Molecular Mechanisms of Pesticide Residues Induced Hepatotoxicity Isolated from Water, Fruits, Rice and Vegetables

A thesis submitted to the

The Maharishi University of Information Technology, Lucknow, Uttar Pradesh

in partial fulfilment of the requirements for the award of the Degree of

DOCTOR OF PHILOSOPHY IN CHEMISTRY

Submitted by

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UNDER THE SUPERVISION OF

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THE SCHOOL OF SCIENCE

SESSION 2017-2020

CERTIFICATE

The research work embodied in the present thesis entitled **"Molecular Mechanisms of Pesticide Residues Induced Hepataotoxicity Isolated From Water, Fruits, Rice And Vegetables"** submitted by **Divyashree J,** for the award of the Degree of Doctor of Philosophy in the Chemistry, has been carried out in, The School of Science, Maharishi University of Information Technology, Lucknow, Uttar Pradesh 226013.

The work reported here in is original and does not form part of any other thesis or dissertation on the basis of which a degree or award was conferred on an earlier occasion or to any other scholar. I understand the University's policy on plagiarism and declare that the thesis and publications are my own work, except where specifically acknowledged and has not been copied from other sources or been previously submitted for award or assessment.

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DECLARATION

I hereby declare that the work embodied in this thesis **"Molecular Mechanisms of Pesticide Residues Induced Hepataotoxicity Isolated From Water, Fruits, Rice And Vegetables",** is a research work done by me under the supervision and guidance of Dr. Sapna Tomar. The thesis or any part thereof has not formed the basis for the award of any Degree, Diploma, Fellowship, or any other similar titles.

Date:

Place:

Divyashree J

ACKNOWLEDGEMENT

The goal of every journey is to reach the destination. Reaching the destination is the most pleasurable moment in one's life. But what makes the journey interesting and memorable is the people who have walked together.

I would first and foremost like to express my deep sense of reverence and gratitude to God almighty. Without His invisible support and guidance this would have been impossible. He has magically unfolded the Chemistry pathway in my mind's eye and empowered me to crease it.

With my deepest gratitude I would like to thank my revered guide, Dr.Sapna Tomar who has inspired, touched and illuminated me. She/he has been a friend, philosopher and guide in the true sense. Here scholarly knowledge added essence to my work. Here persistence and understanding instilled the urge to move on and never give up. Here cheerful and friendly nature let me pour out my ideas, doubts and problems without any inhibition. She/he has been the milestone that gives assurance that one is on the right path and destination is not too far way. She/he has been the backbone, supporting my work in every way possible.

Behind every successful woman there is a man. I would like to thank my dear husband Mr. Ramachandra N R, who provided me with opportunities to explore my potential and never interfered with my academic pursuits. He silently supported my work by providing me with all resources needed to make my journey smooth.

As I travelled through this journey there were lot of hard bends and hurdles. My wonderful family members with their magical want made all of them vanish. I thank them from the bottom of my heart. I would like to lovingly acknowledge their contribution. Especially my mother Vimala devi, father Jayaprakash Narayan H S & Mother-in-law Yashoda N R, who was here at my beck and call to manage my home and children so that I would be able to complete my research.

I would like to thank all the people who have in their own way contributed to this work.

Divyashree J
PhD Research Scholar

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ABSTRACT

The aim of this study is to the Farmers around the world including India use pesticides as a preventive strategy against the possibility of a devastating crop loss from pests and diseases. Pesticides are thus not only used in agriculture for the control and eradication of crop plagues in India for several decades, but in public health for the control of vectors of disease. Cocoa, coffee and cotton, vegetable, fruit and other cereal mixtures (mostly maize), tuber crops, legumes (e.g. cowpeas, beans), sugar cane, rice etc. are used in agricultural pesticides. are also used in cereal and fruit cultivation. In water bodies, especially underwater, pesticide contamination is becoming a serious problem. Several agencies have reported that pesticides are present in underground water in areas that are grown in agriculture. It can be because of direct exhaust, lixiviation, careless disposal of empty containers, washing machinery etc. to reach the aquatic environment. Rice grains are primarily contaminated with pesticides during field sprinkling and storage processes. In storage premises and before being exported to other countries, rice grams are treated with pesticides including organophosphates, carbamates, synthetic pyrethroids and insect growth regulators. In addition to few weeds like Echinocloa, rice is attacked primarily with insect pest and leaf folder, blast and blast diseases.

The growth of agriculture to date has been based mainly on the use of synthetic inputs to supplement or replace natural processes, including pesticides. It has led to a considerable increase in the production of agriculture, but also has had negative effects on the whole ecosystem by entering the food chains and polluting soil, air, soil and water. Pesticide means any substance that may be administered to animals for the purpose of eCS control, prevention, destruction, and attraction, repel or control of any pest, including unwanted species of plants or animals in the process of production, stockpiling, transport, distribution and processing of food, farm goods and food or feed. The term includes materials intended to be used as a controlled plant growth regulator, defoliant, disinfectant, fruit thinning agent or substances used to protect the product from deterioration in storage and transportation before or after harvest. Normally, fertilizers, animale and plant nutrients, food additives and livestock medicines are not included. A number of old times have been recorded with pesticides and fertilizers, which suggest that the use of ecosystems is not a recent cultural feature.

Residue analysis is performed by food and environmental monitoring. Several multiresidue methods were proposed, followed by detections by selective and sensitive detectors, in the identification of organophosphorus, organochlorine and organo nitrogen pesticides in crop materials using gas chromatography. A number of research papers on method development to determine residues in rice grain with traditional gasses (GC with ECD, FID) and sophisticated instruments (GC/MS) with combination of new extraction techniques have been published. The purpose of current research was to assess the concentration of pesticide residues in foodstuffs, to raise awareness of their lethal effects on humans and to estimate the potential consumer health risks associated with pesticide residues. Residues of 15 commonly used pesticides were estimated using GC-ECDINPD detection, namely endosulfan, dieldrin, aldrin, lindane, DDT, chlorpyrifos, profenophos, quinalphos, dimethoate, phorate, cypermethrin, fenvalerate, deltamethrin and carbofurannes. In order to analyze the residuum levels in major feedstuffs in Kerala, samples of rice (main cereal in Kerala), fruit and vegetables have been collected from fields and markets and water samples from wells and streams located in Thiruvananthapuram and Kasargod.

CHAPTER-1

INTRODUCTION

1.1 Introduction:

Pesticides have contributed greatly to the increase of yields in agriculture by controlling pests and also towards ruling out the insect-borne diseases like malaria, dengue, encephalitis, filariasis, etc. (Bhatnagar, 2001; Rekha, 2006). To achieve economic benefit and to make sufficient supply of food to the vast population, the yield per hectare will have to be increased still more and the use of crop protection technology will have to be intensified, not restricted. The steigerning of agriculture that has been achieved so far has been primarily driven by the use of synthesized inputs, including pesticides, to supplement or replace natural processes. It has also shown negative impacts for the whole ecosystems, by entering food residues in and polluting soil, air, ground and surface water (Agnihotra, 1999; ICAR, 1967; UN/DESA, 2002). This model of modern agriculture has enabled a considerable increase in crop yield. Pesticide is any substance which, during production, storage, transport, distribution, transformation and transmission of food, agricultural commodities or feed or which is administerable to the animals to control ektoparasites, is intended to prevent, degrade, attract, repelled or control pests, including undesired plant or animal species (F AO, 1989). It includes substances that are intended to be used as a regulator for plant growth, defoliant and desiccant, as well as thinners for fruits, as well as substances which are used to protect the product from deterioration in storage and transport, both before and after harvest. The term does not generally mean fertilizers, nutrients for plants and animals, food additives and animal drugs (Handa, 1999; WHO 1990).

Ancient times have been recorded with pesticides and fertilizer applications, indicating that management of the ecosystems is not a new cultural attribute. The aims to increase production efficiencies and yields are part of modern agriculture; reduce the costs of food and, above all, increase the availability of grains, fruits and vegetables; improve food quality and losses during transportation and storage; improve soil conservation; and ensure that food supplies are stable and predictable; (NRC 2000). Pesticide residues from foodstuffs have been monitored through their centers in different parts of the country under the Pesticide Residues Coordinated Research Project in India of the Indian Council of Agricultural Research, New Delhi. Food products were found to have contaminated 51 percent with pesticide residues and pesticide

residues were higher than MRL values than 21 percent, with worldwide contamination only 2 percent above MRL (Agnihotri, 1999). A study by the Center for Science and Environment, New Delhi, of bottled water, cassava and other drinks showed very high pesticide residue levels in the samples (Anonymous, 2003 a, b and c).

Historically, chemicals have been used for centuries to kill or control pests. The Chinese use arsenic for insect control, the early Romans use common salt for weeds and sulfur control. The insecticidal properties of pyrethrin were found in the 1800s. Paris green, a mix of copper and arsenic salts, was also a material developed for insecticide control in the 1800s. Bordeaux mixture, lime and copper sulphates were controlled by fungi (Hodgson, 2004). It wasn't until 1900, however, that the pesticide-like compounds we today identify were created. In the early years of DDT, it has been hailed as a miracle chemical for its broad spectrum activity (persistence, insolubility, costlessness and ease of use), and the insecticide was the first major synthetic organic pesticide in Switzerland, dichlorodiphenyltrichlloroethane (DDT), which was first synthesized by the German scientists Ziedler in 1873 (Othmer 1996). (Keneth, 1992). It was used to dislodge soldiers for the control of mosquitoes and vectors of malaria in World War II and in public health. These chemicals have brought huge advantages to increasing agricultural production after World War II (Margaret, 2004, Wangila, 2004).

Following the success of DDT, such other chemicals were synthesized to make this era what Rachel Carson (1962) in her book "The Silent Spring" denoted, as the era of "rain of chemicals" which cites "Pesticide sprays, dusts, and aerosols are now applied almost universally to farms, gardens, forests, and homes - non selective chemicals that have the power to kill every insect, the good and the bad, to still the song of birds and the leaping offish in the streams, to coat the leaves with a deadly film and to linger on soil - all this though the intended target may be only a few weeds or insects. Can anyone believe . .. .. it is possible to lay down such a barrage of poisons on the surface of the earth without making it unfit for life? They should not be called insecticides, but biocides...."

Silent Spring raised public awareness and concerns about hazards associated with uncontrolled use of DDT and other pesticides including wildlife poisoning and human food contamination. The intensive use in agriculture of pesticides is also known to be linked to the "green revolution." There were three important aspects of agricultural practices during the

Green Revolution, including the use of pesticides. In agriculture, pesticide control was always important but, in particular, the green revolution needed more pesticides than conventional agricultural systems, since most high yield varieties were not widely resistant to pests and diseases, and in part due to a monoculture system (Vocke, 1986). Both the efficiency of the cultivation of plants and the amount of food produced has been increased by pesticide use. Alongside the benefits, though, there have been great concerns from consumer groups about the potential impact of trace amounts of remaining pesticide residues on food commodities, demanding that food we eat is safe.

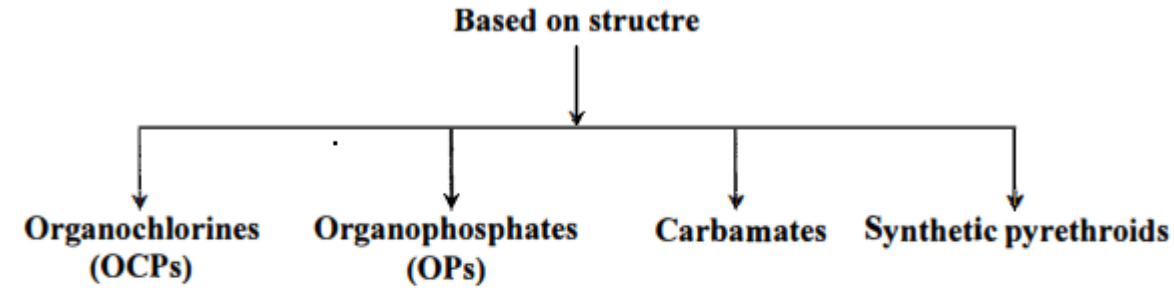
1.2 Classification:

Pesticides can be broadly classified according to their intended target pest and also by their chemical structure and properties (Margaret et al., 2004).

1) Based on the intended target pest, they are mainly classified as follows.

Types of pesticide	**Target organism/pest**
Insecticides	Insects
Herbicides	Weeds
Rodenticides	Rodents
Fungicides	Fungi
Acaricides and miticides	Arachnids such as ticks and mites
Moluscicides	Mollusks
Bactericides	Bacteria
Avicides	Bird pests
Viruses	Virus
Algicides	Algae

2) Based on the chemical structure and properties they are mainly classified as given below.



a) Cyclodiens, aldrin, dieldrin, endrin, heptachlore, endosulfan, and endosulfan sulfate
b) Diphenyl aliphatic such as DDT
c) Hexachlorocyclohexanes αHCH, βHCH, χHCH, and δHCH

1.2.1 Organochlorine Pesticides (OCPs)

Chlorinated orgamic compounds used mainly as insecticides are organic chlorine pesticides. The diphenyl aliphaths are mainly divided into the three categories of aldrin, dieldrin, endrine, heptachlore, endosulphane and endosulfan sulfate. they include the DDT and their metabolites. Hexachlorocyclohexanes are the most common organochlorine insecticides known to exist in many structural isomers like aHCH, PHCH, yHCH and 8HCH (Banu and Semra, 2004). Typically, these pesticides are very persistent to the environment and accumulate in sediments, plants and animals. Most can break down slowly and remain in the environment long after application and long after exposure in organisms (Hodgson, 2004). Organochlorine pesticides are wide-ranging insecticides active against a wide range of pests. Their chemical structures are different. Diphenyliphatics, cyclodiens and hexachloro-cyclohexans are the main categories for the OCPs and their metabolites; (Banu and Semra, 2004).

i] Diphenyl aliphatics include p, p' -DDT (Fig. I. I), p, p' -DDE, p, p' -DDD and methoxychlor. DDT successfully controlled spreading of malaria, a disease still plaguing large parts of the human population in Africa, and crop destroying insects (Ahmed, 2007).

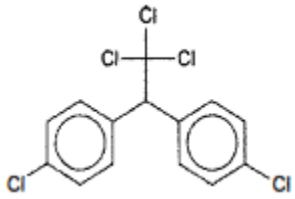


Fig.1.1 structure of DDT

ii] Cyclodiene compounds (Fig. I.2) are Synthetic cyclic hydrocarbon collective group, including aldrin, dieldrin, endrin, endrinaldehyde, heptachlore, heptachlorine epoxide, aendosulfane, endosulphan and endosulfan sulfate. They are especially effective if contact actions and long persistence are necessary; for example, endosulfan acts as a poison of contact with field plants that suck, chew and boron insects (Ahmed, 2007).

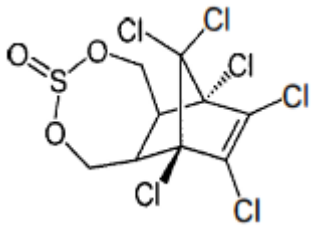


Fig. 1.2 Structure of cyclodienes

iii] Hexachlorocyclohexanes (HCH) are manufactured chemicals that exist in several structural isomers such as aHCH, HCH, yHCH, and oHCH. Only one of these forms, oHCH (commonly called lindane) has insecticidal activity and used as an insecticide on fruits, vegetables, in forestry and animal husbandry.

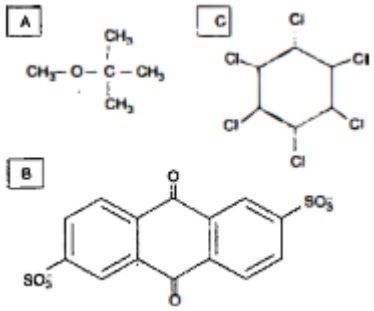


Fig. 1.3 Hexachlorocyclohexanes

1.2.2 Organophosphates (OP)

Compounds of organophosphorus are all chemicals containing carbon and phosphorus (Fig. 1.4). Many of those are not used as pesticides inhibiting the acetylcholine esterase enzymes. In general, OP insecticides have a low mammalian hepatotoxicity and a high level of acute insect hepatotoxicity. This selective hepatotoxicity is designed in the molecule and makes use of differences between mammals and insect pests in the metabolism of OPs. Monocrotophos are highly dangerous pesticides and are continually and indiscriminately used in India, such as phorate, phosphamidon, methyle parathione and dimethoat.

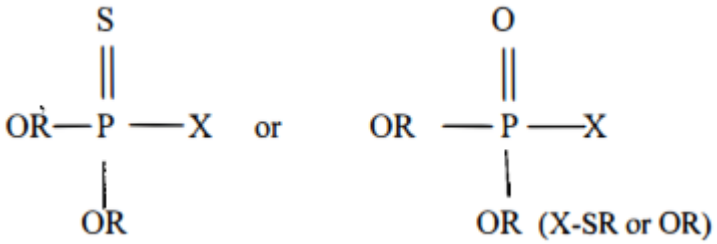


Fig 1.4 structure of organophosphates

1.2.3 Pyrethroids

Pyrethrins are derived from the naturally occurring pyrtherums, which in turn are the oleo-resin extracts of dried chrysanthemum flowers. These pyrethrins owe their insecticidal activity to the keto alcoholic esters of chrysanthemic and pyrethroic acids. In order to at once maintain the fundamental properties of pyrethrins, pyrethrins are synthetic analogs modified by introducing a Biphenoxy Motivity and substituting certain hydrogens for halogens. Pyrethrum flowers are components of insecticidal substances derived from (+) chrysanthemic acid trans and (+) pyrethroic Acid transactions. It is known that pyrethroids have an immediate knockdown on insect pests, low hepatotoxicity of mammals and easy biodegradation. Their environmental persistence takes weeks, unlike organochlorides, which last for years (Elliot, 1976).

Pyrethroids type I "T" are contact pmsons producing ruin, incoordination, prostration, hyperexcitation, aggression, corporeal twists and paralysis in both mammals and in insects (Klaassen, 1996). As for the type II "CS" pyrethroides, type I "T" pyrethroids have no alpha

cyanic group (R-C=O, 0-R). Included in Type I are allethrin, permethrin, bifenthrin resmethrin etc. The CS syndrome of type II contain a cyanoalpha group (RC=O, C=CN, R). Type II: "SS" Syndrome: These pyrethroids are able to disable many species of insects or "knock-down." In household, veterinary and agricultural pesticides Type II "CS" pyrethroids are used (Klaassen 1996). The activity of pyrethroids of TypeII means that they are more toxic than pyrethroids of TypeI. Cyperethrin, cyfluthrin, deltamethrin, fenvalerate, and fipronil are included in Type II "CS" pyrethroids.

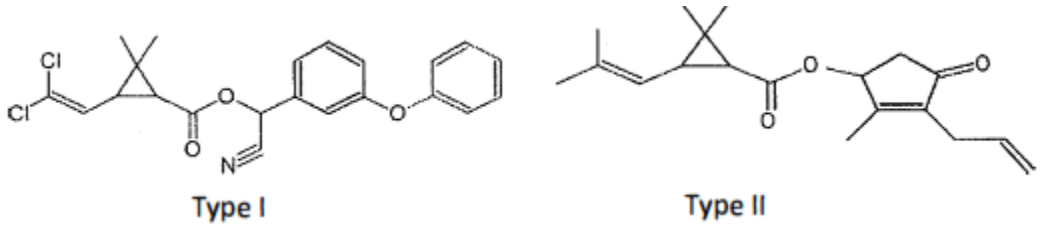


Fig. 1.5 Structure of pyrethroids

1.2.4 Carbamates

Carbamates are organic carbamic acid pesticides. In agriculture, these products are used as inhibitors of insecticides, fungicides, herbicides, nematocides and sprout. They are also used as biocides for industrial or other uses and for domestic products. Public health vector control is potentially used. The structure of R1 as an alcohol group is indicated in Figure 1.5, R2 as methyl group and R3 as a general hydrogen. The inhibitions of carbamate cholinesterase differ from organophosphorus, because it is specific to species and reversible (Drum, 1980). Aldicarb, carbofuran (Furadan), carbaryl (Sevin), ethenocarb, fenobucarb, oxamyl and methomyl are included in this group.

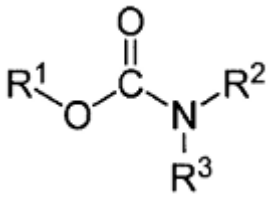


Fig. 1.6 Structure of carbamates

1.3 Pesticide:

Usage Critical to the Indian economy is agriculture. Even if it has declined from about 30% in 1990–91 to less than 15% in 2011–12 in its contribution to the overall gross domestic product (GDP), which is expected to be a trend in the development process of any economy, farming is the cornerstone of development. An average Indian spends nearly half its total cost of food still, while approximately half of India's labor force continues to make a living from farming. An efficient pesticide control is one of the strategies to increase crop productivity because more than 45% of the yearly food production is lost from the infestation of pests. Safe and effective pesticides available and their proper use by the farming community are vital in order for farming production and productivity to continue to increase. Plant loss is even worse in tropical countries because the high temperature and humidity are particularly favorable for the fast growth of plagues (Kannan, 1992; Lakshmi, 1993). Therefore, a wide variety of pesticides are needed to fight pests and vector-borne diseases on crop plants in the tropics. The sporadic use has, however, led not only to terrible effects on public health but also food quality that have an impact on the environment and consequently the development of pest resistance (Agnihotri, 1999). Under the words "if little is good, much more will be better," the widespread use of these chemicals has become a disaster with human and other forms of life. The indiscriminate application in the food chain affects the whole ecosystem and pollutes soil, air, soil and surface water (Agnihotri, 1999; ICAR, 1967; UN/DESA, 2002). Pesticide pollution also affects bird, wildlife, domestic animals, fish and animal life in the local environment (Anony. 1991).

1.4 HEPATOPROTECTIVE ACTIVITY OF COMMONLY CONSUMED VEGETABLES

India is a country with cultural diversity and traditions. Region specific food habits and medical practices are observed. In India, a number of traditional medicine practices such as folk medicine/tribal, Ayurveda, Siddha, Unani etc. take medicinal resources from herbs. However, most edible plants and their components such as fruits, seeds, leaves and rooted substances of medicinal value are also observed to have antioxidants, flavonoids, tannins and other phenolic compounds[1]. In Indian rural and tribal villages, it is estimated that around 7,500 plants are used in the tradition of local health. The real medicinal value of more than

4,000 plants is either unknown to the mainstream population or unknown. Approximately 1.200 plants are used in traditional medicine systems like Ayurveda, Siddha, Amchi, Unani and Tibet. In the maintainance, performance and control of the body's homeostasis the liver places important functions. It involves numerous biochemical growth pathways, disease control, storage of nutrients and supply. It also acts as a metabolism center for nutrients like carbohydrates, protéins, lipids and waste metabolite excretion. One important role in digestion is the bile secreted by the liver. For the overall well-being of a person, maintaining a healthy liver is therefore essential. Liver damage is highly likely because many toxic substances injected through the food have to be detoxified. By producing reactive species, most hepatotoxic chemicals damage liver cells. The free radicals generated are sometimes so high because they are excessively exposed to hepatotoxic chemicals, that they overcome the liver and cause jaundices, circrhosis and fatty liver. Production of reactive species is shown to cause severe liver damage to thiols, buttocks of the tissue, plasma membrane damage, etc. [2]. India is bestowed with diverse climatic conditions, which favour region specific vegetables and their usage. In this review, various studies on fruit vegetables, leaf vegetables and pulses, that are especially consumed in the south India and their hepatoprotective activity against Paracetamol / thioacetamide / Carbon tetrachloride (CCl4) induced Hepatotoxicity by various investigators are reviewed.

Hepatoprotective Activity of Some commonly consumed vegetables (in the form of seeds, roots, leaves and fruits):

In Indian traditional medical practice a variety of food significant plants are used as preventive substances for diverse ailments. Many vegetables are consumed without the realization of the medicinal values. However, a list of plants reported to have significant hepatoprotective activity is shown in Table 1.1, in alphabetical order of their family, together with their scientific names, plant part consumed, type of extract used in assay, Hepatotoxicity inducer, biochemical parameters studied and references.

Biochemical Parameters studied:

Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Estimation of the alanine aminotransferase (ALT),

aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), Total Glyceride (TG), albumin (ALB), glutathione (GSH), Gamma- glutamate transpeptidase (GGTP), malondialdehyde (MDA), Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT), Reactive Oxygen species (ROS) such as superoxide dismutase, catalase with the levels of control animals and micrographs on histopathological changes were used in general as diagnostic tools [3].

