

STATUS OF CHLORINATED PESTICIDE RESIDUE IN SOME SELECTED VEGETABLES

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CERTIFICATE

This is to certify that the thesis entitled "Status Of Chlorinated Pesticide Residue In Some Selected Vegetables" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE IN AGRICULTURAL CHEMISTRY* embodies the result of a piece of *bona fide* research work carried out by Md. Tazul Islam Chowdhury, Registration No. 00757 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any other institutes.

I further certify that such help or sources of information, as have been availed during the course of this investigation have duly been acknowledged.

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STATUS OF CHLORINATED PESTICIDE RESIDUE IN SOME SELECTED VEGETABLES

ABSTRACT

A study was carried out to identify the bioaccumulation and the ascertain level of chlorinated pesticide residues in some vegetables collected from market baskets namely potato, tomato, carrot, red amaranth, spinach and indian spinach. 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 potato, tomato, red amaranth and spinach were contaminated with some chlorinated substances. But Indian spinach and carrot were free of contamination with organochlorine pesticide.

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LIST OF ABBREVIATIONS AND ACRONYMS

GC	= Gas chromatograph
GC-MS	= Gas chromatograph mass spectrometer
OCPs	= Organochlorine pesticides
DDD	= Dichloro-diphenyl-dichloroethane
DDE	= Dichloro-diphenyl-ethane
DDT	= Dichloro-diphenyl-trichloroethane
BHC	= Bengenehexachloride
Pops	= Persistent organic pollutants
HCH	= Hexachlorocyclohexane
HCB	= Hexachlorobenzene
PCBs	= Polychlorinated biphenyls
PCDDs	= Polychlorinated Dibenzo-p-Dioxins
PCDFs	= Polychlorinated Dibenzofurans
EI	= Electron impact ionization
ECD	= Electron capture detector
DOF	= Directorate of fisheries
FAO	= Food and Agriculture Organization
FDA	= Food and Drug Administration
GOB	= Government of Bangladesh
BBS	= Bangladesh Bureau of Statistics
WB	= World Bank
BCSIR	= Bangladesh Council of Scientific & Industrial Research
MLD	= Maximum Lethal Dose
MRL	= Maximum Residue Limit
N ₂	= Nitrogen
NaCl	= Sodium Chloride
ND	= Not detected
NPMP	= National Pest Monitoring Programme
et al.	= Et alibi (and others)



i.e.	= That is
kg	= Kilogram
lb.	= Pound
mg	= Milligram
mm	= Millimeter
cm	= Centimeter
mL	= Milliliter
min	= Minute
KPa	= Kilo Pascal
α	= Alpha
β	= Beta
γ	= Gamma
mt	= Metric-ton
μ L	= Micro liter
μ g	= Micro gram
ng	= Nanogram
No.	= Number
OP	= Organophosphorus
Op	= Ortho para
pp	=Parapara
ppb	= Parts per billion
ppm	= Parts per million
PR	= Pesticide residue
%	= Percentage
>	= Greater than
$^{\circ}$ C	=Degree centigrade
TIC	= Total ion chromatogram
g	= Gram

A 3D graphic of a green arrow that has been bent into a square shape, forming a frame. The arrow's tail is at the top-left, its head is at the top-right, it continues down the right side, and then back up the left side. The arrow is rendered with a light green fill and a dark green outline, giving it a three-dimensional appearance with shadows.

CHAPTER 1
INTRODUCTION

CHAPTER 1

INTRODUCTION

Bangladesh is an agricultural country where more than 80% of the people depend on agriculture for their livelihood. Since there is no scope for horizontal expansion of land area, to achieve the targeted level of food production, the major emphasis has, therefore, been given on increasing the existing level of productivity of different crops through wider adoption of cost effective technologies, bringing more areas under high yielding varieties, hybrid and increasing the cropping intensity with the help of irrigation facility along with use of chemical fertilizers and pesticides (Mukhopadhyay, 2005). 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, organocarbamates and relevantly small volume of pyrethroid compounds have been used (Rahman , *et al.* 1995).

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 (Price, 2008). 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.

Farmers of Bangladesh in general use many types of pesticides to control harmful insects to minimize crop losses. As most of our people are illiterate they use



pesticides indiscriminately. 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 (Khandakar, 1990).

All pesticide compounds potentially pose environmental hazards as they are chemically tailored to be toxic. While causing lethal effect to target pests, these chemicals may evoke acute and chronic toxic effect to non - target organisms. An intensified use of pesticides can cause a serious public health hazard especially in the form of residues in food (Mansingh, *et al.*1996).

Vegetables are very important group of crops and they constitute major part of the human diet contributing nutrients and vitamins. Many farmers in the villages have taken up vegetable production on commercial basis and some grow them in home gardens. But in the urban areas people depend on the market for their vegetable requirements. These market vegetables mostly contain pesticide residue because of their over use in the field, which cause harmful effect for the human health. 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 those vegetables would mostly go above MRL.

Selling of vegetables after 1-2 days of spraying pesticides has become a normal practice in most of the areas of Bangladesh. A few pesticides are available in Bangladesh whose retention period is less than 3-5 days. Macintyre *et al.*, (1989) reported that low level exposure of food products containing pesticides residue to consumers' products over time and again might cause cancer, teratogenesis, genetic

damage and suppression of the immune system. So, it is very important to determine the pesticide residue level in different crops such as tomato, potato etc.

Modern pesticide residue analysis in develop 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.

Therefore, it is very clear that pesticide residue problem is becoming serious focused human health and environmental hazard due to indiscriminate pesticides application. So, it is 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 with the following objective:

- to determine the presence and concentration of persistent organic pollutants (PoPs) in some selected vegetables and
- to determine the presence and concentration of harmful organochlorinated pesticide residues in some selected vegetables.



***CHAPTER 2
REVIEW
OF
LITERATURE***

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 Historical background of usage of pesticides

The evolution of synthetic organic pesticides is a significant event of the twentieth century. In fact, the discovery of the insecticidal properties of DDT in 1939 followed by a gradual but rapid introduction of other members of the organochlorine, organophosphorous, organocarbamate and pyrethroid groups along with compounds with herbicidal and fungicidal properties in the second generation organic pesticides was probably the most revolutionary development in the history of pest control.

Although the first generation inorganic insecticides were available before 1930, none of the substances was ideal for use in agriculture and public health. Some were very poisonous to man, these include arsenic and nicotine. Insecticides of plant origin-pyrethrum and derris were useful particularly for control of mosquitoes and other insects of medical importance, but they were available only in limited quantities and they soon lost their potency when exposed to sunlight and to the atmosphere (Pedigo, 1991).

DDT and other synthetic organic pesticides introduced since the Second World War time and very soon they recognized as wonder pest control chemicals and their increasing uses in the post-war world have significantly contributed in the well being of the mankind. Acute and chronic toxic effects of pesticides on animals are the results of interference with well established biochemical process (Hassall, 1990).

2.2. Pesticide Consumption Status in Bangladesh

Systemic organic pesticides, DDT and other organochlorine compounds, began to be used in the country from early 1950's for both agricultural and public health purposes. Bangladesh has been and still is predominantly an insecticide consuming country. Up

to the present time insecticides belonging to organochlorine, organophosphorus, organocarbamates and relatively small volume of pyrethroid compounds have been used. (Rahman, *et al.* 1995)

Reviewing the consumption of pesticides over the last 45 years it has been observed that during 1950-1960 these crop protectants were distributed free of cost along with spraying services involving equipment by the Agriculture Extension Department. With lifting of subsidy beginning from 1974 till 1979, there was a sharp decline consumption of pesticides. It then started to pick-up gradually.

Agricultural consumption of pesticides was 2510 t (t = 1000 kg) in 1980 which doubled in 1989 with total uses of 5150 t the increase in agricultural consumption of pesticides in the country was not evenly spread over each year (Table-1). In 1984, there was practically no increase in consumption of pesticides. There was a decline of pesticide usage in 1985 and again in 1987 and 1988 due to devastating floods. Annual consumption of pesticides for agriculture, as formulated product, was 7800 t in 1994. The consumption has increased to about 8000 t in 1995 (Matin, 1995).

Table 2.1. Annual Import of Pesticides in Bangladesh

Year	Quantity in Metric Ton (t)	Value in Taka (in *Lakh)	Cost per kg. granular product (in Taka)
1985-86	3064	2229	73
'86 87	2683	1827	68
1987-88	4244	2606	61
1988-89	6604	4520	68
1993-94	8200	10000	121
1994-95	8300	11000	150
1995-96	8500	12000	150

Source: BBS, 1997

2.3. Toxicological considerations of pesticides

All pesticide compounds potentially pose an environmental health hazard as they are chemically tailored to be toxic. While causing lethal effects to target pests these

chemicals may evoke acute or chronic toxic effects to non-target organisms. Acute poisoning of human is usually limited to accidental over-exposure of plant protection personal or persons coming into direct contact with pesticides in agriculture, industry or otherwise. Acute toxicity is the damage done by a single dose, large or small, of a pesticide. Chronic toxicity, on the other hand, is the sort of toxicity which might eventually occur if very small amounts of a pesticide were to get into the body, day by day, week by week, for a long time. Effects are usually diffuse and difficult to pin down to one particular cause, and include damages to the liver (the organ, which tries to detoxify the poison) or to the kidneys (the organs which try to excrete it or its metabolic products). Other possibilities are damage to the circulatory system or to the brain, the development of cancer, malformation of the fetus (teratogenic effects) or damage to chromosomes (mutagenic effects) etc. (Cremlyn, 1980).

2.3.1 Pesticide Residue Tolerances

Acceptable Daily Intake (ADI) values of a number of pesticides have been published jointly by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO). Safety factors, ADIs and the aspects of the evaluations of health hazards have been reviewed by Sharratt (1977) and Vettorazzi (1977). FAO and WHO through their Codex Committee on Pesticide Residues (CCPR) have worked out international pesticide residue tolerances which are intended as guidelines for world-wide national legislation. These tolerances (Maximum Residue Levels-MRLs) are set according to the philosophy that no crop should be treated with pesticides at higher application rates than necessary.

2.3.2 Role of UN Bodies

The UN bodies, FAO, WHO, CCPR evaluate pesticide residues and decides what maximal residue levels (MRLs) of a pesticide should be allowed for each pesticide on each crop. CCPR also establishes an Acceptable Daily Intake (ADI) for each pesticide for an ordinary individual. ADI can be calculated from the analytically determined



amounts of a named pesticide that are present in different foods eaten by any one person, plus a knowledge of the proportion of each type of food in that individuals daily diet. The conclusions of this scientific committee are eventually published by the Codex Alimentarius Commission. More than 200 international standards have been developed and adopted for individual food commodities. They, virtually, cover the whole spectrum of marketable foods milk and milk products, processed fruits and vegetables, quick frozen foods, cocoa and chocolate, sugars, fats and oils, meat and processed meat products, fish and fishery products. Several thousand maximum limits for pesticide residues have been developed for a wide range of pesticide chemicals. An account of CCPR and the methods by which MRLs are set has been described by Boardman (1986).

The maximum levels of pesticide residues (MRLs) that may be allowed in agricultural commodities and foodstuffs are legal matters and need to be regulated. Strict surveillance as part of enforcement of allowable tolerances (MRLs) in food and environment can not be compromised for safe uses of pesticides and human health.

2.4 Review of Insecticide Residues

Their *et al.* (1989) reported that during the past few years' pesticide residues of the German Chemical Society has organized 6 laboratory performance tests in which numerous laboratories were involved. In these tests, the choice of analytical methods for the examination of fats or vegetable substance was free. Organochlorine pesticides at over 0.01 mg/kg were most readily identified, whereas in the analysis of organophosphorus residues often only the classic compound such as Parathion and Diazinon were reported. Many false positive results could have been avoided by using more accurate methods for confirmatory analysis. The quantitative results, however, were generally quite reliable. It can be concluded that the performance of a residue laboratory is not constant, and that it is necessary to assess regularly the quality of the results by participating in such inter laboratory tests.

