International Journal of Ecology and Environmental Sciences 42 (1): 35-37, 2016
ISSN: 2320-5199 (Online)

Short Communication

Determination of Some Pesticide Residues in Cauliflower (*Brassica oleracea var botrytis*) by High Performance Liquid Chromatography

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ABSTRACT

Widespread use of pesticides in vegetables has become a major concern as accumulation of pesticide residues in vegetables causes human health hazards and affects ecological balance. Pesticide residues of chlorpyriphos, endosulfan, carbaryl and piperonyl butoxide were determined in cauliflower samples collected from vegetable market, Dehradun. High Performance Liquid Chromatography (HPLC) with UV detector was used for analysis. Concentration of chlorpyriphos (0.033 mg kg^{-1}), carbaryl (0.026 mg kg^{-1}) and endosulfan (0.104 mg kg^{-1}) was found in cauliflower. Piperonyl butoxide residue was not detected in any sample. The concentrations of pesticide residues were below the MRLs.

Key Words: Chemical Residues; Vegetables; HPLC; Maximum Residue Limits

INTRODUCTION

Vegetables are important ingredient of our food having high nutritional value. Cauliflower is originated from Europe. It is one of the most important vegetables of India. During 2013-14, India produced 8.57 million Mg (= tonnes) of cauliflower from 434,000 ha area with an average productivity of about 19.8 Mg ha^{-1} (Indian Horticulture Database 2015). Cauliflower is low in fat, high in dietary fibre, contains water and vitamin C, possessing a very high nutritional value. It helps in keeping the liver clean and healthy. In the process of enhancing productivity, the use of agro-chemicals became an integral part of the modern agriculture. Approximately 2.5 million Mg pesticides are used in agriculture annually throughout the world (Meena et al. 2008). Pesticide use is increasing in Asia, Africa, Central and South America because of high demand for good quality products and urgent need for self sufficiency in food production (Mansour 2004). For better production and aesthetic value, farmers are using a large amount of pesticides. Owing to this injudicious practice related to pesticide usage, pesticides become the inner part of vegetables, in the shape of residues which could be used by consumers and thus creating health hazards (Agnihotri 1999 and Kumari et al. 2003). The problem of residue accumu-lation needs more attention in vegetables because most of time these are consumed either raw or without much storage time (Kumar et al. 2006).

Besides damage to human health, indiscriminate uses of chemical pesticides over a long run resulted in development of resistance in insect/pests to pesticides (Kranthi et al. 2002) and adversely affect natural diversity that resulted in the reduction of natural enemies (Ranga Rao et al. 2005). For maintaining quality of any commodity, it is essential to keep produce free from any pesticide residues. A zero level residue in the finished product is not only desired but also needed for eco-preservation and human health as well.

Therefore, pesticide residue is becoming a major food safety concern of consumers and Government. Pesticide residue monitoring studies have been reported in many countries of the world on fruits and vegetables (Mukherjee 2003, Wang et al. 2008 and Zhou et al. 2008). In order to remove residual effect of pesticides, which are toxic, we should know the exact dose which should be recommended to the farmer and the interval between pesticide application and harvesting, so that the amount of residual pesticides in vegetable might be lower than the acceptable range.

The extraction and quantification of pesticide residue in food matrix mostly involved liquid-liquid extraction with a great variety of solvents and absorbents for cleanup. Analytical Techniques such as Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) are widely used to monitor the presence of these compounds in water, soil, foods, fruits and vegetables. Several recent papers have reported advances in this field (Randhawa et al. 2007 and Melo et al. 2004).

Present study was conducted to determine the residue of pesticides in cauliflower by High Performance Liquid Chromatography technique. Pesticides studied were Chloropyrifos, Endosulfan, Carbaryl and Piperonyl butoxide.

MATERIAL AND METHODS

Three samples of cauliflower weighing one kg each, were collected from three places of vegetable market (Sabji Mandi) of Dehradun, Uttarakhand, during July (rainy season). These were analysed for four pesticides i.e. chlorpyrifos (organophosphate), endosulfan (organochlorine), carbaryl (carbamate) and piperonyl butoxide (synergist) using HPLC technique with UV detection for separation, identification and quantification of pesticides.