Allium cepa L

Ovion bulbs and their extract were studied with ccl4, ethylacetate and paracetamol and induced liver toxicity by Rawat et.al.[4] in wistar albino rats for their hepatic protection activity. Paracetamol or acetaminope produces N-acetyl- p-benzoquinone-imine (NAPQI) by cytochromes P450 monooxygenase at large amounts of liver necrosis after bio-activation to a toxic electrophile (a microsomal enzyme). This changes the SGOT & SGPT levels. Tetrachloride carbohydrate has significantly increased the seral levels of SGOT, SGPT, ALP and bilirubin, as the structure and function of liver cells is altered thanks to the enzymatic activation of CCl3. In the study the elevated biochemical parameters of the treatment with alcoholic extract (AEAC) and aquatic extract (AQEAC) have been significantly reduced because they interfere with the cytochromome P450 monooxygenase, since it includes saponins, carbohydrates, steroids, flavonoids, thereby reducing the hepatotoxic-free radicals. Hepatoprotective effects of these extracts have also been shown by liver histopathology. The allium cepa aqueous extract (ACE) preventive effect against cadmium induced hepatotoxicity was reported by the Ige et [5], using male Wistar rats as model [6-7]. They showed that cumulative oxidative damage is due to the hepatotoxic effect of Cd. Cadmium is observed in the livers of all groups of rats exposed to Cd to decrease the dismutase (SOD) of superoxides and to increase malondian dehydrate (MDA). Aminotransferase levels of serum aspartate [8]. Hepatocellular damage indicators are ALT, AST, and ALP, the most common enzymes. Hepatic damage causes more activity of these enzymes in the plasma. The mechanism by which AcE improves hepatotoxicity induced by Cd is its ability to preserve the integrity of hepatocytes and ROS scabbing.

Allium sativum L

Supplemented diet of Garlic along with Vitamin-C and its hepatoprotective effect against lead induced hepatotoxicity was studied by Ajayi et.al and Senapati et.al. [9-10] on experimental rats. They observed marked increase of ALT and ALP enzyme levels and decreased level of AST, due to lead treatment, thus suggested hepatic damage. After-leading A. sativum and vitamin C treatment significantly reduced ALT and ALP activities and increased plasma AST activity, such as control activities. Binding of the cysteine-containing enzymes in the sulfhydrate groups is known and complexes are found with amino acids and protein. Since ALT is a liver enzyme the plasma plasma discharge into the blood [11-12] changes the tissue level by disrupting its membrane. In general, the reduced serum activity of ALT and ALP is due to the decreased production of these enzymes, hence the inverse effect of plum hepatotoxicity. Ebenyi et.al. [13] on Paracetamol and Asaduzzaman and other similar studies [14] on Alloxan, and Nasim Ilyas and other [15] on Isoniazid have been carried out.

Amorphophallus paeoniifolius

Pramod J Hurkadale et al. [16] studied methanol and aqueous extracts of the elephant foot yam tuber and their hepatoprotective action on male albino-wistar rats causing hepatic damage to Paracetamol. The reacción metabolite N-acetyl-p-benzoquinoneimine (NAPQI) is caused by paracetamol hepatotoxicity, which causes the oxidative stress and the eradication of lutathione. It is a well-known pyretic and analgesic agent that causes higher doses of hepatic necrosis. Prior to paracetamol administration, pre-treatment of rats with aqueous extract and methanol led to a significant reduction in silymarin and Liv-52 nearly-comparable SGOT, SGPT, SALP and S B values ($P<0.01$). Histopathological examination of the control tissue of the liver and of the treatment animals with amorophallus extract [17-20] confirmed the hepatoprotective act. The report shows flavonoids and steroids may be responsible for a hepatoprotective effect, as the important chemical constituents of the tubers include carbohydrates, sitosterols, stigmasterol, thiamin and riboflavin[21].

Benincasa hispida

Diclofenac hepatotoxicity has been reported to hepatocytes by Bort et.al. [22]. Das, S.K and C. Roy reported hepatoprotective acts of aqueous winter melon extract (Benincasa hispida = BH) to male albino rats against diclofenac-sodium induced liver damage[23]. The hepatoprotective effect was observed by the modulation of antioxidant – mediated mechanism by alterations in serum glutamate oxaloacetate (SGOT), serum glutamate pyruvate transaminase (SGPT), alkalised phosphatase(ALP), superoxide dismutase (SOD) and catalasis (CAT) activities and reduced levels, induced by diclofenac sodium, glutathione (GSH) and lipid peroxidation (LPO). Vitamin E, beta-carotene, flavonoids and flavonols are present in the BH pulp. Thereby the results of SGPT, SGOT, ALP, LPO, CAT, SOD and GSH activities, potentially of vitamin E, beta carotene, flavonols and flavonoids that are present in BH pulpe, are inferred as BH protected rats liver against oxidizing stress. Das and Roy[23] reported hepatoprotection of BH from hepatotoxicity caused by nimesulide as well.

Beta vulgaris

Phytochemical studies of beet root reveal the presence of flavonoids, carbohydrate, betaine, neobetain and anthocyanin pigments. Ranju Pal et.al. [24] evaluated the effect of ethanolic extract of Beta vulgaris (EEBV) root against CCl4-induced hepatic damage in rats. The hepatoprotective activity of EEBV was studied by estimating serum enzyme levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and bilirubin. The treatment with EEBV is observed to ($P<0.01$) reduce the CCl4 induced elevated serum levels of enzyme activities and bilirubin with parallel significant ($P<0.01$) increase in total protein, AST, ALT and ALP, indicating the extract could preserve the normal functional status of the liver. Histology of the liver sections of the animals treated with the extracts were also observed with the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity. The hepatoprotective effect of EEBV may be due to presence of its chemical contents [25-26] using leaves of Beta vulgaris.

Brassica juncea

Agnel Arul John et.al. [27] evaluated the hepatoprotective activity of aqueous extract of Mustard seeds against carbon tetrachloride (CCl4) induced hepatic damage in albino rats [28] evaluated the leaf extract. The Lipid peroxide (LPO) and antioxidants like Superoxide dismutase (SOD) and reduced glutathione (GSH) were estimated in liver tissue homogenate. Hepatotoxicity induced rats were reported to show increased level of LPO and decreased levels of antioxidants like SOD and GSH. The activities of liver marker enzymes such as GOT, GPT, ALP, and GGT in serum were increased. On treatment with BJ extracts, all the biochemical changes observed in the hepatotoxicity induced rats were reversed in a dose dependent manner. It is observed that the presence of terpenoids and flavonoids are the possible reasons for hepatoprotective activity due to their free radical scavenging and antioxidant properties [28-29].

Brassica oleracea

Ethanolic extract of cabbage vegetable and its hepatoprotective activity was studied by Ahmed et.al. against simvastatin induced hepatotoxicity [30]. The increased levels of serum enzymes due to simvastatin treated rats were restored towards normalization in BO. extract (300 mg/kg/p.o and 500 mg/kg/p.o) treated animals. Further it was observed that the hepatoprotection may be due to the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins and terpenoids in BO extracts [31].

Carica papaya

Ethanol and aqueous extracts of Carica papaya has been evaluated for its anti-hepatotoxic activity by Rajkapoor et.al. [32], Md. Zafor Sadeque et.al. [33] and Manikandaselvi et.al. [34] on CCl4 induced hepatotoxicity. The activity was evaluated by using biochemical parameters such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin and gamma-glutamate transpeptidase (GGTP). The histopathological change of liver sample was compared with respect to control and thus hepatoprotection activity was ascertained [35] on aqueous seed extract of Carica papaya. Srinivasan Kantham [36] examined the effect of carica papaya aqueous extract on rat models of paracetamol (PCM) and thioacetamide (TAA) induced hepatic damage. Wistar strain albino rats were prophylactically treated with three doses of CPE (100, 250 and 500mg/kg, p.o) for 10 days and subsequently liver damage was induced. Hepatoprotective potential was evaluated by measuring biomarkers and the hepatoprotection activity was ascertained. It is speculated that the protection may be due to the presence of vitamin - C.

Citrus limon

The ethanol extract of Lemon fruit was evaluated for its effects on induced hepatotoxicity by CCl4 and the ethyl acetate soluble fraction of the extract was evaluated on HepG2 cell line by Bhavsar et.al. [37]. It was observed that, the ethanol extract normalized the elevated levels of liver enzyme, total and direct bilirubin, due to carbon tetrachloride intoxication in rats. In the liver tissue, treatment has significantly reduced the levels of malondialdehyde (MDA), hence the lipid peroxidation, and raised the levels of antioxidant enzymes superoxide dismutase (SOD) and catalase [38]. It improved the reduced glutathione (GSH) levels in treated rats in comparison with CCl4-intoxicated rats. Thus it may be inferred that the hepatoprotection was provided by antioxidant enzymes by their ROS activity. Casimiro et.al., 2010, against acetaminophen-induced liver damage; Karaca [39], using Citrus bergamia.

Colocasia antiquorum

Ethanolic extract obtained from the corms of colocasia was evaluated by Tuse et.al. [40] for hepatoprotective activity using paracetamol and CCl4 intoxicated rats. The protective effect was evident from serum biochemical parameters and histopathological analysis. Ethanolic extract of CA significantly ($P<0.5$) prevented the elevation of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) in paracetamol and CCl4 treated rats as compared to silymarin(Control). These biochemical observations were supplemented by histopathological examination of liver sections showing the prevention of disarrangement and degeneration of hepatic cells induced by paracetamol and CCl4. The activity may be a result of the presence of anthocyanin compounds. Furthermore, acute hepatotoxicity studies showed no signs of hepatotoxicity up to a dose level of 1000 mg/kg. Thus, it may be concluded that ethanolic extract of C. antiquorum possesses significant hepatoprotective properties.

Colocasia esculenta (taro)

The anti-hepatotoxic and hepatoprotective studies were carried against paracetamol and CCl4 by Bhagyashree et.al. [41] using in vitro liver slice method. The free radicals generated by CCl4 and paracetamol cause oxidative stress as well as injury to the hepatocytes. The extent of damage caused by these free radicals and hepatoprotective efficacy was measured using the leakage of marker enzymes of liver function viz AST, ALT and ALP in the incubation medium. There was increase in the levels of marker enzymes indicating hepatotoxicity of these compounds. However the leaf juice of CE remarkably declined the leakage of AST, ALT and ALP in the medium indicating hepatocyte integrity. Thus it was concluded that the CE leaf juice as a whole possesses anti-hepatotoxic and hepatoprotective efficacy when tested in vitro using rat liver slice model [42-44].

Coriandrum sativum (Coriander)

Leena Kansal et al. and Aga [45-46] studied their protective role against plum nitrate induction of oxidative stress and tissue damage in the liver. Some biochemical hepatic

parameters such as AST, ALT, ACP, ALP and total cholesterol showed significant elevation after plum nitrate intoxication. However, a significant ($p<0.001$) decrease in hepatic AST, ALT, AKP, ALP and total cholesterol levels was achieved by the use of aqueous cilantro extract and ethanol extract. Lead management causes reactive species of oxygen (ROS) to overproduce and depletes the cellular capability of antioxidants. Antioxidizer properties like linalol and glycosides like different β-Dglucopyranosides are found in active components in coriander. This may be why hepatoprotection takes place [47-48].

Curcuma longa (Turmeric)

Mahuya sengupta et.al. [49] Evaluated hepatoprotective effects in mice of aqueous C.longa extract against CCl4 and observed that the level of serum and intake of ACL-aqueous extract has increased with SGOT, SGPT and bilirubin and reduced the intake of SGOT, SGPT and bilirubin. CL water extract administration also offered substantial protection against reduced host response parameters such as morphology, phagocytosis, release of nitrogen oxides, release of myeloperoxidases and intracellentic ability to kill peritoneal macrophages [50-51].

Cucumis sativus (Cucumber)

Kamalinejad [52] The antioxidant capacity of Cucumis sativus was studied on induced cytototoxicity and ROS formation in isolated cumene hepatocytes. It was found that the first rapid growth of ROS formation, which was prevented through aqueous extract of CS fruit, was when isolated hepatocytes were incubated with CHP. It has also been shown that Aqueous CS Extract acts as hepatoprotective and CHP antioxidant agents that can easily cross the cell membrane and coping with the intracellular ROS formation of antioxidants and radical scavenging components of CS extract[53-55].

Cuminum cyminum (Cumin)

Arun kumar et.al. [56] has investigated prophenoes, a pesticide organophosphate and its hepatotoxicity in rats. It has also been observed that significant ($p<0.001$) increase in marker SGPT, SGOT and serum bilirubin was the result of hepatic damage caused to profenophos. Oral cumin administration ($p< 0.001$) significantly reduces the levels of the SGPT and SGOT marker enzymes. It also reduces the level of serum bilirubin. In order to enhance profenophon-

induced hepatotoxicity, cytoprotected properties of cumin extracts restore cellular integrity and essential cumin oils have an essential role[57].

Daucus carota sativus (carrot)

Bishayee et.al. has assessed the effects of carrot extraction on the acute liver damage induced by carbon tetrachloride (CCl4). CCl4- Induction was reduced significantly by pre-treatment with the extract, which increased the levels of serum enzymes (e.g. glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, lactate dehydrogenase, alkaline phosphatase, sorbitol and glutamate dehydrogenase). The extract also decreased the high level of CCl4 administration for serum bilirubin and urea. The extract dose-responsive reversed increased activity of hepatic 5'-nucleoteasis, acid phosphatase, acid ribonucleasis and decreasing levels of succinic dehydrogenase, glucose 6-phosphatase and cytochromium P-450. The results of this research showed that carrot could provide significant protection for hepatocellular injury induced by CCl4. The same effects for hepatoprotection against Paracetamol, Isoniazid and alcohol induced hepatotoxicity have been reported by Mani Vasudevan et.al.[59] on Daucus carota seed extracts and Shoba et.al.[60]. The protective effect of carrot against lindane reported by Balasubramaniam et al. [61] has been Hepatotoxicity.

Lagenaria siceraria (Bottle guard)

Lakshmi et.al. [62] evaluated the hepatoprotective activity of Lagenaria siceraria fruit extracts against CCl4 induced hepatotoxicity in rats. Biochemical parameters, hepatic weight and hepatic histopathology studies were examined for the liver function. It is noted that the toxic effect of CCl4 is significantly controlled by restoring the serum of liver bioquemicals to normal levels in animals treated with ethanol extract. The historology of the animals treated with the extracts showed the presence of ordinary liver cords, lack of necrotization and fatty infiltration that further demonstrated the activity of hepatoprotection. It is usually thought to be caused by radical carbon trichloromethyl (CCl•3) peroxidation in lipids. CCl4 is biotransformed to the trichloromethyl-free, radical cytochromium P450 which induce lipid membrane peroxidation and disturbs the hepatocellular injury of Ca2+ homeostasis. The potential hepatoprotective mechanism of extracts can be through cytochrome activity

inhibition P-450, prevention of lipid peroxidation process, hepatocellular membrane stabilisation, and protein synthesis improvement [63-66].

Luffa acutangula

Jadhav et.al. [67] Hydro-alcoholic extract (HAELA) hepatoprotective effect of Ridge guard against CCl4 and hepatotoxicity of Rifampin was studied. Standard drug-silymarine and HAELA administration showed significant protection from CCl4 and hepatotoxicity induced by rat rifampicin. This could be due to lower levels of serum marker enzymes viz. (AST (ALT), ALP and LDH) and increased overall protein in comparison to the control group including an improved histoarchitecture of the hepatic cells of the treated groups. Increased nonenzymatic intracellular antioxidants, glutathione and enzyme antioxidants, catalase and superoxidant dismutases also shown significant decreases in the formation of malondialdehyde (MDA). The results of this study showed that the hepatoprotection activity of HAELA is due to endogenous antioxidants and lipid peroxidation inhibition of the membrane[68-72]. Rawat et al. reported a comparisons with Silymarin for hepatoprotective activity of ethyl acetate extracts of Allium cepa bulbs, Swertia chirata twigs, Silky acutangula leaves and Wood fordia floribunda leaves. Alcoholic and aquatic fruit extracts of Luffa cylindrica linn have a hepatoprotective activities in rats [73].

Macrotyloma uniflorum

Parmar et.al. [74] investigated the hepatoprotective activity of Horse Gram Methanol Extract (MEMUS) in wistar albino rat for hepatotoxicity caused by D-galactosamine and paracetamol. The reference standard was Silymarin. Protection levels of the serum glutamate pyruvate transaminase (SGPL), serum glutamate oxaloacetate transaminase(SGOT), alkaline phosphate (ALP), bilirubin were determined by the estimates of biochemical parameters of the serum (Direct and Total). The histopathological study also supported the test extract hepatoprotective activity. Dose-related decrease in biochemical parameters in D-Glactosamine and in paracetamol as well as in morphological parameters caused hepatotoxicity in rats. Some scientific reports suggest that tannins have a protective effect on the liver due to its antioxidant

properties [75-76]. Some flavonoids, triterpenoids and steroids. Hepatoprotective activity may have been offered by active compound flavonoids and tannins of Macrotyloma uniflorum.

Mentha arvensis (mint)

Aqueous extract of M. arvensis (AEMA) and its protection to alcohol induced liver injury was studied by Radhika et.al. [77]. The ethanol and CCl4 induction caused an elevation in the serum marker enzymes, bilirubin and Lipid peroxide levels confirming liver injury. The treatment of the animals with AEMA depicted the restoration of these parameters in serum and liver. Preliminary phytochemical screening showed the presence of flavonoids. The restoration of the hepatic functioning of the serum and tissue markers suggests the protective activity of M. arvensis which may be attributed to the presence of flavonoids. Kalpana Patil et.al. [78] on similar effect against CCl4 induced hepatotoxicity).

Momordica charantia

In order to evaluate hepatoprotective and hepato-curative effects of Momordica charantia, analysis of various serum enzymes including ALT, AST, ALP and LDH have been carried out. Hepatotoxicity of the liver was induced in rabbits administering acetaminophen, then extracting Momordica and observing hepatocurative effects[79]. The findings revealed that the high enzyme levels of acetaminophen poisoned rabbits decreased significantly. The alloxane-induced hepatotoxicity in Hossain et al. [80]. The hepatoprotection of Momordica Charantia may be attributed to the presence of the saponins, tannins, triterpenes and alkaloids as well as to flavonoids, ascorbic acid, [81-84].

Moringa oleifera

Methanolic extract of Moringa leaves and it’s hepatoprotection against CCl4 induced liver injury was studied by Balamurugan et.al. [85] on albino rats. Ruckmani et.al. [86] reported on Paracetamol induced hepatotoxicity. The potent hepatoprotective activity of leaves of Moringa was found to reduce the level of Total Bilirubin, Direct Bilirubin, SGPT and SGOT in CCl4 treated animals. The Hepatoprotective activity of most plant materials have been attributed to

the presence of flavonoid. The Moringa leaves extracts are powerful and antioxidant against free radicals, prevent the oxidation of major biomolecules and offer significant oxidation protection [87-89]. Ezeonwu et al. [90] reported hepatoprotective activity on CCl4 induced hepatic damage in Albino Rats for the biphenolic extract of Phyllanthus Niruri and Moringa Oliefera.

Murraya koenigii (Curry leaf tree)

Chronic consumption of ethanol reduces cellular antioxidant levels by free radical injuries which cause hepatitis and mortality cirrhosis in severely affected cases. Sadhana Sathaye et.al. [91] has studied the hepatoprotective activity of aqueous extract of curried leaf plants for ethanol-based hepatotoxicity in test animals. Hepatoprotection parameters were studied as anti-lipid peroxidation potential (LPO), antioxidation effects on the content of proteins, liver metabolization enzymes, for example, glutathione (GSH), dismutase superoxide (SOD), catalase and cell morphology. The hepatoprotective activity in tannins and carbazol alkaloids from this watery extract was studied and the normal moral behavior of the cells, even after an ethanolic challenge, was maintained as comparable to the protection provided by the standard medication l-hornithine laspartate (LOLA). The modulatory effects of the aqueously produced extract and isolates on the hepatoprotective activity were shown as a contribution to hepatoprotection[92-94], a reduction in lipid peroxidation and reduced cellular damage.

Musa paradisiaca

The increased liver marker enzymes due to Paracetamol induced liver injury were observed to reduce in serum when M. paradisiaca is fed as supplemented diet to rats [95-97]. Several tropical foods with hepatoprotective properties have been reported[98–99]. This is because of the content of phenolics, alkaloids, coumarins, flavonoids and lignans. This is due to its contents. The hepatoprotective effect of Musa sapientum stem (L) a banana cultivar in rats poisoned by tetrachloride carbon, has been noted by Piyush Dikshit et. al. [100].

Phaseolus vulgaris

Alberto Gabriel Lopez-Reyes et.al. [101] and Parola et.al. [102] have reported anti-fibrotic effects on rats of Methanol extract of P. vulgaris and antioxidants on the model of (CCl4) liver

damage. DR Jose, rguhs.ac.in, reported hepatotoxicity with alcohol, paracetamol, and thioacetamide. Qualitative and quantitative histologic analysis has shown the reduction of type I (44.3% less, P 0.03% less, P IV (68.9% less, P 0.049), and CCl4-injury rats in the hepatic fibrosis index by 18 percent in the black bean extract administration, when compared with positive controls (P 0.006). It is therefore demonstrated that this extract of methanol improves liver fibrosis and the expression of types I and IV of collagen in the animal model used. High levels of P. vulgaris antioxidants [103-107] could have significantly reduced the expression of Type I and IV genes and are also likely to reduce the activation of the hepatic stellar cells, which protect the liver partially from the fibrotic effect induced by CCl4 [108].

Sesamum indicum

Kumar Munisha et.al. studied the hepatoprotective activity of Sesamum ethanol extract against liver injury caused by paracetamol in rats[109]. The paracetamol administration (2g/kg/day, p.o) increased the levels of serum enzyme markers as compared with normal controls, indicating acute hepatocellular damage, decreased the level of Total Protein and Albumine. Before paracetamol was administered, pretreatments of Sesamum indium rats (400mg/kg and 700mg/kg) showed considerable decreases in the serum enzyme level, whereas increases of the total protein and albumin level were almost comparable to those of the Silymarine group (25mg/kg).

Solanum lycopersicum

Weremfo et.al. [111] studied the level of hepatoprotection offered by the Tomato pulp which is known for its rich antioxidants [112] against CCl4 induced hepatotoxicity. Pretreatment of rats with tomato pulp (40 mL/kg) significantly ($P<0.05$) lowered the respective serum AST, ALT and ALP, levels to 281.8 ± 15 U/L, 187.4 ± 6.2 U/L and 613.0 ± 42 U/L. Thus from the results it was revealed that tomato pulp significantly reduced the CCl4 induced hepatotoxicity [113].

Solanum melongena

In terms of the total phenolic and flavonoide content, antioxidant activity [114-1116] (Noda, Y., 2000) and the cytotoxicity of tert- butyl hydroperoxide (t-BuOOH) in human hepatoma, five varieties of the birds (pure colored moderate size, white-green moderate size, green

stretched moderate size and pale-green small size, respectively, called SM1–SM5) were evaluated (Pannarat Akanitapichat, et.al, 2010). The contribution of phenolic antioxidant present in eggplant to its liver-protective effect on t-BuooH-induced hepatotoxicity has been significantly correlated with total phenolic / flavonoid contents and antioxidants' activities.

Spinacia oleracea

The amelioration by Spinacia oleracea L. leaves extract against hepatosuppression induced by carbon tetrachloride (CCl4), which was evaluated in terms of serum marker enzymes. These biochemical parameters were significantly altered by single dose of CCl4, [117]. Pretreatment with S. oleracea L. prior to the administration of CCl4, significantly restored all the serum and liver parameters nearer to the normal levels. Silymarin was used as control; Srivastava et.al. [118]. These indicate hepatoprotection by a possible mechanism to block the P-450 mediated CCl4 bioactivation through selective inhibitors of ROS (reactive oxygen species).