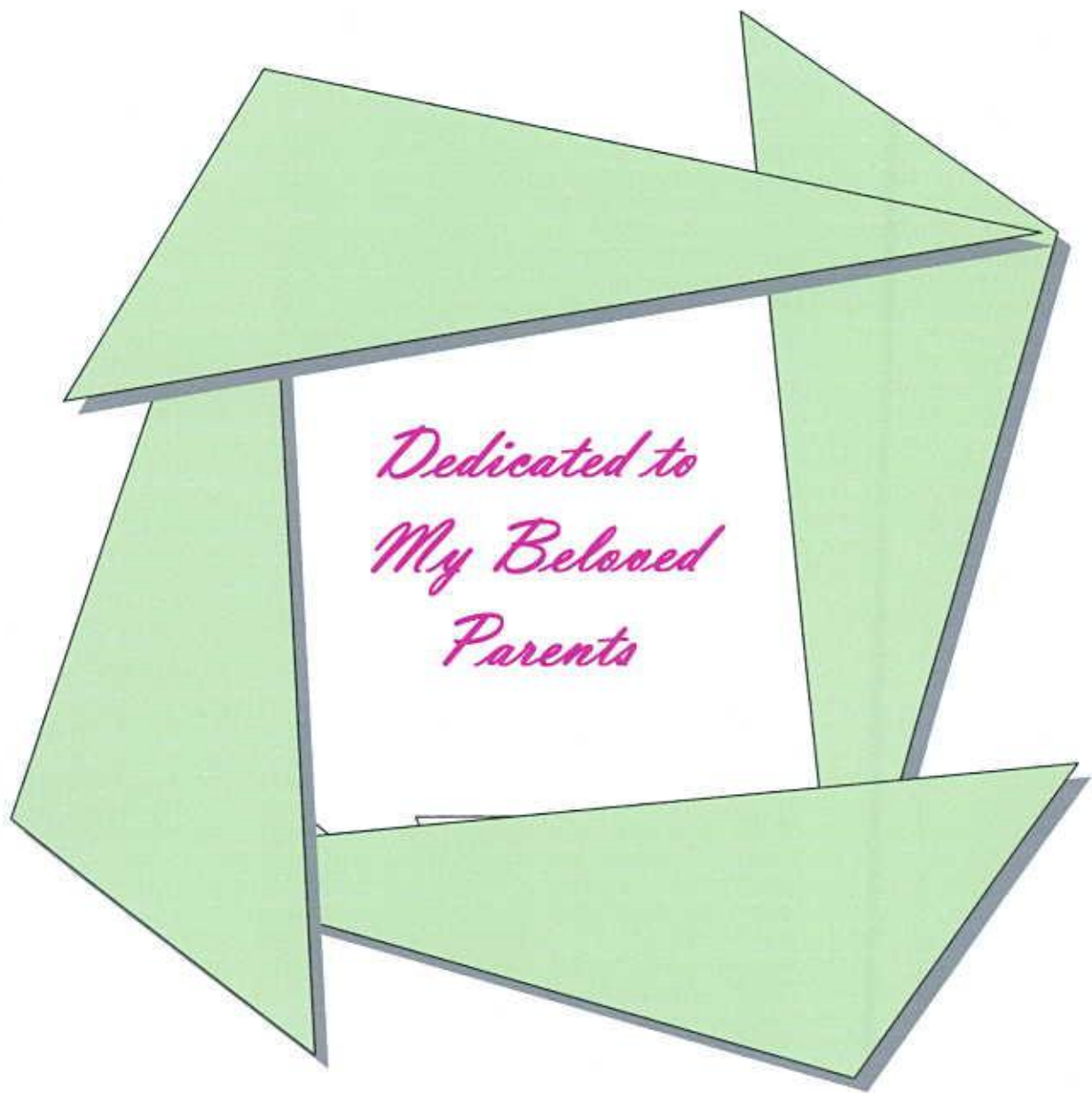
A study was carried out in Ludhiana, India to determine the residues of Permethrin applied for the control of *Leucinodes orbonalis* on eggplant fruit. The compound was sprayed at a rate of 50 g a.i/ha at fortnightly intervals. Initial deposits on fruit range from 1.3 to 0.7 mg/kg and maximum residue level 1, 2, 3 and 10 days after spraying was 0.34, 0.2, 0.11 and 0.07 mg/kg respectively. The half life on insecticides on fruits ranged from 2.1 to 3.0 days. The trans-isomers of Permethrin degraded slightly faster than the cis-isomers in leaves and fruits. A 1-day waiting period is recommended for consumption of fruits (Singh and Kalra, 1989).

According to Singh and Karla (1992) Gas-liquid chromatography determination of Cypermethrin residues in tomato fruits, leaves and soil samples drawn at 0, 1, 2, 5 and 10 days after treatment were analyzed. The analytical methodology used involved acetone-hexane extraction, silica gel column clean-up and gas liquid chromatographic estimation. The minimum limits of cis-cypermethrin and tras-cypermethrin were 0.008 and 0.006 mg/kg. Initial deposit of Cypermethrine on fruit was observed 0.73 mg/kg after 8 sprays @ 50g/kg a.i/ha, which declined to 0.61 mg/kg one day after treatment and then became 0.08 mg/kg after 10 days.

FAO/WHO (1993) reported trials that were conducted in Canada, France and the USA using emulcifiable concentrate, wettable powder and granular formulations of organochlorine insecticides in potatoes. In the US trails a GR or EC pre-planting application at 4.4 kg a.i/ha was followed by five foliar sprays at weekly intervals with WP or EC formulations at the rate of 0.55 kg a.i/ha, the other trials were with granules at 2.25 or 10 kg a.i/ha one month after planting. No residues were detectable (<0.01 mg/kg) in any of the harvested samples except in three trials where residues of 0.01 mg/kg were found.

FAO/WHO (1993) reported that eight trials with summer squash are carried out in the USA using granules pre-planting with an application rate of 4.48 kg a.i/ha and five foliar sprays at weekly intervals with WP or EC formulations at a rate of 0.83 kg a.i/ha. Residues of summer squash 3 to 14 days after the last application were <0.01-0.18 mg/kg.





*Dedicated to
My Beloved
Parents*

The multi-residue method was based on acetone extraction, partitioning with n-hexane/methylene chloride followed by Gel Permeation Chromatography (GPC) clean-up and determination by capillary GC using conventional detectors such as ECD, FPD, NPD and FID. A multi-residue method based on ethyl acetate extraction followed by GPC -clean-up and GC determination using conventional detectors. This is still the main method used for the analysis of fruit and vegetables in Sweden (Akterblom, 1995).

Dethe *et al.* 1995 conducted a study on the residues of commonly used pesticides in/on vegetables in India. Detectable levels or residues were observed in 33.3% of, tomatoes (Diazinon, Endosulfan, Dimethoate and Monocrotophos), 73.3% of eggplant (Endosulfan, Diazinon, Cypermethrin, Fenvalerate, Quinalphos, Dimethoate and Monocrotophos), 14.3% of okras (Endosulfan), 88.9% of cabbage (Endosulfan, Fenvalerate, Cypermethrin, Dimethoate and Monocrotophos). The obtained results of pesticide residues were lower than the maximum residue limits (MRL) prescribed.

Tejada *et al.* (1995) reported that pesticide management survey revealed the crop protection practices of 51 farmers involved in Tomato and Okra production in six provinces of Philippines, market surveys in Laguna and metro Manila showed some samples of tomato and okra containing Triazophos, Carbaryl and Deltamethrin residues. The dissipation of Triazophos and Carbaryl residues in Okra and Tomato were monitored in both supervised and farmer cooperators field trails. Triazophos residues in Okra persisted up to the 7th day while a rapid decline was observed for residue in Tomato. A pre-harvest interval of 5-7 days may be safe for Triazophos use in Tomato Carbaryl degraded rapidly in Tomato and a pre-harvest interval of 3 days may be safe. Farmer practice of insecticide usage in Eggplants generally showed the use of recommended dosage rates and proper observance of recommended pre-harvest intervals. Washing of Tomato is strongly recommended as it reduces Triazophos residues by 20-90%.

Ahuja *et al.* (1998) reported that Cauliflowers, Cabbages, Tomatoes, Brinjal, Okras, Field beans and Cucumbers were monitored for residues of HCH and its isomers, Endosulfan, Dimethoate, Monocrotophos, Quinaiphos, Fenvalerate, Cypermethrin. The residues of alpha, beta, tau isomers of HCH, Endosulfan, monocrotophos, Quinalphos, Dimethoate were detected in most of the samples. However, the residues of Monocrotophos on tomatoes, brinjal and okras and those of Carbendazim on French beans were found to persist over the prescribed maximum residue limit values.

The Malathion was applied at 62.0 kg a.i./ha to lettuce and the plants were harvested 14 days after the last treatment. Malathion represented 36.8%, malathion monocarboxylic acid 12.8% and malaoxon 1.2% of the total radioactivity in the residue. Aqueous extracts contained 44% of the TRR and organic extracts 58% of the TTR (FAO, 1999)

Hanaoka *et al.* (2002) estimated levels of organochlorine residuals in the Japanese population and the contribution of dietary factors to these levels, we determined serum levels of beta-hexachlorocyclohexane (beta-HCH), hexachlorobenzene (HCB), p,p'-dichlorodiphenyldichloroethane (p,p'-DDD), 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (p,p'-DDE) and p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT) in 41 volunteers (14 men and 27 women) in a rural area of Northern Japan. These organochlorine levels were measured using gas-chromatography mass-spectrometry. By a self-administered dietary history questionnaire, the usual dietary intake was estimated. Their median levels (range) were as follows: beta -HCH, 0.50 (0.05-1.50); HCB, 0.20 (0.02-0.70); and total DDT (p,p'-DDE+p,p'-DDT), 5.0 (0.9-31.0) ng/ml serum. Levels of p,p'-DDD were detected in only seven subjects (0.05-0.6 ng/ml serum). The beta -HCH levels were increased with rice and milk intakes, but the least squares means were not simply increased according to the quartile of the intakes. Concerning HCB, fish intake showed a borderline significant correlation (0.20, P=0.052). In terms of total DDT, intakes of meat, fish, vegetable and milk showed a positive relationship, although none of them provided statistically significant results. No other statistically significant relation between any

organochlorines and any food intakes examined was observed in this study. The present study suggests that organochlorine compounds are transported into the human body via foods in the Japanese population. Their effects on health should thus be investigated and monitored.

An-QiOng (2004) observed the residues and distribution of organochlorine pesticides in the soils of the vegetable garden in the southern Jiangsu, China. The effects of soil utilization, cropping system, vegetable species and history of vegetable cultivation on residues of the pesticides were also discussed. A county with long history of vegetable plantation, developed in urbanization and industry, was selected as case study. A total of 89 samples were collected throughout the whole county with global positioning system value for each location. The results showed that organochlorine pesticides were found widely in the soils, with an occurrence of 100%. The mean of the total concentrations (Sigma-OCP) was 174.4 micro g kg⁻¹ with a range of 23.2 micro g kg⁻¹-1126.7 micro g kg⁻¹. p,p'-DDE and p,p'-DDT are the main components and took over 82% of the total. The kailiyard soil had the highest residues of Sigma-DDT, while the paddy soils had the lowest levels. The residues varied significantly with vegetable species in kailiyard soils. The residues in soils growing leaf vegetable were much higher than others. The rotation of vegetable and rice could decrease the risk of organochlorine pollution.

Adeyeye & Osibanjo (1999) estimated residue levels of organochlorine pesticides in raw fruits, vegetables and tubers from markets in Nigeria. In the fruits, total HCH, aldrin and total DDT were detected in 77, 38 and 30% of all samples, respectively. In the vegetables, total HCH, HCB, total DDT and aldrin were detected from 95, 53, 50 and 30%, respectively, of all samples. Aldrin+dieldrin, total HCH, and total DDT were detected from 98, 79 and 49%, respectively, of all tuber samples. Other pesticides were below their detection limits. The average levels were generally low and none were above the FAOs maximum residue limits.

Zidan *et al.* (2000) in a market basket survey found pesticide residues on fresh vegetables and fruits collected from local and public markets in Kalubia governorate in winter crops and summer crops. The detection of various amounts and types of pesticides residues varied according to season and crop. Organochlorine pesticides were the most detected contaminants on vegetables and fruits. Almost all samples contained DDT, HCH isomers, heptachlor, heptachlor-epoxide, aldrin and cis-chlordane. In addition, three organophosphorus (fenitrothion, parathion and profenphos [profenofos]), one dithiocarbamate (thiram), two synthetic pyrethroids (fenpropathrin and s-fenvalerate), fungicide (benalaxyl) and one herbicide were detected in some samples from certain sites. On the other hand, all monitored samples were free from gamma -HCH, p,p-DDD, p,p-DDT, endrin, endrin-ke-ton, mirex, pyrazophos, alpha-methrin and etofenprox pesticides residues. Residues did not exceed the Extraneous Maximum Residues Limits (EMRL) or Maximum Residues Limits (MRLs) established by the Codex Alimentarius Committee on pesticide residues, with few exceptions. As for daily intake of pesticide residues by adult consumers (70 kg average weight), the data indicate that the intakes of pesticide residues in vegetables and fruit from the surveyed areas are generally within the permissible levels of the concerned pesticides except for chlordane and heptachlor-epoxide. The calculated levels for these latter pesticides are higher than the published Acceptable Daily Intake (ADI) in okra, apricot, green bean, jews mallow [*Corchorus olitorius*], and spinach.

Krol *et al.* (2000) in laboratory [USA] initiated a research programme with the objective of examining the effect that rinsing of produce with tap water would have on pesticide residue. Samples were obtained from local markets and/or grown at our experimental farm. Because approximately 35 of produce from retail sources contains pesticide residues, growing and treating produce at an experimental farm had the advantage that all such samples contain pesticide residues. Pesticides were applied under normal field conditions to a variety of food crops and the vegetation was



allowed to undergo natural weathering prior to harvest. The resulting samples contains field-incurred or "field-fortified" residues. The experimental design was employed to mimic as closely as possible real world samples. Crops were treated, harvested, and divided into equal subsamples. One subsample was processed unwashed, whereas the other was rinsed under tap water. The extraction and analysis methods used was a multi-residue method developed in our laboratory. Twelve pesticides were included in this study: the fungicides captan, chlorothalonil, iprodione, and vinclozolin; and the insecticides endosulfan, permethrin, methoxychlor, malathion, diazinon, chlorpyrifos, bifenthrin, and DDE (a soil metabolite of DDT). Statistical analysis of the data using the Wilcoxon signed-rank test showed that rinsing removed residues for nine of the twelve pesticides studied. Residues of vinclozolin, bifenthrin, and chlorpyrifos were not reduced. The rinsability of a pesticide is not correlated with its water solubility.

Zhou ZeYi *et al.* (2001) conducted a survey on the pollution status of pesticides in Beijing's foods. The residual quantities of organophosphorus, organochlorine and others were determined for 16 foods (348 samples) sold in Beijing markets including vegetables, fruits, and meats. The residual quantities of organochlorine pesticides 666 and DDT in 344 samples were lower than their respective standard values, and therefore the pollution of organochlorine pesticides was relatively low in Beijing's foods. The residual quantities of organophosphorus pesticides (methyl parathion [parathion-methyl], parathion, omethoate, malaoxon, phorate, phoxim or trichlorfon) in 49 samples were higher than the standard value, and therefore the residues of organophosphorus pesticides were relatively high in Beijing's foods. Based on these results, some measures for controlling pesticide residues in Beijing foods are presented and discussed briefly.

