HERBICIDE CONTAMINATION IN CARROT GROWN IN PUNJAB, PAKISTAN

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INTRODUCTION

A variety of vegetables are cultivated in Pakistan. Mainly vegetables are concentrated in the vicinities of big urban centers cities like Lahore, Karachi and Peshawar (Bakhsh et al., 2006). In Pakistan, carrot is ranked second among winter vegetables grown throughout the country (MINFAL, 2009) while it ranks as the fifth most consumed vegetable in the European Union (Bressani, 2000). Use of pesticide is widespread throughout the world posing serious health risks to human health (Khan et al., 2009). Consequently, substantial attention is being paid to pesticide residues in the environment. Maximum residue limits standards/ regulations in vegetables and fruits have been established by several nations and organizations. The monitoring of pesticide residue requires real-time, rapid, and highly efficient analytical technology (Kuet and Seng, 2008). Pesticide residues in food ready for consumption usually result from direct application of that pesticide to the crop producing that food commodity. Consumer risk assessment is a crucial element in the approval, registration or licensing of pesticide uses on food crops (Min et al., 2006).

Pakistan is a developing country whose birth rate is among the highest throughout the world (BBC, 2011). To feed this ever increasing population, intensive agricultural production in being practiced using the highest use of chemical fertilizers and pesticides. Vegetable production is also affected by this phenomenon. Among winter vegetables, carrot is produced at larger scale throughout the country. It is a highly nutritious vegetable particularly with reference to carotenoids which are associated with several health promoting attributes. This is cheaply available and is equally consumed by poor and rich people in Pakistan. It has severe weed infestation. To eradicate these weeds, farmers are using herbicides at higher rates. Dual Gold® is being used maximum having S-metolachlor as an active ingredient. It is necessary to understand how much is consumed in order to conduct an assessment of potential risk. The aim of the risk assessment is to compare the dietary intake of the pesticide residue by the consumer with a measure of levels that are acceptable. Therefore, the present study was envisaged to quantify S-metolachlor in carrot collected from leading carrot growing areas of Punjab Province, Pakistan and from the vegetable market in Faisalabad.

MATERIAL AND METHODS

Estimation of pesticide residues in carrot collected from the surveyed areas: Samples of carrot produced in different districts (Faisalabad, Sheikhupura, Nankana Sahib and Gujranwala) and one sample from the Faisalabad market were randomly collected, washed and transported to the laboratory for further analysis. Each sample was analyzed in triplicate.

Extraction of residues: Residues of S-metolachlor were extracted from the homogenized sample using the modified method described by Khan et al. (2009). Carrot samples were chopped into small pieces on clean chopping boards with stainless steel knife to prevent any other contamination. One kilogram of the chopped sample was blended in a
Warring Blender so that homogenous slurry/paste is formed. Ethyl acetate was used as solvent because of its efficient recovery (Banergee et al., 2007). Homogenized samples of 50 g carrot were taken in 250 ml Erlenmeyer flask. Twenty gram anhydrous sodium sulphate (HPLC grade) was added and mixed in homogenized carrot sample in flask to prevent the clod formation. Ten milliliter saturated sodium chloride solution was added in the mixture. Seventy five milliliter ethyl acetate (HPLC grade) was added in the sample. Glass beads were added in mixture to stop bumping. Mixture in flask was shaken at a speed of 240 rpm on a horizontal shaker for 1 hour. Extract was collected in inert plastic bottle. Sample extract was filtered using Whatman’s No. 4 filter paper. Filtered extract was stored at -40°C before further analysis.

**Clean-up of filtered extract: S-metolachlor 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 S-metolachlor were cleaned up using column chromatographic technique as reported by Kumari (2008) and Tuan et al. (2009). Clean glass columns were used for this purpose. Glass wool put at the bottom of column to support the stationary phase. Silica gel and charcoal were activated at 200°C for 24 hours before filling the column. Activated silica gel and charcoal were mixed at a ratio of 5:1 (w/w). A thin layer of anhydrous sodium sulphate was placed on top of glass wool. The activated mixture (12 g) of silica gel and charcoal was placed on sodium sulphate layer. Activated mixture was covered with thin layer of anhydrous sodium sulphate and glass wool respectively. The filled column with adsorbent was washed with acetone (HPLC grade) before loading the sample on column. Flow rate was adjusted at 1 ml/min before loading the target sample. The residue of herbicide was eluted with 50 ml mixture of acetone and hexane (3:7 v/v). The cleaned elute was received in 150 ml round bottom flask. Elute was then concentrated in rotary evaporator at 40°C under vacuum. The concentrated elute (1 ml) was transferred to small vials of volume (1.5 ml) using pasture pipette. The elute in the vial was placed under gentle stream of nitrogen until elute had completely dried.