Table 1.1- List of Commonly consumed Vegetables and their Hepatoprotective assay against hepatotoxic substance

S.No	Name of the Plant	Source of Family	Parts consumed	Hepato-toxicity inducing substances	Extracts studied	Histopathological and Biochemical parameters studied	References
1	*Allium cepa /Onion*	Liliaceae	Bulb, Tender leaves Spring Onion)	CCl_4 & Paracetamol	Aqueous & Ethanolic extract	SGOT, SGPT, ALP, Direct and total bilirubin	Riyaz Shaik *et al.*, 2012
1 a	*Allium cepa /Onion*	Liliaceae	Bulb, Tender leaves	Cadmium	Aqueous extract	ALT, AST,ALP, Total Serum & Protein malondialdehyde (MDA)	Ige et al., 2011
2	*Allium sativum /Garlic*	Liliaceae	Bulbs	Lead	Garlic supplemented diet	ALT, ASP, ALP	G. O. Ajayi, et.al., 2009
3	*Amorphophallus paeoniifolius/yam*	Araceae	Tubers	Paracetamol	Methanol & Aqueous extract	SGPT, SGOT ALP , Serum Bilirubin	Pramod J Hurkadale et al. 2012
4	*Benincasa hispida/winter melon*	Cucurbitaceae	fruits	Diclofenac sodium	aqueous extract	SGOT, SGPT, ALP, SOD, CAT, GSH, LPO	Dr. Shyamal K. Das, et.al. 2011
5	*Beta vulgaris /Beetroot*	Amaranthaceae	Root	CCl_4	Ethanolic extract	AST, ALT, ALP, Total protein, Total bilirubin	Ranju Pal *et al, 2010*
6	*Brassica juncea (Mustard seeds)*	Brassicaceae	seed	CCl_4	Aqueous extract	GOT, GPT, ALP, GGT, LPO, SOD, GSH	Agnel Arul John et. al., 2011
7	*Brassica oleracea/ Cabbage*	Brassicaceae	Leaves	Simvastatin	Ethanolic extract	SGPT, SGOT, ALP, Bilirubin	M.F Ahmed et. al, 2012
8	*Carica papaya*	Caricaceae	Fruits	CCl_4	Ethanol and aqueous extracts	ALT, AST, Alkaline Phosphatase, Total bilirubin & Gamma glutamate transpeptidase (GGTP)	Rajkapoor, B. et.al 2002

8a	*Carica papaya*	Caricaceae	Fruits	Paracetamol & Thioacetamide	Aqueous extract	ALT, AST, ALP and bilirubin	Srinivas Kantham , 2011
9	*Citrus limon* /Lemon	Rutaceae	Fruits / Leaves	CCl_4	Ethanol extract	ASAT, ALAT, ALP, Total and direct bilirubin	Shefalee K. Bhavsar et.al. 2007
10	*Colocasia antiquorum* /Colocasia	Araceae	Tender leaves, Corm	Paracetamol & CCl_4	Ethanolic extract	SGOT, SGPT	T.A. Tuse, et.al., 2009
11	*Colocasia esculenta* /Taro	Araceae	Tender leaves, Corm	CCl_4	Aqueous extract	AST, ALT & ALP	Bhagyashree R. Patil, et.al., 2009
12	*Coriandrum sativum*/Coriander	Umbelliferaeae	Seed	Lead nitrate	Aqueous & Ethanolic extract	AST, ALT, ACP, ALP, Total protein, Cholesterol	Leena Kansal et. al, 2011
13	*Curcuma longa* / Turmeric	Zingiberaceae	Dried Rhizome powder	CCl_4	Aqueous extract	SGOT, SGPT, ALP, Serum bilirubin	Mahuya Sengupta et al. 2011
14	*Cucumis sativus* /Cucumber	Cucurbitaceae	Fruits & Seeds	Cumene hydroperoxide	Aqueous extract	reactive oxygen species (ROS)	H. Heidari, 2012
15	*Cuminum cyminum*/Cumin seeds	Apiaceae	Seed	Profenofos	Aqueous extract	SGPT, SGOT, Bilirubin	Arun kumar et. al. 2011
16	*Daucus carota sativus* / Carrot	Apiaceae	Leaves, Root	CCl_4	Aqueous extract	SGOT, SGPT, ALP, Lactate dehydrogenase, Sorbitol Glutamate dehydrogenase, Serum bilirubin, Urea, Hepatic 5'-nucleotidase, Acid phosphatase, Acid ribonuclease, Succinic dehydrogenase, Glucose-6-phosphatase, Cytochrome P-450.	Bishayee A, et.al., 1995
17	*Lagenaria*	Cucurbitaceae	Fruits	CCl_4	Aqueous and	Total bilirubin, Serum	Lakshmi,

	siceraria / Bottle Gourd				Ethanolic extract	protein, ALP, ALT, AST	BVS. et. al 2011
18	*Luffa acutangula / Ridge Gourd*	Cucurbitaceae	Fruits	CCl_4 & Rifampicin	Hydro-alcoholic extract	• AST, ALT, ALP and LDH total protein	Jadhav VB, et.al., 2012
19	*Macrotyloma uniflorum /Horse gram*	Fabaceae	Seed	Paracetamol & D-Galactosamine	Methanolic extract	• SGPT, SGOT, ALP, Bilirubin (Direct and Total)	H.B. Parmar et. al., 2012
20	*Mentha arvensis / Mint*	Lamiaceae	Leaves and whole plant	CCl_4 & Ethanol	Aqueous extract	• SGPT, SGOT, ALP, Total Bilirubin, Total Protein, Tissue Glycogen	Radhika et. al., 2011
21	*Momordica charantia /Bitter Gourd*	Cucurbitaceae	Fruits	Acetaminophen	Aqueous extract	ALT, AST, ALP, LDH	Zahra.K. et.al, 2012
22	*Moringa oleifera /Drumstick Leaves*	Moringaceae	Leaves, Pod	CCl_4	Methanolic extract	SGPT, SGOT, Total Bilirubin, Direct Bilirubin	B.Balamurugan et.al., 2010
23	*Murraya koenigii /Curry Leaf*	Rutaceae	Leaves	Ethanol	Aqueous extract	• GSH , LPO, CAT , SOD, Total protein	Sadhana Sathaye et. al., 2011
24	*Musa paradisiaca/* Banana	Musaceae	Unripe & Ripe fruits	Paracetamol	Supplemented feed	• ALT, AST,Total protein, • Total Glucose, Total TG, • Total cholesterol, Reduced glutathione, LPO	Iweala, E.E.J., et al., 2011
25	*Phaseolus vulgaris / Kidney Beans*	Fabaceae	Seed	CCl_4	Methanolic extract	• Type I and IV , Collagen	Alberto Gabriel López-Reyes; et.al., 2008
26	*Sesamum indicum/ Sesame seeds*	Pedaliaceae	Seed	Paracetamol	Ethanolic extract	• SGOT, SGPT, ALP, ACP Total Biluribin	Kumar Munish, et. al., 2011
27	*Solanum lycopersicum /Tomato*	Solanaceae	Fruits	CCl_4	Tomato pulp (Aqueous)	AST, ALT, ALP, Total bilirubin	Weremfo, A. et.al., 2011
28	*Solanum melongena /Brinjal*	Solanaceae	Fruits	Tert-butyl hydroperoxide (t-BuOOH)	Methanol extract	Total phenolic, Total flavonoid	Pannarat Akanitapichat, et.al, 2010
29	*Spinacia oleracea/Spinach*	Chenopodiaceae	Leaves	CCl_4	Alcohol extract	• GGT , AST, ALT, ALP, serum- bilirubin, Total protein	R.S. Gupta, et. al. 2006
30	*Vigna mungo /Black Gram*	Fabaceae	Seed	Ethanol	Methanolic extract	SGOT, SGPT, ALP, Total Biluribin	Nitin et. al., 2012
31	*Zingiber officinale /Ginger*	Zingiberaceae	Rhizome	Paracetamol	Aqueous extract	AST, ALT, ALP, Total bilirubin	Siham M.A. El-Shenawy *et al* 2010

The amelioration may be attributed to the combined synergistic effects of its constituents rather than to any single factor as the leaves are rich in carotenoid contents (β-carotene, lutein, zeaxanthine), ascorbic acid, (Ozturk IC, et.al 2009), flavonoids and p-caumaric acid. Bhatia et.al, Otari and Nilesh Kumar et.al. [119-121] reported Ameliorative effects of Spinacia oleracea L. seeds on carbon tetrachloride (CCl4) - induced hepatotoxicity in vitro. Kang et.al. [122] too reported on Spinacia seeds.

Vigna mungo

Nitin et.al. [123] reported that, the hepatoprotective activity of methanolic extract of seeds of Vigna mungo (MEVM) against ethanol-induced hepatic damage in adult albino rats. The liver function parameters were noted to have increased (liver weight and volume), elevated serum enzyme levels (glutathione pyruvate transaminase, oxaloacetate transaminase, alkaline phosphatase and total bilirubin) and increased thiopentone upon exposure to ethanol. When Treatment with MEVM the enzymes SGPT, SGOT, ALP and BIT levels significantly decreased as compared to ethanol control group. The presence of potent diuretic such as saponins and the presence of strong antioxidants like ascorbic acid, total phenolic compounds, tannins, flavonoids etc. in the extract may be responsible for the hepatoprotective activity [124-125]. Anitha et.al. [126] investigated and reported similar hepatoprotective result of Vigna mungo against CCl4 induced hepatotoxicity. Solanki et.al. [127] studied and recorded similar hepatoprotection by Vigna mungo against Acetaminophen and CCl4 induced hepatotoxicity.

Zingiber officinale

The aqueous infusion of ginger was reported to show hepatoprotective effect on the paracetamol induced hepatotoxicity in rats [128]. The liver enzyme levels were reportedly altered and the histopathological studies revealed the tissue architechture damage due to paracetamol hepatotoxicity. However, Examination of liver tissue of rats treated with paracetamol and ginger extract and silymarin before paracetamol administration showed better hepatic architecture [129]. This hepatoprotection ability of ginger extract may be due to Zingerone, polyphenolic components, oleoresin and other antioxidants that are present in them [130-131].

1.5 Benefits of Pesticides:

The main advantages are the impact of the pesticides or the anticipated direct gains from their use. The effect of killing caterpillars on crops, for example, brings the main benefit of higher yields and better cold quality. DDT has been widely used to kill mosquitoes and malaria vectors. The secondary advantages result from the primary benefit, less immediately or less evidently. It can be subtle, less intuitive or long-lasting. Consequently, it is more difficult to establish for secondary benefits but they can nevertheless provide strong justifications for pesticide use.

1.5.1 Improving productivity

The use of pesticides in forestry, in public health and at home and, of course, in agriculture has brought considerable benefits, an area which is heavily dependent on the Indian economy. The production of food grains, which stood at just 50 million tons in 1948-49, had increased from an estimated 169 million hectares of permanently cropped land by the end of 1996-97 by almost fourfold to 198 million tonnes. The results have been achieved through the use of high yield seeds, advanced irrigation and agricultural chemicals (Employment Information: Indian Labour Statistics, 1994). The reduction of losses from weeds, diseases and pests that can significantly reduce the quantity of harvestable products has been an integral part of the process. Weeds reduce dryland production by 37-79 percent (Behera and Singh, 1999). Webster et al. (1999) said that without pesticide use "substantial economic losses" will occur, quantifying the significant increases of pesticide yield and economic margin. In addition, most pesticides are transformed into photochemicals in the environment, producing metabolites that are not toxic both to humans and the environment (Kole et ai., 1999).

1.5.2 Vector disease control

Vector-borne diseases are most effectively tackled by killing the vectors. Insecticides are often the only practical way to control the insects that spread deadly diseases such as malaria, resulting in an estimated 5000 deaths each day (Ross, 2005). In 2004, Bhatia wrote that malaria is one of the leading causes of morbidity and mortality in the developing world and a major public health problem in India. Dr. Arata Kochi, WHO's malaria chief said, "One of the best tools we have against malaria is indoor residual house spraying. Of the dozen insecticides WHO has approved as safe for house spraying, the most effective is DDT" (WHO, 2007). Scientists estimate that DDT and other chemicals in the organophosphate class of pesticides have saved 7 million human lives since 1945 by preventing the transmission of diseases such as malaria, bubonic plague, sleeping sickness, and typhus (Miller, 2004).

1.5.3 Quality of food

In countries of the first world, it has been observed that a diet containing fresh fruit and vegetables' far outweigh potential risks from eating very low re'sidues of pesticides in crops (Brown, 2004). Increasing evidence (Dietary Guidelines, 2005) shows that eating fruit and vegetables regularly reduces the risk of many cancers, high blood pressure, heart disease, diabetes, stroke, and other chronic diseases.

1.5.4 Other areas - transport, sport complex, building

The transport sector makes extensive use of pesticides, particularly herbicides. Herbicides and insecticides are used to maintain the turf on sports pitches, cricket grounds and golf courses. Insecticides protect buildings and other wooden structures from damage by termites and wood boring insects.

1.6 Hazards of Pesticides:

1.6.1 Direct impact on humans

If pesticide credits include improved economic potential for increasing food/fibre production and the improvement of vector-borne diseases, their debts have seriously impacted people's health and their environment. Some of these chemicals now pose a potential risk to people and other forms of life and unwelcome environmental side effects (Forget, 1993; Igbedioh, 1991; Jeyaratnam, 1981; WHO, 1990). There are about 1 million chronic deaths and diseases from pesticide poisoning worldwide per year (Environews Forum, 1999). Overall fatality ranges from 10% to 20%, and the WHO estimates 200 000 people die of pesticide toxicity every year (Phillips, 2002). Production employees, formulators, sprayers, blenders, loaders, and farm workers are among the high-risk groups subject to pesticides. The health effects of pesticides are based on the chemicals' hepatotoxicity and exposure length and extent (Lorenz and Eric, 2009). It has been argued that the long-term low dose exposure of some environmental chemicals, including the pesticides known as endocrine disruptors, has a growing impact on human health such than immune supper SSI On, hormone disorder, reduced intelligence, reproductive abnormalities, etc (Brouwer et al., 1999; Crisp et al., 1998; Hurley et al., 1998). The neurological symptoms (2 1 percent) related to exposure intensity were shown by a study on workers of four units manufacturing HCH in India (N=356) (Nigam et aI., 1993). The National Institute of Occupational Health (NIOH) has evaluated the extent of hepatotoxicity

risks associated with the spraying of methomyl, a carbamate insecticide (Saiyed et al., 1992). The ECG, serum LDH and cholinesterase (ChE) activity in spraymen showed significant changes that indicate the cardiotoxic effects of the methomyI. Health surveillance observational evidence has demonstrated that the generalization of symptoms (headache, nausea, vomiting, fatigue, irritation of skin and eyes) in male formulators engaged in the production of dust and liquid formulations of diverse pesticide types (malathione, methyl parathia, DDT and lindane) is high and generalized (Gupta et al., 1984). Reproductive hepatotoxicity information for 1,106 pairs was obtained by association between males and pesticide sprayed on cotton fields (OC, OP and carbamate) (Rupa et aI., 1991).

Slight headaches, grippe, cutancy, blurred vision and other neurologic disorders, although they are rare, are short-term human health hazards due to misapplication of pesticides (ICAR 1967). Chronic pesticide toxicity has also been attributed to the increased incidence of cancer, chronic kidney diseases, immune system suppression, sterility among males and women, and endocrine disorders, particularly neurological and behavioral disorders among children (Agnihotri, 1999). An herbicide was established with certainty as a result of the dioxin formation in Italy during Seveso 1976 during the production of 2, 4, 5 T (trichloro phenoxy acetic acid) (Pier et al., 1998). Excess death from cardiovascular and respiratory diseases was discovered, probably in addition to chemical contamination, due to the psychosocial consequences of the accident. Results of cancer and mortality follow-ups showed that gastrointestinal and lymphatic and haematopoietic tissue cancer had increased. The results, however, support the notion of dioxins to human beings as carcinogenic and support their association hypotheses with cardiovascular and endochrine effects (Pier et al., 2001). The U.S. military sprayed nearly 19 million gallons of grassland in about 3,6 million hectares of Vietnamese and Laotian soil in Vietnam during the Vietnam war, to remove forest cover, destroy plant crops and clear vegetation on US bases perimetres. The efforts were carried out between 1962 and 1971 and were known as the Ranch Hand operation. Several herbicides were used but the majority were herbicide mixtures, 2, 4-dichlorinated phenoxide acetic acid (2, 4-D), and 2,4, 5-trichlorine acetic phenoxide (2-4-D) (2, 4, 5-T). Men were exposed to defoliant mixtures, including Agent Orange and cancer risk was demonstrated (Frumkin, 2003). The most important method of suicide in India is the consumption of pesticides. In Kerala, 60 percent of the 900 to 1,000 suicides per year are pesticides. Furadan, Malathion and

Rat Poison are the most commonly used (Jayakrishnan 2006). Suicides are usually considered to be a temporary action that might have been prevented if such chemicals are not easily accessible. Experiences with farmers on the health effects of Punjab pesticides revealed that the incidences of cancer, kidney failure, still birth, infertility etc (Kaphalia and Seth, 1983; Singh and Chawla, 1988; Kalra et al., 1994; Battu et al., 2004, 2005). Pesticide intoxication is noted to be deliberate in most cases (85-97 percent).

The first report of pesticide poisoning in India came from Kerala in 1958, with more than 100 deaths after consuming parathionized wheat flour (Karunakaran, 1958). In Kerala itself, 102 lives were reported as a result of careless handling and storage of wheat in that same year. Several human and animal poisonings were subsequently reported, as well as deaths of birds and fish. In general, the cause for death was observed in more than 70% of organophosphorus pesticides (Iyer, 1993; Pandey, 1986). Bhopal was the worst industrial catastrophe in the world in 1984. The Union Carbide plant at Bhopal began to leak 27 tons of deadly methyl isocyanate on the night of 2 and 3 December 1984. (MIC). Half a million people have been exposed to gas and up to now 20,000 died of exposure. More than 120,000 people still have accidents and the resulting pollution at the site. Pesticides have an economic impact estimated at about $8 billion per year in non-target (including humans) countries (Gupta 2006).

1.6.2 Impact through food commodities

The programs entitled 'Monitoring of Pesticide Residues in Products of Plant Origin in the EU' have been established within the European Union since 1996 to establish the extent of pesticide contamination in foodstuffs. An average of approximately 9 700 samples were analyzed, 5,2% of which contained residues and 0,31% had residues higher than the corresponding MRL for that pesticide. In 1997, 13 pesticides (mandarins, pears, bananas, beans and potatoes) were analyzed for about six thousand samples in five commodities. The chlorpyriphus residue most frequently surpassed MRLs (0.24%), followed by methamidophos (0.18%) and Iprodione (0.13 percent). With regard to the products examined, approximately 34% of pesticide residues at or below the MRL and 1% of residues at levels above the MRL were contained. As far as four goods (oranges, peaches, carrots, spinach) investigated in 1998 are concerned, approximately 32% contained pesticide residues at or below the MRR, and 2% above the MRL. For the same 20 pesticides as the 1998 study, a total of 4700 tests were

conducted on four commodities (cauliflower, peppers, wheat grains and melon) (European Commission, 2001). For all the products examined, approximately 22% of samples contained pesticide residues at or below the MRL and 8.7% above the MRL. Exposure to methamidophos ranges between 0.43% of the ADI and 1.4% of the ADI for endosulfan. DDT residues were found in around 82 percent of the 2205 bovine milk samples collected in twelve states in a multi-centric study to evaluate pesticide residues from selected food commodities from various Indian states (Food Contaminant Surveillance in India, 1993). Approximately 37 per cent of the samples had DDT residue above 0.05 mg/kg tolerance (whole milk basis). Found 2.2 mg/kg was the highest DDT residue. In Maharastra (74%) and Gujarat (70%) followed by Andhran Pradesh(57%), Himachal Pradesh(56%), Punjab, the highest percentage was of residue over the tolerance limit (51 percent). This was less than 10% in the remaining states. In approximately 70 and 94 pcs the residues of residue of DDT and HCH isomers, with their maximum levels of 4.3 and 5.7 mg/kg (fat-based), showed figures from 186 samples of 20 commercial brands of infant formulas respectively. The total average adult DDT and BHC was 19,24 mg/day, and 77,15 mg/day (Kashyap et al., 1994). Another study reported that the average Indian daily intake of HCH and DDT was 115 and 48 mg per person, each higher than in many developed countries (Kannan et al., 1992). In a study at Hyderabad, Dasika et aI., (2012) analyzed fruit and vegetable samples before and after washing treatments and detected values above MRL for most commodities using liquid chromatography tandem mass spectrometry.

1.6.3 Impact on environment

Soil, water, turf and other vegetation may be contaminated with pesticides. A number of other organisms including birds, fish, useful insects and non-target plants can also be toxic to the killing of insects or weeds. In general, insecticides are the most highly toxic class of pesticides, but they also pose a risk to non-target organisms (Fig. 1.6). A plant and the soil can run on pesticides to reach surface water. The results of a wide range of studies in major river basin areas throughout the country in the early- to mid-1990s produced surprising results. The US Geological Survey (USGS). Over 90% of water and fish samples in all streams contained one or more pesticides (Kole et al; 2001).

Fig.1.7 Application of an aquatic herbicide

The reports shows that all samples of major rivers with mixed agricultural and urban land use and 99 percent urban stream samples have been found to produce pesticides (Bortleson and Davis, 1987-1995). In water samples from 19 of the 20 studied river basins, trifluralin and 2.4-D were found in 19 (Bevans et al., 1998; Fenelon et al., 1998; Levings et al., 1998; Wall et al., 1998). In general, more pesticides are detected in urban streams than in agricultural streams, according to U.S. Geological Survey and Washington State Department of Ecology (USGS) (Bortleson and Davis, 1987-1995). The organochlorin DDT, endosulfan, endrin, heptachlorine, lindane and their metabolites are hydrophobic, persistent and bioaccumulative pesticides that are strongly bound to earth. Most of them are now prohibited in agriculture, but their residues remain (Andreu and Pico, 2004). Heavy pesticide treatment of soil may cause beneficial soil microorganisms to decrease in populations. Dr. Elaine Ingham, a soil scientist, says, "If the soils degrade, then we lose both bacteria and fungi. The overuse of chemicals and pesticides is similar to the human overuse of antibiotics in soil organisms. The wild use of chemicals could last several years, but after a while, beneficial ground organisms aren't enough to hold on to nutrients "There was a mistake (Savonen, 1997). For example, plants depend on a number of terrestrial microorganisms for nitrates which plants can use in the atmosphere. This is a process which is disrupted by common landschaft herbicides: triclopyr inhibits soil bacteria that are transforming ammonia to nitrite (Pell et al, 1998) and glyphoxy

reduces the growth and the activity of soil free living nitrogen fixing bacteria (Santos and Flors 1995). (Frankenberger et al., 1991, Martens and Bremner, 1993).

The roots of many plants and nutrient intake aid mycorrhizal fungi. One study found that oryzalin and trifluralin both inhibited the development of certain mycorrhizal fungal species (Kelley and South, 1978). In laboratory studies, Roundup has demonstrated toxicity to mycorrhizal fungi, and some damaging effects have been shown at levels below soil concentrations, following traditional applications (Chakravarty and Sidhu, 1987; Estok et al., 1989). Triclopyr was also found to be toxic to many of mycorrhizal fungal species (Chakravarty and Sidhu, 1987) and oxadiazone decreased number (Moorman, 1989). Air, soil and non-targeted plants may be contaminated by a pesticide spray or may be derived from or volatilizing the treatment area (Fig.I. 7). Some pesticides, including ground equipment, are drifting during each application (Glotfelty and Schomburg, 1989). Drift may represent a 2% to 25% loss from the chemical applied, which can spread to several hundred miles over a distance of a few yards. Within a few days of application, 80-90 percent of a pesticide can be volatilized (Majewski, 1995). Many herbicides for esterformulation have shown that the treated plants are volatilized by enough vapor to cause major damage in other plants (Straathoff, 1986). Herbicides including 2, 4-D can harm nearby trees and shrubs when drifting on the leaves or volatilizing (Dreistadt et al., 1994). Herbicide glyphosate exposure can seriously reduce the quality of seed (Locke et al., 1995). It can also make certain plants more susceptible to disease (Brammall and Higgins, 1998).

Figure 1.8 An aircraft spraying pesticides

74 endangered plants which can be affected by gly phosate alone have been identified by the American Fish and Wildlife Service (U.S. EPA- Office of Pesticides and Toxic Substances, 1986). One long-term study investigating atmospheric pesticides in British Columbia (BC), dating back to 1996 (Belzer et al., 1998) revealed that between February 1996 and March 1997 57 chemical substances were investigated at two sampling sites in the Fraser Valley (Agassiz and, abbotsford). Captan and 2, 4-D at these two sites, with diclorvae and diazinon being the highest concentrations and deposition rates (Dosman and Cockcraft, 1989). The air concentration of pesticides currently used in Alberta was investigated in 1999 at four samples selected by geography and by sale of pesticides (Kumar, 2001). The two pesticides mostly detected in the four sites were triallate and trifluralin. Intermittent concentrations in the range 20-780 pg/m3 of insecticides (malathione, chlorpyrifos, diazinone, and endosulfan) were observed.

