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MULTIPLE PESTICIDE RESIDUES;
IN SPINACH AND ASSOCIATED HEALTH RISK

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Novelty Statement: In past, no work has been done regarding the pesticide residues in spinach targeting peri-urban area of district Faisalabad. Keeping in view the persistent nature and cumulative behavior as well potential toxic effects of pesticides as a result of consumption of vegetable this is planned.

ABSTRACT… Background: Food safety is a major public concern worldwide. This is serious problem in the whole world especially in economically poor countries like Pakistan. The production of safe food is an important aspect of food quality assurance as well as human health. The people are demanding fresh, hygienic and quality food. **Objective:** To study the level of pesticide residues in spinach grown in peri-urban area of district Faisalabad and compare the level of contaminants respective their maximum residues limits (MRLS). **Materials and Methods:** Ten farmers were selected randomly for spinach in the peri-urban area of district Faisalabad and vegetable samples were collected at optimum maturity in triplicate and transported to the laboratory of National Institute of Food Science and Technology, University of Agriculture, Faisalabad for further processing. The residues of pesticide were determined by GC-ECD. **Results:** The results regarding pesticide residues in different spinach samples showed variations and residues varied as deltamethrin 0.024 to 0.143 mg kg^{-1}, permethrin 0.159 to 0.573 mg kg^{-1}, cypermethrin 0.098 to 0.503 mg kg^{-1}, profenofos 0.016 to 0.425 mg kg^{-1}, chlorpyrifos 0.009 to 0.212 mg kg^{-1}, bifenthrin 0.012 to 0.623 mg kg^{-1}, endosulfan 0.043 to 0.103 mg kg^{-1}, dimethoate 0.077 to 0.515 mg kg^{-1}, respectively. The chlorpyriphos has no residues in any sample of spinach. **Conclusion:** The spinach samples are found to be contaminated with a number of different pesticides and these pesticides are not applied judiciously according to good agricultural practices and recommended doses. The farmers are unaware of pesticide use and other agricultural practices due to lack of extension as result causes of serious problems to all community.

Key words: Spinach, Pesticide residues, health problems

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INTRODUCTION

Agriculture is backbone of Pakistan's economy and contributes greatly towards GDP in Pakistan. Up to 70 % of our rural population is directly or indirectly engaged in this sector. In Pakistan only 2 percent of the total cultivated area is under vegetable cultivation and it contributes about 0.22% in total world vegetable overall export. The per capita daily intake of vegetables is 134g, which is 33-35 % below the minimum recommended level of 200g per day.[1] The vegetables are a valuable source of minerals, vitamins, fibers and contribute significantly to human health. Vegetables also have some functional constituents such as protein, vitamins and minerals which have health promoting role. Consumer's demand for good quality and safe vegetables is increasing. Cellulose, hemi-cellulose and pectin are the main structural components in vegetables that impart firmness and give texture.[2]

In Pakistan for better production and aesthetic value of vegetables, farmers are being used a huge amount of pesticides during the whole duration of growth of vegetables, even at ripening stage and sometimes farmers also ignored the recommended period between the harvest and last spray. Another injudicious practice associated to pesticide usage, pesticides become the inner part of vegetable and ultimately in the form of

residues creating health hazards when consumed by the consumers. Vegetables are normally used in the form of raw or semi-processed appearance, from that it is assumed they hold elevated amount of pesticides residue in comparison to other food plant origin groups like bread and foodstuffs bases on cereal processing.[3]

Pesticides are the chemicals used by the farmers to protect their crops from the pest attack. Pesticides used in agriculture include insecticides, fungicides, nematicides and herbicides. In vegetables and fruit production, insecticides are used to control the insects and fungicides to control the diseases. These are directly applied to the crops and some pesticides may be present as residues on/in the vegetables and fruits after harvest. The vegetables grown by sewage water contain toxic amounts of heavy metals posing a potential danger to mankind.

The risks of pesticide residues in relation to human exposure are the defects in the central nervous system, haemangiomas, or facial clefts, and urogenital defects, circulatory/respiratory, gastrointestinal and musculoskeletal pathologies. The illiteracy in farmers and the indiscriminate use of insecticides pose a potential threat to local inhabitants, indigenous agro-ecosystem and to export of food commodities. Children have more risk due to chemical are more harmful to developing organs and bodily systems.[4] The illiterate farmers spray vegetables, even after harvesting, usually to force pests inside not to peep out which might reduce the market price. They are often harvested on daily basis and in many cases these are sprayed almost daily. This may create a serious problem to the health of the consumers. To sensate the consumer on the health consequence of high pesticide residue on vegetables, this project will document the pesticide residue level on selected fresh vegetables.