Dogheim *et al.* (2001) in samples of the most common fruits and vegetables were collected from 8 local markets in 6 governorates. These 1579 samples were analysed

for residues of 53 pesticides, which included organophosphorus and organonitrogen compounds and some synthetic pyrethroids. Samples were also analysed for residues of organochlorine pesticides, although they had been prohibited from use several years ago. Only 510 of the 1579 samples were analysed for dithiocarbamate pesticide residues, which were determined as CS₂. Overall, 76.1% of the total analysed samples had no detectable residues, 23.9% contained detectable residues, and 2.59% contained residues that exceeded maximum residue limits. For individual crops, contaminated samples ranged from 0 to 96% of the number of samples analysed. However, the highest violative percentage for samples of individual crops was 12.5. Chlorpyrifos, carbaryl, dimethoate, bromopropylate, and profenofos were the violative pesticides determined in fruit and vegetable samples. The results of the current study demonstrated that no restricted or banned pesticides such as DDT, HCH, and their isomers were found in any of the samples analysed. Dithiocarbamate residues were detected in 9.4% of the 510 samples analysed, with a violative percentage of 0.39, representing one grape sample and one peach sample.

Neela *et al.* (2002) found considerable quantities of organochlorine insecticides are still used worldwide and their persistent residues are widely distributed throughout the environment. Various researchers from different parts of world investigate the magnitude of contamination of organochlorine insecticides in vegetables which were brought for sale to the consumers in the local. Samples of vegetables (potato, tomato, cabbage, cauliflower, spinach and okra) were collected at the beginning, middle and end of the seasons with respect to different vegetables. Organochlorine levels were assessed using gas liquid chromatography equipped with electron capture detector. Most of the collected samples were contaminated with residues of DDT and its metabolites (DDD [TDE], DDE), isomers of HCH (alpha -, beta - and gamma -HCH [lindane]), heptachlor, heptachlor epoxide and aldrin. Some of the detected insecticides exceeded the limit of tolerance prescribed by WHO/FAO. Results of studies on seasonal variations in residue levels are presented.

Jha & Mishra (2005) showed the side effects of pesticides application on microbial population under different cropping systems (vegetable, rice, wheat, sugarcane and pulse-based) were studied in Bihar, India. Enumeration of microorganisms included viable counts for bacteria, fungi, actinomycetes, cyanobacteria, Rhizobium and Azotobacter and most probable number for Azospirillum, Nitrosomonas and Nitrobacter. All the thirty-two soil samples from different cropping systems analysed were found contaminated with HCH or DDT or endosulfan or all three. The average concentration of total HCH was 0.046, 0.030, 0.027 and 0.009 micro g/g in vegetable, rice-wheat, sugarcane and pulse growing soils, respectively. Total DDT concentration was more than total HCH in all cropping systems. Endosulfan had the least value. Pesticide residues in soil had definite influence on microbial population. Vegetable soils had the least microbial population and the highest pesticide residue. The highest microbial population was recorded in pulse growing soils, which contain the least pesticide residues. Actinomycetes were the least affected by the pesticide residues. Domination of Rhizobium in pulse soil, Azospirillum in sugarcane soils and cyanobacteria in rice-wheat soils, irrespective of pesticide residue level, suggests the influence of cropping systems. An additional field experiment was conducted to evaluate the effect of insecticides (endosulfan, cypermethrin and chloropyriphos) on soil microorganisms and its residue in/on brinjal [aubergine] fruit. Rapid succession of pesticide applications drastically reduced the microbial population. Cypermethrin had the least effect on microbial population, while chloropyriphos had the major effect. The insecticide residues in/on brinjal fruits showed a continuous decline.

Gao (2005) in an experiment determine the residual levels and bioaccumulation of organochlorine pesticides (OCPs) in representative vegetables, i.e. carrot, radish, lettuce, cabbage, celery cabbage, garlic leaf, leek, spinach, tomato and pumpkin, from Nanjing, Jiangsu, China. 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. Carrot and cabbage were used as reference matrices for the recovery assay. DDT and its metabolites DDE and DDD gave better



recoveries in carrot and cabbage than HCH and HCB. 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 total OCPs residual level in carrot was more than 100 ng/g, which was the highest residual level among all the tested vegetables. The residual levels of OCPs in garlic leaf (96.2 ng/g) and spinach (95.3 ng/g) were slightly less than that in carrot. However, the residual levels in the other vegetables were relatively low and had no significant difference. It narrowly ranged from 56.8 to 64.0 ng/g, accounting for 60-70% of the total OCP residual levels in carrot, garlic leaf and spinach. DDTs and HCHs were the main OCP residues, which accounted for 50-75 and 14-28%, respectively, of all OCP residues detected. The bioaccumulation factor (BAF) of total OCPs in the vegetables was between 0.41 and 0.78. Moreover, BAF in carrot, garlic and spinach was higher than 0.7. The ratios of γ -HCH and DDT/DDE in the vegetables ranged from 1.0 to 2.5 and 1.0 to 4.2, respectively.

Pany and Pattnaik (2006) evaluated 92 total diet samples from average diet consumed in the localities consisting of vegetables, fish, egg and meat meals collected from in and around Bhubaneswar and Cuttack (Orissa, India) during 2001-02. From 53% of the samples, contaminated with pesticide residues were detected. The magnitude of contamination was higher in non-vegetarian diets than in vegetarian diets. Insecticides such as α -HCH, β -HCH, γ -HCH, τ -HCH, aldrin and dieldrin were the main contaminants. However, DDT, endosulfan and their metabolites could not be detected. The mean residues of total HCH were 0.005, 0.0085 and 0.008 ppm in vegetables, fish and egg meals, respectively. Only 22% of the vegetarian diets were contaminated whereas 82-100% contamination was detected in the case of non-vegetarian diet. With respect to contamination in non-vegetarian diet, the order was fish > egg > meat. The actual daily intake of organochlorine compounds was well within their respective acceptable daily intake (ADIs) reported by FAO/WHO. The average contamination level of various diets tested was believed to be harmless.

Yang Xing Lun (2007) found the leaf-air transfer of organochlorine pesticides (OCPs) in three kinds of vegetables, namely lettuce, romaine and garlic leaves was investigated. It was found that although the uptake of OCPs by the three selected vegetables was similar under controlled conditions, the values varied significantly among chemicals and plant species in terms of elimination rate, final residue of each OCPs, as well as the effect of temperature on the residue of OCPs in the vegetables. The results indicated that neither QCB nor HCB could be trapped tightly by any of the three selected vegetables, in contrast, p,p'-DDT could be retained effectively by all of them; the retainment of alpha -HCH, gamma -HCH, p,p'-DDE, was dependent on the vegetable species, of which the garlic leaf had the biggest ability to trap them.

Zhang Mao Sheng (2008) found a new approach for the extraction of nine kinds of organochlorine pesticides (OCPs) from vegetable samples coupling single-drop microextraction with gas chromatography-mass spectrometry was presented. Experimental parameters, such as organic solvent, exposure time, agitation and organic drop volume were controlled and optimized. An effective extraction was achieved by suspending a 1.00 micro L mixed drop of p-xylene and acetone (8:2, v/v) to the tip of a microsyringe immersed in a 2 mL donor aqueous solution and stirred at 400 rpm. The approach was applied to the determination of OCPs in vegetable samples with a linearity range of 0.05-20 mg/ml for alpha- hexachlorobenzene, beta-hexachlorobenzene, gamma-hexachlorobenzene, delta-hexachlorobenzene (BHC) and dicofol, 0.5-20 mg/ml for dieldrin and 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD) or 0.5-50 mg mL⁻¹ for 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE) and 2-(2-chlorophenyl)-2 (4-chlorophenyl)-1,1,1-trichloroethane (p,p'-DDT). Correspondingly, the determination limit at an S/N of 3 ranged from 0.05 mg mL⁻¹ for alpha -, beta -, gamma -, delta -BHC to 2.0 mg/ml for dicofol, dieldrin or p,p'-DDT. The relative recoveries were from 63.3 to 100%, with repeatability ranging from 8.74 to 18.9% (relative standard deviation, R.S.D.). The single-drop

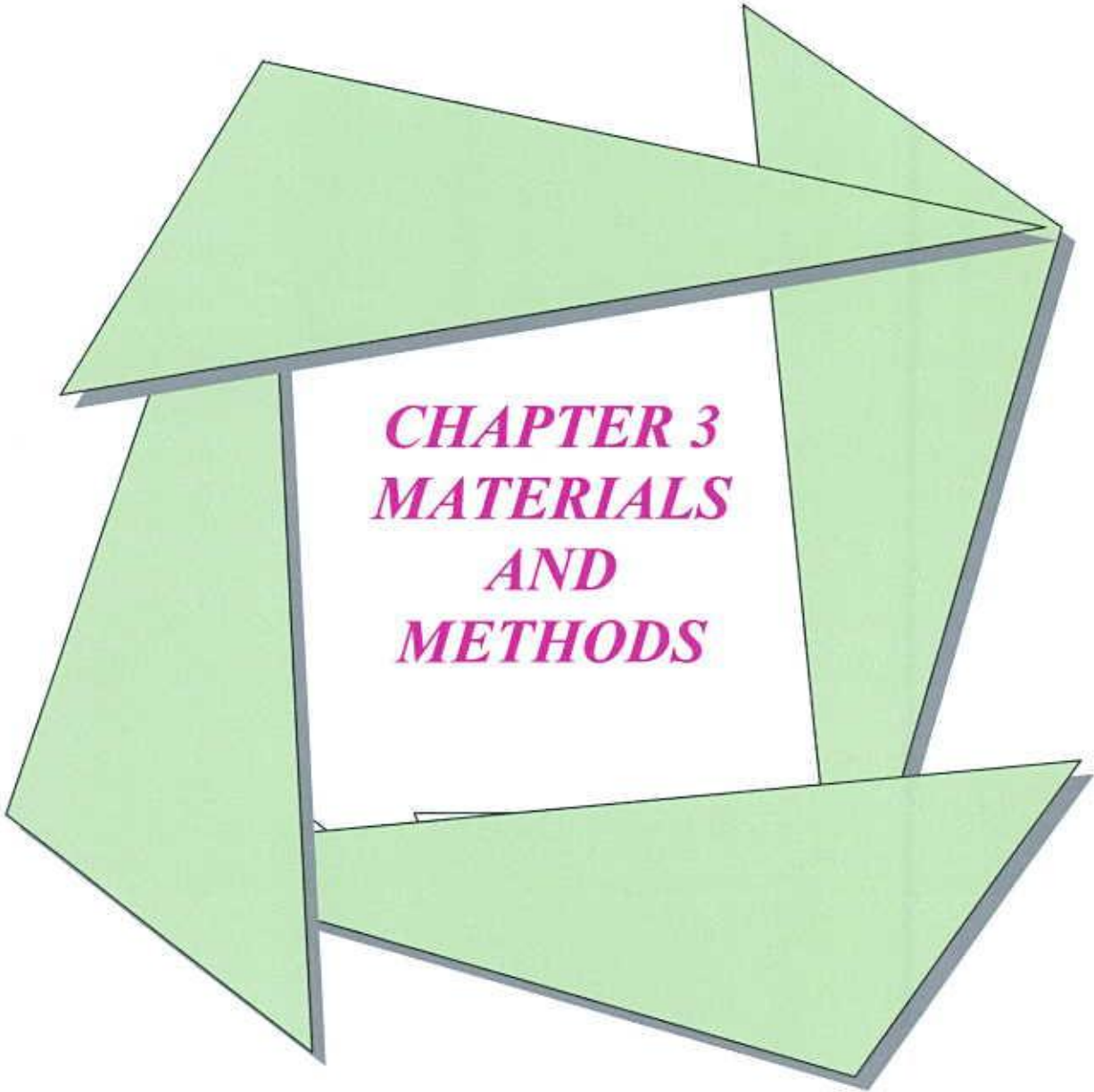
microextraction was proved to be a fast and simple approach for the pre-concentration of organochlorine pesticides in vegetable samples.

Shao-Xue-Xin (2008) observed the distribution, potential sources and risks of organochlorine pesticides (DDT and HCH) in the soils of the Xixi National Wetland Park, Zhejiang, China. Results showed that HCH and DDT could be detected in all the topsoil samples. The σ -HCHs and σ -DDTs residues were similar with mean content being 18.44 and 20.80 ng g⁻¹, respectively. HCH residues in the soils of persimmon orchards, bamboo gardens, bulrush bottomlands, vegetable farms and others were similar, while DDT residues in the soils of vegetable farm were higher than in the others. σ -HCHs and σ -DDTs in the soils were lower than the most stringent criteria (the first grade criterion, 50 ng g⁻¹) of the Soil Environmental Quality Standard of China (GB 15618-1995). In comparison with other places of the country, Xixi is relatively lower in σ -DDTs, but higher in σ -HCHs. The eco-risk analysis shows that HCH residues in the soils do not pose much risk to the geobiont therein, while DDTs do have some risk to the birds and geobiont.