**GC-ECD analysis:** The cleaned extracts were analyzed on Agilent 6890N GC system equipped with capillary column using Ni$^{63}$ electron capture detector (Tuan et al., 2009). The separation of pesticides was done on a column (30 meter length, 0.25 mm internal diameter and 0.25 µm film thickness). Helium was used as the carrier gas at 9.6 pound per square inch (PSI) pressure with flow rate 1 ml/min.. The injector and detector temperatures were set at 250°C and 300°C respectively. The initial oven temperature was 110°C (3 min isothermal) to 275°C (at 10°C min$^{-1}$) isothermal for 15 minutes.

One µL was injected into the GC-MS system and chromatograms were obtained from the system. The GC-ECD chromatogram of standard solution of S-metolachlor (0.5 µg/ml) with an internal standard of dieldrin (0.90 µg/ml) was shown in Fig. 2. Retention time for S-metolachlor was 28.36 minutes. An internal standard of Dieldrin (0.90 µg/ml) was added in the sample to check the efficiency of GC system. Its retention time was 22.63 minutes. The quantification of pesticide residues was done using the peak area of standard (Fig.2) and the samples (Fig. 3 & 4).
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Statistical analysis: Analysis of variance of the data was computed using the Statistica® Computer Program. The Least Significant Difference test at 5% level of probability was used to test the differences among mean values (Steel et al., 1997).

RESULTS AND DISCUSSION

Statistical analysis showed significant differences in S-metolachlor concentration in carrot samples collected from different locations. In all the analyzed samples, the maximum S-metolachlor (0.73 µg/ml) concentration was observed in district Faisalabad while the lowest one (0.45 µg/ml) was in Sheikhupura. However, all the other three locations were statistically similar in this regard with 0.58 µg/ml in Gujranwala, 0.56 µg/ml in Faisalabad market and 0.54 µg/ml Nankana, respectively (Fig. 1).

Pesticides and herbicides are certainly hazardous to human health. Among many problems, pesticides cause heart congestion, lung and kidney damage, low blood pressure, muscle damage, weight loss and damage to adrenal glands. The herbicides alachlor, acetochlor, butachlor, pretilachlor, metolachlor, dimethenamid, propachlor, napropamide, propanil, atrazine, and metribuzin have been widely used in different crops (Zhang, 2002). Metolachlor induces cytotoxic and genotoxic effects in human lymphocytes (Rollof et al., 1992). Dual gold® is a very common herbicide being used in Pakistan to control weeds in vegetable crops. Maximum residual limit of S-metolachlor in carrot sample is 0.4 µg/ml (HCPMRA, 2011) while in the present study it ranges between 0.45-0.73 µg/ml in different carrot growing regions. In USA, it has been detected in ground and surface water with a range of 0.08 to 4.5 parts per billion (Pothuluri et al., 1992) and United States Environmental Protection Agency (USEPA) classified it as a Category C pesticide which indicates limited evidence of carcinogenicity (USEPA, 1987) This higher concentration might be due to the unprecedented use of herbicide for better crop productivity. Secondly, higher level of S-metolachlor residues might be multiple sprays at high concentration as farmers reported that they used to spray on their crop after an interval of 2-3 days due to the increased growth of weeds (Baig et al., 2009). On the other hand, mostly farmers are un-educated and they do not know that which chemical should be used to control target insect pest/weeds and at what concentration it should be sprayed (Khan et al., 2009).

In Pakistan, the persons applying pesticides do not care about the precautions given on the label of pesticide packages as was observed during farmer’s selection. Results of present study are supported by Randhawa et al. (2007) who quantified 3,5,6-trichloro-2-pyridinol and chlorpyrifos residues in processed as well as fresh vegetables and found the higher pesticide levels in them. Charan et al. (2010) also found similar results of pesticide residues in different vegetables. The findings of the present study depicted that the content of pesticides in vegetable crops does not only depend on the sprayed amount over them but also on the content present in soil or water used for irrigation. Therefore, there is also dire need to know the level of various pesticide residues in soil and water directly.

Conclusion: Present results showed higher levels of S-metolachlor contamination in all the carrot samples analyzed that was above the permissible limits. To cope up this alarming issue, there is a dire need to streamline the application of pesticide residues for safer food production as well as to protect the consumers from the harmful health effects of pesticide residues.
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REFERENCES