Acute pesticide poisoning result in symptoms like nausea, abdominal cramps, diarrhea, dizziness, anxiety and confusion, which can be quite severe but are often reversible. Chronic pesticide poisoning is associated with respiratory problems, memory disorders, skin conditions, depression, miscarriage, birth defects, cancer and neurological conditions such as Parkinson disease. Fetuses, infants, growing children, pregnant and nursing mothers and women childbearing age are most at risk for adverse outcomes from exposure to pesticides.[5]

As several insect pests attack the vegetables, hence they are produced under very high input pressure for better yield and quality; insecticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage. These pesticides are absorbed by vegetables and upon consumption in minute amount may cause deleterious problems for human health as well as animals and birds.[6]

Keeping in view the persistent nature and cumulative behavior as well potential toxic effects of pesticide residues as a result of consumption of spinach, the present study has been designed to determine the residues of pesticide in spinach to explore the Maximum residues limits and their health effects on human health.

MATERIAL AND METHODS

Selection of farmers

Ten farmers were selected randomly for spinach in the peri-urban area of district Faisalabad.

Collection of samples

The vegetable samples were collected at optimum maturity stage in triplicate from the peri-urban areas of district Faisalabad. The samples of spinach were procured from the selected farmers in three replicates and were kept in polyethylene air tight bags and transported to the laboratory of National Institute of Food Science and Technology, University of Agriculture, Faisalabad for further processing.

SAMPLES PREPARATION FOR PESTICIDE RESIDUES

Extraction of residues

The residues of different pesticides were

extracted from the homogenized sample using the method of Kadenezki et al., (1992)[7] with some modifications reported by Khan et al., (2009).[8] The vegetable samples were chopped into small pieces on clean chopping boards with stainless steel knife to prevent any other contamination. One kg of the chopped sample was blended in a Blender so that homogenous slurry/paste is formed. Ethyl acetate was used as 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 vegetable sample in 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 in order to facilitate the extraction process. The mixture in flask was shaken at a speed of 240 rpm on a horizontal mechanical shaker for time period of 1 hr. The extract was collected in inert plastic bottle. The sample extract was filtered using What man (No.4) filter paper. The filtered extract was stored at -40oC before further analysis.

Clean-up of filtered extract

The pesticide residues analysis requires high sensitivity as these are present in traces. Therefore, to get high sensitivity, cleanup operation was carried out so that interfering substances in the extract could be removed and precise measurement of residues could be done. For this reason, residues of different pesticides were cleaned up using column chromatographic technique as reported by Kumari, (2008).[9]

The glass wool was used to support the column and that was located at the bottom of column. The silica gel and charcoal were activated at 200oC for 24 hours before the filling of column. The activated charcoal and silica gel were mixed at 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 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 was received in 150 ml round bottom flask. Elute was then concentrated in rotary evaporator at 40°C up to 1-1.5 ml. The concentrated elute was transferred to small vials of volume 1.5 ml by using glass suckers for this purpose. The elute in the vial was placed under gentle stream of nitrogen until elute had completely dried.

ANALYSIS OF PESTICIDE RESIDUES

Determination of different pesticide residues were done by using GC-ECD analysis as illustrated by Chandra et al., (2010).[10]

RESULTS

The statistical results for all pesticide residues regarding spinach collected from district Faisalabad has been shown in Table-I. The results indicated that the pesticide residues differed significantly among different samples obtained from different farmers. The results pertaining to mean values of all pesticide residues detected in spinach are revealed in Table-II. The results pertaining pesticide residues in different spinach samples showed variations and residues varied as deltamethrin 0.024 to 0.143 mg kg^{-1}, permethrin 0.159 to 0.573 mg kg^{-1}, cypermethrin 0.098 to 0.503 mg kg^{-1}, profenofos 0.016 to 0.425 mg kg^{-1}, chlorpyrifos 0.009 to 0.212 mg kg^{-1}, bifenthrin 0.012 to 0.623 mg kg^{-1}, endosulfan 0.043 to 0.103 mg kg^{-1}, dimethoate 0.077 to 0.515 mg kg^{-1}, respectively. The chlorpyriphos has no residues in any sample of spinach. It is also evident that among spinach samples, three samples were found exceeding their MRLs i.e 0.1 mg kg^{-1} showing that 30% of the spinach samples tested had deltamethrin residue level