Birds may also suffer from herbicides. Exposure to 2, 4-D, eggs reduced successful chicken egg hatching and/or sterilization in faster chicks (Dufford et coll., 1981). (Lutz et al., 1972). The treatment with glyphosate clearly caused dramatic decreases in bird populations (MacKinnon et al., 1993). India has become one of the largest producers and consumers of organic chlorine pesticides in the last few years. As a result, large quantities of OC pesticides are exposed to wild birds in India (Tanabe et al., 1998; Grewal, 1990). Some studies were also conducted on the decrease of OC exposure populations in different parts of the world (Altenbach et al, 1979; Clark, 1976; Clark, 1983; Clark, 1981; Geluso et al., 1976; Jefferies, 1976; Thies and Mc Bee, 1994), showing that bats can accumulate high levels of OCs and are susceptible of being affected by potential toxic effects in the bats. Insects are an important diet for many bats, which allows OCs to pass through your body (Mc Bee et al., 1992). Several studies have identified OC pesticides and PCBs in levers and bird eggs in developed countries (Becker, 1989; Bemardz et al., 1990; Cade et al., 1989; Castillo et al., 1994; Mora, 1996; Mora, 1997). Clorpyrifos are a common contaminant of urban streams and have been highly toxic to fish and have been responsible for killing fish, near treated fields or buildings in waterways (US EPA, 2000). The Ronstar and Roundup weed killers are also highly poisonous for fish (Folmar et al., 1979; Shafiei and Costa, 1990). Increased concentrations of persistent

organic pollutants accumulate in aquatic mammals such as dolphins due to their high trophic level in the nutrition chain and relatively low drug metabolisation activity (Tanabe et al., 1988) and are vulnerable to contaminant exposure to toxic effects. In addition to habitat degradation, such as dam building, fishing, incidental and intentional deaths and chemical pollution, the health of river dolphins has also been threatened (Senthilkumar et al., 1999; Kannan et al., 1997, 1994, 1993b; Reeves and Leatherwood, 1994). Previous studies showed heavy metal concentrations (Kannan et al., 1993), organo-chlorine pesticides in Ganges River dolphine and poly-chlorinated biphenyls (PCBs) (Kannan et al., 1994) and butyltines (Kannan et al., 1997). The ongoing application of organochlorine and PCB in India is extremely worrying (Tanabe et al., 1998; Kannan et al., 1997a, b, 1992). The River Ganges is heavily populated and polluted with fertilizers, pesticides and industrial and household effluents (Mohan, 1989). The exposure to high concentrations of persistent, bioaccumulative, or toxic contaminants such as DDT (l, 1,I-trichloro-2,2-bis,[p-chlorophenyl] ethane) and PCBs has shown adverse effects in captive and wild aquatic mammals on reproductive and immunological functions (Colborn and Smolen, 1996; Ross et al., 1995; Kannan et al., 1993; Martineau et al., 1987; Reijnders, 1986; Helle et al., 1976).

Non-target birds can also be killed when poisoned beans are baited for pigeons and rodents (US EPA, 1998). Ingestion potential for non-target grain feeding birds is large for Avitrol, commonly used pigeon bait. Small seed-fed birds can become deadly (Extoxnet, 1996). The bird's Brodifacoum is highly toxic, a common rodenticide. It also poses a secondary risk of poisoning of birds which can feed toxic rodents (USEPA, 1998).

1.7 PESTICIDE USAGE IN INDIA

The Indian pesticide manufacturing industry began with the creation at Rishra near Kolkata in 1952 of a BHC technical plant. Today there are over 125 basic, large and medium producers and over 500 pesticide formulations in the Indian pesticide industry. According to a Green Peace Report, the largest industry in Asia and 12th in the world is now producing 90,000 metric tons of pesticides. In India, pesticide consumption per hectare is 381 g lower than the world average of 500g.

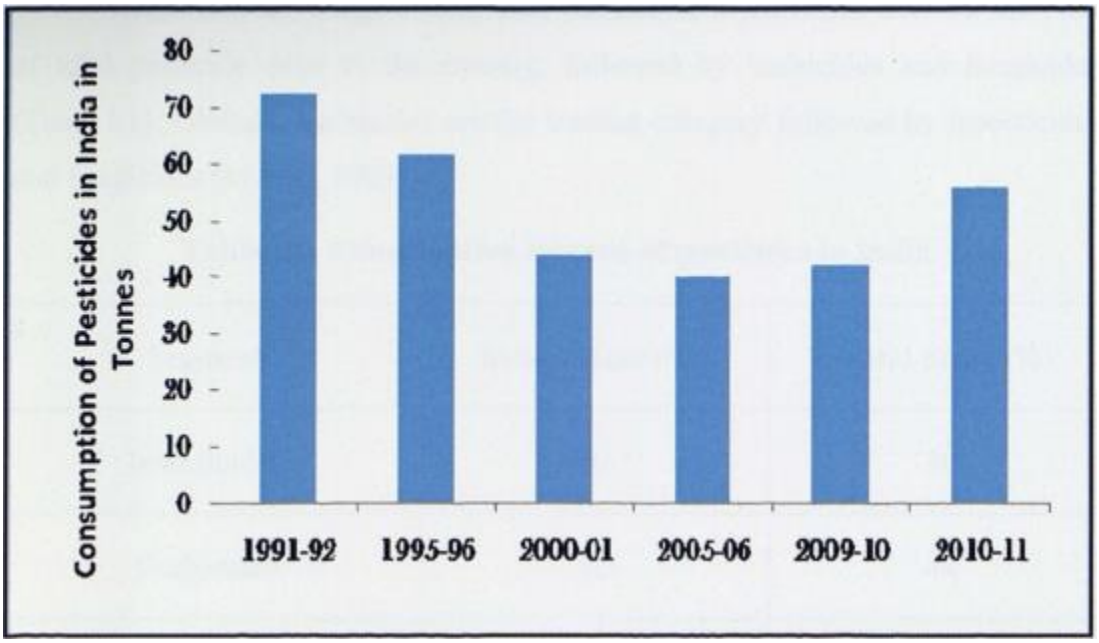


Fig. 1.9: Consumption pattern of Pesticides in India

The existence of fragmented land holdings, dependence on moonsons, insufficient awareness among fanners can be attributed in India to a low consumtion. The pattern of pesticide use shows that use decreases until 2005-06 but is subsequently high (Fig 1.7). Cumulative pesticide consumption in India up to i985,000 tons of hexachlorocyclohexane (HCH), and around 45,000 tons of HCHs have since been applied each year since then (Voldner 1995). Until recently, DDT and HCH were used (Gupta, 2004; 2006). India, apart from the US and its agricultural and malaria control programs, has been the only country to have used more than 100,000 tons of DDT since its formulation until the ban for agricultural use in 1989, (Kannan,1995). Although the use of technique RCH was totally prohibited by the government of India in 1977 (Kannan, 1997; Li, 1999), it was promoted to replace it with Lindane (gHCH), an isomer with all dangerous properties of HCH. While DDT is prohibited for agriculture, India requested exemption from use in the public health sector under the Stockholm Convention for 10,000 Tons DDT (Lallas, 2001). For residual spraying in rural and peripheral regions the National Malaria Program (NAMP) used 3750 tons of DDT in 2001. (Gupta, 2004).

With regards to use of technically based pesticides, 76% are insecticides, followed by herbicides and fungicides, of the total pesticide used in the country (Table 1.2). In the world, insecticides and fungicides are the leading categories (MAFF, 1999-00).

Table 1.2: Consumption Pattern of pesticides in India

Segment	Indian Share (%)	World Share (%)
Insecticides	76	30
Herbicides	13	44
Fungicides	10	21
Fumigants	1	5

Of the various pesticides used in India, 40% are organochlorinated (FAG, 2005; 1994) by chemical pesticides. The other main category is pesticides for organophosphates. Currently, organophosphorus compounds have been overtaken by organochlorine compounds in recent decades as the most commonly used insecticides (Gupta 2006). Several reports are available in different environmental compartments in India for the occurrence of pesticides. Different worker surveys found the existence of different pesticides in water samples (Amaraneni, 2006; Rehana 1996, 1995; Misra, 1994; Sarkar 1989) as well as soil and sediment (Guzella et al., 2005; Sankaramakrishnan and others, 2005; Bhattacharya et al., 2003.; Pandit et al., 2001; Kumar et al., 2001; Singhet and al., 2004. The finding of persistent organochlorine residues such as DDTs, HCHs, and PCBs in human milk is particularly serious since children can be sensitive to the toxic implications of any toxicant other than adults (Sanghi et al., 2003, Kunisue et al., 2002; Kumar et al., 2006; Banergee et al., 1997, Tanab et al., 1990; Dikshith et al., 1989; Siddique et al., 1981). Furthermore, the health implications of pesticide workers and sensitive general populations in India are presented as numbers of recent studies (Singh 2007;

Ahamed et al., 2006; Singh and Unnikrishnan 2006; UNEP 2004; Dewan et al., 2004; Rupa et al., 1991; Kashyap 1986; Waghray et al., 1986).

1.8 Molecular Biology and Biotechnology for Fresh Fruit Quality:

For both scientific and societal reasons, the mechanisms of fruit maturation have received great attention for many years. The emphasis nowadays is on the nutritional value and safety of the human health, not just the sensory properties of fruits. As the majority of quality attributes change during the maturation procedure, the mechanisms underlying fruit maturation were always considered as essential to better understand. In the last decade, great steps have been taken to clarify the molecular mechanisms to control the fruit maturation programme. In particular, there has been extensive study of the role of ethylene perception and the transduction signal. Research has now extended to understand I the amplification and diversification of the ethylene signal and (ii) the interaction between hormones for the development of the maturing programme. Our laboratory's recent results in this area are presented here. Biotechnology and genetics may contribute to improving the sensory and food quality of horticultural crops and shelf life after harvest. Progress in biotechnology post-harvest depends greatly on knowledge of fundamental mechanisms in fruit maturation and of the methods of metabolic synthesization of compounds that are important for the flavor and the nutritional value. The colored compounds (carotenoids), the aromatic amino acids, fatty acids, the sulphoid and sulfo- galactolipids and the fragrance of volatiles take place in chromoplasty some of the most significant modifications during fruit ripening. We report here on the role of chromoplasts in the ripening of tomatoes using proteomic methods. We report here.

In consumer purchasing decisions, the appearance and duration of fresh fruits and vegetables are important criteria. Consumers want better texture, taste and aroma, however. They value nutrition and are concerned about chemical residues. In view of these criteria, an ideal vegetable product must appear attractive, long-lasting, delicious, health-efficient and free of toxic residues. During the maturation process, most of the quality characteristics develop. The mechanisms that underlie fruit maturation therefore have to be better understood. Fruit maturation is a process that changes biochemistry, physiology and organ structure from a

scientific perspective to affect appearance, texture, flavor and aroma (Giovanonni, 2004). Fruit ripening corresponds to a series of chemical and structural changes to make fruit comestible and attractive for consumption for the consumers and for the distribution chain partners. There has been great progress in the isolation and characterization of genes involved in specific aspects of maturation (e.g. dismantling of the cell wall, production of volatile aroma) and the processes of maturation control (e.g., ethylene perception and transduction pathways). Present efforts are focused on the transcriptional control of the maturation process and are aimed at functionally determining transcription factors which control specific maturation characteristics. There have been novel methodologies that allow the global changes in gene expression (genomics), proteins, (proteomics) and metabolites following mainly tomatoes as model fruits (metabolomics). Here we report some of our work on the transcription factors at the intersection of ethylene and auxine that control certain aspect of the nutritional and sensory quality of the fruit. As many of the compounds which contribute to the fruit's sensory and nutritional quality are synthesized in chromoplastic compounds during their chloroplastic differentiation, we have carried out a research project to study the role of chromoplasts in quality development. Some of our preliminary results have also been reported in this field.

Progress in Molecular Biology of Fruit Ripening

Fruit maturing is seen by molecular biologists as a genetically-programmed development process involving the expression of genes associated with maturation (Fig. 1.10). The first gene cloned was the gene that encoded avocado cellulase (Bennett and Christoffersen, 1986). Since then a number of genes have been isolated and characterized in the maturing process (Cara and Giovannoni, 2008). The finding of the ethylene biosynthesis and the pathways of perception and transduction (Lelièvre et al., 1997), Alecander and Grierson (2002), Cara and Giovannoni (2008), have made significant progress in understanding the regulatory mechanisms for fruit matureness. The expression of ripening-related genes is controlled by nuclear transcription factors that interact with gene-cis elements to stimulate (or inhibit) gene expression (Fig. 1.10). The ethylene transduction route leads to the expression of the EIN3-LIKE (EIL) familial primary transcription elements that recognize ERE cis elements (Ethylene-Responsive Elements). A second set of transcription factor factors, the ERFs, is induced by binding the Ethylene-Response Factors to the GCC box in the ethylene-responsive

genes Subsequently (Cara and Giovannoni, 2008). Several ERF members express themselves in tomato fruit (Tournier et al., 2003). Ethylene is, however, not the only sign in the development of the fruit maturation program (Pech et al., 2008). Others, whether or not hormonal (eg, IAA) signals play a role, but they are much less well understood as their mechanism of action in fruit maturation. Moreover, there is now proof that the different signals interact. Below are some of our results relating to transcriptional interactions between ethylene and auxin.

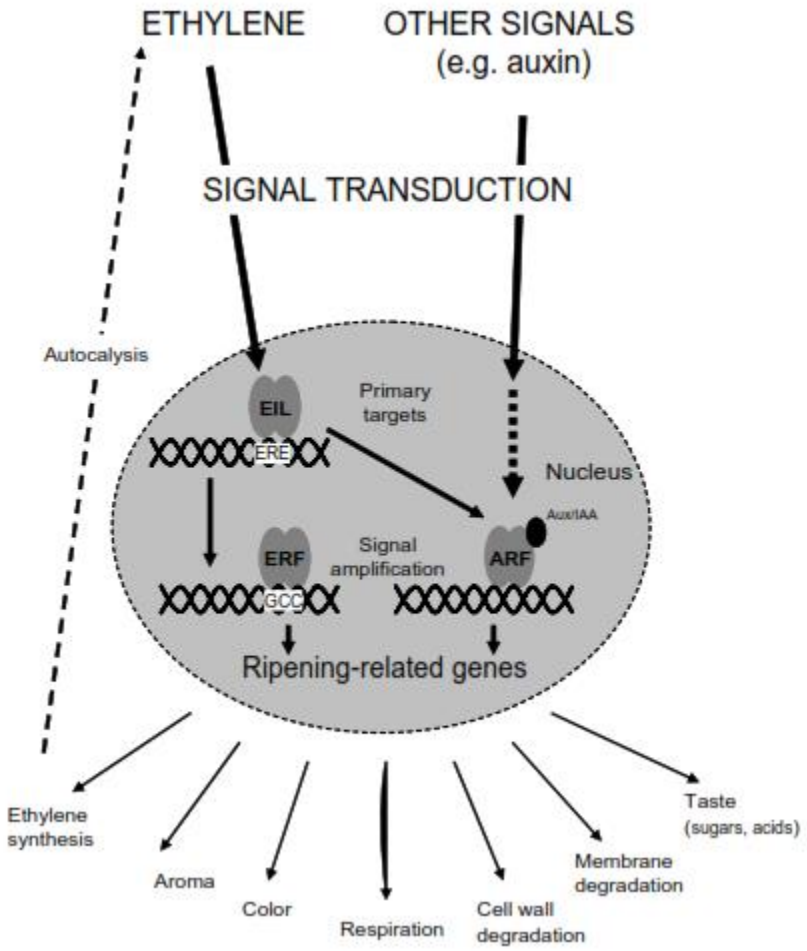


Fig. 1.10 Schematic representation of the transcription regulation of fruit ripening. The ethylene transduction signal leads to the expression of EIN3-Like (EIL) transcription factors that induce (i) primary target genes and (ii) ethylene-response factors (ERFs) which stimulate the expression of secondary ripening-related genes. Other signals, such as auxin, are also involved in the process. Some Auxin Response Factors (ARFs) and Aux/IAA transcription factors are also ethyleneresponsive and participate in the expression of ripening-related genes.

Milestones in the Advancement of Biotechnology for Improving Fruit Quality

Biotechnology is a technology that is extremely recent. In 1983 and 1984 were published the first legacy reports on a gene made for plants (Herrera-Estrella et al., 1983; Horsch et al., 1984). In 1994, the Flavor-Savr tomato was the first transgenic product to be sold. Since then, GMOs have been expanding very quickly. 2007 coincides with the twelfth year in which GM or transgenic crops are commercialized. The International Agri-Biotech Application Acquisition Service (ISAAA), a non-profit association, has provided information showing that farmers from 23 nations planted more than 114 million ha of biotechnological crops in 2007. The production accounts for over 99 percent of four main crops (soybean, maize, cotton and canola), and two characteristics (herbicides and insect resistance). The report by the ISAAA does not mention fruits or vegetables, which show that the production of GM crops is confidential. Bt potatoes and virus-resistant papayas and squashes are among the horticultural products that are currently grown on recordable surfaces. The position of leader in plant biotechnology at both the fundamental and application levels has been post-harvest biotechnology. Anti-sense PG tomatoes were the first fresh transgenic product on the market (Kramer and Redenbaugh, 1994). It was sold in 2500 markets in the United States in 1995, but in 1996 it was removed from the market for a variety of reasons including inadequate agronomic and sensory quality. In addition, post-harvest biotechnology has provided the first example that antisense technology can be used to identify the function of genes in higher plants (Sheely et al., 1988; Smith et al., 1988; Hamilton et al., 1990). A limited number of fruits, including tomatoes, melons, squash and papayas have been treated as genetic transformation. Examples of genetic transformation have begun to develop in perennial fruit

plants. Apple fruits were produced for example with reduced ethylene production (Dandekar et al., 2004).

Transcriptional Regulation of Fruit Ripening Interactions between Hormones

The role of plant hormone ethylene in driving climate fruit ripening such as melon and tomato was clearly confirmed in recent years in physiological and reverse genetics approaches. However, changes in plant hormone levels during fruit growth and development strongly indicate their dynamic participation in this development process. We have used the mRNA differential display check procedure to isolate developmental regulated genes (DR) which show altered expression of tomato fruit over the critical transitions leading to the increase of capacity to autonomously mature, in order to gain more insight into factors that work in combining with ethylene, to regulate late stages in tomato-fruit development (Zegzouti et al., 1999; Jones et al., 2002). Among the DR clones were identified several putative transcription factors. Note, few DR genes have been identified as putative encoding factors for auxin transcription. DR12, an ARF homologous tomato, and DR1, DR3, DR4, DR8 were among the first characteristically similar to the Aux/IAA genes in several plant species. Expression studies indicated that ethylene in the tomato fruit but not in the leaves regulates mRNA accumulation for the Aux/IAA-like genes. This suggests that these suspicious auxin reaction components participate also in tissue-specific regulation of gene expression based on ethylene (Fig. 1 and Jones et al., 2002). Reverse genetic approaches have examined the functional importance of DR12, an ARF-like gene. The DR12 transgenic plants showed a pleiotropic phenotype, including a dark-green immature fruit, an extraordinary division of cells into the fruit pericarp, blotched ripening, enhanced fruit firmness, upward curling of leaves and increased hypocotyl and cotyledon growth (Jones et al., 2002). The down-control of another Aux/IAA family auxin transcription factor, originally named DR4 and now rebranded as Sl-IAA9, has led to a pleiotropic phenotype. The inhibited Sl-IAA9 lines show the morphological changes of the vegetative parts (single leaves, reduced apical predominance, etc.) but most of them give rise to parthenocarpic fruits at an early age. Moreover, fruits have more sugar than controls (Wang et al., 2005).

Role of Chromoplast in the Development of Fruit Sensory and Nutritional Quality

The degreening and synthesis of pigments like carotenoids is one of the most remarkable changes during the fruit maturing. These changes occur during differentiation between chloroplasts and chromoplasts (Camara et al., 1995). In spite of significant advances in the understanding of the metabolic pathways relating to fruit sensory and nutritional quality, the chloroplastic / chromoplastic transition molecular foundation remains unclear. Biogenesis of Chromoplast is coordinated using a wide array of proteins encoded with the nuclear genome and its very genome (84 proteins) (2300 to 3000 proteins) (Soll, 2002). We have intact chromoplasts of tomato fruit in our laboratory at the full red maturation stage. Western blot analysis confirmed the high purity of the preparation. Extracted, separated by SDS-PAGE and identified by a powerful OrbiTrap nano LC, MS/MS machine were solvent and insoluble chromoplastic proteins. We have identified 2233 proteins from which 532 were found in the soluble and 629 were found in the insoluble fraction using the EST tomato database. The two fractions shared most of the proteins (1072). Approximately 5 percent of total proteins were poorly represented in non-plastidial proteins (<100 proteins) and showed that the contamination was low. This category is the most important protein for folded, assembled, interchanged and stored proteins (~250 proteins) with genetic expression proteins and photosynthesis (~200), transportation (~170), heat shock (~150), amino acid metabolism (~150), carbohydrocarbon metabolism (~140), signaling (~120), redox (~100) and metabolism of the lipids (~100). The structure (~40), nucleotide metabolism (~30), aroma biosynthesis (~25) and carotenoid metabolism (~20) were also less abundant categories. The chromoplastic functions of more than 250 proteins were not known. The chromo-plastic proteome analysis confirms its participation in biosyntheses of aromatic amino acids, vitamins, terpenoids and carotenoids, hormones, fatty acids, aroma and sulphuric acids and galactolipids, and brings new information on post-transcriptional, post-translational, trafficking and subcellular localization and organelle features. The large majority of protein found in chromoplast is also found in chchloroplast, contradicting the common belief that a change in plastid structure is the result of the transition from chloroplast to chromoplast, which is based on major changes in protein levels.

OBJECTIVE (S) /NEED OF STUDY

The main objectives of the study are as follows:

- To study the Analysis of pesticide residues in water, fruits, rice and vegetables

- To study the Analysis of Organochlorines and synthetic parathyroid pesticides in water, fruits, rice and vegetables

- To study the Calculation of total residues that can be accumulated from all the food varieties

HYPOTHESIS

The hypotheses generated for the present study are as follows.

H1: The Analysis of Organochlorines and synthetic parathyroid pesticides in water, fruits, rice and vegetables

H2: Analysis of pesticide residues in water, fruits, rice and vegetables

CHAPTER-2

PESTICIDE RESIDUES FROM WATER, FRUITS, AND VEGETABLES

Toxicity of the liver faces a major global health challenge. Many medicines are removed from the market for drug-induced hepatotoxicity by the Food and Drug Administration (FDA). The present study has investigated in vitro ameliorative activity on carbon tetrachloride (ccl4)-induced hepatotoxicity of methanoic extracts of various parts (peel, pulp and seed) of exotic fruits and vegetables. For lipid peroxidation (LPO), free radical scavonization and sodium dismutase (SOD) testing, CCl4-treated liver cell suspension was used in chicken. The effect was potent with antioxidants in extracts of different areas of the exotic fruits and common vegetables. Methanolyte extracts of certain unused parts (peel & seed) of fruit and vegetables even showed hepatoprotective action in the present study but the notable hepatoprotective action was demonstrated between all those bottles of gourd pulp (vegetable) and passive fruit pulp (exotic fruit). Therefore it has been concluded that hepatotoxicity was observed with methanoic extract of exotic fruit and vegetable induced by the CCl4.

The liver is the largest glands and main organ in the body to preserve the internal surroundings of the body. Almost every organ of the body is supported in some facets by the liver and essential for survival. Some of these important functions include protein, carbohydrate and fat metabolism, detoxification, bile sequestration and vitamin storage. It performs a number of critical functions to keep the body clean through the filtration of toxins and many medicines;

processes and excretes. Maintaining a healthy liver is therefore crucial to human health and well-being as a whole. Although the liver is enormously regenerative, the liver damage morbidity has increased steadily. Drug-inducing liver injury is prominent among all causes of hepatotoxicity. It accounts for about 20% of ALF in children and a higher percentage of ALF among adults, and an annual incidence estimated between 10 and 15 per 10,000 and 100,000 persons exposed to prescription medicinal products and drugs induced acute liver failure (ALF). Hepatotoxicity is therefore one of the major concerns about pharmacovigilance today and the leading cause for drug withdrawal for reasons of safety. Often used as model substances in vivo and in vitro conditions for experimental Hepatocyte damage to chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide). Most hepatotoxic chemicals damage lipid peroxidation to the hepatocytes. The best-studied model of liver cirrhosis is probably liver cirrhosis induced by CCl4. The CCl4 was the first toxin to which a free-radical mechanism has been shown to mediate the injury generated largely or completely. The cytochrome P450 metabolizes the CCl4, which is believed to give the radical, carbon-centered trichloromethyl. The initial step in a chain of events leading to membranous lipid peroxidation (LPO) and, ultimately, cell apoptosis and necrosis, is considered the covalent binding of trichloromethyl free radicals into cellular proteins. High levels of ROS damage cells and involve several diseases such as liver cirrhosis and fibrosis in humans. Reactive aldehydes, malondialdehyde (MDA) and 4-hydroxynonenal are among the degradation products of fatty acids. These products are easily binding to protein working groups and inhibit significant enzyme activity. Stressed cellular processes are most likely due to increased membrane permeability, a loss of cell protection as a result of membrane breakdown and a result of all these changes – death of the body cells, and the increased level of thiobarbituric acid reactive species (TBARS), lactate dehydrogenase (LDH) leakage. The 2nd phase of hepatotoxicity induced by CCl4 involves activating cells of Kupffer, together with producing mediators. Therefore, CCl4 – hepatotoxicity induced, acts as a great model for the study of molecular, cellular and morphological liver changes. Therefore, hepatoprotection can be shown in extracts with high free radical breakage/antioxidants. Antioxidants also transform free radicals into by-products of waste that are eventually removed. They can also repair cell damage previously. Fruit and vegetables intakes are known, however, to reduce the risk of multiple diseases, and health benefits are imposed mainly on the presence of

phytochemicals such as polyphenols, carotenoids and vitamins E and C. About five thousand plant phenolics are known and model studies have shown that many of them have antioxidants. Ayurveda, Siddha, Unani etc. are practiced in India and depend entirely on the plant source for these practices. Naturally, in these traditional medicines, the consumption of fruit and vegetables has shown therapeutic values. In addition, it is noted that the majority of edible plants and their parts such as fruit, seeds, leaves and roots have medicinal value agents such as antioxidants, flavonoids, tannins and other phenolic compounds. We have therefore tried different extracts of exotic fruits and common vegetables for hepatoprotection in the present study.