The organic solvents, acetonitrile and ethyl acetate (HPLC grade) and anhydrous sodium sulphate were obtained from E. Merck whereas the technical grade pesticide standards were of AccuStandard, Inc. (New Haven, CT, USA). Standards were stored in a freezer at -5^{0}C. Water (HPLC grade) was purchased from Renkem.

For preparing stock solution, chlorpyrifos standard was dissolved in acetonitrile and carbaryl, endosulfan and piperonyl butoxide standards were dissolved in methanol. These standards were stored at 4^{0}C in dark.

Extraction and Clean up

Cauliflower was cut into small pieces and homogenized in kitchen blender and kept in freezer in an airtight polythene bag (zip lock). Seventy five gram of blended cauliflower sample was mixed with 50 g of anhydrous sodium sulphate and extracted with ethyl acetate (200 mL) in a conical flask. The content was allowed to settle down for about 30 minutes and then ethyl acetate extract was filtered through a Buchner funnel fitted with filter paper. After filtration, the extract was evaporated to dryness and redissolved in acetonitrile (5 mL) and finally the volume was made upto 2 mL using rotary evaporator. These solutions were centrifuged and filtered. The clean organics layers analysed by High Performance Liquid Chromatography (HPLC) with UV detector.

HPLC System

Waters 1500 series (Milford, MA, USA) HPLC system with UV detector (Waters 2487) was used for identification and quantification of pesticides. Analysis was performed in isocratic system using a reserve phase C-18 column (25 cm × 4.6 mm, id). Samples were injected in manual injector. For separation of chlor-yrifos, acetonitrile plus water (70:30), for carbaryl, methanol plus water (60: 40), for endosulfan, methanol plus water (70:30) and for piperonyl butoxide- aceto-nitrile plus water (55:45) were used as mobile phase. Flow rate was 1.0 mL min^{-1}, injection volume 20 µL and the wavelength of UV detector was fixed at 230 nm for chlorpyrifos, 280 nm for carbaryl, 214 nm for endosulfan and 295 nm for piperonyl butoxide.

Identification and Quantification

Compounds were identified by comparing its retention time with respect to technical grade reference standards. Quantitative determination was carried out with the help of a calibration curve drawn from chromatographic experiments with standard solution of different concentrations. Mean values and standard deviation were calculated by using simple descriptive statistics.

RESULT AND DISCUSSION

Results of the present study regarding concentration of residues, retention time and maximum residual limit (MRL) of chlorpyrifos, carbaryl, endosulfan and piperonyl butoxide in cauliflower samples are presented in Table 1. The results indicate that the concentrations of chlorpyrifos, carbaryl, endosulfan and piperonyl butoxide residues in cauliflower were detected below MRLs. This may be due to application of low doses of these pesticides in vegetable crops by growers between sowing to harvesting period or it may be due to photo degradation of some pesticide residues in environment. Chlorpyrifos, when exposed to sunlight, undergoes hydrolysis in presence of water to liberate 3,5,6-trichloro-2 pyridinol, which is further degraded to 3, 5, 6-trichloro-2 methoxy pyridine and CO_2 (Racke 1993).

Table 1. Residues, Retention Time and Maximum Residue Limit for cauliflower samples collected from Dehradun market.

Pesticides	Residue (mg kg^{-1})	Retention time (min)	MRL (mg kg^{-1})
Chloropyriphos	0.033	12.6	0.05
Carbaryl	0.026	6.5	5.00
Endosulfan	0.104	20.0	0.50
Piperonyl butoxide	ND	23.0	0.10

It was further observed that the concentration of endosulfan residue in cauliflower was more than that of other pesticides. This may be explained by the fact that organochlorine pesticides have long persistence and high stability in environment (Abou-Arab and Abou-Donia 2001). The concentration of piperonyl butoxide residue was not detected in cauliflower, because it is short lived in the environment and possessing very low toxicity (Amold 1998).

