Journal of Ecobiotechnology 1/1: 041-045, 2009
ISSN 2077-0464

Journal of Ecobiotechnology
http://journal-ecobiotechnology.com/

Determining the status of chlorinated pesticide residue in some leafy vegetables

M.T.I. Chowdhury[1], A.H.M. Solaiman[2], Naheed Zeba[3], Mirza Hasanuzzaman[4], S. S. B. Mustafiz[5], Kamrun Nahar[6]*

[1]*Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh*
[2]*Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh*
[3]*Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh*
[4]*Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh*
[5]*Bangladesh Sugar & Food Industries Corporation (BSFIC), Dhaka, Bangladesh*
[6]*Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh*

**Corresponding Author, Email:mhzsauag@yahoo.com*

Keywords

Organochlorinated Pesticide
Phytochemistry
Plant Analysis
Phytotoxicity
Vegetables

Abstract

A study was carried out to identify the bioaccumulation and the ascertain level of chlorinated pesticide residues in three leafy vegetables viz. red amaranth, spinach and Indian spinach collected from city market. The samples were randomly collected from different shops and analyzed by capillary column of Gas Chromatograph Mass Spectrometry (GCMS) with Electron Impact Ionization (EI) method for the detection of chlorinated pesticide. The results of the study revealed that collected samples of red amaranth and spinach were contaminated with some chlorinated substances. But Indian spinach was free of contamination with organochlorine pesticide.

1. Introduction

Now-a-days the use of pesticides has become indispensable in increasing vegetable crop production because of its rapid effect, ease of application and availability. The use of synthetic organic pesticides, DDT and other organochlorine compounds, began in this country in early 1950's for both agriculture and public health purposes. Bangladesh has been and still is, predominantly, an insecticide consuming country. Up to the present time pesticides belonging to organochlorines, organophosphates, organo carbamates and relevantly small volume of pyrethroid compounds have been used [1].

Over use of pesticides in crop fields has lead to decreased biodiversity of flora and fauna. Residues are present in all compartments of agro-ecosystems, but perhaps the most real risk of human is through consumption of residues in food as vegetables [2]. Reliable data are needed concerning the presence of pesticides outside the targets in order to set priorities for actions to remedy and prevent pollution and to follow the effect of action taken.

The indiscriminate use of pesticides against the pest cause several problems viz., insecticide resistance, toxic residues in vegetables, killing of natural enemies and ultimately pest resurgence. These harmful pesticides are dissolved in our water system and ultimately enter into the system of human, fishes and many other animals and cause severe damage to their health [3]. An intensified use of pesticides can cause a serious public health hazard especially in the form of residues in food [4].

Vegetables are very important group of crops and they constitute major part of the human diet contributing nutrients and vitamins. In Bangladesh, most of the market vegetables contain pesticide residue because of their over use in the field. To assure safety of the consumers, many of the developed countries have set maximum residue limit (MRL) based on the acceptable daily intake (ADI) and potential daily intake (PDI) which should not be exceeded in food item. In Bangladesh context, since harvesting and selling of vegetables of economic importance are done without bothering for the postharvest interval of insecticide use, insecticide residue levels in in those vegetables would mostly go

above MRL. A few pesticides are available in Bangladesh whose retention period is less than 3-5 days.

Modern pesticide residue analysis in developing countries is focusing more and more on subtle problems, such as looking for very low concentrations of pesticides in the environment. For this, complicated and expensive equipments like- gas chromatograph, high performance liquid chromatograph, mass spectrometer etc are being used.

It is very clear that pesticide residue problem is becoming serious focused human health and environmental hazards due to indiscriminate pesticides application. However, importance that for the safe uses of vegetables and other crops a regular, it is very essential a residue analysis program of pesticides is crying need of the day in Bangladesh. The present study was therefore undertaken to determine the presence and concentration of persistent organic pollutants (PoPs) in three leafy vegetables and to determine the presence and concentration of harmful organochlorinated pesticide residues in those vegetables.

2. Materials and Methods

The research work was performed in the laboratory of the Analytical Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh.