above MRLs proposed by FAO. However, in five spinach samples deltamethrin residues were not detected while two samples contained pesticide residues which fall below the MRLs. The results further showed that out of ten spinach samples, two samples possessed residue limit exceeding their MRLs of 2 mg kg^{-1}. This showed that 20% spinach tested samples possessed residue level above MRLs presented by FAO. On the other hand, seven spinach samples were found to have no permethrin residues and one sample contained pesticide residues falling below the MRLs. Only one sample exceeded their MRLs of 0.5 mg kg^{-1} indicating that 10% spinach sample had residue level above MRLs. However, in six spinach samples, cypermethrin residues could not be detected and three samples possessed pesticide residues which were below the MRLs. The result further showed that out of ten samples of spinach, no sample possessed residue limit exceeding their MRLs of 0.5 mg kg^{-1}. On the other hand, six samples were found to have no profenofos residues and four samples possessed pesticide residues falling below the MRLs. The results further showed that out of ten samples spinach, no sample exceeded their MRLs of 0.5 mg kg^{-1}. On the other hand, six samples were not contaminated with chlorpyriphos residues while four samples were contaminated with pesticide residues which fall below the maximum resides limits. It is also evident that among spinach samples, one sample was found exceeding their MRLs of 0.4 mg kg^{-1}showing that 10% tested samples had residue level above MRLs proposed by FAO. However, in five spinach samples bifenthrin residues were not detected while four samples contained pesticide residues which fall below the MRLs. Only one sample exceeded their MRLs of 0.1 mg kg^{-1} indicating that 10% spinach samples had residue level above MRLs proposed by FAO. On the other hand, six samples were found to have no endosulfan residues while three samples contained pesticide residues which fall below the MRLs. The result further showed that out of ten samples of spinach, three samples possessed dimethoate residue limit exceeding their MRLs of 0.2 mg kg^{-1}. This showed that 30% tested samples possessed residue level above MRLs proposed by FAO. On the other hand, five samples were found to have no dimethoate residues and two samples possessed pesticide residues falling below MRLs.

DISCUSSION

The vegetables are an important part of a healthy diet because they are a vital source of vitamins

	Mean squares								
	Deltamethrin	Permethrin	Cypermethrin	Profenofos	Triazophos	Chlorpyrifos	Bifenthrin	Endosulfan	Dimethoate
Source of variation									
Farmer (9)#	0.01005**	0.152640*	0.075850*	0.078080**	-	0.014490**	0.111780**	0.005640**	0.08341**
Error (29)	0.00004	0.000002	0.000001	0.000004	-	0.000001	0.000002	0.000002	0.00001

Table-I. Analysis of variance for pesticide residues in spinach collected from Faisalabad

*# values within the braces are degrees of freedom; ** = Highly significant (P<0.01)*

Farmer	Deltamethrin	Permethrin	Cypermethrin	Profenofos	Triazophos	Chlorpyrifos	Bifenthrin	Endosulfan	Dimethoate
FS-1	0.123b	ND	ND	0.425a	ND	0.212a	0.623a	ND	0.080d
FS-2	ND	0.573a	ND	ND	ND	ND	ND	0.103a	ND
FS-3	ND	0.508b	0.098d	ND	ND	ND	ND	ND	0.077d
FS-4	0.042d	ND	ND	0.145c	ND	ND	0.033d	ND	ND
FS-5	ND	ND	0.503a	ND	ND	ND	0.012e	0.093b	ND
FS-6	0.024e	ND	0.133c	0.345b	ND	0.088b	ND	ND	0.224b
FS-7	ND	0.159c	ND	ND	ND	0.073c	ND	ND	0.215c
FS-8	0.106c	ND	ND	ND	ND	ND	0.045c	0.043d	ND
FS-9	ND	ND	ND	0.016d	ND	ND	0.073b	ND	ND
FS10	0.143a	ND	0.172b	ND	ND	ND	ND	0.076c	0.515a

Table-II. Mean values for pesticide residues in spinach collected from Faisalabad (mg/kg^{-1})