Skrbic & Predojevic (2008) founds the levels of 16 organochlorine pesticides (OCPs) were investigated in 39 composite samples of agricultural crops, related by-products, and foodstuffs collected in Vojvodina, Serbia, in 2002 through 2004. After extraction and cleanup, OCPs were determined by capillary gas chromatography using electron-capture detection. The highest mean level of 0.971 ng/g whole weight (ww) was found for alpha -HCH in wheat flour samples. OCPs levels were well lower than the respective maximum residue limits set by current European and Serbian regulations. Mean OCP levels were low (<1 ng/g ww) for all sample types. The most frequently determined residue was 4,4'-DDT (identified in 76.9% of all samples analyzed), followed by gamma -HCH (66.7%), beta -HCH (48.7%), and endosulfan II (41.0%). OCP levels were compared with data from other international surveys. Calculated daily intakes of OCPs by way of consumption of the crop products included in this

study according to data of the Serbian National Institute for Statistics were compared with the acceptable daily intakes established by the Food and Agriculture Organization/World Health Organization. The average level of contamination of the Vojvodina diet was believed to be harmless regarding the studied food commodities.

Kotey *et al.* (2008) in a survey involving 60 farmers was conducted in Keta district, Volta region, Ghana, during 2006 to determine the sources, types, formulations, rates and application frequency of pesticides used on vegetables in this region. Shallots, okra, pepper (*Capsicum* sp.), tomato and aubergine were cultivated alternately in the area. Most pesticides were obtained from pesticide retailers. Extension agents and non-governmental organizations provided pesticides to some farmers. Information on pesticides and their uses was obtained mainly from friend farmers and extension agents. Nine pesticide active ingredients in 4 chemical classes were used, and these were mainly in WHO toxicity classes I (moderately hazardous) and III (slightly hazardous). The majority of the depression and lagoon area respondents (71 and 58%, respectively) stated that they used more than one active ingredient per crop cycle (approximately 2 months). More than 70% of these farmers said that they applied these active ingredients in mixtures of 2 or more active ingredients. The farmers generally failed to protect themselves from contamination during pesticide application. The farmers could not state precise application rates to use for specific pests and crop stages, and were unable to calibrate application equipment. They estimated the amount of pesticide to add from an estimation of the volume of the lid of the pesticide container and knowing the volume of the mixing container. Chlorpyrifos, DDT, cypermethrin and dimethoate residues were detected in more than 90% of the samples submitted for gas chromatography. More than 60 and 50% of the samples from depression and lagoon areas, respectively, had chlorpyrifos residue levels above the Codex and EU maximum residue limit of 0.05 mg/kg.



***CHAPTER 3
MATERIALS
AND
METHODS***

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Laboratory

The present research work was performed in the laboratory of the Analytical Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Qudrat-e-Khuda Road, Dhanmondi, Dhaka-1205.

3.2 Selection of Vegetable Samples

To detect the residues of pesticides in vegetables, one marketing location was selected namely New Market of Dhaka. Samples were collected from different vendors. About 1kg each of six different vegetables were purchased from different vendors to prepare composite samples for detection of the presence of organochlorinated pesticides if any there in. The collected vegetables were tomato, potato, carrot, spinach, indian spinach and red amaranth.

Table 3.1. The local name, scientific name and location of collection and growing of the collected vegetable samples

Local Name	English Name	Scientific Name	Location of collection	Growing Area
Tomato	Tomato	<i>Lycopersicon esculentum</i>	Dhaka New Market	Chandina, Comilla
Aalu	Potato	<i>Solanum melongena</i>	Dhaka New Market	Munshigonj
Gazor	Carrot	<i>Daucus carota</i>	Dhaka New Market	Dhamrai
Lal Shak	Red Amaranth	<i>Amaranthus gangeticus</i>	Dhaka New Market	Karanigonj
Pui Shak	Indian spinach	<i>Basella Alba</i>	Dhaka New Market	Savar
Palon Shak	Spinach	<i>Spinach oleracea</i>	Dhaka New Market	Dhamrai



Plate 1. Collected tomato sample



Plate 2. Collected potato sample



Plate 3. Collected carrot sample



Plate 4. Collected red amaranth sample



Plate 5. Collected indian spinach sample



Plate 6. Collected spinach sample

3.3 Instruments and chemicals

For the identification of pesticides residues in vegetables, equipments and chemicals used as follows

3.3.1 Equipments

1. Oven (WTB binder, TUTTLINGEN, GERMANY)
2. Deep refrigerators
3. Digital analytical balance (SCIENTECH, SA 210)
4. Rotary vacuum evaporator (EUCLA, RIKAKIKAI CO. LTD, JAPAN)
5. Vortex mixture
6. Centrifuge machine (ROTO FIX, Nr. 9468. V-220-240)
7. Gas chromatograph mass spectrometer (GCMS-QP5050A Shimadzu)

3.3.2 GCMS conditions (EI mode)

- Column : DB-1 MS
- Column Condition: Slightly polar
- Detector EI : Electron impact ionization
- Capillary: 30m (length of column) X 0.252mm (internal diameter)
X0.25 um (inner surface coated film thickness of the column)
- Temperature Time: -60°C to 340°C (340°C Pgm)

3.3.3 Specification of the capillary column

- Oven temperature : Initial 80°C and final 250°C.
- Injection temperature : 230°C
- Interface temperature : 250°C
- Initial temperature : 80° C, stay at 2min
- Incremental temperature time : 3°C/min
- Oven equilibrium time : 3 min.
- Gas flow rate : Helium as carrier, 50 mL/min

- Column Pressure : 100 (KPa).
- Column Flow : 50 ml/min.
- Injection volume : 0.4 μ l
- Linear velocity : 44.7
- Split ratio : 30

3.3.4 Gas Flow

- About 99.9997 % pure helium were used.
- About 99.9998 % pure nitrogen were used.

3.3.5 Chemicals

- Ethanol GPRTM (BDH Laboratories, England).
- Sea sand (GR, Purified by acid and Calcined, India)
- Anhydrous Na₂SO₄ food grade (Extra pure, Merck, Germany)
- Sulphuric acid, GPRTM, about 98% (BDH Laboratories, England).
- n-Hexane GPRTM (BDH Laboratories, England).
- Aceton GPRTM (BDH Laboratories, England).
- Distilled water

3.3.6 Extracting Solvents

- Ethanol, for pesticide residue analysis grade.
- n-Hexane, distilled in reagent grade.

3.4 Analytical Standard

The standard was procured from SIGMA Chemicals Co, Catalog No.49151, USA. The standard composed of 2, 4-DDT (0.09 μ g/mL); 4, 4-DDD (0.076 μ g/mL); 4, 4-DDT (0.104 μ g/mL); 2, 4-DDD (0.08 μ g/mL); 4, 4-DDE (0.04 μ g/ml); Endrin (0.08 μ g/mL); Dieldrin (0.048 μ g/mL); Heptachlor epoxides isomer B (0.032 μ g/mL) ; Aldrin (0.02 μ g/mL) : Heptachlor (0.01 μ g/mL); β -BHC (0.04 μ g/mL); γ -BHC

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(0.01 μ g/mL) and α -BHC (0.01 μ g/mL) [Analytical standards (~99.5) supplied by AQC Company] in distilled n-hexane, use as mixed standards were prepared in the laboratory.

3.5 Glassware and other small items

- Porcelain mortar
- Beaker (10-250 ml)
- Conical flask (50-500 ml)
- Quick fit conical flask
- Funnel
- Glass wool (Filter grade)
- Filter paper (100 Circles 1 ,What man, England)
- Glass rod
- Separating funnel
- Centrifuge tuber
- Screw-cap test tubes
- Measuring test tubes
- Disposable tips
- Measuring Cylinder (20-100ml)
- Reagent Bottles
- Micro-pipette
- Pipette
- Round bottom flask
- Column
- Vials (10ml)



Plate 7. Filtration of the blended sample for extract preparation



Plate 8. Collected filtrate of different vegetables



Plate 9. Separating of the organic part by separating funnel



Plate 10. Evaporation by rotary evaporator





Plate 11. Concentrated sample after evaporating



Plate 12. Collection of eluate from column



Plate 13. Separating organic part with a separating funnel

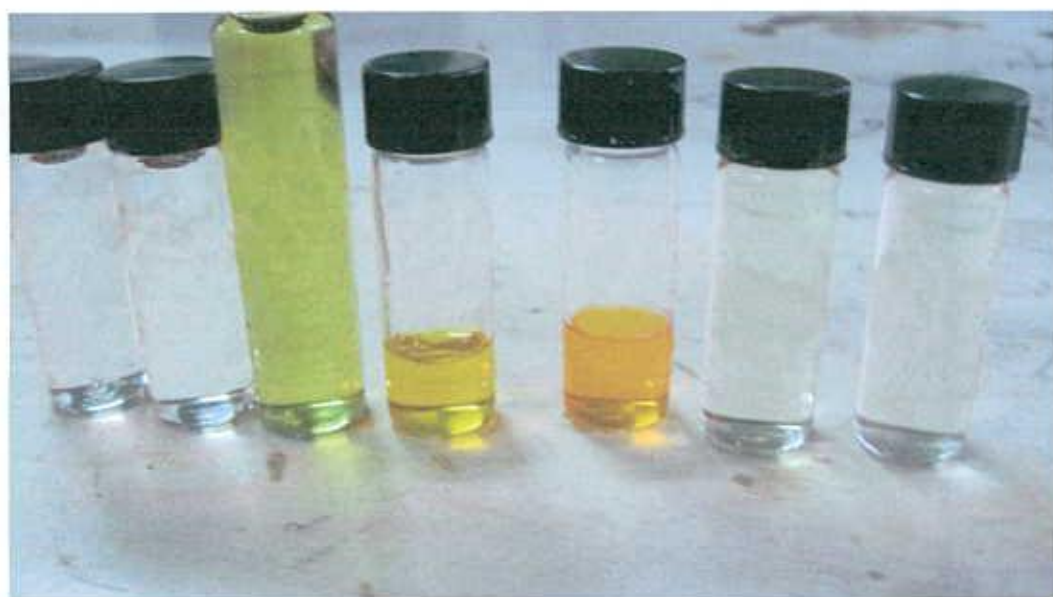


Plate 14. Finally prepared sample for analysis in GCMS



Plate15. GCMS Machine

3.6 Accessories

- Ice-box
- Aluminum foil
- Brush and detergent
- Centrifuge tube holders
- Micro syringe : 10 μ ml capacity
- Chopping board
- Cotton and tissue paper
- Desiccators
- Forceps
- Gloves
- Knife
- Mask
- Scissors
- Scotch tape
- Stands and holders
- Spoon
- Clean cloth.

3.7 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 (1993).

3.7.1 Analysis of vegetables sample

3.7.1.1 Sample preparation

Exactly 250 gm 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.

3.7.1.2 Mortar grinding of the sample

Exactly 250 gm of each sample was macerated in a mortar.

3.7.1.3 Extraction of the sample

250 gm of ground samples was 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 (Gohlki and McLafferty, 1993).

3.7.1.4 Filtration of the extracted sample

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.

3.7.1.5 Evaporation of the extracted solvent

50 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 gm 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.

3.7.1.6 Precaution for Clean-up

- Hexane was distilled at 78°C. The boiling point of Hexane is 78°C.
- All kinds of apparatus were firstly washed by detergent and then rinsed with distilled water.
- All the apparatus were kept into oven for drying.
- All the apparatus were rinsed 2 times with acetone.
- Fresh reagents were used.
- Micro-crying was rinsed by Hexane, before injecting to GCMS.
- After running each sample, GCMS column was cleaned by heat at 300°C.

3.7.1.7 Injection into GCMS

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 (Plate 24).

3.7.1.8. Detection

For organochlorine pesticide detection EI 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.

3.7.1.9. Efficiency of extraction and clean-up

Efficiency of extracts and clean up which was easy seen by clean Chromatogram, peak of the solvent, area of the solvent, height of the solvent and retention time.



***CHAPTER 4
RESULTS
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Chapter 4

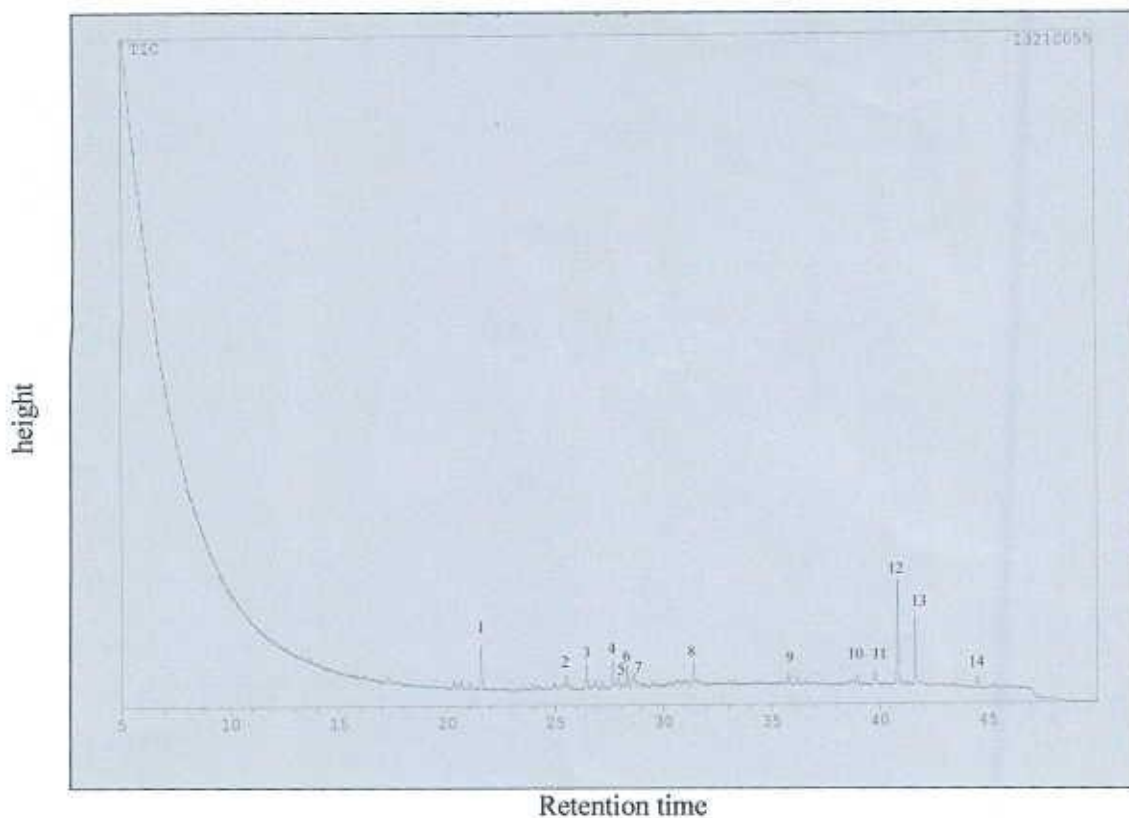
RESULTS AND DISCUSSION

4.A Results

Status of chlorinated pesticides residue in potato, tomato, carrot, indian spinach, spinach and red amaranth sample was analyzed and detected by Gas chromatograph Mass spectrometer (GCMS) and their results are described in the following headings.

