International Journal of Agricultural and Life Sciences- IJALS (2015), Volume 1 (4) pp.7-11
http://doi.org.in/10.9379-sfjal-122059-002-0081-x

Available online at http://www.skyfox.co
ISSN: 2454-6127.

RESEARCH ARTICLE Open Access

Organochlorine and Organophosphorus Pesticide Residues analysis by GC-MS/MS in Raw Cow Milk

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Received: Oct 2015 / Accepted: Nov 2015/ Published: Dec 2015

Abstract: Different pesticides have been used in tropical countries to control agricultural pests. Problem of the presence of pesticides in milk contributes to the serious issue of human health. The identification and quantification of Organophosphorus (Methamidophos, Acephate, Omethoate, Monocrotophous, Diazinon, Chlorpyrifos, Parathion methyl, Malathion and Ethion) and Organochlorine (Atrazine, Lindane, Dieldrin, Heptachlor, Chlordane, DDE p, p', DDT, p, p', Endosulphan and Imazalil) pesticides in cow milk collected and pooled from different localities of Thanjavur, Tamil Nadu was performed. Extraction was performed with 1:2 ratios of Dichloromethane and Hexane. The analysis showed that most of the pesticides were presence in the selected cow milk sample. Confirmation of pesticides was done through Scion 436 - GC Bruker with BR-5MS GC-MS/MS. Most of the Organochlorine and Organophosphorus present in milk samples. The result of the analysis showed that the cow may be exposed to pesticide rich feed which consequences the presence in milk. Hence, the intake of such milk might pose serious health hazardous to humans in this locality known tropical fruit which has been used in India since 1300 AD. Grape juice is well relished by all age groups of the society. It was reported that consumption of grape juice at moderate level helps in prevention of aging related diseases. The aim of this review, a summary of the nutrient content which is available in grape juice and to obtain a further understanding of the reported beneficial health effects of the naturally occurring nutrient content in the grape juice. It has been systematically studied for various biological activities with particular reference to clinical effects. Several other potential beneficial properties in grape juice like flavonoids, polyphenols, antioxidants, anthocyanins, and resveratrol have since been ascertained. We review the potential clinical applications of these fascinating natural substances.

Keyword: Pesticides, Organochlorine, Organophosphorus, GC-MS/MS, Contamination, Methamidophos, Acephate, Omethoate, Monocrotophous, Diazinon, Chlorpyrifos, Parathion methyl, Malathion ,Ethion , Atrazine, Lindane, Dieldrin, Heptachlor, Chlordane, DDE p, p', DDT, p, p', Endosulphan and Imazalil.

INTRODUCTION

Fluazifop- Pesticides are used worldwide to increase the production but many persistent residues of pesticides cause problem in environmental contamination and human health. India is the largest user of pesticides. Several pesticides contain the noxious substances that persist in environment for a long time [8].

Different types of organophosprous and organochlorine pesticides have been screened in milk sample. These pesticides can be absorbed by cows through their feed. Problem of the presence of pesticides in milk contributes to the serious issue of human health. Pesticides work to kill the pests or make them ineffective. In the same way, pesticides can affect the unintended individual, such as human. Pesticide residues have greater impact on human diet so contamination with these residues checked with greater concern in milk and milk products. [11,12].

To control the presence of pesticide residues in fresh milk and milk products is a big issue for producer, consumer and government due to the potential risk. Human milk as well as animal milk is contaminated through the contaminated food. These residues are too much persistent. They accumulate in body fat even in breast milk. These pesticide residues that move in human through milk samples cause different heart problems, endocrine disrupt and cancer. Applications of the regular pesticides firstly cause the acute effect then produce the chronic effect and may also cause death [3,7,16].

Intensive use of pesticides in agriculture as well as in the community health sector is the major cause of contamination of environment. Accumulation of these dangerous pesticides causes serious health issues in humans. Evidences show the presence of different organochlorine

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ISSN: 2454-6127.

pesticides in crops, human fluids and also in meat. These pesticides also cause little sperm count, increased testicular cancer, different birth defects and different other reproductive defects. A broad range of pesticides are being used around the world by farmers because of their wide spectrum activity and greater efficiency. Low cost is also another factor for their use. Because of the harmful effects of organochlorine pesticides these were banned in different time periods in different countries. The main problem of organochlorine pesticides was that they stick within the environment [1,15,17].

India is basically an agricultural country. Livestock is playing a vital role in its economy. Almost 40-45 million peoples are engaged with livestock. On the average each family in rural areas has 5-6 cattle's, 5-6 goats and buffalo. People are deriving 30-40% of their income from these animals. Buffalo contributing 68% of milk production, cow 27% and remaining 5% is from sheep's, goats and camel. Buffalo is the major dairy animal of Pakistan and contributing maximum milk production. Pesticides may be classed into two classes' synthetic pesticides and the biological pesticides. Wide spectrum pesticides can kill any species while selected pesticides only kill the selected species for which it has been made. Systemic pesticides are those which are absorbed by the plant. These pesticides come inside the plant circulation. DDT was very famous insecticide and in past its use was very common. 75 % pesticides are being in use by the developed countries. Pesticides save farmer money by killing the unwanted insects or pests in the crops by increasing production yield [2,17].