2.1 PESTICIDES REACH NON TARGET SITES

Pesticides may interact with plant surfaces after they are applied on the crops; they may be exposed to environmental factors like the wind and the sun; they may be washed away during precipitation. The plant surface (waxy cuticule and root surfaces) can absorb and enter the system for plant transport (systemic) or stay on the plant surface (contact). During the rainfall on the surface of the crop, some chemicals are necessarily washed away from the floor surface and transformed by sunlight.

Soil-applied pesticides generally first interact with humidity between and around the soil. Through various processes these chemicals enter the aquatic environment.

1) Sorption is the process of transferring pesticides to soil between solid matter and water. Pesticides that are strongly sorbed in soil particles have reduced mobility; therefore, groundwater is less susceptible to contamination and a long environmental persistence.

2) Microbial degradation is a process of transformation that leads to a partial or complete metabolization of a pesticide into carbon dioxide, water and methane by soil microorganisms (bacteria and fungi).

3) The breakdown by non-biological reactions of pesticides, including hydrolysis and photodegradation, is abiotic (chemical) degradation

(4) Volatilization involves the evaporation into the atmosphere as a gas of a solid or liquid. It will be less likely to volatilize a pesticide that is firmly sorbed to soil.

5) The term "leaching" is used to transmit pesticides to small pores in soil for downward movements (infiltrations) in water.

6) Runoff is a water flow through the soil surface when water collects at a rate faster than it can infiltrate the land (because of precipitation, irrigation or snow melting).

7) Erosion is the lateral dislocation by water of small parts of the earth. Surface rushes and erosion move pesticides and other pollutants side by side from higher altitudes to lower altitudes collecting points (rivers, rivers, lakes).

2.2 PESTICIDES IN WATER

Pesticide losses from the area in which non-target sites like surface and ground water have been applied and contaminated are a monitory loss for the farmer and an environment danger. Because a considerable number of the pesticides applied are often found in the soil, they are likely to build up. Rivers and canals constitute one of both agriculture and drinking water's most important water sources. Many pesticides have been used in rice-producing countries such as India, Pakistan, Japan, the Philippines and Bangladesh. As a result, many pesticides and their metabolites may be found in river and canal water. Chemicals pollution of rivers and rivers has become one of the century's most critical environmental problems. 10 million people are estimated to die of drinking contaminated water each year. India has contaminated its surface water resources and an increasing number of its reserves through biological, organic and inorganic pollutants. The pollution of rivers near urban areas in South Asian countries like Nepal, India and Bangladesh is more severe and critical because of large amounts of pollution caused by urban activities (F AO, 2005).

1. International scenario

Fluvial contamination from various parts of the world has been reported (Varsa, 2011; Oliver et al., 2011; Schulz, 2001a, b). A pesticide can dissolve into water (solubility) and environmental factors, like land, weather, season, and water sources from the spray location of pesticides, are influenced by several factors that affect the potential of a pesticide to contaminate water (Gustafson et al., 1993). The Danish National Borehole Database data is used to predict the vulnerability of potable water to pesticide contamination in Zealand, Denmark. Results show that pools in urban areas are more susceptible to contamination than wells in non-urban areas (Malaguerra, 2012).

In Netherlands, 771 groundwater samples were taken from wells in 2006 and analysed for a broad list of pesticides. Pesticide were detected in 27% of samples, while in 11 %, the limit was exceeded (Schipper et al., 2008). In the Pondicherry Region, nineteen water samples were collected and analysed for different pesticide residues like HCH, Aldrin and DDT. In the groundwater sources of agricultural areas the residual levels of organochlorine pesticides were three to four times higher than in urban areas and indicates the impact of pesticide use on agriculture (Sivasankaran et aI., 2007). International reports show pesticide residues in drinking and ground water of developed countries like USA California (Ensminger, 2012 ; Richard, 2012; Starner, 2012; Smalling and Orlando 2011, Stamer et aI., 2011, Stamer 2011, Zhang et al., 2011; Robert Budd, 2011; Phillips et aI., 2010, Anderson et al.,2010; Starner and Goh, in press; Oki and Haver, 2009; Weston et al., 2009; Orlando et al., 2008; Hunt et al., 2006; Weston et aI., 2005), France (Legrand et aI., 1991), Australia (Ang et al., 1989), Denmark (Felding, 1992) and Japan (Tanabe et al., 2001) have been reported. From Pakisthan, pesticide residues in drinking water in Karachi (Parveen and Masud 1988, De Carcia and Marchetti, 1991; Davi et al., 1992), shallow ground water in Samundri, Faisalabad and NWFP (Ahad et al., 2000, Ali and Jabbar 1991, Brooks et al., 1990), waste water samples from Lahore (Feihn and Jekel, 1996; Lacorte and Barcelo, 1996, Anony. 1993) have also been reported. A broad variety of organic chemicals have been detected in surface (Fernandez et al., 1998; Aguilar et al., 1999), costal (Zhou et aI., 1998) and marine waters (Utvik et al., 1999) by SPE (Font et aI., 1993, Benfenati et aI., 1990). Substantial amounts of pesticides have been detected in lakes (Buser, 1990), sea water (Sehulz et al., 1995) and rain water (Bester et aI., 1995, Baumann et al., 1995, Albanis et aI., 1998).

2. Indian scenario

A number of researchers in various parts of India have reported pesticides in drinking and groundwater (Bouwer, 1989; Dikshit et al., 1990; Jani et aI., 1991; Kumar, 1995; Bansal and Gupta, 2000). Pesticides are mainly used as a crops runoff in surfaces and groundwater and are most common in farming areas (Pimental et al., 1991). Freshwater bodies such as rivers, lakes and estuaries are widely contaminated with pesticides. Currently, approximately 10% of waste water produced from different sources is processed; the remainder is discharged to water bodies. Unchecked entry of pollutants into groundwater, rivers and other waters. This water is often heavily contaminated with chemicals and disease-causing microbes, which ultimately is used in families. The water from the river fields carries fertilizers and pesticides (Barbash et al., 1996). Pestizid products may cause a severe environmental contamination, especially in the water, with a human safety risk since they will be applied directly to the soil or sprayed onto crop fields and thus released into the environment. The levels of its residue in potable water shall be of concern to all public under the Directive on water quality in the European Union; O.llg/L for each individual substance and 0.5Ilg/L for the sum of pesticides is the maximum permissible concentration for pesticides (Anony. 1998). These chemicals can either be found in milk or present in meat or eggs from animal bodies resulting from contaminated food commodities.

Waters Pollution is a grave problem, as nearly 70% and an increasing number of Indian ground water reserves have been contaminated with biological, organic and inorganic pollutants (Thirumala, 2012). The water supplied to Delhi is groundwater, while the Yamuna River accounts for another 70 percent. DDT residues from four different Yamuna sites in Delhi from 1976 to 1978 have been monitored in water, bottom sediments and certain not target organisms. 0.04 Ilg/L, 3.42 Ilg/L (water), 0.007 to 5.63 mg/Kg (bottom sediments), 0.05 to 15.24 mg/ kg (invertebrates), and 0.54 to 56.31 mg/Kg have been found to contain total DDT residues; (fishes). In Wazirabad upstream (where the Yamuna river remains separated from all city drains) total DDT concentration was 0.24mg/L compared to 0.558mg/L downstream of Wazirabad, which receives water from the drain of Najafgarh. This shows clearly that DDT in Delhi is highly consumed (Mutiyar et al., 2011, Aggarwal et al., 1986). Another studies of water samples ollected from various river Yamuna sites showed that aldrin

and dieldrin residue ranged from 0.0005 to 0.05 lg/mL (upstream) and between 0.0001 – 0.1 lg/mL respectively (downstream) (Nair et ai., 1991, Mohapatra et ai.,1994; Agnihotri, 1994; Agarwal, 1997).

Bhagalpur River water and sediments were collected seasonally for a 2-year period and high methyl parathione, endosulfan and DDT concentrations were observed (Singh et ai., 2012). In the Ganga river basin covering Uttarakhand, Uttar Pradesh and Bihar, organochlorine pesticides (OCPs) occurs. The findings show that various OCP types dominated in various strets in accordance with the practices of land use and farming, i.e. higher elevation (mountain); endosulfan (UP stretch) and Aldrin (BR stretch) (Mutiyar and Mittal, 2012). In 1991-92, Ganga River Water and Groundwater in a rural Farrukabad area in Northern India, multiple organophosphorus pesticides residues were monitored (Agnihotri et ai., 1994; Ahmad et ai., 1996). The average levels of organochlorine and organophosphorus in water samples ranged between 0.258-0.829 mg/l and 0.143-0.294 mg/l respectively from about 147 water samples gathered in and around Lucknow City (Chaturvedi et ai., 2011). In Aligarh, groundwater samples were found to be organochlorine and organophosphorous pesticide residue (Ray, 1992). Water samples were evaluated and found contaminated with high levels of HCH on the banks of the Rapti and the Gandak and Gomti rivers. The residue of a-, p- and DDT residues in the tissues were found in 80 adult fishes of 5 different river species (Prasad 2000, Malik et ai., 2009). A-BHC residue (ranges from 8.1 to 26.8!lg/L) were found in the Hindon River, India, with P-BHC, y-BHC, aldrin, endosulfan, dide, ddd, and methoxychlor (lmran Ali et ai., 2008). Residues of fenitrothion, 2, 4-DDT and diazinone were reported in the water samples collected at Rawal and Simly lakes and 4, 4-DDT (Shazia Iram, 2009). The presence of the DDT (6,054-31,336 Ilg/l) and the HCH (3,121-8,656 Ilg/l) water samples from five lakes used for drinking and domestic use was reported despite the fact that no insecticide was being used for the vector control program in the vicins of lakes (Dua et aI., 1998). This means that DDT and HCH are highly mobile. In Keoladeo National Park and in Bharatpur, Rajasthan and 3.86 Ilg/L were also found to have residuals of BHC and DDT (Muralidharan, 2000). A survey in Kanpur, Northern India found that the surface water samples collected from the River Ganges in Kanpur showed high concentrations of y-HCH (0,259 Ilg/L) and malathion (2,618 Il g/L). Dieldrin was also found in samples of groundwater, except for y-

HCH and malathion. 0.900, 29.835 and 16.227 Ilg/L were respectively the highest concentration values of Y-HCH malathion and dieldrin (Sankararamakrishnan et at., 2005).

Astonishing facts about the extent of pesticide contamination sold in Indian soft drinks and bottle water during 2003 were released by the Center for Science and Environment (CSE) Pollution Monitoring Laboratory (PML). Their levels were high enough to cause cancer, nervous and reproductive system damage, birth defects and severe disorder of the immune system. On average, all the PepsiCo products have 0.0180 mg/L total pesticides, which was 36 times higher than the EEC limit for total pesticides (0.0005 mg/L) and 0.0150 mg/L. CSE also tested two brands of soft drinks sold in the US, but could not detect residues showing that businesses follow double standards (Mathur et at., 2003). The study investigated 17 bottled water brands in Delhi and around 13 brands across the region of Mumbai including Pure Life by Nestle, Pepsico Aquafina and Coca Cola Kinley. Of the twelve organochlorines tested on the various brands of water in the bottlenecks, 91% HCH, 70.6% DDT, and 85.3% of mineral water samples analyzed for pesticide residues have been detected, respectively. It has been found that only Evian (the French foreign brand) is free of pesticides.

2.3 THE RESIDUES IN CEREALS

Total food grain production in 2011-2012 is estimated at a record 250.42 million metric tons, up 5.64 million tons from last year's output (State of Indian Agriculture, 2011-12). Rice grain is usually contaminated by pesticides via two main sources: pesticide residues from field spray and residues that are accumulated during storage by pesticide treatment (Jamil et al., 2005; Iqbal and Ali, 2006). Rice grain shall be treated in storage sites and before shipment to other countries with pesticides, including organophosphates, carbamates, synthetic pyrethroids and insect-growth regulators (Khan et aI., 2007a).

1 International scenario

Munshi et aI., (2011) reported the analysis of 1000 samples of rice collected at the GCECD Scientific and Industrial Research Board of Pakistan from different export-ready vessels. LDDT and LHCH waste were found to be 0,001 mglkg and 0,005 mglkg respectively, in 30% and 45% of samples in 2005. Residue was observed. In 2006, the level of LDDT and LHCH

increased in 0,071 mglkg and 0,191 mglkg, respectively. The residual level of 0.003 and 0.002 mglkg of Lheptachlor and LChlordane was 0.191 mglkg and 00.005 mglkg respectively, respectively, in 2005.

Noher study was conducted in Basmati 385 and IRRI-6, in major rice-growing areas of Punjab, Pakistan for research into pesticide residues in husked and husked rice (Oryza sativa L.). Gas Chromatography with fire ionization detection has been used to determine the concentre (GC-FID). Four pesticides in all 400 samples were found at concentrations between 19 and 148 ppm, i.e. Karate, Malathion, Novacran and Padan. In all samples the median concentrations of pesticides were below or only the TLV values were crossed (Ahmad, 2008). A statistical study of pesticide residue in 598 domestic and 612 imported samples of rice was conducted in 1993-1994 in the U.S. Food and Drug Administration (FDA). Domestic residues were detectable for 56 percent and for imports of 12 percent. Malathion occurred in both groups of rice most frequently (Roy, 1997). Another analysis for organochlorine, organophosphorus and pyrethroids on domestic and imported wheat was conducted. 22.5% of imported wheat was found contaminated with organophosphorus and pyrethroid (chlorpyrifos 0.073-0.230 I-lb/g, malathion 0.0419–0.1003 I-lg/g) while domestic wheat contacted pyrethroid samples of 6.7% (delthroid 0.0650–1.2903 I-lg/g) Only pyrethroid contaminated (deltamethrin 0.0650-1.2903 I-lg/g) was observed with 22.5% of the wheature imported (Uddin et al., 2011)

2 Indian scenarios

Fifteen rice varieties were collected in a study by Assam, Titabar of Assam Agricultural University, in 2002 from the Regional Agricultural Research Station (RARS). Each variety has been tested for the presence of pesticide residues in polished, unpolished and parboiled form. HCH and DDT were detected in rice even if the values were less than MRL, endosulfan, cyperetherin, cyhalothrin, and chlorpyrifi (Deka et aI., 2004). A study of the fields soil, water and rice grain samples under IPM and non-IPM modules for pendimethalin, lindane, chlorpyriphos, and carbendazim was conducted in Kaithal (Haryana) and in dehradun (Uttarakhand) regions in 2008. Residues of pesticides have been detected but are below the boundary in Kaithal soil and water samples. Residues of carbendazin were found in rice grains at considerable levels from the Dehradun region.

3 Pictures in Kerala

Almost 90 per cent of farmers in Kuttanad, known as the Kerala rice bowl, sow high yield varieties, which require high levels of chemical inputs. In addition, the area is susceptible to pests like Brown Plant Hopper. The Kerala University of Agriculture restricts the use of some rice chemicals (methyl parathion and BHC). It is worth mentioning that methyl parathione and monocrotophos were the most common chemicals in the region (Devi, 2007). Organophosphates and carbamates are considered less persistent than organochlorines (which are more persistent). The study found that 40% of spreading is performed to protect the crop from the pest of the Brown Plant Hopper, 17% from the rice bug and 16% from the leaf folder (Devi, 2007). In 21% of scraping events in the area of investigation, extremely toxic, marked red chemicals are seen in use. The rate of use of toxic chemicals (yellow mark) is 51.25%; moderately toxic (blue marked) chemical products are 22.55%, whereas the remaining chemicals are relatively safer.

As pesticide consumption has been reported to be very high, several mass media reports have been published about its effects on the ecosystem. However, there are few scientists trying to quantify these externalities. The externalities of the estuarine ecosystem in Kuttanad have been examined by Rakesh (1999). The resistant externality cost and its influencing variables were also analysed. The survey showed that the pesticide poisoning resulted in explicit and implicit healthcare costs for the applicator/farmer. Most farmers (60%) have been reported to experience pesticide-related health problems. In Kuttanad, skin allergy and headache were among the health dangers induced by pesticides, and even genetic diseases were noted in Kuttanad (Krishna 200 I). Besides these few studies, there are no reports in Kerala about the level in rice or other cereals of pesticide contamination, although rice is our main food.

2.3 PESTICIDES IN FRUITS AND VEGETABLES

In addition to preventing consequences because of vitamin deficiency, the incidence of major diseases, such as cancer, cardiovascular diseases and obesity was also encouraged to consumes a high level of fruit and vegetable consumption (five portions or more per day). Food consumption (based on mass) of fruits and vegetables is averaged by 30%, according to the World Health Organization (WHO 2003). Next to China, India is the world's second largest

fruit and vegetable manufacturer. Out of a surface area of approximately 6 million hectares and 75.8 millions tons of fruit from 6.6 millions hectares, it produces around 92 million tonnes. Like other crops, pests and diseases attack fruit and vegetables, leading to damage that reduces quality and yield, in both production and storage. Pesticides are used in combination with other pest control techniques for the cultivation of fruit and vegetable harvests to destroy pests and prevent illnesses in order to reduce loss and maintain quality. Pesticides have been used more quickly, reduced by food-infecting organisms' toxins and are less labor intensive than other pest management methods. The indiscriminate use of chemicals particularly in fruiting and failure to adopt safe periods of waiting lead to a buildup of residues of pesticides in vegetables. Given the fact that the majority of pesticides are toxic in their nature, they can build up in body tissue with a serious negative health effect even in trace amounts (Handa et ai., 1999). Pesticide residues are assumed to be more exposed than on other routes of exposure, such as air and drinking water by five orders in magnitude (Juraske et ai., 2009). In Indian export consignments, the occurrence of pesticide residues has often caused the importing countries reject such consignments.

1. International scenario

Studies from different regions of Pakisthan reported occurrence of high amount of different residues in the samples of fruits and vegetables analyzed eventhough some samples were at or below the MRLs (Latif, 2011; Khan, 2011; Anwar et ai., 2004; Parveen et ai., 2005; Masud and Hassan, 1995a, b; Tari, 1993). Many recent reports are also available from different parts of the world showing pesticide residues in the food stuffs examined including Bangladesh (Chowdhury et al., 2011), Venezuela (Quintero, 2008), Ghana (Darko, 2008) and China (Li, 2011; Owago, 2008; Wang L, 2008). In 1991 the US National Cancer Institute launched a national "Five Aday for Better Health" campaign aimed at enhancing fruit and vegetable consumption in America (Beuchat, 1997). In 2001, the surveillance of pesticide residues by the US FDA showed that domestic fruits and vegetables violation rates were 1,5% and 1,1% respectively. However, 6.4% of the vegetables imported and 2.8% of the fruits imported were in violation of this rule. In fresh fruits and vegetables surveys on the Sydney Market, 98.1% of samples did not identify or meet pesticide residues at Maximum Residue Limit (PRL). Only 7 (1.9 percent) samples contained more than MRL pesticide residues.

2. Indian scenario

While analyzes of pesticide residues in foodstuffs are conducted in most developed countries over the decades, fruit and vegetables in India are less investigated from the point of view of the pollution of pesticide (Claeys et al., 2011; Fontcuberta et al., 2008; Barria-Pereira et al., 2005; Tadeoa et al., 2000). A complete survey of the pesticide residues in foodstuffs was not carried out, apart from a few monitoring studies (Hussain et al., 2002; Tahir et al., 2001; Masud and Hassan, 1995). In its own monitoring report (Pesticides Residues Coordinated Research Project All India, or the AICRPPR) the government of India has indicated that 59% of the food samples being tested are contaminated, 20% above 'MRL.' The above observation has been confirmed by the AICRPPR (1999) report, which shows 20% more than MRL samples. Samples of winter fruit were examined for pesticide resids in another study (apple, grapes, banana cheeku, papaya, lemon). The majority of the samples were tested in nine pesticides, whereas BHC, endosulfan and dieldrin were of very high toleration (Guptha et al., 2012). The purpose of the survey was to evaluate the residues of five commonly used vegetable pesticides, namely endosulfan, carbendazim, chlorpyrifos, cypermethrin and imidaclopride, from the five different districts of Uttarakhand during 2009–2010. The analysis showed that most vegetables are residuals of endosulfane above MRL and followed by carbendazim, chlorpyrifos, imidachloprid and cypermthrin (Chauhan et al., 2012). Fruits and vegetable samples from Delhi are being monitored regularly and the reports showing the presence of residues in more than permissible level (Kumar 2011, Srivastava et al., 2011; Sardana et al., 2005, Irani, 2003; Sasi and Sanghi 2001; Reddy et al., 1998; Reddy, 1998; Mukherjee and Gopal 1996; Dethe et al., 1995; Kashyap, 1994, Kannan, 1992; Awasthi 1989a; Nair 1989; Kaphalia et al., 1985; Mukherjee, 1980). Bhattacharya et al., (2005) studied the residue levels in samples from West Bengal and revealed that in some samples, the residue levels of OCs were much high, even above the acceptable daily intake value and few of them contained residue levels above the tolerance levels.

ICMR has also carried out a multi-centered study to evaluate pesticide residue in selected food commodities from different states of the state. DDT residues were found in approximately 82 percent of the 2205 cattle samples collected from 12 countries (ICMR, 1983, 1993). About 37% of the samples had DDT residues above 0.05 mg/kg tolerance. The sample percentage

was highest in Maharastra (74%), followed by Gujarat (70%), Andhra Pradesh (57%), Himachal Pradesh (56%) and Punjab, respectively (51 percent). This was less than 10% in the remaining states. In 186 samples, the residues of the DDT and HCH isomers were shown in approximately 70 and 94 per cent of samples with their respective maximum 4.3 and 5.7 mg/kg levels of the commercial markings. In addition, Kumarin et al. (2006, 2003, and 1996), GC-ECD/NPD and capillary pillars showed contamination by OC, synthetic pyrethroid, and OP insecticides, and analysed the samples of fruits, grapes and guava from Hisar, Haryana, India for pesticider residues. Almost every sample was detected with OCs, HCH, DDT and Endosulfan.

3. Picture in Kerala

Alarming high values of endosulfan residues (ppm) for blood, fruits and tissues were detected from samples of Padre village in Kasargod district of Kerala (CSE, 2001). All farmers regularly practice commercial farming using preventive methods for the protection of chemical plants. The application of the pesticide was found to be started from transplantation according to another study carried out in Kerala (Devi, 2010). Of the approximately 15 chemicals in bitter gourd, eight were insecticides, four were fungicides and 1 was weedicide and the rest were stimulators for plant growth. During the seedlings transplantiation, phorate granules were found to be placed in the pit. At an interval of two weeks the prophylactic use of pesticides was initially reversed, which fell to two days as the crop became flowering and the fruit set. Furthermore, Acetamaprid, Phorate and Dimethoate were sprayed six times, Quinalphos and Indoxacarb, four times each and the remaining three to four times each. Farmers used pesticides as many as 50 times in bitter gourdes during a 90-day crop cycle. Even if there are many reports from various parts of the globe, including from India, very few areas are examined in comparison to the large number of sensitive areas. Moreover the studies in India are mostly concentrated on major cities or industrialized areas avoiding the most susceptible rural areas including fields. Besides the studies show that the farmers are extremely unaware of the precautions or incapable to follow supervised trials of pesticide applications. In the case of Kerala, the studies are too less or practically nil for developing a database of the usage of different deadly chemicals other than a few attempts including the analysis of soil and food materials in Kasargod. It is obvious that there is a need to analyze

food and environmental samples primarily in order to control the use of pesticides in agriculture and, indeed by using pesticides contaminated products, to limit the risks of human residues intake. This study has revealed the pesticide load imposed by pollution of water, rice, vegetables and fruits on people in Kerala. We assumed that this study would help to raise consumer and producers' awareness of the levels and possible health risks associated with contamination of our food and environment.