4.1. Chromatograph of Tomato

The tomato (*Lycopersicon esculentum*) 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 in tomato sample have been presented in Fig 4.1. The major organic compounds present in tomato samples according to their peak were phytol; hexadecanoic acid, ethyl ester; phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl); 2,2-thiobis. [4-(1,1,3,3-tetramethylbutyl) phenol]; N-(1-benzyl-3-chloro-2-oxopropyl)-4-iodobenzen sulfonamide; 1,2-benzenedicarboxylic acid, diisooethyl ester; Di-n-oethyl phthalate; phthalic acid, diisooethyl ester; Aspidofractinine-3-methanol (2 alpha, 3-beta, 5-alpha); Cholest-5-en-3-ol, carbonochloridate; Dis (5-tert-butyl-6-methoxy-m-talyl) sultide; Cholesta-3,5-diene; (Z) 14-tricosenyl formale; Hexadecane acid dodecyl ester. Among the 14 compounds detected only Cholest-5-en-3-ol, carbonochloridate was found as chlorinated compound in tomato samples (Table 4.1)



Peak found (Tomato)

**** Peak Report ****

PKNO	R.Time	I.Time	F.Time	Area	Height	A/B(sec)	MK	%Total
1	22.244	22.233	22.342	26911695	4245983	6.338	V	2.06
2	26.392	26.383	26.433	11675409	3944183	2.960	V	0.90
3	27.462	27.433	27.533	22511023	3843900	5.856	V	1.73
4	27.553	27.533	27.608	16045808	3633864	4.416	V	1.23
5	28.617	27.608	28.900	56878629	3545250	16.044	V	4.36
6	28.911	28.900	28.050	25600502	3005199	8.519	V	1.96
7	29.097	29.050	29.242	29431583	2680415	10.980	V	2.26
8	32.253	32.242	32.500	35236306	2452440	14.368	V	2.70
9	35.639	35.625	35.717	10535852	2001656	5.264	V	0.81
10	38.727	38.717	38.842	13358421	1879843	7.106	V	1.02
11	39.123	39.042	39.200	13306646	1429761	9.307	V	1.02
12	40.213	39.200	39.333	10185992	1367884	7.447	V	0.78
13	41.422	39.408	39.575	10526140	1161108	9.066	V	0.81
14	44.834	40.733	40.942	10526800	2189425	4.808	V	0.81

Peak report of tomato

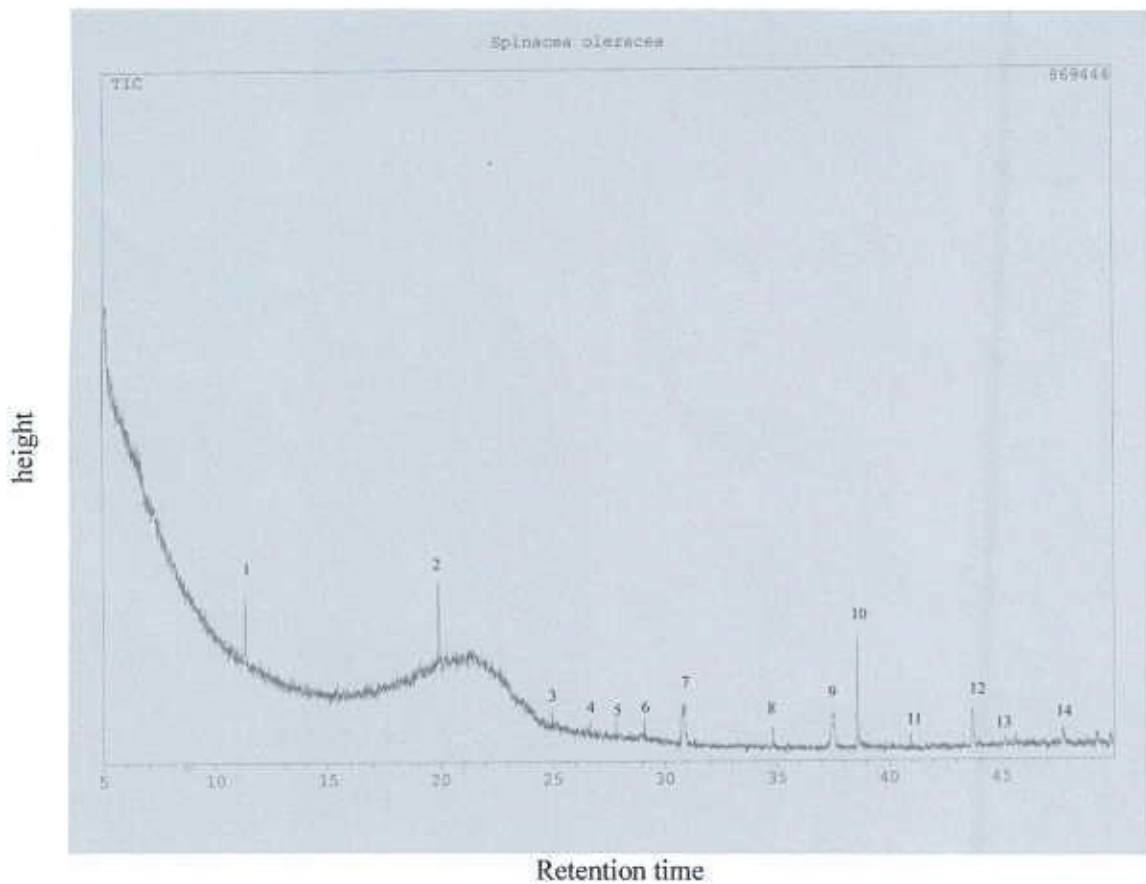
Figure 4.1. Chromatogram of tomato in GCMS

Table 4.1. List of detected organic compounds with chlorinated pesticides in Tomato (*Lycopersicon esculantum*) sample

Peak	Name of Compound	Mol wt.	Mol From	Chlorinated Compound
1.	Phytol	296	C ₂₀ H ₄₀ O	–
2.	Hexadecanoic acid ethyl ester	284	C ₁₈ H ₃₆ O ₂	–
3.	Phenol, 2,6-bis (1,1-dimethylethyl)-4-(-1-methyl-1-phenylethyl)	324	C ₂₃ H ₃₂ O	–
4.	2,2-Thiobis. [4-(1,1,3,3-tetramethylbutyl) phenol]	384	C ₂₈ H ₄₂ O ₂ S	–
5.	N-(1-Benzyl-3-chloro-2-oxopropyl)-4-iodobenzen sulfonamide.	463	C ₁₆ H ₁₅ ClNO ₃ S	–
6.	1,2-Benzenedicarboxylic acid, diisooetyl ester	390	C ₂₄ H ₃₈ O ₄	–
7.	Di-n-oetyl phthalate	390	C ₂₄ H ₃₈ O ₄	–
8.	Phthalic acid, diisooetyl ester	390	C ₂₄ H ₃₈ O ₄	–
9.	Aspidofractinine-3-methanol (2 alpha, 3-beta, 5-alpha)	310	C ₂₀ H ₂₆ N ₂ O	–
10.	Cholest-5-en-3-ol carbonochloridate	448	C ₂₈ H ₄₅ Cl ₂	√
11.	Dis (5-tert-butyl-6-methoxy-m-talyl) sultide	386	C ₂₄ H ₃₄ O ₂ S	–
12.	Cholesta-3,5-diene	368	C ₂₇ H ₄₄	–
13.	(Z) 14-tricosenyl formale	366	C ₂₄ H ₄₆ O ₂	–
14.	Hexadecane acid dodeyl ester	424	C ₂₈ H ₅₆ O ₂	–

4.2. 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 4.2. 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-tetrahydrocyclohexanone, 2, 6- di- t- butyl- 4- hydroxymethylene ; decane, 1,1-dioxybis -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; nonanoyl chloride-SS. Among the 14 compounds three samples contained 1-chloro heptacosane; pentane, 3-(2,2-dichloro-3-methylcyclopropyl and nonanoyl chloride-SS respectively was chlorinated compound in spinach (Table 4.2)



Peak found (spinach)

**** Peak Report ****

PKNO	R.Time	I.Time	-F.Time	Area	Height	A/H(sec)	MK	%Total
1.	11.180	11.150	-11.283	15254172	2018646	7.557	V	0.53
2.	19.102	19.08	-19.14	26290138	3207450	8.197	V	0.91
3.	25.139	25.067	-25.190	2833490	1436976	1.972	S	0.10
4.	26.806	16.600	-26.850	4155531	1418086	2.930	S	0.14
5.	26.915	26.650	-26.992	10891774	1300151	8.377	S	0.38
6.	29.321	29.292	-29.467	11743458	1171449	10.025	SV	0.41
7.	31.452	31.367	-32.508	8135923	1003288	8.109	SV	0.28
8.	35.081	35.008	-35.267	7758637	862871	8.992	SV	0.27
9.	37.563	37.367	-12.408	5886075	725616	8.112	SV	0.20
10.	39.352	39.345	-12.525	4268108	674532	6.328	SV	0.15
11.	40.173	39.125	-40.258	2321870	758758	3.060	TV	0.08
12.	43.090	43.017	-43.233	1405931	475744	2.955	SV	0.05
13.	46.678	46.550	-46.825	2007585	546769	3.672	V	0.07
14.	48.871	48.775	-49.100	1548986	380726	4.069		0.05

Peak report of spinach

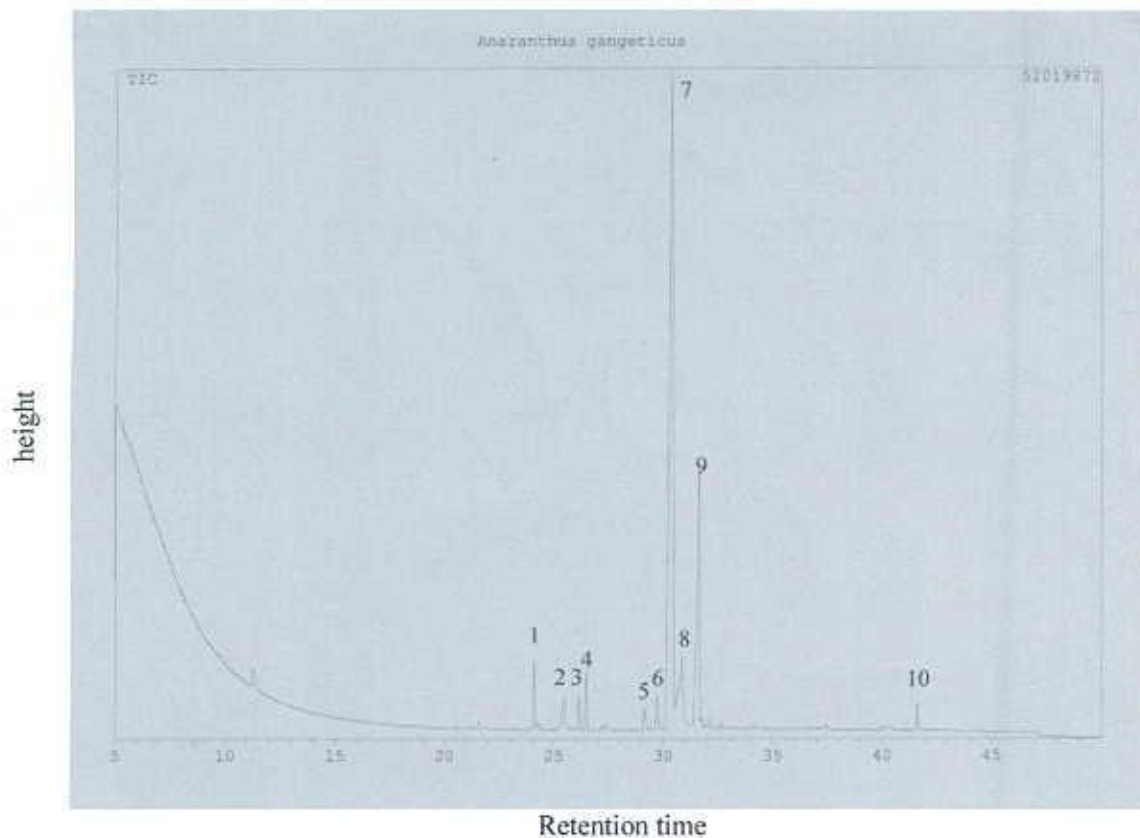
Figure 4.2. Chromatogram of spinach in GCMS

Table 4. 2. List of detected organic compounds with chlorinated pesticides in spinach (*Spinacea oleracea*) sample

