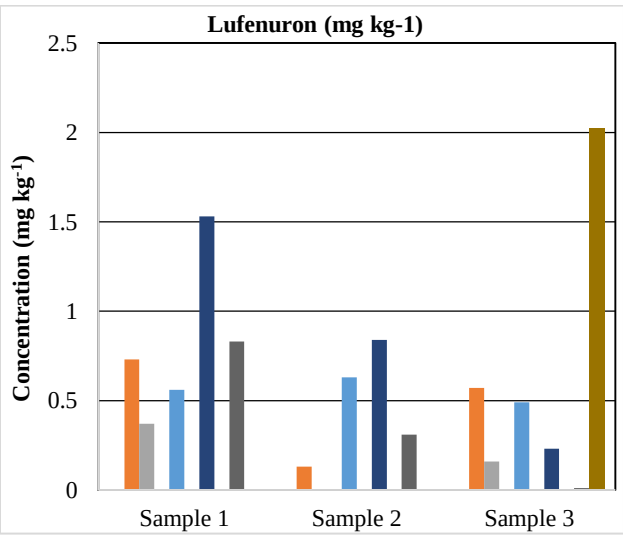

Fig. 3. Concentration of lufenuron mg kg^{-1} in grape samples of different cities.

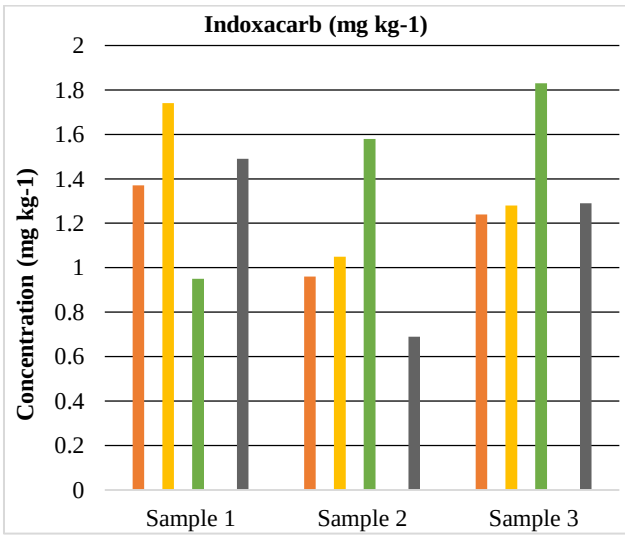

Fig. 4. Concentration of indoxacarb mg kg^{-1} in grape samples of different cities.

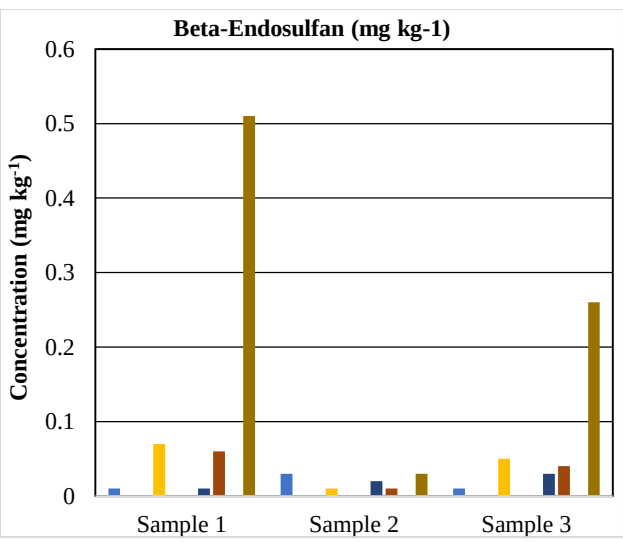

Fig. 5. Concentration of beta-endosulfan mg kg^{-1}in grape samples of different cities.

Conclusion

Contamination of different pesticide residues are found on samples of grapes with not recommended doses, and they are not sprayed thoughtfully according to good agriculture practices (GAPs). These issues are being raised due to lack of extension work and unawareness about the hazards of pesticides, causing a serious threat to human beings, animals and the environment. Nevertheless, such improvements need to produce and implement in developing countries like Pakistan so that consumer have a safer side to protect them from these serious threats. The human creature is a community of excellent resource. It can be achieved by individual and communal efforts to make their resource and environment healthy. Now developed nations are working on social, biological and environmental sciences to improve the lifestyle of their citizens and even protecting their surroundings. The basic aim of this research was to find out the possible solution that eliminates this factor from

our community, and it can only be possible by educating and creating awareness among farmers. They can give their nations pesticide-free food to make them more protective and healthier.

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