Assay for Lipid Peroxidation:

Lipid peroxidation was estimated colorimetrically by measuring malondialdehyde (MDA). In brief, 0.5 ml of homogenate treated with 0.5 ml carbon tetrachloride and 100µg/ml of fruit/vegetable extract along with 2 ml of a 1:1:1 ratio of TBA-TCA-HCl (TBA 0.6%, TCA 15%, HCl 0.25 N) reagent added and placed in water bath at 65°C for 15 min, cooled, and centrifuged at 5,000 rpm for 10 min at room temperature. The optical density of the clear supernatant was measured at 535 nm. Controls were prepared similarly without CCL4 for each extract. The MDA formed was calculated by using the molar extinction coefficient of thiobarbituric acid reactants (TBARS; 1.56 x 105 l/mole cm−1). The product of LPO was expressed as nmol of MDA formed per g of tissue.

Free Radical Scavenging Assay:

Free radical scavenging activity was measured by a decrease in absorbance at 517 nm of a solution of coloured 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) in methanol brought about by the sample. To the 1ml methanolic solution of DPPH, 0.5 ml of homogenate treated with 0.5 ml carbon tetrachloride and 100µg/ml of fruit/vegetable extract was added. The reaction mixture was allowed to incubate for 30 min at room temperature in the dark and the absorbance of the resulting mixture was measured at 517 nm against ascorbic acid as standard. Controls were prepared similarly without CCL4 for each extract.

Superoxide Dismutase (SOD) assay:

The assay of SOD is based on the inhibition of the formation of NBT- diformazan which is measured at 560nm. Cuvets containing 1.17X1O-6M riboflavin, 0.01 M methionine, $2X10^{-5}$M sodium cyanide, 5.6 X 10^{-5}M NBT, and 0.05 M potassium phosphate at pH 7.8 were illuminated and the absorbance was read at 560nm[24] . Controls were prepared similarly without CCL4 for each extract.

2.4 FRUIT AND VEGETABLE EXTRACT PREPARATION:

Fresh exotic fruit (Table 2.1a) and vegetables (Table 2.1b) were purchased from hopcoms and were washed under running tap water and their peel, pulp and the seeds were separated. They were shade dried separately for over a month. The dried fruit parts were ground using a mechanical grinder. Each of the samples was approximately weighed and subjected to extraction by cold maceration with 80% methanol at room temperature. The macerate was allowed to soak in 80% methanol for 72 hours. The samples were filtered after 72 hours using regular filter paper. The methanolic extracts thus obtained was collected in the vials and stored at 4°C.

Table 2.1a: Exotic Fruits Used In Experiment

Exotic fruits	Scientific Name
Date Palm	*Phoenix Dactylifera*
Grape Vine	*Vitis Vinifera*
Passion Fruit	*Passiflora Edulis*
California Wild Grape	*Vitis Californica*
Kiwi Fruit	*Actinidia Deliciosa*
Wood Apple	*Limonia Acidissima*
Indian Jujube	*Ziziphus Mauritiana*
Avocado	*Persea Americana*
Litchi	*Litchi Chinensis*
Java Plum/Black Palm	*Syzygium Cumini*
Rose Apple	*Syzygium Jambos*
Soursop	*Annona Muricata*

Table 2.1 b: Common Vegetables Used In Experiment

Common Vegetables	Scientific Name
Eggplant	*Solanum melongena*
Bitter Gourd	*Momordica charantia*
Ivy Gourd	*Coccinia grandis*
Tomato	*Solanum lycopersicum*
Green chilli	*Capsicum frutescens*
Ladies finger	*Abelmoschus esculentus*
Cucumber	*Cucumis sativus*
Bottle Gourd	*Lagenaria siceraria*
Bell peppers	*Capsicum annuum*
Snake Gourd	*Trichosanthes cucumerina*

Liver is the very important part of our organism which is responsible for maximum metabolic and secretarian activities and is therefore a sensitive target location for biotransformation modulating substances. Liver is also involved in the detox of xenobiotics, drugs, viral infections, and chronic alcoholism from exogenous and endogenous challenges. In an animal model that is closely analogous to human hepatotoxicities, CCl4 has been extensively used to study liver injury induced by ROS. CCl4's toxic effects are the result of free radical production. These peroxy radicaux in turn propagate the process of LPO, apart from neutralizing them by radical scavengers, with abstract hydrogen atoms of other lipid molecules. A biochemical parameter for oxidative stress indicators and its improvement by different fruits and vegetables was planned for assessing the toxic effects of carbon tetrachloride in vitro.

There were significant values in all the plant samples containing different fruit and vegetable extracts. The extract from peel of litchi-fruit and california grapes has been found to very

efficiently inhibit lipid peroxidation compared to other fruit extracts. The efficiency was reduced with Black plum and passionate fruit. In comparison to peel extracts from other people, peroxidation has been found to be extremely inhibited by vegetable peeling of Brinjal and Cucumber, but ladies' fingers have little effect. The pulp extracts showed LPO inhibitions in addition to the peel extract. A stable free, free radical DPPH was used to determine the radical scavenging abundance of fruit and vegetables. The present study showed high levels of free radical scavenging among all other vegan vegetables: snake gourd seed, bottles of gourd pulp and brinjal peel; the free radical activity against ascorbic acid was a positive standard in the fruit, litchic peel, passion fruit pulp and rose apple seas. It is well documented that many forms of life have enzyme-based and non-enzyme antioxidants, among which there are self-defensive mechanisms and Super oxide dismutases, capable of protecting the body against damages caused by free radicals. Dismutase of superoxide is an antioxidant enzyme that is used to metabolize the toxic intermediate oxidative. SOD calculates the translation into hydrogen peroxide of superoxide radicals. There is therefore a reduction in SOD activity in the accumulation of peroxide or its decomposition products. This study showed high SOD activity for passion fruit pulp in the fruit and bottle of gourd pulp.

CHAPTER-3

MATERIALS AND METHODS

3.1 MATERIALS

Chemicals

Pesticide mixtures for residue analysis were obtained from agrochemical devision, Vellanikkara, Thrissur. Phorate 94.6% pure, Endosulfan 93.7% pure and Fenvalerate 95% pure were obtained from Agrochemical Division, Indian Agriculture Research Institute (JARI, New Delhi, India). All other reagents were obtained from sigma chemicals and Merck and of analytical grade. Acetone, nhexane, dichloromethane and methanol were Super Purity Solvents from Sigma chemicals. Ethyl acetate (PAR) for instrumental analysis was from Pancreac (Barcelona, Spain). Alumina for column chromatography was from Sigma (St. Louis, USA). Homogenizer-FOSS 2096 based on Tecator Technology. Centrifuge - CRi multifunction was from Thermo Electron Industries SAS, (France), Rotary vacuum evaporator - Buchi RE-200 was from Buchi Labortechnic AG, (Postfach, Switzerland).

Normal melting agarose (NMA), low melting agarose (LMA), Triton X100, ethylenediamine tetraacetic acid (EDTA), N-Iauryl sarcosme, tris(hydroxymethyl)-amino-methane, and ethidium bromide were purchased from Sigma Chemicals, St. Louis, MO. Sodium chloride, di-sodium hydrogen phosphate, sodium di-hydrogen phosphate, sodium hydroxide, sodium acetate, dimethylsulfoxide, ethanol, and xylene were purchased from Merck, Germany. P53 detection kit (including anti-p53 polyclonal antibody, secondary antibody) was from Oncogene (San Diego, CA); polyclonal goat anti-rat/mouse Bcl-2 and polyclonal goat anti-rat/mouse Bax antibodies, horseradish peroxidaseconjugated donkey anti-goat secondary antibody were from Santa Cruz Biotechnology (Santa Cruz, CA, USA)..Nitrocellulose membrane (0.2 mm pore size) was obtained from Bio-Rad Laboratories (Hercules, CA). AnnexinV-FITC kit, PI and all other biochemicals were purchased from sigma chemicals.co USA. The kit for TUNEL staining was purchased from BD Biosciences Clontech, PaloAlto, CA, USA.

Equipment's

Gas Chromatograph used for pesticide residue analysis was Thermo quest Trace GC with the 63Ni selective Electron-Capture Detectors with advanced software (Chromcard-32 bit Ver 1.06 October 98) and Nucon -GC- 5765 series equipped with Nitogen Phosphorus detector. GC column employed was capillary column, DB-17, J & W make and DB-5, J & W make (for cross verification). A 10-IlL syringe from Hamilton Co. was employed.

3.2 METHODS

Analysis of pesticide residues

Pesticides impart a vital role for the enhancement of food production according to the requirement of world population but the residues left in the food chain pose a lot of problems for human beings as well as the environment. In the present study, water, fruits and vegetable samples were collected to assess the pesticide concentrations of pesticide residues.

3.3 SAMPLING

Water Sampling

Worldwide pesticides are used for crop protection and production. Some polar pesticides have good tendency to leach down and run-off with rain water from field area and contaminated the fresh waters like canals, streams/rivers and well water. The attention was focused to do sampling from wells and streams near the sprayed areas of rice and vegetables. In order to assess possible impact of pesticides on aquatic ecosystems and drinking water supplies, water samples were taken from Trivandrum and Kasargod, the southern and northernmost districts in Kerala state.

Well water sampling

Water sampling was carried out using the procedure of Knedel, 1999 and Tanabe et aI., 2000. The samples were collected from wells of Palode (WW1) and kallara (WW2) areas of Trivandrum and Periya (WW3) and Cheemeni (WW4) of Kasargod. Samples were stored at -4°C before processing further.

Stream/River water sampling

In Trivandrum, the samples were collected from Aruvikkara (WS1) dam and fresh-water lake at Vellayani (WS2) (potential water sources for Trivandrum city). From Kasergod, the samples were collected from Kodenkari stream (WS3) and Shiriya (WS4) river. Samples of water were taken following the same procedure in triplicate in glass bottles, marked properly for identification.

Rice sampling

In Trivandrum, the nee samples for analysis were collected from Connemara market (RM1) and Chalai market (RM2) and also from paddy fields from Palode (RF1) and Kallara (RF2). From Kasargod, the rice samples for analysis were collected from Periya (RM3) and Cheemeni markets (RM4) and also from paddy fields RF3 and RF4 respectively from the same areas.

Vegetables / Fruits sampling

To get more yields of good quality vegetables, farmers are using pesticides to protect them from different infestations. Fresh fruits/vegetables were collected from farmer's fields and markets in triplicates. All samples were washed with tap water, rinsed with distilled water and dried for some time, packed in plastic bags and stored at _4° C. In Trivandrum, samples of fruits and vegetables were collected from Connemara and Chalai markets. From Kasargod, samples of fruits and vegetables were also collected from the Periya and Cheemeni markets.

Analysis of Pesticide residues from samples

A constant broadening range of chemical classes and increased diversity of structure and properties made it difficult to develop methods that can determine all pesticides in different matrices. Many steps are involved to analyze pesticides in different selected fruits, vegetables, and water samples. After a sample is composited, the sequence of steps in pesticide residue analysis usually involves: (l) extraction of the pesticide residues from the sample matrix, (2) removal of coextracted water from the extract, (3) cleanup of the extract, and (4) analytical determination.

Extraction of pesticide residues from samples

Different methods are used to extract the pesticide residues from water, rice, fruits and vegetable samples collected from various localities.

Extraction of pesticide residues from Water

The collected samples were extracted through liquid removal (Ahad et aI., 2005). A separating burner collected a liter of water sample and 75 mL of dichloromethane was used as a pesticide. With the aid of a dispenser bottle dichloromethane (25 mL) had been added. By placing the deck on it, the separating funnel was closed. In and out of the movements of the separator funnel, the solvents were mixed softly (not strongly). Three times has been mixing. Every time the stopper was opened, the gases were removed from the separation funnel. In a round bottom flak, a small funnel with a small cotton plug and anhydrous sodium sulphate at its openings was held in place in 10 minutes to separate the two phases. The lower oily layer was collected by a small funnel. The use of pasture pipettes for efficient separation has been used to add sodium chloride. Dichloromethane addition (25 mL) and the lower organic layer separation have been repeated three times. There were a few drops of propylene glycol and approximately 3 ~ 4 glass beads in ethyl acetate solution (l: 1). The contents were evaporated at a vacuum speed of 40°C at rotary evaporator. Nitrogen stream was used for full dryness. In ImL ethyl acetate the contents were reconstituted. Gas chromatography (GC) analyzes were conducted using the Ahad et al method (2000).

Extraction of pesticide residues from rice

Analytical method was based on AOAC (2005). 20-50 g duplicate ground sample of rice was blended with 350 mL H20-CH3CN (7+13) mixture at high speed in a stainless steel blender for 05 min to get a well, homogenized blend. Filtered with suction through Buckner funnel in to 500 mL suction flask and transferred the filtrate to Petroleum ether. The measured volume (with a measuring cylinder) of extract/filtrate was added to 100 mL petroleum and transferred to 1 L separator. It was shaken vigorously for 1-2 min and added 10 mL saturated sodium chloride (NaCl) and 600 ml H20. 15 g anhydrous sodium sulfate (Na2S04) was added and shaked vigorously. Filtrate was concentrated to 15-10 mL in K-D concentrator by starting evaporation

and transferred solution directly to Florisil column for cleanup prior to determination (Griffith, 1983).

Extraction of pesticide residues from Fruits and vegetables

The most commonly used approach is to remove non-ionic pesticides from the plant matrix with an organic solvent with a blender or a homogenizing device. Ethyl acetate has been shown to be a good solvent for the extraction of residues of various pesticides from fruits and vegetables in relation to other solvents because of its high polarity and thermally volatile and labile compound. In this study, Kadenezki et al, (1992) have made little change in the extraction of pesticide residues from fruit and plant samples because it is faster, less laborious, environmentally friendly and less expensive.

One kilogram of each sample was cut and thoroughly mixed with a knife. About 200 grams of the chopped sample have been transferred for a homogenous sample to a fast blender. A sample of 50 g in a 250 mL Erlenmeyer extraction flake and a horizontal shaker shaking for 2 hours was added to a 75-mL ethyl acetate and 25g anhydrous sodium sulphate. A Whatman No.4 filter filter filtered the ethyl acetate extract.

Sample clean-up

The non-selective nature of the exhaustive extraction procedures and the complexity of sample matrices result in complex extracts that require further purification to remove any interfering substances co-extracted with pesticide residues. For this purpose, a florisil column adsorption chromatography was used (Krinitsky et al., 1988).

Florisil column adsorption chromatography

Glass wool was placed at the bottom of a 15 em x 25mm chromatographic column fitted with draw-off valve and was used for clean-up. The column was then fitted with activated florisil (l5g) that had been activated at 150°C for at least 24 hours followed by charcoal (3g) and anhydrous sodium sulphate (5gm). Just prior to use, the column containing adsorbents was washed with ethyl acetate before loading the sample and flow rate of 5mL/min was adjusted.

After preparation of the column, the sample extract was transferred to the column. The eluate was collected in a 100mL round bottom flask and was concentrated first with a rotary evaporator and then under gentle stream of N2 to dryness. The dried extract was dissolved in exactly ImL acetone and then analyzed using GC.

Analytical determination

Analytical methods employed for qualitative and quantitative determination of pesticides and their metabolites in water and food samples playa significant role in evaluation and interpretation of data. When sample analysis was carried out, it is necessary to validate the analytical methods and provide appropriate validation information for the reliability of data (Shah et ai., 1992). For the studies, food and water extracts were analyzed by GC method using ECD and NPD detectors on capillary columns. Stock standard solution of pesticides was prepared in n-hexane and those pesticides, which were not soluble in nhexane, were first dissolved in acetone and then made up the volume by n-hexane. Working standard solutions were prepared in hexane. These solutions were all stable for at least 1 month ifstored in the dark at 4°C.

3.3 ANALYSIS OF ORGANOCHLORINES AND SYNTHETIC PYRETHROID PESTICIDE RESIDUES

Organochlorine and synthetic pyrethroides were analysed by Gas Chromatograph (Thermoquest-Trace GC) with the 63Ni selective ElectronCapture Detector. This detector allows the detection of contaminants at trace level concentrations in the lower ppb range in the presence of a multitude of compounds extracted from the matrix to which the detector does not respond. The capillary column used was DB-I7-coated with 50% phenyl, 50% methyl polysiloxane (length 30m, ID 0.25 mm and film 0.25Ilm). The carrier gas and the makeup gas was nitrogen with a 0.4 mUmin and 60 mUmin flow rate respectively employing the split less mode. 2.01lL of the final extract as injected at a temperature of 270°C. The oven temperature was kept at 120°C with a hold time of 1 minute, then from 120°C to 205°C at a rate of 25°C/ minute with a hold time of 1 minute then finally from 205 to 290°C at a rate of 2°C / minute with a hold time of 12 min. The total run length was 59.9 minutes. The detector was maintained at 320°C. Peak identification was performed by the GC software (Chromcard-32 bit Ver 1.06 October 98) calibration table set up with a relative retention time window of 0.65%.

Analysis of Organophosphorous pesticides

Organophosphorus pesticides were analysed by Gas Chromatograph (Nucon -5765 series equipped with Nitogen Phosphorus detector). The capillary column used was another GLC capillary column - DB- 17- coated with 50% phenyl, 50% methyl polysiloxane (length 30m, ID 0.25 mm and film 0.25llm). The carrier gas and the makeup gas was nitrogen with a 1.3 mL/min and 25- mL/min flow rate respectively, Hydrogen at 8mL/min and Air at 80mL/min respectively employing the split less mode. 2.01lL of the final extract was injected at a temperature of 270°C. The oven temperature was kept at 120°C with a hold time of 1 minute, then from 120°C to 205°C at a rate of 25°C/minute with a hold time of 1 minute then finally from 205 to 270°C at a rate of 2°C / minute with a hold time of 1 min. The total run length was 38.90 minutes. The detector was maintained at 300°C.The samples were calibrated (retention time, area count) against standard mixture of known concentration of 12 organophosphorus pesticides. Each peak was characterized by comparing relative retention time with those of standards.

Quantitation

An external method was employed in the determination of the quantities of residues in the sample extracts. A standard mixture of known concentration of pesticide was run and the response of the detector for each compound ascertained. The area of the corresponding peak in the sample was compared with that of the standard. All analyses were carried out in triplicates and the mean concentrations computed.

$$\text{Concentration of Pesticide Residues in mg/Kg} = \frac{\text{Area of sample peak}}{\text{Area of std peak}} \times \frac{\text{Final volume}}{\text{gm of sample taken}} \times \frac{\mu\text{l of std injected}}{\mu\text{L of sampling}} \times \text{Conc. of std}$$

Recovery studies

The analysis method was standardised by the processing of spiked samples in triplicates of each fruit individually at different levels of fortification range from 0.01 to 0.50 mg/g in order to provide quality assurance before analysis of tests. A pesticide-free sample of rice grain was ground on a mechanical hand grinder and fortified by adding known volumes of mixed

standard solutions of the pesticides to the quantities of each pesticide studied. At least 24 hours before extraction, the fortified rice kernel samples have been left to absorb pesticides into the kernel and are more accurate with commercially treated grain. These enhanced samples were then treated triple and finally analysed by gas chromatography in response to a blank and control sample (free of pesticides). Similar processes were also carried out for fruit and vegetable samples for recovery studies.

Toxicological studies

Pesticides in general induce oxidative stress leading to generation of free radicals and alterations in antioxidant and scavengers of oxygen free radicals. The present study was undertaken to investigate the effect of sub-chronic exposure of the insecticides endosulfan, phorate and fenvalerate (each from organochlorines, organophosphates and Pyrethroids respectively) in causation of oxidative stress mediated hepatotoxicity in liver tissues of male Sprague Dawley rats. The hepatotoxicity level was measured by assessing the antioxidant status, cytochrome P450, different biochemical parameters and tissue residual concentrations following repeated oral administration of different doses for 90 days in rats.

Animals

Female Sprague-Dawley rats, weighing between 150 to 200 g, of the same breed were used for this study. They were housed in stainless cages and maintained under a controlled environment (temp 25±2°C, light cycle 6 a.m. to 6 p.m.) used for the experiment. They were provided with a standard laboratory diet (Hindustan Lever Lab diet) and water ad libitum throughout the experimental period. All procedures with animals were conducted strictly in accordance with guidelines approved by the University Animal Ethical Committee IAEC-KU11/06-07/BC-AH(S) according to the Government oflndia accepted principles for animal use and care.

3.4 EXPERIMENTAL DESIGN

Rats were randomly divided into ten groups (1 control and 9 treated groups) each containing six animals. Different doses of Endosulfan, Phorate and Fenvalerate were administered orally (using 22-gauge oral feeding needles) for 90 - days. The treatment dose was determined

according to the data obtained from Chapter IV and the grouping is given in detail in section V.2.1. Rats. were

euthanized at the end of treatment period. Blood was collected in test tubes from each animal, was kept under refrigeration (4 °C) for separation of serum and utilized for estimation of activities of aspartate transaminases (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), acid phosphatase (ACP) and total protein (TP). Portions of liver, from all groups were collected in 10% formalin solution for histopathology. One portion of liver was washed in physiological saline, homogenized and the homogenate was kept for estimation of catalase activity (CAT), levels of reduced glutathione (GSH), malondialdehyde (MDA), and tissue total protein. Another portion of liver was collected in ice-cold 1.15% KCl, homogenized within 10 min. and centrifuged. Microsomal pellets were separated and used for estimation of superoxide dismutase (SOD), and cytochrome P450 contents.

Preparation of liver microsomal fraction

The livers were perfused in situ with homoge.nizing buffer A (Tris-HCL + EDT A + BHT) by single pass injection through the portal vein and dissected out, placed in ice-cold KCl (1.15%). All the subsequent steps in the preparation of microsomal fraction were carried out at 0-4°C. Then the livers were minced and mixed with four volumes of Buffer A and homogenized in a mechanically driven Teflon glass homogenizer (Remi RQ 127 A). The homogenate was centrifuged at 10000 g in an automatic high-speed cold centrifuge (Hitachi-SCR 20B) using the rotor RPR 20-2 for 30 min. The supernatant was recentrifuged at 105000 g for one hour in an automatic preparative ultracentrifuge (Hitachi 70 P-72, Japan) using rotor RP-65T to yield microsomal pellet. Microsomal pellet was suspended in Buffer B (potassium pyrophosphate + EDT A + BHT) and homogenized with four passes of mechanically driven Teflon glass homogenizer (Remi RQ 127A) and again centrifuged at 104000g for 1 hour. The supernatant fraction was decanted and the microsomal pellet was resuspended in a minimum volume of buffer C (Tris-HCl +EDT A + glycerol) and stored at -20°C till further use. The pellet was used for estimating SOD activity and cytochrome P450 levels.

Assay of Antioxidant Enzymes

1 Assay of Superoxide dismutase [SOD; EC 1.15.1.1]

Superoxide dismutase was determined by the method described by Kakkar et al., 1984.

Reagents

1. Sucrose (0.25 M)

2. Sodium pyrophosphate buffer (pH 8.3, 0.052M)

3. PMS (1.2 µM)

4. NBT (300µM)

5. NADH (780µM)

6. Glacial acetic acid

7. n-Butanol

Procedure:

The tissues were homogenized in 0.25 M sucrose at 4 °C and centrifuged at 5,000 rpm. The supernatant was used as the enzyme source. The assay mixture contained 12 mL sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml NBT, 0.2 ml of NADH approximately diluted the enzyme preparation and water to a total volume of 3 mL. The reaction was started by the addition of 0.2 ml of NADH. After incubation at 30°C for 90 seconds the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The mixture was allowed to stand for 10 minutes, centrifuged and butanol layer was taken out. Colour intensity of chromogen was measured at 560 nm against butanol blank, which was stable upto 48 hours. A system devoid of enzyme served as control.

One unit of enzyme activity is defined as the enzyme concentration required for the inhibition of chromogen production by 50% in one minute under the assay conditions and expressed as specific activity in units/mg protein.

Assay of Catalase [EC 1.11.1.6]

Catalase was assayed by the method of Maehly and Chance, 1954.

Reagents

1. Ml 15 Phosphate buffer pH 7. 0

2. H202- Phosphate buffer- Diluted 0.16 mL H202 (30% w/v) to 100 mL with buffer

Procedure:

The tissue was homogenized in 2 ml phosphate buffer (0.01 M) at 4 °C and centrifuged at 5000 rpm. The estimation was done spectrophotometrically followed by decrease in absorbance at 230 nm. Pipetted into experimental cuvette and 3 ml H202-phosphate buffer and about 0.01 ml of enzyme solution. Read after every 10 seconds for about 2 minutes against a control cuvette containing enzyme solution in H202 free phosphate buffer. Specific activity is expressed in terms of units per mg protein.

Estimation of Glutathione Content

The glutathione content was determined by the procedure described by Patterson and Lazarow, 1955.