Endosulphan and organophosphate pesticides block the nerve impulses by reducing the acetyl cholinesterase activity. Most insecticides act on the nervous system of the insect's. Although there is remarkable difference between the nervous system of insects and the mammals but the toxicity mechanism is same in both Different tests are used to check the maximum residue limits of pesticides.

Individual government and the international government set standards for the maximum residue limits. Environmental and the agricultural conditions affect maximum residue limits as these factors are not same in every country [9,11].

In this monitory study, milk samples collected and pooled from different cows were analyzed for the presence of pesticides residues. The purpose of this study was to check whether milk was contaminated with pesticides or not and what was the limit of contamination with pesticides residues. This study will be helpful for general public and farmers that they should use pesticides with caution.

MATERIALS AND METHODS

Collection and storage of cow milk sample

The fresh cow milk was collected from the local animal farm in Thanjavur district, Tamil Nadu. The milk sample was received in a glass bottle and immediately cooled at -20°C until analyzed.

SAMPLE PREPARATION

The sample was prepared as per United States Environment Protection Agency Method (US-EPA) (16). The raw milk sample of 5 gm was mixed with 15 ml of distilled water. Twenty milliliters of 1:2 ratio of DCM: Hexane was added to the sample mixture. Centrifuged at 5000 rpm for 20 minutes at room temperature. The centrifuged sample aqueous layer was freezed by placing at deep freezer. The organic layer was collected and filtered with sodium sulphate. The sample quantity of 2µl was injected into GC-MS/MS.

Pesticide Residues Analysis through GC-MS/MS: The pesticide screening of raw cow milk sample was investigated through Gas Chromatography-Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436- GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30m; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The column oven temperature program was as follows: 80 °C hold for 2 min, Up to 160 °C at the rate of 20 °C/min-No hold, Up to 280 °C at the rate of 5 °C/min-No hold, Up to 300 °C at the rate of 20 °C/min- 10 min hold, Injector temperature 280 °C and total GC running time was 41 min [14]. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan interval of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 280 °C, source temperature 250 °C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was MS Work station 8. The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The GC-MS/MS was performed by Food Safety and Quality Testing Laboratory, Institute of crop processing technology, Thanjavur.

RESULTS

The raw cow milk sample was pooled and analyzed for the presence of selected Organophosphorus (Methamidophos, Acephate, Omethoate, Monocrotophous, Diazinon, Chlorpyrifos, Parathion methyl, Malathion and Ethion) and Organochlorine (Atrazine, Lindane, Dieldrin, Heptachlor, Chlordane, DDE p, p', DDT, p, p', Endosulphan and Imazalil) pesticides. The result interpretation was done through scanning the quantifier and qualifier ions of pesticide mass spectra with that of analyzed milk sample. The pesticide residue analysis confirmed that the mil sample was contaminated with the pesticides. The study shows that the presence of organochlorine pesticides likes chlordane and endosulphan and organophosporous pesticides like monocrotophous in the milk sample. The list of compounds screened for their presence in milk sample was provided in Table 1.

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ISSN: 2454-6127.

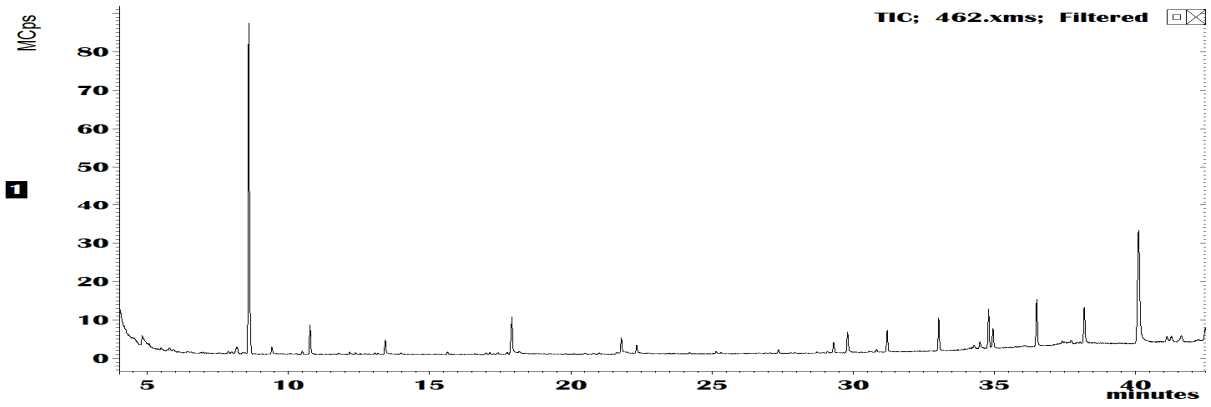
Table: 1. Organochlorine Pesticides screened in the Cow's Raw Milk