Peak	Name of Compound	Mol. Wt.	Mol. From.	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 ₉ H ₁₆ Cl ₂	√
06.	1,3 -cyclopentanediol, cis-SS	102	C ₅ H 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 ₉ H ₁₇ ClO	√

4.3. Chromatogram of Red amaranth

The red amaranth (*Amaranthus 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 4.3. The major organic compounds found in red amaranth sample were 2-Pentadecanone, 6,10,14-trimethyl; Trans- undec- 4 -enal; hexadecanoic acid, ethyl ester; 2-hydroxy 1,1,10 - trimethyl-6,9- epidioxydecal; Cyclopentanol 2,4,4 - trimethyl; Phytol; cyclopentanone, 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-tetramethyl-1-hexadecen-1-ol; chdestan-3-ol,2-methylone (3.beta.5.alpha). Among the 14 compounds only heptacosane, 1-chloro -SS was found as chlorinated compound in red amaranth samples (Table 4.3)



Peak found (red amaranth)

**** Peak Report ****

PKNO	R.Time	I.Time - F.Time	Area	Height	A/R(sec)	MK	%Total
1.	24.123	23.942 - 24.433	21493604	4594281	4.678	SV	0.60
2.	25.489	25.400 - 25.600	10219019	2122213	4.825	V	0.29
3.	26.489	26.400 - 26.593	10319019	2112213	4.885	V	0.29
4.	27.609	27.400 - 27.639	10327019	2132213	4.345	V	0.29
5.	29.715	29.633 - 29.858	11903021	2152989	5.529	V	0.33
6.	30.325	30.067 - 31.325	239136531	30243949	7.907	SV	6.72
7.	30.893	30.817 - 31.000	11096087	2740551	4.049	TV	0.31
8.	31.443	31.325 - 31.500	23768623	4907993	4.843	V	0.67
9.	31.546	31.500 - 31.650	13836151	3498525	3.955	V	0.39
10	41.653	41.575 - 41.808	12286594	1400510	8.773	V	0.35

Peak report of red amaranth

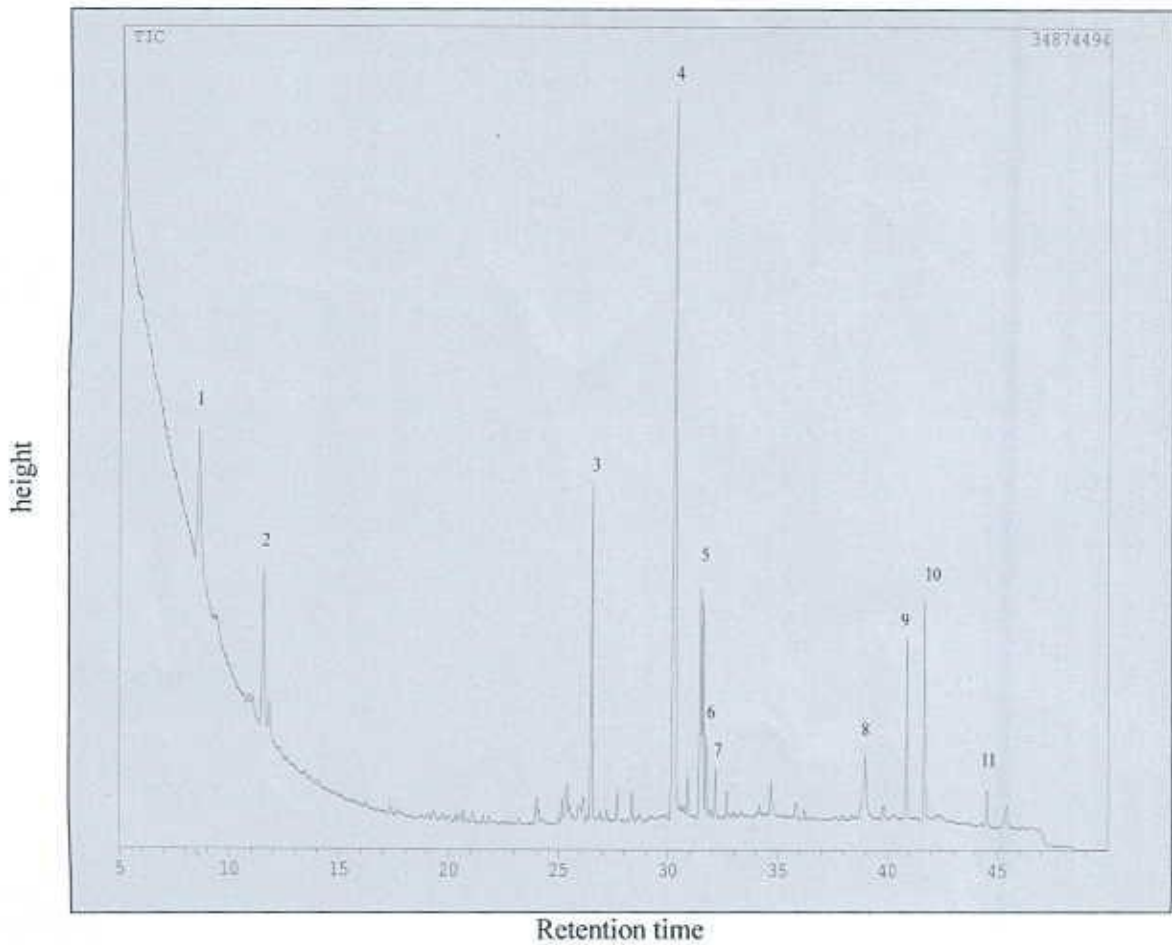
Figure 4.3 Chromatogram of red amaranth in GCMS

Table 4.3. List of detected organic compounds with chlorinated pesticides in red amaranth (*Amaranthus gangeticus*) sample

Peak	Name of Compound	Mol. wt.	Mol. From	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 ester	284	C ₁₈ H ₃₆ O ₂	–
04	2-Hydroxy 1,1,10 – trimethyl-6,9-epidioxydecal	226	C ₁₃ H ₂₂ O ₃	–
05	Cyclopentanol 2,4,4 –trimethyl	128	C ₈ H 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-tetramethyl-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	–

4.4. Chromatogram of carrot

The carrot (*Daucus carota*) 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 4.4. The major organic compounds found in carrot sample were caryophyllene; bicyclo(3,1,1) hept-2-ene-2,6-dimethyl -6 - (4- methyl- 3- pentenyl) -SS; phytol; hexadecanoic acid, ethyl ester-SS ; phenol, 2,4-bis (1-phenylethyl)-SS ;phenol-2,6-bis (1,1-dimethylethyl)-4-(1methyl-1-phenylethyl)-SS; phytal; linoleic acid, ethyl ester; 2,4-bis (dimethyl benzyl)-6-butyl phenol-SS; 1,2-benzenedicarboxylic acid, diisooctyl ester; di-n-octyl phthalate. Among the 11 organic compounds present in carrot samples no one was found as chlorinated (Table 4.4)



Peak found (carrot)

**** Peak Report ****

PKNO	R.Time	I.Time	F.Time	Area	Height	A/H(sec)	MK	%Total	Name
17	8.070	8.050	8.342	148026498	9085538	16.293	V	3.68	
22	11.529	11.325	11.717	61468131	7298847	8.422	V	1.53	
23	26.529	26.392	30.050	104209754	14463442	7.205	DV	2.59	
24	30.319	30.050	31.325	271200115	30907237	8.775	DV	6.74	
25	31.473	31.325	31.525	54681136	10107052	5.410	V	1.36	
26	40.886	40.750	41.567	50109613	8003814	6.261	SV	1.24	
18	8.585	8.408	8.958	283244112	12971290	21.836	V	7.04	
19	8.968	8.958	9.125	55805593	5924078	9.420	V	1.39	
20	9.213	9.200	9.358	47499912	5131696	9.256	V	1.18	
21	9.388	9.358	9.592	64593443	5065192	12.752	V	1.60	
27	41.686	41.567	41.808	45892793	9600059	4.780	V	1.14	

Peak report of carrot

Figure 4.4. Chromatogram of carrot in GCMS

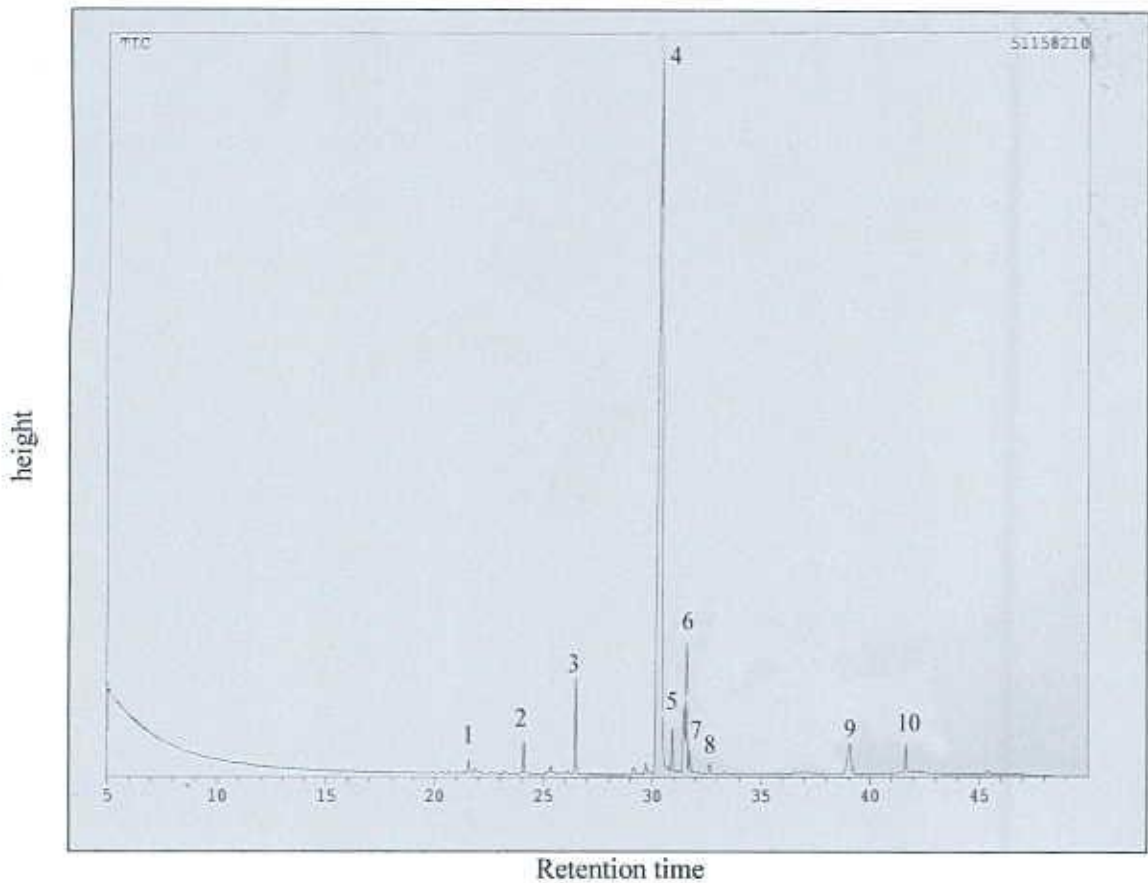
Table 4.4. List of detected organic compounds with chlorinated pesticides in carrot (*Daucus corota*) sample

Peak	Name of Compound	Mol wgt	Mol From	Chlorinated Compound
1.	Caryophyllene	204	C ₁₅ H ₂₄	-
2.	Bicyclo(3,1,1)hept-2-ene-2,6-dimethyl -6-(4-methyl-3-pentenyl)-SS	204	C ₁₅ H ₂₄	-
3.	Phytol	296	C ₂₀ H ₄₀ O	-
4.	Hexadecanoic acid, ethyl ester SS	284	C ₁₈ H ₃₆ O ₂	-
5.	Phenol, 2,4-bis (1-phenylethyl)-SS	302	C ₂₂ H ₂₂ O	-
6.	Phenol-2,6-bis (1,1-dimethylethyl)-4-(1methyl-1-phenylethyl)-SS	324	C ₂₃ H ₃₂ O	-
7.	Phytal	296	C ₂₀ H ₄₀ O	-
8.	Linoleic acid, ethyl ester	308	C ₂₀ H ₃₆ O ₂	-
9.	2,4-bis (dimethyl benzyl)-6-butyl phenol SS	386	C ₂₈ H ₃₄ O	-
10.	1,2-benzencdicarboxylic acid, diisooetyl ester	390	C ₂₄ H ₃₈ O ₄	-
11.	Di-n-octyl phthalate	390	C ₂₄ H ₃₈ O ₄	-



4.5. 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 4.5. 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-octadecatrienoic acid, methyl ester; 2,6,10,14,18-Pentamethyl-2,6,10,1418-cicas; 1,2-Benzenedicarboxylic acid; nonadecane SS-n-nonadecaness; O-mannitol 1, 1- 1, 16- hexadecanediylbis- 111- trifluoro hexadecane-2- one- SS; 9-bromonoralddehydess; (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 4.5)



Peak found (indian spinach)

**** Peak Report ****

PKNO	R.Time	I.Time	F.Time	Area	Height	A/H(sec)	MK	Total	Name
1	10.189	10.175	10.775	40588783	1435279	28.279	V	1.72	
2	26.477	26.350	30.033	47657196	9085270	5.246	SV	2.02	
3	30.162	30.033	31.300	39471304	7500161	5.263	SV	1.67	
4	31.408	31.300	31.467	22556841	4530774	4.979	V	0.96	
5	31.527	31.467	31.600	24333667	6261934	3.886	V	1.03	
6	39.086	38.775	39.117	37226972	3977972	9.358	V	1.58	
7	39.127	39.117	40.733	27235212	3750800	7.261	SV	1.15	
8	40.837	40.733	40.942	14971448	3606771	4.151	V	0.63	
9	40.837	40.733	40.942	14971448	3606771	4.151	V	0.63	
10	41.661	41.450	44.625	36468853	5317495	6.858	SV	1.55	