2.1. Experimental materials

Samples were collected from different vendors of vegetable market. About 1.0 kg each of three leafy vegetables viz. red amaranth (*Amaranthus gangeticus*), indian spinach (*Basella alba*) and spinach (*Spinach oleracea*) were purchased from different vendors to prepare composite samples for detection of the presence of organochlorinated pesticides if any there in.

2.2. Analytical Methods

For identification of organochlorine pesticides (OCP) and persistent organic pollutants (PoPs) in vegetables following analytical extraction methods were used proposed by Gohlki and Mclafferty [5].

Exactly 250 g of each item of vegetable was taken for analysis. Each sample was sliced on chopping board and tissues were dissected from the vegetable. Chopped in small pieces and mixed thoroughly. Each sample was macerated in a mortar. Ground samples were taken in a conical flask. 250 mL of each solvent, n-Hexane and 95% ethyl alcohol was added and the mixture was shaken, preferably mechanically for 1 hour. 50 gm of anhydrous sodium sulfate was added before mixing to remove excess water [5].The extract was filtered through the filter paper, until no solvent remained into the conical flask. The amount of extracted solution was about 100ml. The liquid phase was transfer to a large separating funnel and diluted with 250 mL of water, shaken for 10 minuets and allowed the phases to separate. In the first rinse, the aqueous phase contains about 30 percent alcohol, in which solvents such as hexane were soluble only to about 1 percent. The various insecticides distribute themselves in favor of the water insoluble phase, which contained only about 3 percent of alcohol. This small amount of alcohol was removed by the second rinse.

Fifty mL of the extract was transferred to a round bottom flask and the volume was reduced to about 5 ml with a rotary evaporator and water bath at 37°C. 5 g of fine sea sand was added to the flask and continue the evaporation with constant stirring to ensure that the plant extractives represent were left in a thin coat on the sand. 5 mL of acetonitrile was added to the beaker and placed on a hot plate. The mixture was heated and stirred to the boiling point. 6 ml of water was added to the hot solution immediately after boiling and then was allowed cooling. The entire volume was added to the column. The beaker was rinsed with hot 40 percent acetonitrile and was placed in the column. Above air pressure was applied to the column to the column to hasten the entry of the 60 percent acetonitrile and 40 percent distilled water solution of the extract. When the liquid level just reached the top of the granular packing the pressure was released and 100 mL of developing eluent was added. The pressure was applied again and the eluate was collected at a rate not exceeding 1 mL per minute. The eluate was placed into a 500mL separator funnel, 50 mL of water was added, shaken, 100mL of hexane was added, shaken thoroughly, and allowed to separate. The extraction was repeated with an additional 25 mL of hexane. The Hexane layer was combined to make up to a definite volume. This solution could be used for the chemical test.

Extract 0.4 μL of sample in volume was taken with a micro syringe and injected into injection port of the Gas Chromatograph Mass Spectrometer capillary column, which was fitted with Electron Impact Ionization.

2.3. Detection of pesticides

For organochlorine pesticide detection E1 mode of Gas Chromatograph Mass Spectrometer was used. Identification of the suspected pesticide was carried out to retention time of the pure analytical standards.

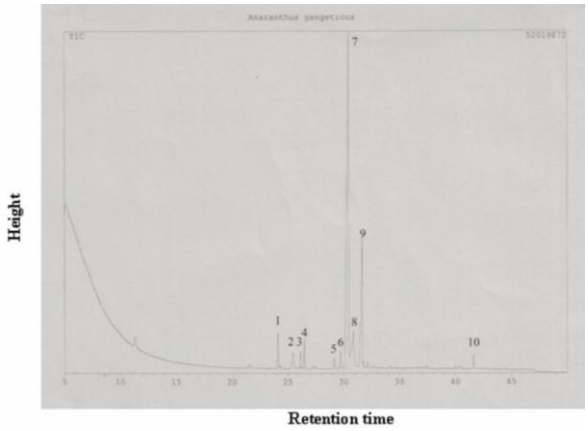
The function of Gas Chromatograph Mass Spectrometer is identification, quantification and analysis of a compound. Name, molecular formula, molecular structure, molecular weight and fragmentation pattern can also be detected by GCMS.