Reagents

1. Precipitating solution- 1.6 g glacial metaphosphoric acid, 0.2g of disodium or dipotassium EDTA and 30gm of NaCl were placed in a lOOmL flask and brought to volume with distilled water. This solution is stable for weeks at 4°c.

2. Phosphate solution (0.3M)

3. DTNB solution- 40mg of 5, 5'- dithiobisnitrobenzoic acid in 100 ml of 1 % sodium citrate was placed in a 100 mL volumetric flask and brought to volume with a solution of sodium citrate (lg/dL). The solution is stable for weeks.

4. GSH standard: 50mg/dL solution was prepared.

Procedure:

500 mg of the tissue was homogenized in 4 ml of the precipitating solution. After mixing, the solution was allowed to stand for 5 minutes and filtered. To 2 ml of the filtrate added 3 ml of phosphate solution (0.3 M Na2HP04). To all the tubes, 1 ml of DTNB solution was added mixed well and the OD was measured at 412 nm. Blank was prepared by substituting the sample with water and following the entire procedure for test. The quantity of reduced glutathione is expressed in mg/g tissue.

Estimation of Malondialdehyde (Thiobarbituric acid reactive substance)

Lipid peroxidation (LPO) was measured in hepatic tissue homogenates according to the method (Ohkawa et al., 1979) based on the formation of thiobarbituric acid reactive substances (TBARs) and expressed as the extent of MDA production.

Reagents

1. TCA-TBA-HCl reagent: 15% (w/v) TCA and 0.375 (w/v) TBA in 0.25 NHCl.

Procedure:

The tissue was homogenized in 0.1 M Tris-HCl buffer, pH 7.5 and allowed to stand for 5 minutes. Centrifuged and 1 m.L supernatant was combined with 2 mL of TBA-TCA-HCl reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling the flocculent, precipitate was removed by centrifugation at 1000 g for 10 minutes. Absorbance of the sample was read at 535 nm against the blank that contained no tissue homogenate. The extinction coefficient of TBARS is 1.56xl0 5 M-1 cm-1.

Hepatotoxicity marker enzymes

Assay of Alkaline Phosphatase (ALP; EC 3.1.3.1)

Quantitative determination of alkaline phosphatase was done usmg enzyme kit of Dr.Reddy's laboratories, Hyderabad, India (Kind and King's method, 1954).

Procedure:

Pipetted 1 mL substrate (pH 10.0) into a series of test tubes m'arked as blank, standard, control and test. 0.3mL distilled water was added and incubated for 3 minutes at 37° C. To 'test' added 0.1ml serum and to standard added O.lmL phenol standard (10 mg%) and to 'blank' added 0.1ml distilled water. Incubated for 15 minutes at 37° C. 2 mL colour reagent was added to all the tubes followed by 0.lmL serum to 'control'. Mixed well after each addition of reagent and measured the absorbance at 510nm.

Acid Phosphatase (ACP; EC 3.1.3.2)

Procedure was same in the case of alkaline phosphatase except that the citric acid-sodium citrate buffer was used for preparation of buffered substrate and incubation for lhour. For developing the colour with aminoantipyrine, lmL sodium bicarbonate was added. Values are expressed in King Amstrong units/ 100mL serum.

Assay of serum glutamate oxaloacetate transaminase (Aspartate amino-transferase; EC 2.6.1.1]

Serum glutamate oxaloacetate tramsaminase (AST) was assayed by DNPH method (Reitman and Frankel, 1957) using the enzyme kit from CML Biotech (P) Ltd, Ernakulam, India.

Reagents

1. O.lM Phosphate buffer, pH 7.4.

2. Substrate; Dissolved 13.3 g of aspartic acid in 90 mL of IN NaOH. Added 0.146 g of a-keto glutaric acid and dissolved it by adding little more NaOH. Adjusted the pH to 7.4 and made upto 500mL with phosphate buffer.

3. Standard: Dissolved 22mg sodium pyruvate in lOOmL buffer.

4. 0.4NaOH

5. DNPH colour reagent; Disolved 19.8 mg of 2, 4- DNPH m 10mL concentrated HCl and made up to 100mL with water.

Procedure:

Marked four test tubes as blank, standard, control and test. Added 0.5 mL substrate reagent to all the four test tubes. Added 0.1 mL deionised water to the test tube labelled 'blank'. 0.1 mL standard solution was added to the test tube labelled 'standard'. 0.1 mL serum was added to the tube labelled 'test'. Mixed well and incubated all the tubes at 37° C for 60 minutes. Added SGOT colour reagent (3mL) to all the tubes. 0.1 mL serum was added to the tube labelled 'control'. Mixed and incubated at room temperature for 20 minutes. Read the absorbance of all tubes against distilled water at 505 nm.

Assay of Serum glutamate pyruvate transaminase [Alanine aminotransferase; EC 2.6.1.2]

Serum glutamate pyruvate tramsaminase (ALT) was assayed by DNPH method (Reitman and Frankel, 1957) using the enzyme kit from CML Biotech (P) Ltd, Ernakulam, India.

Reagents

1. 0.lM Phosphate buffer, pH 7.4

2. Substrate; Dissolved 9 g of alanine in 90 mL of water and 2.5mL IN NaOH. Added 0.146 g of a-keto glutaric acid and dissolved it by adding little more NaOH. Adjusted the pH to 7.4 and made upto 500mL with phosphate buffer.

3. Standard: Dissolved 22mg sodium pyruvate in 100mL buffer.

4. 0.4NaOH

5. DNPH colour reagent; Disolved 19.8 mg of 2, 4- dinitrophenyl hydrazine in lOmL concentrated HCl and made upto 100ml with water.

Procedure:

Marked four test tubes as blank, standard, control and test. Added 0.5 mL substrate reagent to all the four test tubes. Added 0.1 mL deionised water to the test tube labelled 'blank'. 0.1 mL standard solution was added to the test tube labelled 'standard'. 0.1 mL serum was added to the tube labelled 'test'. Mixed well and incubated all the tubes at 37°C for 60 minutes. Added

SGPT colour reagent (3mL) to all the tubes. 0.1 mL serum was added to the tube labelled 'control'. Mixed and incubated at room temperature for 20 minutes. Read the absorbance of all tubes against distilled water at 505 nm.

Estimation of y glutamyl transpeptidase (yGGT)

GGT in serum was assayed by the method of Tietz (1986). 100µL serum and lmL buffer were incubated at 37°C. 0.lmL substrate was mixed and monitored the reaction continuously at 405 in 1cm cuvette so as to obtain the change in absorbance per minute. Values are expressed in µg/ min/ 100mL serum.

Lactate Dehydrogenase (EC 1.1.1.27)

The serum LDH was determined by the method of Martinek (1972).

Procedure

Pipetted out lmL of buffer substrate and 20µ1 of serum into each of the two tubes designated as test and blank, added 0.2 mL of NAD solution to the test and shaken well. After 15 min, added 1ml DNPH solution to both the tubes and mixed and kept in a water bath for another 20min. After removal from the bath, to each tube, added 10mL of 0.4N sodium hydroxide and the absorbance was read at 440nm within 5min. Sodium pyruvate solution containing 1-M of pyruvate/mL was used as the standard. Values are expressed in IU/100mL serum.

Estimation of Cyt.P450

The microsomal suspension containing 0.5-2.0 mg/mL protein prepared in 0.1 M phosphate buffer pH 7.2 was used for the estimation of Cytochrome P 450 concentration by the method of Omura and Sato (1964). 3.0 mL suspension was taken into sample and reference cuvettes and 7-8 mg of sodium dithionite was added to both. The baseline was adjusted setting the instrument at zero. The sample cuvette was gassed with carbon monoxide for 30 seconds. The difference in O.D. was measured at 450 and 490 nm in an UV-VIS spectrophotometer. Concentration of Cyt. P450 was presented as number of moles of Cyt P/mg protein calculated on the basis of milli molar extinction coefficient of 91m mole "cm". Protein was estimated by the method of Bradshaw (1966) in microsomal and cytosolic preparations.

Assay of ROS level

Reagents

1. Locke's buffer (154mM NaCl, 5.6mM KCl, 3.6mM NaHC03, 2.0mM CaC12, lOmM d-glucose, and 5mM HEPES, pH7.4

2. DCFH-DA (2',i-dichlorodihydro fluorescein diacetate)

ROS was measured based on the oxidation of 2' f-dichlorodihydro fluorescein diacetate (DCFH-DA) to ii- dichloro-fluorescein (DCF). Briefly, the homogenate was diluted 1 :20 times with ice-cold Locke's buffer to obtain a concentration of 5mg tissue/mL. The reaction mixture (lmL) containing Locke's buffer (pH7.4), 0.2mL homogenate and lOmL of DCFH-DA (5mM) was incubated for 15min at room temperature to allow the DCFH- DA to be incorporated into any membrane-bound vesicles and the diacetate group cleaved by esterases. After 30min of further incubation, the conversion of DCFH-DA to the fluorescent product DCF was measured using a spectrofluorometer with excitation at 484nm and emission at 530nm. Background fluorescence (conversion of DCFH-DA in the absence of homogenate) was corrected by the inclusion of parallel blanks. ROS formation was quantified from a DCF-standard curve and data are expressed as pmol DCF formed/ min/mg protein.

Estimation of proteins

Proteins were estimated by the method of Lowry et al., 1951

Reagents

1. Reagent A: 2gm sodium carbonate was dissolved in lOOmL O.IN NaOH solution.

2. Reagent B: Solution 1: 500mg copper sulphate was dissolved in 50 mL water. Solution 2: l gm sodium potassium tartarate was dissolved in 50 mL water. Mixed 0.5 mL solution 1 and 0.5mL solution 2.

3. Reagent C: 50mL of reagent A and lmL of reagent B were mixed fresh at the time of use.

4. Follin-Ciocalteau reagent: Commercially produced reagent was diluted in the 1 :2 ratio with distilled water.

5. Protein standard: 4mg BSA was dissolved in 10mL of distilled water to make protein standard solution of 400µg/L.

Procedure:

An aliquot of 0.1 mL sample was taken and made up to 0.5 mL alkaline copper reagent was added and mixed well. After allowing to stand for 10mins at room temperature, 0.5 mL of Folin's reagent was added, mixed and optical density was read at 600nm after 20 mins in dark. A standard curve was prepared by using different concentrations of BSA.

Tissue residue level

The tissue residue levels of Endosulfan, Phorate and Fenvalerate in brain, lungs, liver, heart, and kidney were estimated by the method of Marie et al., modified by Mandal et al., 1992.

Tissue sample preparation

Tissues (2g) were extracted for 4 minutes with acetonitrile (25mL) and anhydrous sodium sulphate (0.5g) using a homogenizer. The extract was filtered through anhydrous sodium sulphate (0.5g) and the tissues were re-extracted twice with acetonitrile (first by 25 and then by 12mL). The extract was clarified by centrifugation and filtered through anhydrous sodium sulphate. The combined acetonitrile extracts were concentrated to 20mL and partitioned with hexane (2 x10 mL). The hexane phases were discarded and the acetonitrile phase was evaporated to dryness using a rotary vacuum evaporator at 40°C. The volume was finally made up to 5 mL with acetone for GLC estimation.

Calibration

Stock solutions of 1 mg/L of the pesticides (analytical grade) were prepared as external standards. The data were recorded in a HP 3392A integrator.

Apparatus

A Hewlett Packard (USA) model 5890A gas chromatograph coupled with a 3392 A (HP) integrator and equipped with a 63Ni electron capture detector was used for analysis the pesticides in tissues.

Operational parameters were:

Injector temperature - 275°C

Oven temperature - 255^0c

Detector temperature - 275°C

Flow rate of carrier gas N2 - 70 mL per minute.

Column: A 1.8 x 2 mm I.D. glass column packed with 3% OV-101 on chromosorb W.H.P. (80-100 mesh) was used. Injection: Standard and samples (2 µL) were injected into gas liquid chromatograph With 10 µL Capacity Hamilton Syringe.

Histopathological examination

The liver was excised and fixed in neutral buffered formalin (10%); the organs were routinely processed and sectioned at 4 to 5 µm thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained with Hematoxylin and eosin stain, bromophenol blue. The sections were then observed under microscope at 400X magnification.

Measurement of Genotoxicity

The current study is devoted to determine the in-vivo genotoxic effects of the exposure of three insecticides, endosulfan, phorate and fenvalerate in rat liver tissues by measuring the impact on the level of DNA damage in rat liver tissues.

Experimental design

Rats were randomly divided into ten groups (1 control and 9 treated groups) each containing six animals. Different doses of Endosulfan, Phorate and Fenvalerate were administered orally (using

22-gauge oral feeding needles) for 30 days. At the specified times after treatment, animals were anaesthetized using diethyl ether and were sacrificed by cervical dislocation. Liver was rapidly removed, quickly minced and suspended in chilled homogenization buffer (2gm of liver in 2 mL of cold HBSS containing 20 mM. EDT A, and 10 % DMSO), and then homogenized gently. Then, 1.5 ml cell sample in micro-centrifuge tube was centrifuged for 5 min at 5°C, at 3500 rpm.

Determination of DNA single strand breaks (DNA-SSBs) by Alkaline Elution method

Reagents

l. Homogenizing buffer: 0.075 M NaCl containing 0.024 M EDTA, pH 7.5

2. Elution solution: 0.1 % sodium dodecyl sulfate and 20 mM NaiEDTA adjusted to pH 12.3

3. Lysing solution: 2% w/v sodium dodecyl sulfate (SDS) and 25 mM Nc4EDTA, pH 10.4

4. Bovine serum albumin solution (2 mg/mL)

The DNA damage was measured as single strand breaks in the hepatic nuclei by the alkaline elution method as modified by Bagchi et al., (1992a). The liver was homogenized in 2 g/8 mL buffer, and centrifuged at 480g for 15 min. The nuclear pellets were washed once and were resuspended in homogenizing buffer. Isolated hepatic nuclei (0.1 mL of nuclear homogenate) were lysed on a 5 µm membrane filter with a solution containing 2% w/v sodium dodecyl sulfate (SDS) and 25 mM NEDTA, pH 10.4, for 20min. DNA was then eluted with an elution solution containing 0.1 % sodium dodecyl sulfate and 20 mM NEDTA adjusted to pH 12.3 with tetraethyl ammonium hydroxide, at a flow rate of 0.1 mL/min. DNA was precipitated from the collected fractions by the addition of 0.1 mL bovine serum albumin solution (2 mg/mL) and 0.5 mL of 40% (w/v) trichloroacetic acid. The fractions were then kept in a refrigerator for 2-3 h, washed once with 3.6 mL ethanol solution containing HCl (36: 1 v/v) and allowed to dry overnight. DNA content was measured microfluorometrically (excitation wavelength 436 nm, emission wavelength 521 nm) on a spectrofluorometer (American Instrument Co., Silver Springs, MD) after addition of 3, 5-diamino benzoic acid dihydrochloride as the fluorogen and incubated for 45 min at 60°C. The elution constants (k) were determined by plotting the log DNA remaining on the filter against the volume of eluate, where k = -2.3 x slope of the plot.

Comet assay

Reagents

1. Homogenizing buffer: 0.075 M NaCl containing 0.024 M EDTA, pH 7.5

2. Normal melting agarose: 1 % prepared in 0.1 M sodium phosphate buffer, pH 7.2 containing 0.9% NaCl

3. Low melting agarose : 2% in phosphate buffer saline.

4. Lysis buffer : containing 0.25 M NaCl, 100 mM EDT A, 10 mM Trizma base, 1 % sarcosine, pH 10.0 adjusted with 10 N NaOH. 5% DMSO and 1 % Triton X-100 was added just before use.

5. Electrophoresis buffer (300 mM sodium hydroxide and 1 mM EDT A, pH 13.0).

6. Neutralizing buffer (0.4 M Tris-HCl, pH 7.4).

7. Ethidium bromide (20 µg/ mL)

Single strand breaks were measured by alkaline comet assay as described by Sasaki et al., (1997). Fresh tissues were collected and homogenates (25% w/v) were prepared in homogenizing buffer with a single stroke. To obtain nuclei, homogenates were centrifuged at 700.3 g for 10 min and the resulting pellets were gently resuspended in 4.0 mL of chilled homogenizing buffer. 75µL of normal melting agarose (1 % prepared in 0.1 M sodium phosphate buffer, pH 7.2 containing 0.9% NaCl) was quickly layered on an end frosted slide, covered gently with another slide, and allowed to solidify. The upper slide was gently removed, and the precoated slide was coated with 100 µL of mixture containing equal volumes of sample (nuclei preparation) and low melting agarose (2% in phosphate buffer saline). Slides were then immersed in the lysis buffer for 1 hour at 48C in the dark. After lysis, the slides were rinsed with chilled distilled water, transferred on a horizontal electrophoresis platform and immersed in electrophoresis buffer for 20 min for unwinding of DNA. Electrophoresis was performed for 15 min at constant voltage (1 V /cm and 300 mA). After electrophoresis, the slides were washed thrice with neutralizing buffer (0.4 M Tris-HCI, pH 7.4) for 5 min each to remove excess alkali and detergents. Slides were dehydrated in absolute methanol for 10 min and left at room

temperature to dry. The whole procedure was performed in dim light to minimize artifactual DNA damage. Just before visualization, each slide was stained with 50 µL of ethidium bromide (20 µg/ mL), rinsed with water, and covered with a cover slip. Slides were stored at 4 ° C in sealed boxes until analysis.

Evaluation of DNA damage

After application, the cover slip was removed, each slide was examined at 1200X magnification in a fluorescence microscope {excitation filter: 400 nm, with barrier filter: 590 nm}. The endpoint, which was identified in this study, was the percentage of DNA in the tailor {the percentage of DNA damage}. A total of 100 randomly observed comets on each slide {3 slides/individual rat liver; 300 comets per individual rat liver sample; 900 comets per treatment}; were observed at a constant depth of the gel, avoiding the edges of the gel on each of three replicate slides.

Apoptosis

It is important to study the exact mechanism(s) involved in the hepatotoxicity of these pesticides. Thus, the present investigation was also designed to study the pathway(s) involved in pesticide induced apoptotic cell death in rat liver.

Experimental design

Rats were randomly divided into ten groups (1 control and 9 treated groups) each containing six animals. Different doses of Endosulfan, Phorate and Fenvalerate were administered orally (using 22-gauge oral feeding needles) for 60 days. At the specified times after treatment, animals were anaesthetized using diethyl ether and were sacrificed by cervical dislocation. Liver was rapidly removed, quickly minced and the samples were frozen in the liquid nitrogen immediately and stored in the -80°C refrigerator for the followed study.

DNA fragmentation analysis

DNA fragmentation analysis was done by method of Sambrook (1989).

Reagents

1. Tris-HCl : 100 mM (pH 8.0)

2. EDTA: 25 mM

3. Proteinase K: 0.1 Ilg/mL.

4. Lysis buffer: containing 0.25 M NaCl, 100 mM EDTA, 10 mM Trizma base, 1% sarcosine, pH 10.0 adjusted with 10 N NaOH. 5% DMSO and 1% Triton X-lOa was added just before use.

5. 1.5 % agarose gel.

Liver tissues were homogenized and incubated in 100 mM Tris-HCl (pH 8.0),25 mM EDTA, 0.5 % SDS, and 0.1 Ilg/mL proteinase K at 60 °C for 3 hour. DNA was extracted with phenol/chloroform (1: 1) and chloroform/isoamyl alcohol (l :24). The extracted DNA was precipitated and digested in 10 mM Tris-HCl (pH 5.0) containing 1 mM EDTA and 10 Ilg RNase for 1 h at 37°C. Five Ilg of DNA per sample were electrophoretically separated on 1.5 % agarose gel containing 0.5 Ilg/mL ethidium bromide. The DNA pattern was examined with a UV illumination in Gel Documentation System.

TUNEL assay

TUNEL detection was based on method by Loo and Rillema, 1998.

Reagents

1. Phosphate-buffered saline (PBS), pH 7.4.

2. 4% buffered formaldehyde: dilute high quality formaldehyde (v/v) in PBS prior to use.

3. 20 Ilg/mL proteinase K (Roche Diagnostics Corp.). Stock solution may be stored at -20°C for several months.

4. 95, 90, 80, and 70% ethanol in Coplin jars 69

5. 2% hydrogen peroxide. Prepare fresh from hydrogen peroxide reagent stock prior to use.

6. 2% BSA solution: 2% BSA (w/v) dissolved in PBS and passed through a 0.45 11m filter. Sterile stock solution may be stored at 4°C for several weeks.

7. 2x SSC buffer: 300 mM NaCl, 30 mM sodium citrate. Stock solution may be stored at room temperature for several months.

8. TdT Equilibration Buffer: 2.5 mM Tris-HCl (pH 6.6), 0.2 M potassium cacodylate, 2.5 mM CoC12, 0.25 mg/mL BSA. Prepare from stock solutions. Aliquots may be stored at -20°C for several months.

9. TdT Reaction Buffer: TdT Equilibration Buffer containing 0.5 U/IlL of TdT enzyme and 40 pmoll/-lL biotinylated-dUTP (Roche Diagnostics Corp.). Prepare fresh from stock solutions prior to use.

10. 3, 3'-Diaminobenzidine (DAB) staining solution.

11. TdT staining buffer: 4x saline-sodium citrate (0.6 M NaCl, 60 mM sodium citrate), 2.5 /-lg/mL fluorescein isothiocyanate-conjugated avidin, 0.1% Triton X-I 00, and 1% BSA. Prepare fresh from stock solutions prior to use.

Procedure

1. Fix tissue samples in 4% formaldehyde in PBS for 24 h and embed in paraffin. Adhere 4-6 /-lm paraffin sections to glass slides pretreated with 0.01% aqueous solution of poly-L-Iysine.

2. Deparaffinize sections by heating the slides for 30 min at 60°C (or 10 min at 70°C) followed by two 5 min incubations in a xylene bath at room temperature in Coplin jars. Rehydrate the tissue samples by transferring the slides through a graded ethanol series: 2 x 3 min 96% ethanol, 1 x 3 min 90% ethanol, 1 x 3 min 80% ethanol, 1 x 3 min 70% ethanol, 1 x 3 min double-distilled water (DDW).

3. Carefully blot away excess water and pipet 20 ~g/mL proteinase K solution to cover sections. Incubate 15 min at room temperature.

4. Following proteinase K treatment, wash slides 3 x 5 min with DDW.

5. Carefully blot away excess water then cover sections with TdT equilibration buffer for 10 min at room temperature. While the sections are equilibrating, thaw the Biotinylated Nucleotide Mix on ice.

6. Remove TdT equilibration buffer and cover sections with TdT reaction buffer. Incubate slides in a humidified chamber for 30 min at 37°C. In order to conserve reagents a reduced volume of TdT buffer may be carefully covered with a glass coverslip during the incubation. Take care to avoid trapping air bubbles which may lead to staining artifacts.

7. Stop reaction by incubating slides 2 x 10 min in 2xSSC.

8. Rinse slides in PBS then block nonspecific binding by covering tissue sections with 2% BSA solution for 30-60 min at room temperature.

9. Wash slides 2 x 5 min in PBS then cover tissue sections with TdT staining buffer. Incubate slides at room temperature for 30 min in the dark.

10. Wash slides 2 x 5 min in PBS.

11. The incorporated biotinylated-dUTP may be visualized following staining on a fluorescent microscopy equipped with a standard fluorescein filter (520nm ± 20 nm).

SDS-PAGE

SDS-PAGE was done according to the method of Laemmli, 1970.

Reagents

1. ABA: Acrylamide Bis acrylamide (30: 0.8 w/w in 100 mL distilled water)

2. 10% SDS

3. Electrode buffer- Tris glycine buffer (0.05 M, pH 8.3, 1%SDS)

4. Separating Buffer - Tris HCI buffer (3M, pH 8.8)

5. Stacking Buffer - Tris HCI buffer (0.05M, pH 6.8)

6. Sample Buffer (2x) - Stacking buffer - 1.0 mL, water- 0.2mL, glycerol - 0.8 mL, 10%SDS - 1.6 mL, 2-mercaptoethanol - 0.4 mL, bromophenol blue -1.0mg

7. Staining solution - Methanol (50%), glacial acetic acid (7%), Coomassie brilliant blue R-250 (0.2%) in distilled water.

8. Destaining solution - Methanol (50%) and glacial acetic acid (70%) in distilled water.

9. Composition of stacking gel: ABA - 0.68 mL, Stacking buffer - 0.5 mL, water-2.72 mL, 10%SDS -0.04 mL, 10% APS - 0.04 mL, TEMED - 0.004 mL.

