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Estimation of Persistent Organochlorine Pesticide Residues in Selected Vegetables

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Abstract

The vegetable samples were tested for its content in persistent organochlorine pesticides. Using QuEChERS method, analyzed five vegetable samples to evaluate some methodologies and to obtain consensus values for selected organochlorine pesticides. The obtained results contribute to the accuracy and precession for organochlorine pesticides quantification in vegetable samples. The limit of quantification (LOQ) was 0.3 to 1.5 µg /kg, whereas limit of detection (LOD) was 0.1to 0.5 µg /kg found respectively. Vegetable tested in our analysis do not contain any quantities of pesticide residues representing & hazard to the humans.

Keywords: Organochlorine, pesticide, Analysis, vegetables, maximum residues limit.

Introduction

During the last few decades numerous research efforts to identified and adverse health affect the environment fate of persistent organic populants (Pops) have resulted in a variety of field method for the sampling and analysis of vegetables. The roles of organochlorine pesticides have been very vital in public health and agriculture production in developing countries including India. Further the enormous uses of organochlorine pesticides (OCPs) in developing countries have been of serious concern because of there persistent in nature. Large amount of pesticide are used in agriculture sector and public health programmers every year [1,2]**.**Continuous use of OCPs lead to there presence in water, soil, air, crop plant and biological tissue. A multi residue method for determining pesticide residues in large no of vegetable samples was studied [3].Although pesticide residue analysis has been done in several food/product [4-5]However, pesticide residues analysis in vegetables was carried out in recent years [6].

This paper describes the preliminary observation about the presence of organochlorine pesticide in daily used vegetables. The present study also deals with the analysis of organochlorine pesticide residues in above vegetables to about evaluate the presence of these commonly used persistent OCPs. The presence of organochlorine pesticide (OCPs) in terrestrial and aquatic environment may leaves to toxicological implication [7-10]. Organochlorine pesticides are widely used in agriculture as insecticides leaves residues to varying extent in agricultural product such as vegetables and fruits. Due to there toxic property and potential risk to consumer, there residuals in food commodities is an issue of public concern and controlled by legislation**.**

Results and Discussion

Result revealed from Table 2 that level of butachlor & endosulphan higher in almost all the items except in tomato higher level of butachlor was found in cauliflower (13.83ppb); lowest level of butachlor was recorded in tomato (5.26ppb).Butachlor residues also detected in water, soil and rice [12].However, endosulphan concentration is highest in brinjal (4.81ppb) and lowest in tomato (3.49ppb).Previous studies shows that isomers of endosulphon are also present in tomato [13]. Though contamination of chlordane maximum in cauliflower(7.92ppb) & lowest in okra(4.13ppb).In view of the fact that toxicity of chlordane and its ability to bioaccumulate, the chlordane and its isomer were banned in so many countries but still be detected [14,15].

It is also evident from Table 2 that residues of total HCH were more than residues of total DDT in all position except in cauliflower & okra where DDT residues 35.5&6.2ppb was more than that of HCH (9.48&3.4ppb).The contamination of HCH was 23.1ppb in both brinjal ,cabbage & DDT(7.9 & 7.8ppb)respectively .The residues level pattern in brinjal , cabbage , tomato is HCH>DDT, contrary to this in okra & cauliflower DDT>HCH. As given in Table 2 the level of beta HCH was higher in all isomer of HCH in brinjal, cabbage, cauliflower, tomato, okra. Although pesticide like HCH & DDT are well known toxic chemicals [16]. Report have shown that gamma isomer of HCH is more susceptible to degrade in biological environment [17-18]. It is evident from Table 2 the level of both the isomers of DDT was highest in cauliflower only ,DDT undergoes metabolic conversions & dehydrochloroination [19].The presence of p-p DDE & p-p DDD in present study might be due to such metabolic conversion .

Studies also shows that level of alchalor was found maximum in cabbage (5.45ppb) & lowest in bringal (2.41ppb).Although quantitative assessment of the mutagenic potential of environment degradative products of Alachlor was studied [20]. Through organochlorine pesticide are present in nature, even organochlorine pesticide were also detected in human diet [21] and drinking water [10].Certain physical & chemical conditions like heat treatment, streaming treatment with supercritical carbon dioxide etc are known to reduces the level of Ocps [22-23] .The contamination level of Aldrin was also found to be maximum in cauliflower(4.29ppb) and least in Cabbage (2.80ppb) .The contamination level dicofol (highest in Cauliflower 5.19 ppb and lowest in Brinjal 3.53 ppb) slightly higher than aldrin(max. 4.29ppb in cauliflower & min. in cabbage 2.80ppb).Although pesticide like aldrin and dicofol are known as toxic chemicals [24-25] .