3. Results and Discussion

3.1. Chromatogram of Red amaranth

The red amaranth (*Anaranthus gangeticus*) samples were analyzed and chlorinated pesticide residues were detected by GCMS for this study. The chromatograph shows the presence of different organic compounds in vegetable samples. Detected peaks due to different organic compounds present in spinach sample have been presented in Fig 1. The major organic compounds found in red amaranth sample were 2-Pentadecanone, 6,10,14-trimethyl; Trans-undec-4–enal; hexadecanoic acid, ethyl caster; 2-hydroxy 1,1,10– trimethyl-6,9- cpidioxydecal; Cyclopentanol 2,4,4 -trimethyl; Phytol; cyclo pentanone, 2 (2-octenyl); 3, 7,11, 15–tetramethyl-2 hexadecen-1-ol.; heptacosane, 1-chloro–SS; Tritetra contanne; Phytol; 11-oxa-dispiro(4.0.4.1) udecan-1-ol; 3,7,11,15-tretramethyl-1-hexadecen-1-ol; chdestan-3-ol, 2-methylone (3.beta. 5alpha). Among the 14 compounds only heptacosane, 1-chloro–SS was found as chlorinated compound in red amaranth samples (Table 1).

Figure 1. Chromatogram of red amaranth in GCMS



From the chromatograph of red amaranth (*Amaranthus gangeticum*) Fig. 1 and Table 1 it appears that the tissue contained 14 organic compounds. Among those one compound, (Heptacosane, 1-chloro–SS) was chlorinated, which is harmful for human body. Jha & Mishra [6] showed the side effects of pesticides application on vegetable. The average concentration of total HCH was 0.046, 0.030, 0.027 and 0.009 micro g/g in vegetables, sugarcane, rice and pulse growing soils, respectively. Total DDT concentration was more than total HCH in all cropping systems. Endosulfan had the least value.

Table 1. List of detected organic compounds with chlorinated pesticides in red amaranth (*Amaranthus gangeticus*) sample

Peak	Name of Compound	Mol. wt.	Mol. Fromula	Chlorinated Compound
01	2-Pentadecanone, 6,10,14-trimethyl	268	$C_{18}H_{36}O$	–
02	Trans- undec- 4 –enal	168	$C_{11}H_{20}O$	–
03	Hexadecanoic acid, ethyl caster	284	$C_{18}H_{36}O_2$	–
04	2-Hydroxy 1,1,10 – trimethyl-6,9- cpidioxydecal	226	$C_{13}H_{22}O_3$	–
05	Cyclopentanol 2,4,4 –trimethyl	128	$C_8H_{16}O$	–
06	Phytol	296	$C_{20}H_{40}O$	–
07	Cyclopentanone, 2 (2-octenyl)	194	$C_{13}H_{22}O$	–
08	3, 7,11, 15 – tetramethyl -2 hexadecen -1-ol.	296	$C_{20}H_{40}O$	–
09	Heptacosane, 1-chloro –SS	414	$C_{27}H_{55}Cl$	√
10	Tritetra contanne	604	$C_{43}H_{88}O$	–
11.	Phytol	296	$C_{20}H_{40}O$	–
12	11-oxa-dispiro(4.0.4. 1) udecan-1-ol	168	$C_{10}H_{16}O_2$	–
13.	3,7,11,15-tretramethyl-1-hexadecen-1-ol	296	$C_{20}H_{40}O$	–
14.	chdestan-3-ol,2-methylone (3.beta 5alpha)	400	$C_{28}H_{48}O$	–