Peak report of indian spinach

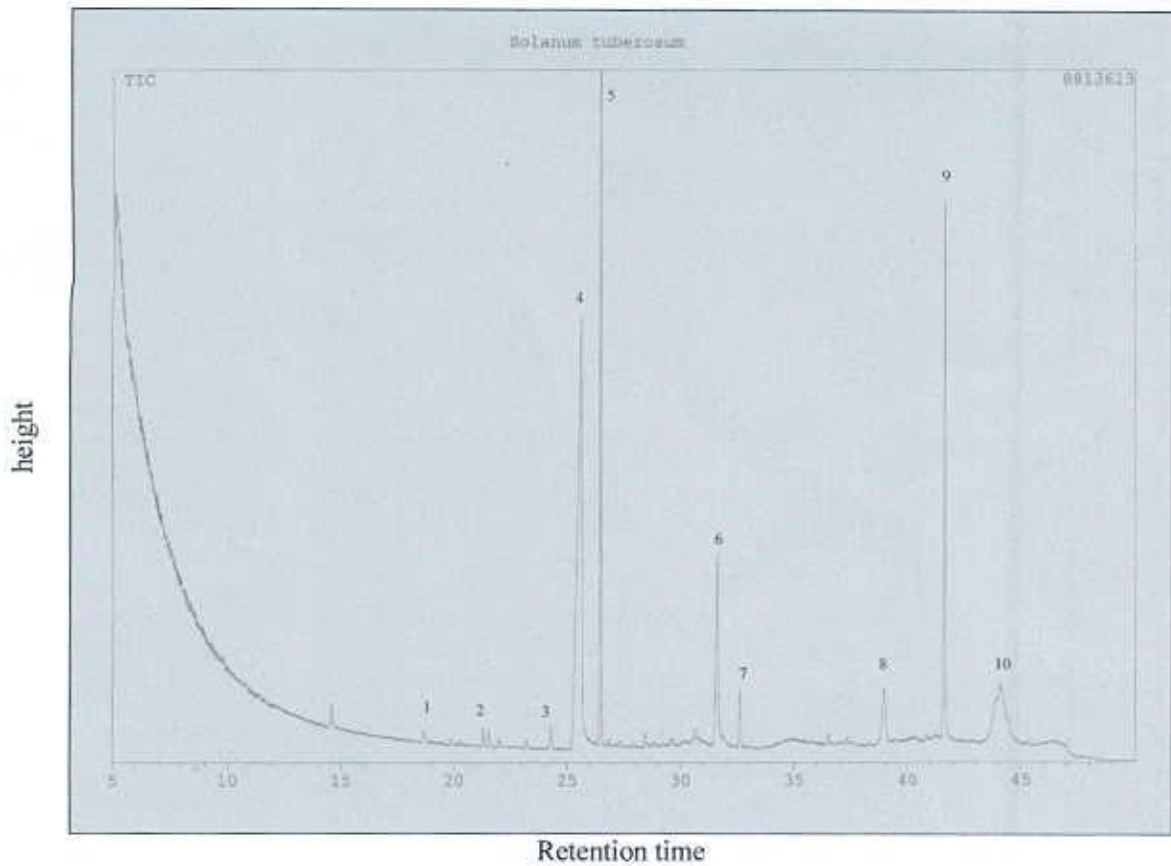
Figure 4.5. Chromatogram of indian spinach in GCMS

Table 4.5. List of detected organic compounds with chlorinated pesticides in indian spinach (*Basella alba*) sample

Peak	Name of Compound	Mol. Wt.	Mol From	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-octadecatrienoic 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-hexadecanediybis-	586	C ₂₈ H ₅₈ O ₂	
11.	111-Trifluoro hexadecane-2-one-SS	308	C ₁₇ H ₃₁ FO	
12.	9-Bromonoraldehyde-SS	220	C ₉ H 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-Neoleana-12,14-diene, (3xi,5,alpha)	408	C ₃₀ H ₄₈	

4.6. Chromatogram of potato

The potato (*Solanum tuberosum*) samples collected were analyzed to detect chlorinated pesticide residues if any there in. The chromatograph of the samples extract shows the presence of 10 organic compounds in (Fig 4.6). The major organic compounds found in potato sample were 1,16-cyclocorynan -17- oic acid, 19,20-didhydro methyl ester-SS; 1,3-propanediol, 22-diethyl -SS; n-hexadecanoic acid; hexadecanoic acid, ethyl esters; 9-octadecenoic acid (z)- hexylester-SS; nonadecanoic acid, ethyl ester-SS; 1,2-benzenedicarboxylic acid, disooctylester; di-n-octyl phthalate; heptocosane.1-chloro-SS; chloromethyl-5-chloroundecanoate. Among the 10 compounds heptocosane 1-chloro-SS and chloromethyl-5-chloroundecanoate were detected to be chlorinated compound contained in potato samples (Table 4.6)



**** Peak Report ****

PKNO	R.Time	I.Time	F.Time
1	10.189	10.175	10.775
2	26.477	26.350	30.033
3	30.162	30.033	31.300
4	31.408	31.300	31.467
5	31.527	31.467	31.600
6	39.086	38.775	39.117
7	39.127	39.117	40.733
8	40.837	40.733	40.942
9	40.837	40.733	40.942
10	41.661	41.450	44.625

Peak found (potato)

Area	Height	A/H(sec)	MK	%Total	Name
40588783	1435279	28.279	V	1.72	
47657196	9085270	5.246	SV	2.02	
39471304	7500161	5.263	SV	1.67	
22556841	4530774	4.979	V	0.96	
24333667	6261934	3.886	V	1.03	
37226972	3977972	9.358	V	1.58	
27235212	3750800	7.261	SV	1.15	
14971448	3606771	4.151	V	0.63	
14971448	3606771	4.151	V	0.63	
36468853	5317495	6.858	SV	1.55	

Peak report of potato

Figure 4.6. Chromatogram of Potato in GCMS

Table 4.6. List of detected organic compounds with chlorinated pesticides in potato (*Solanum tuberosum*) sample

Peak	Name of Compound	Mol wt.	Mol From	Chlorinated Compound
01.	1,16-cyclocorynan-17-oic Acid, 19,20-didhydro methyl ester-SS	322	C ₂₀ H ₂₂ N ₂ O ₂	-
02.	1,3-propanediol, 22-diethyl-SS	132	C ₇ H ₁₆ O ₂	-
03.	n-hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂	-
04.	Hexadecanoic acid, ethyl ester-SS	284	C ₁₈ H ₃₆ O ₂	-
05.	9-octadecenoic acid (z)- hexylester-SS	366	C ₂₄ H ₄₆ O ₂	-
06.	Nonadecanoic acid, ethyl ester-SS	326	C ₂₁ H ₄₂ O ₂	-
07.	1,2-benzenedicarboxylic acid, disooctylester	390	C ₂₄ H ₃₈ O ₄	-
08.	Di-n-octyl phthalate	390	C ₂₄ H ₃₈ O ₄	-
09.	Heptacosane.1-chloro-SS	414	C ₂₇ H ₅₅ Cl	√
10.	Chloromethyl-5-chloroundecanoate	268	C ₁₂ H ₂₂ Cl ₂ O ₂	√

4.B Discussion

The present study was undertaken for measuring the pesticide residue and persistent organic pollutants (PoPs) in potato, tomato, carrot, red amaranth, indian spinach and spinach. These vegetables were collected from different vegetable sellers of New Market, Dhaka.

The collected vegetable samples contained some chlorinated hydrocarbons, as have been detected through gas chromatograph and mass spectrometer.

From the study of chromatograph of tomato (*Solanum melongena*) samples (Fig. 4.1) it is evident that a tomato sample contains 14 organic compounds of which 1 (one) compound (Cholest-5-en-3-ol, carbonochloridate) was marked as chlorinated compound (Table 4.1). This chlorinated hydrocarbone damage and eventually depletes the serotomn in the central nervous system (EHP, 2006). Singh and Karla (1992) observed 0.73 mg/kg of chlorinated compound after 8 sprays @ 50g/kg a.i/ha, which declined to 0.61 mg/kg one day after treatment and then became 0.08 mg/kg after 10 days. Dethe *et al.* (1995) found detectable levels of residues of 33.3% in tomatoes. Tejada and Columpang (1995) in market surveys found residue of chlorinated pesticide in tomato by 20-90%. They recommended that a pre-harvest interval of 5-7 days would be safe in tomato that degraded pesticide residues. Ahuja *et al.* (1998) observed tomatoes for residues of HCH and reported that the residues of monocrotophos on tomatoes persisted over the prescribed maximum residue limit values.

The chromatograph of spinach (*Spinacea oleracea*) samples (Fig. 4.2) shows the presence of 14 organic compounds in tissues, of which 3 were chlorinated compounds viz. pentane, 3-(2,2-dichloro-3-methylcyclopropyl, 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 (2005) 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.

From the chromatograph of red amaranth (*Amaranthus gangeticum*) Fig. 4.3 and Table 4.3 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 (2005) 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.

It appears from the chromatograph of carrot (*Daucus carota*) samples that there were as much as 11 organic compounds present there in Fig. 4.4 and Table 4.4.. But there were no chlorinated compounds detected, some of the compounds were fat and some were byproducts of enzymes. Gao *et al.* (2005) found bioaccumulation of organochlorine pesticides (OCPs) in carrot. These authors maintained that carrot was used as reference matrices for the recovery assay. DDT and its metabolites DDE and DDD gave better recoveries in carrot than HCH and HCB. 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 total OCPs residual level in carrot was more than 100 mg/g. The ratios of gamma /

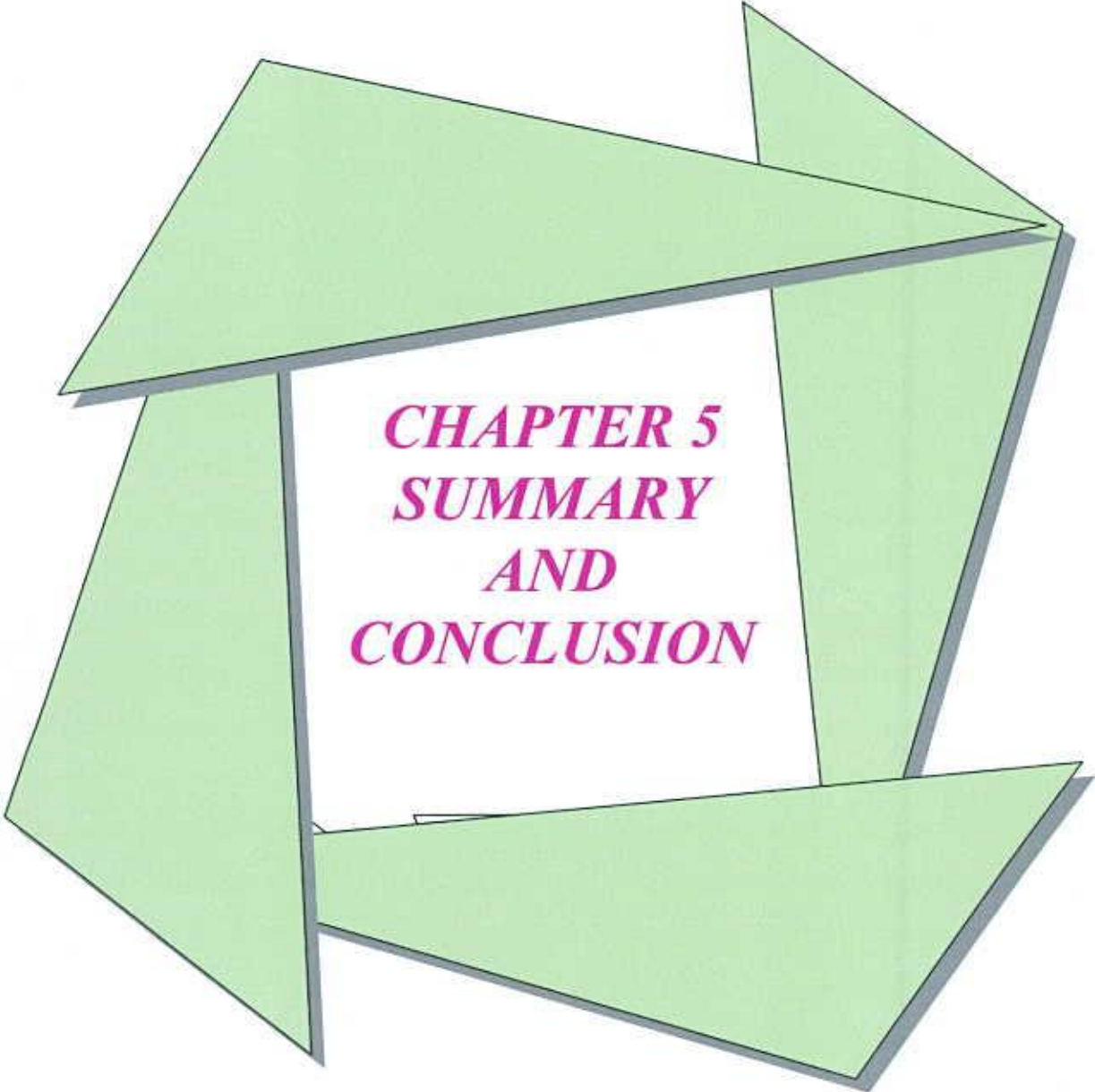
alpha -HCH and DDT/DDE in the vegetables ranged from 1.0 to 2.5 and 1.0 to 4.2, respectively.