Table 2 Level of Organochlorine pesticide residues (µg/kg) in vegetables

	Vegetables				
Name of pesticide	Brinjal	Cabbage	Cauliflower	Okra	Tomato
Alachlor	2.41 (1.55-4.01)	5.45 (1.21-9.82)	3.16 (1.19-6.92)	4.37 (1.25-8.04)	2.69 (0.96-4.65)
Aldrin	3.90 (1.26-6.90)	2.80 (0.69-4.01)	4.29 (1.58-6.23)	3.48 (1.90-4.10)	3.02 (1.09-6.10)
Butachlor	12.08 (5.53-22.01)	9.37 (2.96-14.32)	13.83 (4.80-19.70)	11.74 (2.33-21.60)	5.26 (1.86-9.02)
Chlordane	6.05 (3.20-6.47)	5.21 (2.06-6.17)	7.92 (5.60-10.06)	4.13 (3.01-8.30)	5.04 (0.98-8.79)
Dicofol	3.53 (0.78-4.07)	4.38 (1.05-7.80)	5.19 (1.03-8.20)	4.71 (2.95-6.83)	3.89 (1.79-7.82)
Endosulphan	4.81 (2.84-7.09)	3.59 (1.72-1.036)	4.05 (1.81-6.19)	4.32 (0.87-9.09)	3.49 (1.77-4.21)
Alpha HCH	3.20 (0.63-7.21)	4.20 (2.88-7.13)	1.60 (0.40-2.90)	0.60 (0.12-1.22)	0.40 (0.08-0.80)
Beta HCH	11.50 (2.30-16.89)	10.40 (3.80-14.13)	4.80 (2.90-5.23)	1.70 (0.45-3.33)	4.40 (1.83-6.27)
GammaHCH	3.0 (0.68-6.87)	4.50 (2.01-7.95)	1.30 (0.50-2.59)	0.60 (0.18-1.27)	0.60 (0.20-1.10)
delta-HCH	5.40 (1.10-8.70)	3.60 (1.08-4.39)	2.20 (0.56-4.76)	0.60 (0.17-1.24)	0.20 (0.04-0.40)
HCH Total	23.1 (4.71-39.67)	23.1 (9.77-33.6)	9.80 (4.36-15.48)	3.40 (0.92-7.03)	5.60 (2.15-7.85)
p-p DDE	2.10 (0.49-2.71)	0.80 (0.24-1.53)	5.20 (2.03-7.72)	0.40 (0.09-0.66)	0.30 (0.06-0.46)
p-p DDD	2.40 (0.55-2.60)	0.40 (0.07-0.78)	12.60 (3.61-15.42)	'- '	'- '
Op -DDT	'- '	'- '	10.30 (2.02-16.45)	5.80 (1.36-6.10)	0.60 (0.15-1.11)
p-p-DDT	3.40 (0.75-6.73)	6.60 (1.34-12.62)	8.40 (4.70-12.68)	'- '	'- '
Total DDT	7.90 "-"-12.04	7.80 "-"-14.93	35.50 12.36-52.30	6.20 "-"-6.67	0.90 "-"-1.57

'-' = Not detected; value are the mean of five samples; values in parenthesis are range

Materials

- **(a)** Fluorinated ethylene propylene (FEP) centrifuge tube, - 50 mL.
- **(b)** Spatula / spoon and funnel: For transferring sample into centrifuge tubes.
- **(c)** Solvent dispenser and 1-4 L solvent bottle. - For transferring 15 ml 1% of HOAc in MeCN per 15 gram samples in FEP centrifuge tubes or bottles.
- **(d)** Centrifuge tubes (optional). - 10-15 ml graduated. For evaporation and /or dispersive –SPE.
- **(e)** Mini centrifuge tubes (optional) – 2ml for dispersive –SPE (use tubes with o – ring – sealed caps to avoid leaks)
- **(f)** Repeating or volumetric pipettes.-Capable of accurately transferring 0.5-8ml solvents.
- **(g)** Container.- Graduated cylinders volumetric flasks, vials ,and other general containers in which to contains samples ,extracts , solution, standards ,and reagents.
- **(h)** Balance(s). - Capable of accurately measuring weight from 0.05-100g with in ± 0.01g.

(i) Freezer. - Capable of continuous operation <20 ^{O}C.
(j) Food chopper and /or blender. - Preferable S blade vertical cutter and probe blender.
(k) 50 ml Teflon centrifuge tube with screw caps (e.g. Oak –ridge Nalgene 3414 -0050).