3.2. Chromatogram of Spinach

The spinach (*Spinach oleracea*) samples were analyzed and chlorinated pesticide residues were detected by GCMS for this study. The chromatograph shows the presence of different organic compounds in vegetable samples. Detected peaks due to different organic compounds present in spinach sample have been presented in Fig. 2. The major organic compounds found in spinach samples were cyclopentane-1,2,3,4,5- pentamethyl; 1-chloro heptacosane; decane, 5- cyclohexyl; Tetratriacontane; pentane, 3- (2, 2-dichloro-3- methylcyclopropyl; 1, 3-cyclopentanediol, cis-SS; 2, 3, 5, 6-detetrahydro cyclohexanoe, 2, 6- di-t-butyl- 4-hydroxymethylene ; decan, 1,1-oxybis–SS; 3,7,11,15-tetramethyl-2-hexadecen-1-ol. 1–octene 3,4–dimethyl-SS; octadecane, 5-methyl; 9-octadecenoic acid (z), hexyl ester; hexyl octyl ether–SS; nomanoyl chloride-SS. Among the 14 compounds three samples contained 1-chloro heptacosane; pentane, 3-(2,2-dichloro-3-methylcyclopropyl and nomanoyl chloride-SS respectively was chlorinated compound in spinach (Table 2).

The chromatograph of spinach (*Spinacea oleracea*) samples (Fig. 2) shows the presence of 14 organic compounds in tissues, of which 3 were chlorinated compounds viz. pentane, 3-(2,2-dichloro-3-methyl cyclopropyl, 1-chloroheptacosane and nomanoyl chloride-SS (Table 4.1). These compounds are very much harmful for our health. The findings of the present study keep in with the study of Gao [7] who found the residue and bioaccumulation of organochlorine pesticides (OCPs) in spinach. The tested OCPs included o,p'-DDT, o,p'-DDE, p,p'-DDT, p,p'-DDE, p,p'-DDD, alpha -HCH, beta -HCH, sigma -HCH, gamma -HCH [lindane], HCB, dieldrin and endrin. Most of the OCPs gave recoveries between 80 and 120%, their relative

standard deviation ranged from 3 to 15% while the recoveries of dieldrin and endrin were only between 20 and 50%. The residual levels of OCPs in spinach (95.3 mg/g) were slightly less. DDTs and HCHs were the main OCP residues, which accounted for 50-75 and 14-28%, respectively, of all OCP residues detected. The ratios of gamma / alpha -HCH and DDT/DDE in the vegetables ranged from 1.0 to 2.5 and 1.0 to 4.2, respectively.

Figure 2. Chromatogram of spinach in GCMS

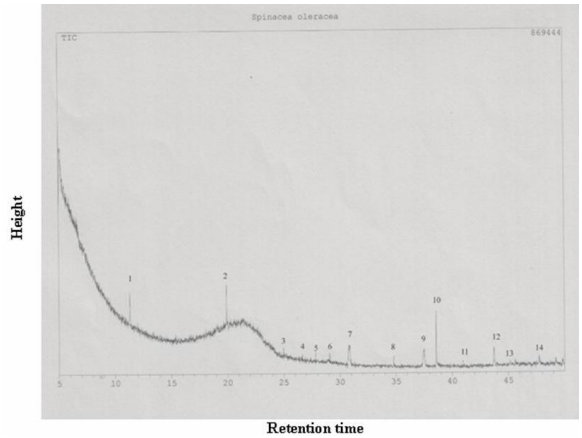


Table 2. List of detected organic compounds with chlorinated pesticides in spinach (*Spinacea oleracea*) sample

Peak	Name of Compound	Mol. Wt.	Mol. Fromula	Chlorinated Compound
01.	Cyclopentane-1,2,3,4,5- pentamethyl	140	$C_{10}H_{20}$	–
02.	1-chloro heptacosane	414	$C_{27}H_{55}Cl$	√
03.	Decane, 5- cyclohexyl	224	$C_{16}H_{32}$	–
04.	Tetratriacontane	478	$C_{34}H_{70}$	–
05.	Pentane,3-(2,2-dichloro-3-methylcyclopropyl	194	$C_9H_{16}Cl_2$	√
06.	1,3-cyclopentanediol, cis-SS	102	$C_5H_{10}O_2$	–
07.	2,3,5,6-Detetrahydrocyclohexanoe, 2,6-di-t-butyl-4-hydroxymethylene	234	$C_{15}H_{22}O_2$	–
08.	Decan, 1,1-oxybis –SS	298	$C_{20}H_{42}O$	–
09.	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{42}O$	–
10.	1 – Octene 3,4 –dimethyl SS	140	$C_{10}H_{20}$	–
11.	Octadecane, 5-methyl	268	$C_{19}H_{40}O$	–
12.	9-Octadecenoic acid (z), hexyl ester	366	$C_{24}H_{46}O_2$	–
13.	Hexyl octyl ether –SS	214	$C_{14}H_{30}O$	–
14.	Nomanoyl chloride-SS	176	$C_9H_{17}ClO$	√