Means sharing similar letter in a column are statistically non-significant (P>0.05)
FS = Faisalabad spinach (1-10 = shows farmers from spinach samples were collected)
ND = Not detected

and minerals. The quality of these crops may be affected by pest under the natural profile. However, these pests can be controlled by pesticides.[11] The pesticide no doubt increased the agricultural production but persistent residues of these chemicals have tremendous harmful impact on environment and human health. A considerable attention has been focused on the threat to human life from the diet food, drinking water and residential risk caused by the residue of pesticides. The pesticide and their contamination is regulates by through maximum residues limits (MRLs). The MRL is the maximum concentration of pesticides residue resulting from the use of pesticides according to good agricultural practice (GAP). It is the limit that is legally permitted to recognize as acceptable in or on food, agricultural commodities and animal food. In Pakistan there is no practice to fix the MRL with in the country but for the export MRL are used as standard recommended by international agencies like FAO and WHO.

It is clear from the data obtained from present study that the residue of pesticides including deltamethrin, permethrin, cypermethrin, profenofos, triazophos, chlorpyriphos, bifenthrin, endosulfan and dimethoate exceeded their MRLs in spinach samples collected from farmer fields. The pesticide belongs to different groups like organochlorine, organophosphate and pyrethroids, which have different Physiochemical properties, stability and persistence as well as differences in degradation behavior. However, the farmer's community in the especially developing countries is mostly illiterate and unaware of the potential hazards of pesticide residue in food. In Pakistan the subtropical climatic conditions are prevailing as a result proliferation of insects is very high which also require heavy use of pesticides. Therefore, for a better crop yields, insecticides are applied repeatedly during the entire period of growth, even at the stage of fruiting.[6,12] Moreover, sometimes farmers even spray or dip their produce especially vegetables in pesticide solution for enhancing the market value after the harvesting of crop.

The present study also suggested that one of the possible solutions of injudicious pesticide use is to convince the farmers by presenting evidence through cost-benefit analysis of alternative production techniques that high use of pesticide does not assure higher level of benefit and on the other side to create awareness among consumers about the hazardous effect of pesticide residuals in vegetables. The judicious use of chemicals is an approach to agriculture that emphasizes protection of the environment, animal welfare, food quality and health, sustainable use of resources and social justice using the market to help support the goals and compensate the internalization of externalities. The interest in food products with low chemical/non use of chemicals is increasing worldwide, especially in developed in response to concerns about intensive farming practices and their high amount of chemical use and their potential effect on human health and on the environment. However, such awareness needs to generate in developing country like Pakistan so that consumers have better vision about negative impact of pesticide residues in food chain. The findings of the present investigations are very close to previous data of Tahir et al (2001)[13] who found the pesticide residue in cauliflower collected from the Islamabad market. The results regarding pesticide residues detected in cauliflower samples are identically correlated with the findings of Randhawa et al. (2007)[14] who observed the vegetable samples for chlorpyrifos residues collected from the farmer's field around Faisalabad city and found that 84% of the samples contained detectable chlorpyrifos residues. The with respect to pesticide residues in the present study are similar to the earlier studies predicted by Baig et al. (2009)[15] who reported organophosphorus pesticide residues in vegetables grown in southern Punjab.

The data acquired from present study have similar findings suggested by Mukherjee (2003),[16] who reported similar results while studying pesticide residues in vegetables growing around Delhi. He reported 100% contamination rate in cauliflower by endosulfan and residues of methyl-parathion, cypermethrin, malathion, chlorpyrifos,

fevvalerate, chlorothalonil and quinalphos. Moreover, out of seven samples analyzed, the residues of cypermathrin and chlorpyrifos wre above MRL values in one sample each. The results presented in theis study are also consistent with the findings of Masud and Hasan (1992)[17] who illustrated that out of 6 samples of cauliflower, 5 wre contaminated by different pesticides and the residues of lindane, methyl-parathion, thiometon, malathion and methamidophos wre higher than the MRL limits set by FAO.

CONCLUSION

The spinach samples are found to be contaminated with a number of different pesticides and these pesticides are not applied judiciously according to good agricultural practices and recommended doses. The farmers are unaware of pesticide use and other agricultural practices due to lack of extension as result causes of serious problems to all community. However, such awareness needs to generate in developing country like Pakistan so that consumers have better vision about negative impact of pesticide residues in food chain. Human beings are a nation's greatest resource. Individual and collective progress can be achieved only if this resource is healthy, enlightened, and well adjust to the environment. Progressive nations spend large amounts on research in health, education, social and biological sciences to maximize the human potential of their citizens. Most of the nation's problems are rooted in its own culture, context and environment. The mission of present research is to help find indigenous solutions to these problems. This is only possible to aware the farmers and consumers about the negative effects of pesticide residues on human health.