Reagents:

(a) Anhydrous magnesium sulphate ($MgSO_4$). - Powder form; purity > 98%.
(b) Acetonitrile (MeCN). - Quality of sufficient purity that is free of interfering compounds.
(c) Acetic acid (HOAc). - Glacial; quality of sufficient purity that is free of interfering compounds.
(d) 1% HOAc in MeCN. - Prepared on a v/v basis (e.g.10 ml glacial HOAc in a 1 L MeCN solution).
(e) Anhydrous sodium acetate (NaOAc). Powder form.
(f) Primary secondary amine (PSA) sorbent. - 40 µm particle size.
(g) C-18 sorbent (Optional). - 40 mm particle size, if sample contain >1 % fats.
(h) Graphical corbon black (GCB) Sorbent (optionl). - 120/400 meshes size, if no structurally planer pesticides are included among the analytes.

Sample collection

Samples of five different varieties of vegetables were collected from local market of Kanpur, Unnao, Lucknow, Kannuj, Fatehpur (U.P. India) for the analysis.

Standard preparation

High purity reference standards of the pesticide analytes , and quality control (QC) and internal standard (ISs) obtained from M/s Phosphorous Limited Mumbai, India and prepared at highly concentrated stock solution in MeCN with 0.1% HOAc .Stored in a dark vials in the freezer .

Sample preparation

Vegetable samples accurately weight 10 gram into a 50 ml centrifuge tube (in triplicate having screw caps) were add to 10 ml of acetonitrile and shake vigorously for one minute. Add 4 g $MgSO_4$, 1g NaCl, 1g Na_3 Citrate dehydrate and 0.5 g Na_2H Citrat sesquihydrates, shake each tube directly after the salt addition shortly ,shake vigorously for 1 min and then centrifuge for 5 min at 3000 U/min. For citrus vegetable co –extracted wax is removed overnight in the refrigerator .5 ml of extract are transferred into a PP single use centrifuging tube, which contains 5 to 25 mg PSA and 150 mg $MgSO_4$ .Shake the mixture for 30 sec and centrifuge it for 5 min at 3000 U/min.5 ml of the extract are transferred into screw cup vial and acidified with 5 into 10 µl 5% formic acid in acetonitrile (10 µl /extract).The cleaned and acidified extract are transferred into auto sampler vials and use for the residues determination by GC techniques[11].

Instrumentation

Analysis was carried out by using a pre calibrated GC machine(Perkin Elmer)with Ni electron capture detector .A stainless steel column (30m×22mm.id)packed with 1.5% ov-17 P+ 1.95 Q f -1 on 100 to 120 mesh chromosorb was used .Operation temperature were programmed at 220,280,300^{O}C for column, injector, detector, respectively. For specially OCPs, the GC oven temperature was programmed at an initial temperature of 80 ^{O}C with the hold time 2 min, increased to 175 ^{O}C at 16 ^{O}C /min and then further increased to 220 ^{O}C at 6 ^{O}C /min and hold for 5 min. Purified nitrogen gas was passing through silica gel and a molecular sieve was used as carrier gas at flow rate of 60ml.per minute.

Quality control

Each congener was identified by matching the retention time in the sample with that in the standard. Procedural blank, consisting of all reagents and glass ware used during the analysis, were periodically determine to check the cross contamination. Since no compound that interfered with is detected the sample values were not corrected for procedural blank.

Table 1 Limit of detection and Limit of quantification

Name of pesticide	Limit of detection (µg/kg)	Limit of quantification(µg/kg)
Alachlor	0.20	0.8
Aldrin	0.20	0.8
Butachlor	0.20	1.0
Chlordane	0.10	0.3
Dicofol	0.20	0.8
Endosulphan	0.10	0.3
Alpha HCH	0.50	1.5
Beta HCH	0.20	0.8
GammaHCH	0.50	1.5
delta-HCH	0.50	1.5
p-p DDE	0.30	1.0
P-P DDD	0.20	0.8
Op -DDT	0.10	0.3
pp-DDT	0.20	0.6

Recovery studies with fortified sample have indicated that overall recovery values exceeded 86%.For 10 -mg vegetable samples the limit of detection (LOD) was about 0.1 to 0.5 µg/kg (Table 1) whereas limit of quantification (LOQ) was about 0.3 to1.5 µg/kg(Table 1). In any case, it is highly desirable to improve the accuracy and precision for OCPs quantification in vegetable samples .One of the most important way to established a common basis for accuracy measurement and quantification is the existence of a reliable certified reference material (CRM).The objective of the present analysis was to test the hypothesis that commercially available vegetable samples may contains amount of OCPs that can be reliably quantified.

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