No.	RT	Name of the Pesticide	Group	MS Mass Number	MS Quantifier Ion	Qualifier Ions	Presence/ Absence
1.	10.54	Atrazine	Organo Chlorine Pesticides	200, 215	215 > 173	215 > 200	Absence
2.	10.81	Lindane		181, 183	180 > 144	218 > 180	Absence
3.	12.25	Dieldrin		79, 81	277 > 241	277 > 170	Absence
4.	13.58	Heptachlor		100, 272	272 > 237	272 > 117	Absence
5.	14.89	Chlordane		375, 373	372 > 265	372 > 267	Presence
6.	15.32	DDE p, p'		246, 318	245 > 175	317 > 245	Absence
7.	16.20	DDT, p, p'		235, 237	234 > 164	234 > 164	Absence
8.	16.85	Endosulphan		195, 197	271 > 236	273 > 238	Presence
9.	18.25	Imazalil		41, 215	173 > 145	215 > 173	Absence

Table: 2. Organophosporous Pesticides screened in the Cow's Raw Milk

No.	RT	Name of the Pesticide	Group	MS Mass Number	MS Quantifier Ion	Qualifier Ions	Presence/ Absence
1.	5.87	Methamidophos	Organo Phosphate Pesticides	94, 95	141 > 95	141 > 126	Absence
2.	7.50	Acephate		136, 142	136 > 42	136 > 94	Absence
3.	9.00	Omethoate		156, 110	110 > 79	156 > 110	Absence
4.	9.80	Monocrotophous		127, 67	127 > 95	127 > 109	Presence
5.	10.88	Diazinon		179, 137	137 > 84	304 > 179	Absence
6.	12.08	Chlorpyrifos		97, 197	124 > 78	285 > 92	Absence
7.	12.22	Parathion methyl		291, 109	263 > 109	263 > 246	Absence
8.	12.92	Malathion		125, 173	127 > 99	173 > 127	Absence
9.	16.12	Ethion		231, 97	230 > 202	383 > 230	Absence

GC-MS/MS Chromatogram of Raw Cow's milk sample



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DISCUSSION:

The major source of contamination of dairy products by different hazardous pesticides is the presence of their residues in animal feed stuffs. Other factors that may also contribute to this sort of contamination include the application of pesticides on farm animals, environmental contamination and accidental spills [4]. Milk contamination with the pesticides residues can be controlled by preventing the contamination of feedstuffs.

In this monitory study, residues of different pesticides were determined by GC-MS/MS analysis which showed presence of Organophosphorus (Methamidophos, Acephate, Omethoate, Monocrotophous, Diazinon, Chlorpyrifos, Parathion methyl, Malathion and Ethion) and Organochlorine (Atrazine, Lindane, Dieldrin, Heptachlor, Chlordane, DDE p, p', DDT, p, p', Endosulphan and Imazalil).

Organophosphate and organophosphate pesticides residues were also detected in the milk samples collected by Indraningsih et al., 2004 [5]. A similar study was done on dairy sheep's and goat milk and feed samples for the detection of pesticides residues in Greece by Tsiplakou et al. (2010) [14]. Endosulfan was the main pesticide residue which was detected in the feed samples with the average of 0.10 mg/kg and this level was lower than the maximum residue level. In sheep and goat milk samples no pesticides were detected and this milk was considered to be safe for human use.

CONCLUSION

In this study, the pooled cow milk samples collected around Thanjavur area were subjected for GC-MS/MS analysis and examined for the presence of Organophosphorus (Methamidophos, Acephate, Omethoate, Monocrotophous, Diazinon, Chlorpyrifos, Parathion methyl, Malathion and Ethion) and Organochlorine (Atrazine, Lindane, Dieldrin, Heptachlor, Chlordane, DDE p, p', DDT, p, p', Endosulphan and Imazalil) pesticides. The study findings revealed that the organochlorine pesticides namely chlordane and endosulphan and organophosporous pesticides namely monocrotophous were found presence in the selected raw cow milk sample. The current method with optimized parameters for GC-MS/MS analysis can be used for the screening of pesticides residues in any milk samples. The findings of the study might help in extending awareness in dairy farmers and local people about pesticides and their hazardous effects on humans.

ACKNOWLEDGMENT

The authors are grateful to Dr. K. Singaravadivel, Director, Indian Institute of Crop Processing Technology, Thanjavur for providing all the facilities, encouragement and support used to carry out the work.

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How to cite this article

Karthikeyan Ravichandran* • Srinivasan Krishnamoorthi • Muthukumaran Pakkirisamy • Prithiviraj Pandiyarajan • Sadhana Sekar• Kumaravel Shanmugam (2015): Organochlorine and Organophosphorus Pesticide Residues analysis by GC-MS/MS in Raw Cow Milk. In International Journal of Agricultural and Life sciences 1 (4), pp. 7–11. Available online at 10.9379/sfjal-122059-002-0081-x.

CONFLICTS OF INTEREST

"The authors declare no conflict of interest".