The chromatograph prepared with the extract of the collected samples of indian spinach (*Basella alba*) (Fig. 4.5) 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 4.5). An-QiOng *et al.* (2004) observed that the residues of organochlorine pesticides was widely distributed in indian spinace, with an occurrence of 100%.

The chromatogram of collected potato (*Solanum melongena*) samples indicated the presence of 10 organic compounds of which 2 samples contained chlorinated compounds (Chloromethyl-5-chloroundecanoate and Chloromethyl-5-chloroundecanoate) Fig. 4.1 and Table 4.1. According to Ehi, 2006 this two compounds are carcinogenic to human, used as an intermediate in making insecticide, herbicide, pharmaceuticals, food flavorings, dyes, rubber chemicals, adhesives, paints, explosives and disinfectants. Adeyeye and Osibanjo (1999) estimated residue levels of organochlorine pesticides in raw tubers from markets. Aldrin+dielddrin, total HCH, and total DDT detected were 98, 79 and 49% respectively in all tuber samples. Other pesticides were below their detection limits. The average levels were generally low and none were above the FAOs maximum residue limits.

Pesticide residues have a relation with lipid content of vegetables. Lipids and fats act as a storage depot of organic toxic compounds (Doull *et al.* 1975). 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 (Reinert, 1970) and different parts of the plants contain different amount of pesticide residues (Elzorgani *et al.* 1979); But presence of pesticides in vegetables depends on the biodegradation of pesticide in the agricultural lands.





***CHAPTER 5
SUMMARY
AND
CONCLUSION***

SUMMARY AND CONCLUSION

There were in total six winter vegetables namely potato, tomato, carrot, spinach, red amaranth and indian spinach selected to undertake a study about the use or content of organic compounds notably organochlorinated ones, if there was any. About 1 kg of each of the vegetables was collected from vendors selling those vegetables to the consumers at Newmarket kancha bazaar area of Dhaka city. Exactly 250 gm of each of the vegetables was chopped and then macerated in a mortar. The macerated vegetable samples were extracted with solvents like n-hexane and 95% ethyl alcohol following the extraction procedure proposed by (Gohlki and McLafferty (1993). The extract of different vegetables was passed through a gas chromatograph mass spectrometer for identification of organic compounds notably organochlorinated compounds / persistent organic pollutants (PoPs). The extraction and identification of organic compounds were performed at the laboratory of the Analytical Research Division of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka.

The analytical observations indicated that the 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 collected tomato samples contained 14 organic compounds of which only one compound was detected to be chlorinated and it was identified as Cholest-5-en-3-ol, carbonochloridate.

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 heptacosane and nomanoyl chloride-SS.

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 the extract of carrot 11 compounds were found to be organic but none was detected as chlorinated compound.

As regard to the extract of indian spinach there were 14 compounds detected to be organic. But not a single one was chlorinated, while in the extract of potato samples 10 compounds were detected to be organic in nature. Among them only 2 were chlorinated compounds and were identified as, heptacosane 1-chloro -SS, chloromethyl-5-chloroundecanoate.

Conclusion:

There is a public concern through out the country that farmers use different harmful pesticides in their crops for higher yield and more profit. A few samples were studied within limited time which is not focused overall status of persistent organic pollutants. The study revealed that the vegetables available in the market contained some organic compounds and some have been identified as chlorinated compounds whether they were originated from any pesticide if were applied could not be ascertained. Since use of chlorinated compounds in agriculture is banded / restricted in Bangladesh like elsewhere due to several reasons, the sources and the quantity of the detected chlorinated compounds need to be search out with emphasis. Persistent organic pollutants (POPs) such as chlorinated pesticides are of global concern due to their wide spread occurrence, persistence, bioaccumulation and toxicity to human and animals. So for the betterment of the world and food safety the sources of organic pollutant should be identified and eliminated.

It is therefore suggested that:

- regulatory authority must be strict to control unauthorized use of pesticides.
- the knowledge of health hazards arising from pesticide residue should widely be circulated through mass media to develop awareness of random use of pesticides.
- integrated pest management, cultural practices, biological and biotechnical control of insect diseases, pathogens and weeds may be encouraged.





CHAPTER 6
REFERENCES

REFERENCES

- Adeyeye, A; Osibanjo, O. (1999). Residues of organochlorine pesticides in fruits, vegetables and tubers from Nigerian markets. *Science of the Total Environment*. 231(2/3): 227-233
- Ahuja, A. K.; Soudamini, M., Debi, S., Awasthi, M. D., Mohapatra, S., Sharma, D., Reddy, P. P., Kumar N. K. K. and Verghese, A. (1998). Monitoring of vegetables of insecticide residue contamination at harvest. *Advances in IPM for horticultural crops. Proceeding of the First National Symposium on Pest Management in Horticultural Crops: Environmental implications and thrusts, Bangalore, India, 15-17 October 1997, 243-246.*
- Akerblom, M. (1985). Draft Environmental Monitoring of pesticides Residues, Gridlines for the SADC Region. Sweden, 132-136.
- An-QiOng; Dong-YuanHua; Wang-Hui; Wang-Xia; Wang-MeiNong; Guo-ZongXiang. (2004). Organochlorine pesticide residues in cultivated soils, in the South of Jiangsu, China. *Acta Pedologica Sinica.*; 41(3): 414-419
- Broadman, R. (1986). In: "Pesticides in world Agriculture", Macmillan, Basing stoke, UK. Codex Alimentarius commission. 1994 status of codex maximum Residue Limits for pesticides in foods and Animal-food, Codex committee on pesticide residues, 26th session, Hague, 11-18 April.
- Cremlyn, R. (1980). Pesticides in the Environment, In: 'pesticides: preparation and mode of Action'. (John willey and sons published.) 210-221.
- Dethe, M. D., Kale, V.D and Rane, S.D. (1995). Insecticide residues in/on farm gate samples of vegetables. *Pest management in Horticultural Ecosystem*. 1 (1): 49-53.
- Dogheim, S. M; Gad Alla, S. A; El Marsafy, A. M. (2001). Monitoring of pesticide residues in Egyptian fruits and vegetables during 1996. *Journal of AOAC International.*; 84(2): 519-531
- Doull, J., Klaassen. C.D. and AMDUR- M.O. (1975). *Toxicology The Basic Science of Poison*. 2nd ed. Macmillan publishing Co. Inc. New York.
- Elzorgani, G.A., Abdullah, A.M. and Ali, M.E.T. (1979). Residues of organochlorine insecticides in fish in Lake Nubia *Bull. Environ. Contam. Taxicol*. 22:44.

- EHP, (2006). Comparative Metabolism of Chloroacetamide Herbicides and Selected Metabolites in Human and Rat Liver Microsomes. *Environmental Health Perspectives*. Environmental and Occupational Toxicology Division- Bureau of Chemical Hazards, Environmental Health Directorate, Department of National Health Canada, Environmental Health Centre, Room B35, Tunney's Pasture. Ottawa. Ontario KIA OL2. Canada. EHP 12(108). January 2006.
- FAO. (1999). International Code of Conduct on the Distribution and use of Pesticides (amended version) , FAO, Rome.
- FAO/WHO. (1993). Insecticide residues in food. Joint report of the FAO Working Party on Insecticide Residues and the WHO Expert Committee on Insecticide Residues. FAO Plant Production and Protection Paper # 103/1.
- FAO/WHO. (1993). Insecticide Residues in food. Part I Residues. Food & Agriculture Organization. pp. 257-287.
- Gao, H. J; Jiang, X; Wang, F; Wang, D. Z; Bian, Y R. (2005). Residual levels and bioaccumulation of chlorinated persistent organic pollutants (POPs) in vegetables from suburb of Nanjing, People's Republic of China. *Bulletin of Environmental Contamination and Toxicology*. 74(4): 673-680
- Gohlki, R. S. and McLafferty, F. K. 1993. Early gas chromatography/mass spectrometry. *Journal of the American Society for Mass Spectrometry* 4 (5): 367-371.
- Hanaoka, T; Takahashi, Y; Kobayashi, M; Sasaki, S; Usuda, M; Okubo, S; Hayashi, M; Tsugane, S. (2002). Residuals of beta-hexachlorocyclohexane, dichlorodiphenyltrichloroethane, and hexachlorobenzene in serum, and relations with consumption of dietary components in rural residents in Japan. *Science of the Total Environment*.; 286(1/3): 119-127
- Hassall, K. A. (1990). In: *The Biochemistry and uses of Pesticides,* Second Edition, ELBS/Macmillan. 125-128.
- Jha, M N; Mishra, S K. (2005). Decrease in microbial biomass due to pesticide application/residues in soils under different cropping systems.
- Khandakar, K. (1990). Population, Agriculture And Environmental Degradation. An article published in the daily "The Bangladesh Observer" July 28, 1990.
- Kotey, D. A; Gbewonyo, W.S.K.; Afreh-Nuamah, K. (2008). High chlorpyrifos levels on vegetables in Ghana. *Pesticides News*. 2008; (80): 4-6

- Krol, W. J; Arsenault, T. L; Pylypiw, H. M Jr; Mattina, M. J. I. (2000). Reduction of pesticide residues on produce by rinsing. *Journal of Agricultural and Food Chemistry.*; 48(10): 4666-4670
- MacIntyre, A.N.N., Allison, N. and Penman, D.R. (1989). Insecticides issues and options for New Zealand Ministry of the Environment, Wellington, New Zealand.
- Mansingh, A. Robinson, D.E, Walker N., and Thomas, C. (1996). Distribution, fate and effects of pesticides in tropical Marine environment, presented at the third IAEA-MEL Res. Coordination meeting Heredia, Costa Rica, 9-13 September.
- Matin, M.A. (1995). Environmental Pollution and its control in Bangladesh. *Trends Anal. Chem.* 14 (10):468-473.
- Mukhopadhyay. K., Bera. R. and Roy, R. (2005). Modern agriculture and environmental pollution. *Everyman 's Science (XL) 3*: pp. 186.
- Neela Bakore; John, P. J; Pradeep Bhatnagar. (2002). Evaluation of organochlorine insecticide residue levels in locally marketed vegetables of Jaipur city, Rajasthan, India. *Journal of Environmental Biology.*; 23(3): 247-252
- Pany, B K; Pattnaik, M R. (2006). Monitoring of total diet for organochlorine insecticide residues and their metabolites. *Journal of Plant Protection and Environment.*; 3(2): 34-38
- Pedigo, L.P. (1991). *Entomology and pest Management.* Maxwell Macmillan Int., New York.
- Price. C. (2008). Implications of Pesticide residues in inter-rated ditch-duke farminL), systems. *Central Thailand. Aauaculture News 32*: nn. 23.
- Rahman, M.S. Malek, M.A., and Matin, M.A (1995). Trend of pesticide usage in Bangladesh. *Sci. Total Environ.* 156, 33-35
- Reinert, R. W., Menzel, D. B., Martin, D.T. and Olcott, H.S. (1970). DDT residues in pacific sea birds, a persistence insecticide in marine food chain. *Nature, London* 216:589-590.
- Sarratt, M. (1977). In: *The Evaluation of Toxicological Data for the Protection of Public Health*", Published for the commission of the European Communities, Pergamon, Oxford. p-105



- Shao XueXin; Wu Ming; Jiang KeYi. (2008). Distribution and risk assessment of organochlorine pesticides in the soils of Xixi Wetland Park. *Journal of Ecology and Rural Environment*; 24(1): 55-58
- Singh, I.P. and Kalra, R.L., (1989). Dissipation of permethrin in brinjal fruits. Leaves and soil. *Indian Journal of Entomology*. 51 (3) : 246-252
- Singh, I.P. and Kalra, R.L., (1992). Determination of residues of cypermethrin in brinjal fruits, leaves and soil. *Indian Journal of Entomology*. 54 (2) : 207-216.
- Skrbic,-B; Predojevic,-Z. (2008). Levels of organochlorine pesticides in crops and related products from Vojvodina, Serbia: estimated dietary intake. *Archives of Environmental Contamination and Toxicology*. 54(4): 628-636
- Tejada, A.W. and Calumpang, S.M.F. (1995). Insecticide Residue in Field Sprayed Eggplant. *The Philippine Agriculturist*. 78.(2): 119-128.
- Their, H.P; Stijve-T; Specht-W. (1989). Quality assurance in insecticide residue analysis. Experience gathered during inter laboratory tests. *Fresenius-zeitschriftfur-Analytische-chemie*. 334 - 6, 545-549.
- Yang XingLun; Jiang Xin; Yu GuiFen; Yao FenXia; Bian YongRong; Wang Fang. (2007). Leaf air transfer of organochlorine pesticides from three selected vegetables.
- Zhang MaoSheng; Huang JiaRong; Wei ChangLin; Yu BinBin; Yang XiaoQing; Chen Xi. (2008). Mixed liquids for single-drop microextraction of organochlorine pesticides in vegetables. *Talanta*. 74(4): 599-604
- Zhang XiaoLin; Wang HongHua; Pan YanHua; Yang MingBin; Yan TingTing; Sha LingJie; Mi YanHua; Yang WenHong. (2006). Dynamic monitoring and the results analysis of the environmental quality on three basic farmland in Yunnan. *Southwest China Journal of Agricultural Sciences*.; 19(4): 616-620
- Zhou-ZeYi; Liang-JianPing; Gai-LiangJing. (2001). Pesticide pollution in food and control countermeasure in Beijing. *Beijing Agricultural Sciences*. 19(3): 13-16
- Zidan, Z. H.; Mohamed, K. A.; Bayoumi, A. E. (2000). Market basket monitoring of pesticides residues on vegetables and fruits from Kalubia governorate, Egypt.