Since these pesticides residues are not degraded into non-toxic products in short period of time, they persist in vegetables. So the dose of pesticide used by farmers should be lower than recommended to control pests and the time interval between the spray of pesticides and harvesting of vegetables should be longer.

ACKNOWLEDGEMENTS

The financial support from Wildlife Institute of India, Dehradun, is thankfully acknowledged. I would like to thank the Director of the Institute and In-charge, Central Laboratory, for providing all essential facilities to carry out this study.

REFERENCES

Abou-Arab, A.A.K. and Abou-Donia, M.A.B. 2001. Pesticide residues in some Egyptian spices and medicinal plants as affected by processing. Food chemistry 72: 439-445.

Agnihotri, N.P. 1999. Pesticide Safety Evaluation and Monitoring. Report, All India-coordinated Research Project on Pesticide Residues. Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi.

Amold, D. J. 1998. The fate and behaviour of Piperonoyl Butoxide in the environment. Pages 105-119, In: Jones, D.G. (Editor) Piperonyl butoxide: The Insecticide Synergist. Academic Press, San Diego, CA.

Indian Horticulture Database 2015. Crop-wise area, production and productivity of major vegetable crops in India during 2008-09. National Horticulture Board, Ministry of Agriculture, Government of India, Gurgaon 122 015, India

Kranthi, K. R.; Jadhav, D.R.; Kranti, S.; Wanjari, R.R.; Ali, S. S. and Russel, D. A. 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Protection 21: 449-460.

Kumar, M.; Gupta, S.K.; Garg, S.K. and Kumar, A. 2006. Bio-degradation of hexachlorocyclohexane-isomers in contaminated soils. Soil Biology and Biochemistry 38: 2318-2327.

Kumari, B.; Kumar, R.; Madan, V.K.; Singh, R.; Singh, J. and Kathpal, T.S. 2003. Magnitude of pesticidal contamination in winter vegetables from Hissar, Haryana, Environment Monitoring and Assessment 87: 311-318.

Mansour, S. A. 2004. Pesticide exposure: Egyptian scene. Toxicology 198: 91-115.

Meena, R.P.; Meena, S.S. and Meena, S.R. 2008. Use of agro-chemicals in agriculture, their consequences and possible solution. Green Farming 1: 52-55.

Melo, L.F.C.; Collins, C. H. and Jardim, I.C.S.F. 2004. New materials for solid-phase extraction and multiclass high-performance liquid chromatographic analysis of pesticides in grapes, Journal of Chromatography, A 1032: 51-58.

Mukherjee, I. 2003. Pesticides residues in vegetables in and around Delhi. Environment Monitoring and Assessment 86: 265-271.

Racke, K.D. 1993. Environmental fate of Chloropyriphos. Reviews of Environmental Contamination and Toxicology 131: 1-154.

Randhawa, M. A.; Anjum, F. M.; Asi, M. R.; Butt, M. S.; Ahmed, A. and Randhawa, M. S. 2007. Removal of endosulfan residue from vegetables by household processing. Journal of Scientific and Industrial Research 66: 849-852.

Ranga Rao, G.V.; Rameshwar Rao, V.; Reddy, Y.V.R. and Murthy, K.S.R.K. 2005. Status of safety in pesticides use in sia: Options for improvement. Proceedings of the National Seminar on Pesticides Residues and their Risk Assessment: 129-136. January 20-21, 2005; Food and Drug Toxicology Research Centre, National Institute of Nutrition, Hyderabad .

Wang, L.; Liang, Y. and Jiang, X. 2008. Analysis of eight organo-phosphorus pesticides residues in fresh vegetables retailed in agricultural product markets of Nanjing, China. Bulletin of Environment Contamination and Toxicology 81: 377-382.

Zhou, Q.; Bai, H.; Xei, G. and Xiao, J. 2008. Trace determination of organophosphorus pesticides in environmental samples by temperature controlled ionic liquid dispersive liquid-phase microextraction. Journal of Chromatography A. 1188: 148-153.

Received 28 January 2016
Accepted 8 March 2016