REFERENCES

1. GOP (Government of Pakistan). 2012. Economic Survey. **Economic Advisor Wing, Financial Division, Govt. of Pakistan**, Islamabad, Pakistan 2011-2012.

2. Marwat, S.K., M. A. Khan, M. A. Khan, M. Ahmad, M. Zafar, F. Rehman and S. Sultana. **Vegetables Mentioned in Holy Quran and Aadith and their Ethnomedicinal Studies in Dera Ismail Khan, N.W.F.P., Pakistan.** Pakistan Journal of Nutrition, 2009; 8: 530-538.

3. Tariq, M.I., S. Afzal, I. Hussain and N. Sultana. **Pesticides exposure in Pakistan: A review**. Environment Internarional, 2007; 33:1107-1122.

4. Karam, **Determination of insecticide residues in groundwater of Mardan Division, NWFP, Pakistan: A case study**. Water Research Commission. 2000; Vol. 26, No. 3.

5. Bouchard, M.F. Bellinger, D.C. Wright, R.O. and Weisskopf, M.G. **Attention deficit/ hyperactivity disorder and urinary metabolites of organophosphate pesticides,** Pediatrics, 2010; 1270-7.

6. Kumari B, Madan VK, Singh J, Singh S and Kathpal TS. **Monitoring of pesticidal contamination of farmgate vegetables from Hisar**. Environmental Monitoring and Assessment, 2004; 90: 65-71.

7. Kadenezki, L., Arpad, Z. Gardi, I. Ambrus, A. Gyorfi, L. Reese, G. and Ebing, W. **Column extraction of residues of several pesticides from fruits and vegetables; a simple multi residue analysis method**. Journal of AOAC International, 1992; 75:53-61.

8. Khan, S., S. Rehman, A.Z. Khan, M.A. Khan and M.T. Shah. **Soil and vegetables enrichment with heavy metals from geological sources in Gilgit, northern Pakistan** Journal of Ecotoxicology and Environmental Safety 2010; 73: 1820-1827.

9. Kumari, B. **Effects household processing on reduction of pesticide residues in vegetables**. ARPN Journal Agricultural and Biological Sciences, 2008; 3:46-51.

10. Chandra, S., Mahindrakar, A.N. and Shinde, L.P. **Determination of cypermethrin and chlorpyrifos in vegetables by GC-ECD**. International Journal of Chemtec Research, 2010; 2: 908-911.

11. Dayan, H., Abrajano, T. Sturchio, N.C. and Winsor. L. **Carbon isotopic fractionation during reductive dehalogenation of chlorinated ethenes by metallic iron**. Org Geochem. 1999; 30: 755-763.

12. Juhler, R. K., Larsen, S.B. Meyer, O. Jensen, N.D. Spano, M. Giwercman, A.and Bonde, J.P. **Human semen quality in relation to dietary pesticide exposure and organic diet.** Archive of Environmental Contamination and Toxicolology, 1999; 37: 415-423.

13. Tahir, S., T. Anwar, I. Ahmad, S. Aziz, A. Mohammad and K. Ahad. **Determination of pesticide residues in fruits and vegetables in Islamabad market**. Journal of Environmental Biology, 2001; 22: 71-74.

14. Randhawa, M.A., F.M. Anjum, A. Ahmed and S. Randhawa. **Field incurred Chlorpyrifos and 3,5,6-trichloro-2-pyridinol residues in fresh and**

processed vegetables. Food Chemistry, 2007; 103: 1016-1023.

15. Baig, S.A., Akhter, N.A. Ashfaq, M. and Asi, M.R. **Determination of the Organophosphorus Pesticide in Vegetables by High-Performance Liquid Chromatography**. American-Eurasian Journal of Agriculture and Environmental Sciences, 2009;6 (5): 513-519.

"Strive not to be a success, but rather to be of value."

Albert Einstein

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Rai Muhammad Amir	Ph. D student prepare research paper after completion of his Ph. D research project.	
2	Prof. Dr. Faqir Muhammad Anjum	Supervisor under his supervision and guidance reasearcher completed his research work and research article.	
3	Dr. Muhammad Atif Randhawa	He provided technical assistance in designing reaserch project and research article.	
4	Prof. Dr. Muhammad Sarwar	He provided the valuable suggestion for the improvement of research work and research article.	