3.3. Chromatogram of indian spinach

The indian spinach (*Basella alba*) samples were analyzed and chlorinated pesticide residues were detected by GCMS for this study. The chromatograph shows the presence of different organic compounds in vegetable samples. Detected peaks due to different organic compounds present in spinach sample have been presented in Fig. 3. The major organic compounds found in indian spinach sample were phytol; hexadecanoic acid, ethyl ester-SS; phytol; cyclopentanone, 2-(5-oxyheayl-SS; dinoleic acid, ethyl ester; 9,12,15-octadeeatrienoic acid, methyl ester; 2,6,10,14,18-Pentamethyl-2,6,10,1418-cicas; 1,2-Benzenedicarboxylic acid; nonadecane SS-n- nona decaness; O-mannitol 1, 1-1, 16- hexadecanediylbis-111-trifluoro hexadecane-2-one-SS; 9-bromono raldehydess; (2-methoxy-1,3,2-thiozin) (5,10,9), pregnan -21-ol-3,11,20 trione SS; AD-neooleana-12,14-diene, (3xi,5,alpha). Among the 14 compounds none was found to be chlorinated (Table 3)

Figure 3. Chromatogram of indian spinach in GCMS

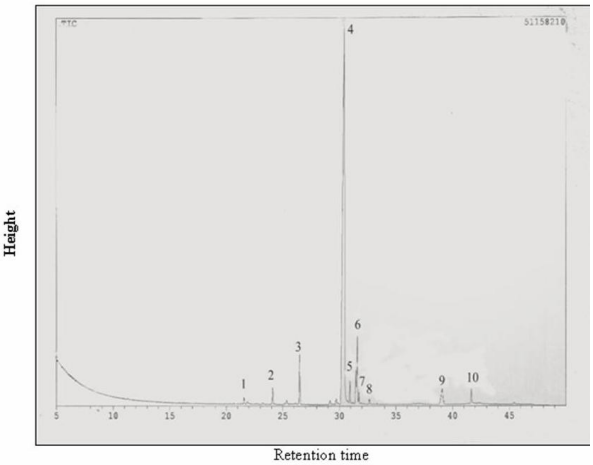


Table 3. List of detected organic compounds with chlorinated pesticides in indian spinach (*Basella alba*) sample

Peak	Name of Compound	Mol. Wt.	Mol. Fromula	Chlorinated Compound
1.	Phytol	296	$C_{20}H_{40}O$	-
2.	Hexadecanoic acid, ethyl ester SS	284	$C_{18}H_{36}O_2$	-
3.	Phytol	296	$C_{20}H_{40}O$	-
4.	Cyclopentanone, 2-(5-oxyheayl-SS	182	$C_{11}H_{24}$	-
5.	dinoleic acid, ethyl ester	308	$C_{20}H_{36}O_2$	-
6.	9,12,15-octadeeatrienoic acid, methyl ester	292	$C_{19}H_{32}O_2$	-
7.	2,6,10,14,18-Pentamethyl-2,6,10,1418-cicas	342	$C_{25}H_{42}$	-
8.	1,2-Benzenedicarboxylic acid	390	$C_{24}H_{38}O_4$	-
9.	Nonadecane SS-n- Nonadecane-SS	268	$C_{19}H_{40}$	-
10.	O-Mannitol 1,1-01,16-hexadecanediylbis-	586	$C_{28}H_{58}O_2$	-
11.	111-Trifluoro hexadecane-2-one-SS	308	$C_{17}H_{31}FO$	-
12.	9-Bromoneraldehyde-SS	220	$C_9H_{17}BO$	-
13.	(2-Methoxy-1,3,2-thiozin) (5,10,9), pregnan -21-ol-3,11,20 trione SS	433	$C_{23}H_{31}NO_{55}$	-
14.	AD-Neooleana-12,14-diene, (3xi,5,alpha)	408	$C_{30}H_{48}$	-

The chromatograph prepared with the extract of the collected samples of indian spinach (*Basella alba*) (Fig. 3) shows that there were as much as 14 organic compounds in tissues of the samples. Among these compounds not a single chlorinated compound could be detected (Table 3). An-QiOng *et al.* [8] observed that the residues of organochlorine pesticides was widely distributed in indian spinace, with an occurrence of 100%.

Pesticide residues have a relation with lipid content of vegetables. Lipids and fats act as a storage depot for organic toxic compounds [9]. Pesticides may enter into vegetables through the initial stage of food chain. The presence of pesticide residues in

fresh vegetables is an important factor for pesticide residues content in vegetable [10] and different parts of the plants contain different amount of pesticide residues [11] but presence of pesticides in vegetables depends on the biodegradation of pesticide in the agricultural lands.

Conclusion

The analytical observations indicated that the leafy vegetables samples contained different types of organic compounds. In some vegetables a single or a few organochlorinated compounds could be detected. But due to some inconveniences the amount of those chlorinated compounds and whether they are originated from any pesticide could not be ascertained. However, the organochlorinated compounds detected in different vegetables can be summarized as follows. The extract of red amaranth samples had also 14 compounds detected as organic and among them one compound - heptacosane, 1-chloro –SS was detected to be chlorinated. In indian spinach also 14 compounds were detected as organic of which 3 were chlorinated and they were pentane, 3-(2,2-dichloro-3-methylcyclopropyl), 1-chloro hepta cosane and nomanoyl chloride-SS. As regard to the extract of indian spinach there were 14 compounds detected to be organic. But not a single one was chlorinated.

References

[1]. Rahman, M.S., M.A. Malek and M.A. Matin, 1995. Trend of pesticide usage in Bangladesh. *Sci. Total Environ.*, 156, 33-35

[2]. Price, C., 2008. Implications of Pesticide residues in interrelated ditch-duke farming systems. Central Thailand. Aquaculture News 32: 23.

[3]. Khandakar, K., 1990. Population, Agriculture And Environmental Degradation. An article published in the daily "The Bangladesh Observer" July 28, 1990.

[4]. Mansingh, A., D.E. Robinson, N. Walker and C. Thomas, 1996. Distribution, fate and effects of pesticides in tropical Marine environment, presented at he third IAEA-MEL Res. Coordination meeting Heredia, Costa Rica, 9-13 September, 1996.

[5]. Gohlki, R.S. and F.K. Mclafferty, 1993. Early gas chromatography/mass spectrometry. *J. Amer. Soc. Mass Spectromet*., 4 (5): 367-371.

[6]. Jha, M.N. and S.K. Mishra, 2005. Decrease in microbial biomass due to pesticide application/residues in soils under different cropping systems. *Earth Environ. Sci.* 75(2): 316-323.

[7]. Gao, H.J., X. Jiang, F. Wang, D., Z. Wang and Y. R. Bian, 2005. Residual levels and bioaccumulation of chlorinated persistent organic pollutants (POPs) in vegetables from suburb of Nanjing, People's Republic of China. *Bull. Environ. Contam. Toxicol.*, 74(4): 673-680.

[8]. An-QiOng, D.Y. Hua, W. Hui, W. Xia, W.M. Nong and G.Z. Xiang, 2004. Organochlorine pesticide residues in cultivated soils, in the South of Jiangsu, China. *Acta Pedol. Sinica*., 41(3): 414-419.

[9]. Doull, J., C.D. Klaassen and M.O. Amdur, 1975. *Toxicology The Basic Science of Poison*. 2nd ed. Macmillan publishing Co. Inc. New York.

[10]. Reinert, R.W., D.B. Menzel, D.T. Martin and H.S. Olcott, 1970. DDT residues in pacific sea birds, a persistence insecticide in marine food chain. *Nature*. 216:589-590.

[11]. Elzorgani, G.A., A.M. Abdullah and M.E.T. Ali, 1979. Residues of organochlorine insecticides in fish in Lake Nubia. *Bull. Environ. Contam. Toxicol.*, 22:44.