10. Composition of resolving gel (mL):

Reagents	Concentration		
	8%	10%	12%
ABA	1.325	1.675	2.0
Separating Buffer	1.25	1.25	1.25
Water	2.325	1.975	1.65
10% SDS	0.05	0.05	0.05
10%APS	0.05	0.05	0.05
TEMED	0.002	0.002	0.002

Procedure

Protein samples of electrophoretic analysis were mixed with sample buffer (1: I) and boiled for 2-3 minutes for heat denaturation. Samples were briefely centrifuged to remove particulates and loaded to 12% gels. Stacking was carried at 7 mA constant current and resolving at 100 V constant voltages. Electrophoretic separation ws monitored by the movement of tracking dye (bromophenol blue). Gel was placed in staining solution for 1 hr and destained until a clear gel with distinct bands was obtained.

Western Blot Analysis

Reagents

1. Transfer buffer : 0.025 M Tris, 0.15 M glycine, pH 8.3 + 10% methanol

2. Lysis buffer : 50 mmol /L Tris, 15 mmol/L EDT A, 150 mmol/L NaCl, 0.1 % Triton X-100, pH 8.0)

3. Protease inhibitors: 10 mg/mL Aprotinin, 10 mg/mL Leupeptin and 1 mmol/L PMSF

4. Substrate Buffer: 5-Bromo- 4-chloro-3-indolyl phosphate/ nitro blue tetrazolium (BCIP/NBT) liquid substrate system.

5. Blocking buffer: 5% defatted milk in PBS containing 0.1 % Tween20

Procedure

For western blotting sample preparation, frozen pieces of rat liver or cell pellets were resuspended in lysis buffer (50 mmol/L Tris, 15 mmol/L EDTA, 150 mmol/L NaCl, 0.1 % Triton X-100, pH 8.0) containing protease inhibitors (10 mg/mL Aprotinin, 10 mg/mL Leupeptin and 1 mmol/L PMSF). Liver tissues were homogenized in lysis buffer at a ratio of 1: 15 w/v. Cell pellet was solubilized in 100 uL lysis buffer and put on ice for 30 min. After centrifugation at 12,000g (4°C) for 20 min to remove debris, the supernatant was carefully recovered. Protein concentrations were determined using the Bradford assay (Bradford, 1976). All samples were stored at 70°C prior to electrophoresis. Aliquots from supernatant containing 50 mg of proteins were mixed with equal volume of 2 x sample buffer. The samples were boiled for 5 min and subjected to 12.5% SDSPAGE. After electrophoresis, the resolved proteins were transferred to nitrocellulose membrane. The filter buffer, membrane, gel, and filter paper were arranged in that order on the anode of semidry apparatus in the form of a sandwich carefully avoiding air bubbles. Membranes were blocked at room temperature for 1 hr in blocking buffer containing 5% defatted milk in PBS containing 0.1 % Tween20, to prevent non-specific binding of reagents, and then incubated with anti-p53 (1 :2500 dilution), anti-Bcl-2 (1 :500 dilution) or anti-Bax (1:500 dilution) at 4 °C overnight. The membranes were washed in TBST (50 mmol/L Tris-Cl,

pH 7.6, 150 mmol/L NaCl, 0.1% Tween 20) for 30 min and incubated with HRP conjugated secondary antibody (1 :25,000 dilution) for 1 hour at room temperature. The membranes were washed three times in TBST and exposed to 4 mL ECL chemiluminescence reagents for 1 min. Blots were exposed to X-ray film for radiographic detection of the bands. The autoradiograms were scanned and the level of p53, Bcl-2 and Bax expression was quantified by densitometry.

Translocation Studies

Reagents

1. Suspension buffer : 20 mmol/L HEPES, pH 7.4, 1.5 mmol/L MgCh, 10 mmol/L KCl, 1.0 mmol/L EDTA, 1.0 mmol/L EGTA, 1.0 mmol/L dithiothreitol, and 1 % proteinase inhibitor.

2. Sucrose: 0.25 mol/L
3. anti-Bax and anti-cytochrome-c primary antibodies

4. Blocking solution: 2.5% BSA in 0.05% Tween 20

Procedure

Translocation of Bax protein from cytosol to mitochondria and cytochrome-c from mitochondria to cytosol due to the pesticide induced mitochondrial membrane permeabilization (MMP) was studied by Western blot analysis using specific anti-Bax and anti-cytochrome-c primary antibodies. Processed for isolation of mitochondrial and cytosolic fractions using the protocols of Ghosh et al., (2007) and Waterhouse et al., (2004), respectively. Fresh liver tissues were homogenized gently with a glass tissue grinder in a suspension buffer (20 mmol/L HEPES, pH 7.4, 1.5 mmol/L MgCh, 10 mmol/L KCl, 1.0 mmol/L EDT A, 1.0 mmol/L EGT A, 1.0 mmol/L dithiothreitol, and 1 % proteinase inhibitor cocktail) with 0.25 mol/L of sucrose. The crude homogenate was centrifuged at 750 x g for 10 minutes at 4 °C and then at 8000 x g for 20 minutes at 4 °C. The 8000 x g pellet was homogenized with Teflon homogenizer in the suspension buffer without sucrose and used as the mitochondrial fraction. The cross-contamination of cytosolic protein in the mitochondrial fraction and vice versa was also assessed by running separate blots using antibodies specific to mitochondria and cytosolic proteins.

Statistical analysis

The results were analyzed using a statistical program SPSS/PC+ version 11.0 (SPSS Inc., Chicago, IL, USA). One-way Anova was employed for comparison test of significant differences among groups were determined. Pair fed comparison between the groups was made by Duncan's multiple range tests.

CHAPTER-4

ANALYSIS OF PESTICIDE RESIDUES

4.1 INTRODUCTION

Farmers around the world, including India, are using pesticides to prevent the risk of devastating crop losses caused by pests and diseases. Pesticides have thus been used in agriculture for several decades in India, not just for the control and eradication of crop pests but for the vector control of disease in the public-health sector. Cocoa, coffee and coton, vegetables and fruit crops as well as other cereal (primarily maize), tuber (e.g. Yam, Cassava), pulp products (e.g., cowpeas, drums), sugar cane, rice etc. are used for the production of agricultural pesticides. Agriculture pesticides

In waterbodies, especially underwater, pesticide contamination is becoming a major concern. Several agencies have reported that there are pesticides in the underground water of those areas developed in agriculture. Pesticides may be affected by a direct runoff, sprinkling, careless disposal of vacuum containers, washing equipment etc (Ahad et al., 2000, 2001 Tariq et al., 2004 a, b and 2006). Rice grains are primarily contaminated with pesticides through field spraying and storage treatment. In storage facilities and before shipment to other countries, Rice grams are treated with pesticides including organophosphates, carbamats, synthetic pyrethroids and insect growth regulators (Khan et al., 2007a). In addition to few weeds such as Echinocloa, rice is attacked mostly by stem and leaf folder insect pests, blasts and black disease (Garg et al., 2004).

Pesticide residue analysis

Residue testing is performed by food and environmental monitoring. Several multi residue methods for organophosphorus, organic chlorine or organo-nitrogen pesticides have been proposed in cultures which are subject to the gas chromatography for the separation of individual compounds (Berrada et al., 2010; Menezes Filho et al., 2010; Pose-Juan et al., 2009, 2006; Ba1inova et aI., 2007; Rial-Otero et al., 2005; Albero et al., 2003). Many research papers on method development for rice grain residue determination using traditional gas chromatographic techniques (GC with ECD, FID) and smart instruments (GC/MS) with the combination of new extraction techniques have been published. Dong et al. 2008, Aldana-madrid etc. 2008, Ping et al. 2008, Bottomley and Baker 1984, Zhang et al. 2006, 2008, 2014

and 2000. Today's investigation was conducted to assess the concentration and awareness of pesticide residues in foodstuffs as well as to estimate the potential health risks associated with the residue of pesticides for consumers. The estimation of residues of fifteen commonly used pesticides, namely endosulfan, dieldrin, aldrin, lindane, DDT, chlorpyrifos, profenophos, quinalphos, dimethoate, phorate, cypermethrin, fenvalerate, deltamethrin and carbofuran was done using GC-ECDINPD detection. The samples of rice (main cereal used in Kerala), fruits and vegetables were collected from the fields and markets and water samples from wells and streams located in Thiruvananthapuram and Kasargod region for analysing the levels ofthe residues in major food items of people in Kerala.

4.2 MATERIALS AND METHODS

The water and food samples collected were analysed for the pesticide residues and the quantities were estimated using GC-ECD/NPD. Recovery studies were done initially to check the efficiency of the system for the residues in each type of samples. Finally the health risk was estimated according to the life time exposure for each of the pesticides analyzed.

4.3 RESULTS

Recovery study

Rice and water samples

Recovery and precision (expressed as relative standard deviation) were calculated for three replicate samples and the data are presented in IV.l. The data showed reveals that the average range of recovery for 0.1 and 0.5 Ilg/g fortification level of the method fell within 75.2%-93.2% with % RSD in the range of 4.90-12.42 and within 81.8%-96.9% with %RSD in the range of 2.41- 9.00 respectively. The table shows that the recovery rate for fifteen pesticides were within acceptable range.

Table 4.l; Analytical recoveries (%) ±SD of pesticide residues in water and rice samples at different fortification levels

Pesticides	Rice (mg/Kg)		Water (mg/L)	
	0.1	0.5	0.1	0.5
Endosulfan	82.4±0.03	95.2±0.06	78.5±0.04	88.1±0.06
Dieldrin	86.6±0.04	88.5±0.04	93.2±0.05	94.1±0.08
Aldrin	85.8±0.02	89.3±0.05	84.6±0.06	95.2±0.05
Lindane	85.2±0.05	86.8±0.06	80.8±0.04	86.2±0.04
DDT	89.4±0.03	89.8±0.05	75.2±0.07	84.5±0.06
Chlorpyrifos	84.5±0.02	89 .8±0.03	76.5±0.08	88.6±0.08
Profenophos	82±0.06	94.8±0.05	79.2±0.06	82.5±0.05
Chlordane	78.5±0.03	93.8±0.02	85.5±0.05	94.4±0.08
Phorate	83.2±0.05	86.8±0.05	79.2±0.02	88.2±0.03
Quinalphos	92.5±0.06	96.3±0.04	85.8±0.05	93.8±0.04
Cismethrin	82.2±0.09	86.4±0.07	93.2±0.03	96.9±0.09
Cypermethrin	79.5±0.03	94.8±0.06	81.3±0.06	84.5±0.07
Fenvalerate	90.6±0.07	81.8±0.05	85±0.04	88±0.05
Deltamethrin	95.9±0.06	96.7±0.05	82.9±0.05	87.6±0.08
Carbofuran	92.7±0.08	94.8±0.06	90.2±0.08	93.8±0.09

Fruits/ Vegetable samples

Recovery and precision (expressed as relative standard deviation) were calculated for three replicate samples of vegetables and fruits and the data are presented in Table 4.2. The recovery rates for the pesticides were within acceptable range. Retention times and peak areas of the studied pesticides were comparable with the relative standards. Detection limit(s) of the method were also assessed based on the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC; which were 0.001 g-1 for OC, 0.005Ilgg-1 for SP and 0.01Ilgg-1 for OP compounds. Blank analyses were also carried in order to check any interfering species in the reagents.

Table 4.2: Analytical recoveries (%)±SD of pesticide residues in vegetables and fruit samples at different fortification levels

Pesticides	Tomato (mg/kg)			Grapes (mg/kg)			Carrot (mg/kg)		
	0.05	0.1	0.5	0.05	0.1	0.5	0.05	0.1	0.5
Endosulfan	85.3 ±0.03	87.2 ±0.04	88.5 ±0.04	86.1 ±0.02	88.5 ±0.04	96.1 ±0.07	87.2 ±0.06	91.3 ±0.02	96.5 ±0.03
Dieldrin	78.2 ±0.04	88.5 ±0.02	96.2 ±0.06	86.6 ±0.013	90.6 ±0.01	98.8 ±0.03	82.3 ±0.06	97.2 ±0.02	96.6 ±0.02
Lindane	79.3 ±0.03	87.1 ±0.04	98.1 ±0.05	88.8 ±0.005	92.5 ±0.006	91.5 ±0.04	88.1 ±0.04	92.4 ±0.05	93.4 ±0.04
DDT	85.1 ±0.06	91.3 ±0.02	95.6 ±0.04	79.2 ±0.02	84.7 ±0.005	89.3 ±0.01	78.4 ±0.07	88.6 ±0.03	87.9 ±0.04
Chlorpyrifos	90.1 ±0.02	92.4 ±0.04	94.2 ±0.02	81.2 ±0.015	88.4 ±0.004	93.8 ±0.08	79.5 ±0.02	89.8 ±0.04	87.2 ±0.03
Profenophos	84.7 ±0.04	89.2 ±0.03	93.9 ±0.014	79.2 ±0.031	88.8 ±0.03	96.3 ±0.04	87.6 ±0.01	96.9 ±0.05	97.5 ±0.04
Chlordane	90.1 ±0.06	90.4 ±0.05	95.1 ±0.032	81.3 ±0.06	89.9 ±0.043	93.2 ±0.01	86.8 ±0.05	88.8 ±0.04	87.6 ±0.08
Phorate	88.3 ±0.03	89.6 ±0.02	89.1 ±0.031	84.5 ±0.021	88.1 ±0.041	95.5 ±0.03	81.9 ±0.06	87.2 ±0.03	95.1 ±0.05
Cypermethrin	86.5 ±0.07	90.1 ±0.06	93.2 ±0.023	87.9 ±0.032	91.4 ±0.032	94.6 ±0.05	83.7 ±0.02	86.5 ±0.04	88.6 ±0.09
Fenvalerate	80.1 ±0.06	91.2 ±0.03	94.5 ±0.021	85.8 ±0.041	92.2 ±0.021	94.1 ±0.07	85.5 ±0.04	89.1 ±0.05	90.4 ±0.07
Deltamethrin	79.2 ±0.01	84.24 ±0.05	91.5 ±0.013	78.8 ±0.026	82.3 ±0.03	89.2 ±0.07	79.1 ±0.03	86.1 ±0.04	88.6 ±0.08

Pesticide residues in water and food samples

Freshly collected samples of fruits and vegetables from the selected areas were analyzed to assess the residue levels of different insecticides on these crops. The samples were extracted, cleaned up and were analyzed using GC. Since no MRLs have been so far fixed for any pesticides in fruits, vegetables and other food commodities in India, we had to compare our results with MRLs established by the FAD/ WHO CODEX Alimentarius Commission. Residue levels of less than minimum detectable quantities are not reported and are indicated as zero.

Pesticide residues in Water samples collected from Trivandrum

In Trivandrum, the samples were collected from Aruvikkara (WS I) dam and fresh-water lake at Vellayani (WS2) (potential water sources for Trivandrum city). The well samples were collected from wells of Palode (WWI) and Kallara (WW2) areas of Trivandrum. The samples are analyzed by gas chromatography equipped with ECD and NPD detectors. A standard

mixture containing insecticides (DCs, OPs, Pyrethroids and Carbamates) was run before the actual samples. The concentrations of individual pesticides in water samples collected from different locations of Trivandrum are given in Table 4.3. A comparison of residues in well and river water samples is made in the figure 4.1. In water sample WWI, the residues of all the organochlorines analyzed were found to be present except aldrin. But the level of endosulfan only was found to be above MRL. Among organophosphates, dimethoate and chlorpyrifos could be detected but the values were well below the residue limit. All of the pyrethroids analyzed were present in the samples. The concentration of carbofuran exceeded the limit in the samples. In water sample WW2, among the organochlorines analyzed, residues of endosulfan, lindane and DDT could be detected eventhough the concentrations of lindane exceeded in the case of lindane. Among organophosphates, profenofos, dimethoate and quinalphos could be detected but the values were well below the residue limits except in quinalphos. All of the pyrethroids analyzed were present in the samples except cismethrin. The concentration of carbofuran exceeded the limit in the samples similar to WWI.

In water sample WS 1, among the organochlorines analyzed, residues of dieldrin and DDT only could be detected and the concentrations exceeded MRL in the case of dieldrin in the samples. Among organophosphates, profenofos, quinalphos and chlorpyrifos could be detected and the concentration of profenofos and quinalphos only was found to be above MRL. All of the pyrethroids analyzed were present in the samples except cismethrin. But the values were in the limit except of carbofuran. In water sample WS2, residues of endosulfan could only be detected among organochlorine pesticides, but the value was below the residue limit. All the organophosphates analyzed were found to be present except profenophos. Moreover the concentration of chlorpyrifos and dimethoate were found to be above MRL. All of the pyrethroids analyzed were present in the samples except deltamethrin and cismethrin. The cdncentrations of fenvalerate and carbofuran exceeded the limit in the samples.

Compared to the well water, the residues of organophosphates and pyrethroids were found to be detected more and which was above the limits in the samples from the stream and lake. The result shows that about 75% of the samples were contaminated with either one or another pesticide and sometimes with more than one type. Also it is evident from the table that about 25% of the samples were found to be containing the pesticides above the MRLs

Table 4.3: Pesticide residues analyzed from the water samples (mg/L) collected from Trivandrum

Pesticides	WW1	WW2	WS1	WS2
Endosulfan	0.018*±0.08	0.003±0.005	0	0.002±0.008
Lindane	0.013±0.005	0.018*±0.04	0	0
Dieldrin	0.007±0.003	0	0.024*±0.005	0
Aldrin	0	0	0	0
p,p'-DDT	0.023±0.005	0.01±0.005	0.017±0.008	0.019±0.008
Chlorpyrifos	0.013±0.002	0	0.033±0.004	0.045±0.003
Dimethoate	0.021±0.004	0.003±0.008	0	0.016±0.005
Quinalphos	0	0.037*±0.003	0.032*±0.006	0.018*±0.04
Profenophos	0	0.013±0.004	0.027*±0.05	0
Phorate	0	0	0	0.035*±0.02
Cypermethrin	0.038±0.006	0.024±0.005	0.053±0.03	0.004±0.006
Fenvalerate	0.005*±0.009	0.019*±0.04	0.042*±0.005	0.048*±0.008
Deltamethrin	0.002±0.002	0.023±0.009	0.018±0.006	0
Cismethrin	0.008±0.003	0	0	0
carbofuran	0.028*±0.004	0.02*±0.01	0.038*±0.004	0.056*±0.006
∑Mean level	0.018±0.005	0.018±0.03	0.018±0.08	0.02±0.004

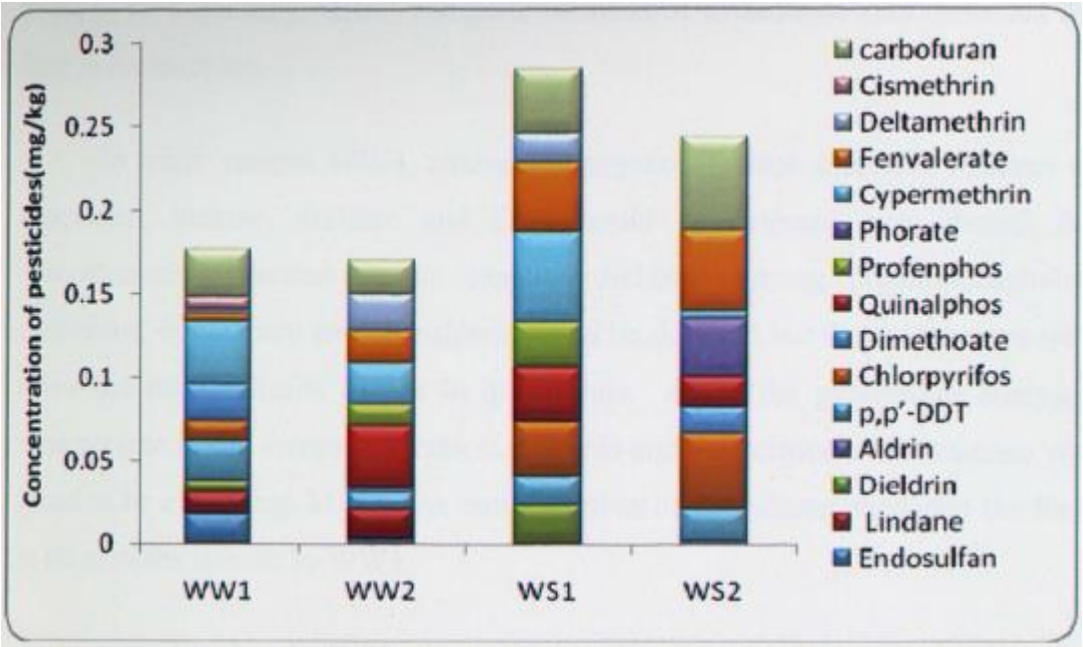


Figure 4.1: Comparative level of pesticide contamination in the well and stream water samples (mgIL) collected from Trivandrum

WWl, WW2, WS1 and WS2 denote water samples from wells of Palode, Kallara areas and from Aruvikkara dam and fresh-water lake at Vellayani in Trivandrwn were analyzed for pesticide residues by Gas Chromatography.

Pesticide residues in water samples from Kasargod

From Kasargod, the samples were collected from Kodenkari stream (WS3) and Shiriya (WS4) river. The well samples were collected from wells Periya (y{W3) and Cheemeni (WW4) of Kasargod. The concentrations of individual pesticides in water samples collected from different locations of Kasargod are given in Table 4. 4. A comparison ofresidues in well and river water samples is made in the figure. 4. 2.

In water sample WW3, the residues of all the organochlorines analyzed were found to be present except aldrin. But the concentration of endosulfan only was found to be above MRL. Among organophosphates, dimethoate and quinalfos could be detected but the values were well below the residue limit. All of the pyrethroids analyzed were present in the samples and the residues of fenvalerate found to be exceeding MRL. The concentration of carbofuran also exceeded the limit in the samples. In water sample WW 4, among the organochlorines analyzed, residues of endosulfan, lindane, dieldrin and DDT could be detected even though the concentrations exceeded in the case of dieldrin. Among organophosphates, profenofos, dimethoate and quinalphos could be detected but the values were well below the residue limits except in quinalphos. All of the pyrethroids analyzed were present in the samples except cismethrin and the residue of fenvalerate was found to be exceeding MRL. The concentration of carbofuran exceeded the limit in the samples similar to WWI.

In water sample WS3, among the organochlorines analyzed, residues of endosulfan, lindane and DDT could be detected and the concentrations exceeded MRL in the case of lindane in the samples. Among organophosphates, Profenofos and chlorpyrifos could be detected but the concentration of profenofos only was found to be above MRL. All of the pyrethroids analyzed were present in the samples except cismethrin. But the values were within the limit. The

concentration of carbofuran exceeded the limit in the samples. In water sample WS4, residues of endosulfan, lindane and pp-DDT could be detected among organochlorine pesticides, but the values were below the residue limit except of lindane. The organophosphates which were found to be present were dimethoate and chlorpyrifos and dimethoate was found to be above MRL. All of the pyrethroids analyzed were present in the samples except deltamethrin and cismethrin and the concentration of fenvalerate exceeded the limit in the samples. The carbamate, carbofuran could not be traced out in the samples.

Table 4.4: Pesticide residues analyzed from the water samples (mg/L) collected from Kasargod

Pesticides	WW3	WW4	WS3	WS4
Endosulfan	0.019*±0.004	0	0.011±0.026	0.009±0.01
Lindane	0.006±0.008	0.012*±0.025	0.042±0.028	0.028±0.003
Dieldrin	0.005±0.023	0.018*±0.032	0	0
Aldrin	0	0	0	0

p,p'-DDT	0.012±0.008	0.01±0.031	0.037±0.019	0.042±0.02
Chlorpyrifos	0	0	0.033±0.017	0.025±0.006
Dimethoate	0.011±0.003	0.009±0.024	0	0.056*±0.004
Quinalphos	0.003±0.005	0.008±0.006	0	0
Profenphos	0	0.015±0.006	0.017*±0.008	0
Phorate	0	0	0	0
Cypermethrin	0.018±0.007	0.004±0.007	0.053±0.007	0.004±0.03
Fenvalerate	0.015*±0.005	0.019*±0.008	0.042*±0.008	0.018*±0.04
Deltamethrin	0.002±0.006	0.023±0.005	0.018±0.005	0
Cismethrin	0.008±0.003	0	0	0
carbofuran	0.028*±0.004	0.02*±0.002	0.038*±0.006	0
∑Mean level	0.009	0.008	0.6	0.018

NB; WS3, WS4, WW3 and WW4 denote samples collected from Kodenkari stream, Shiriya river and from wells of Periya and Cheemeni areas in Kasargod. Values are the mean of three samples anal yzed in duplicate collected from each locations. * values above corresponding MRL. The MRLs (mg/kg) for endosulfan: 0.02, lindane: 0.02, dieldrin; 0.01, aldrin: 0.01, DDT: 0.05, chlorpyrifos: 0.5, dimethoate: 0.02, quinalphos: 0.05, profenophos: 0.02, phorate: 0.02, cypermethrin: 0.5, fenvalerate 0.02, deltamethrin 0.05 cismethrin: 0.05 and carbofuran: 0.02.

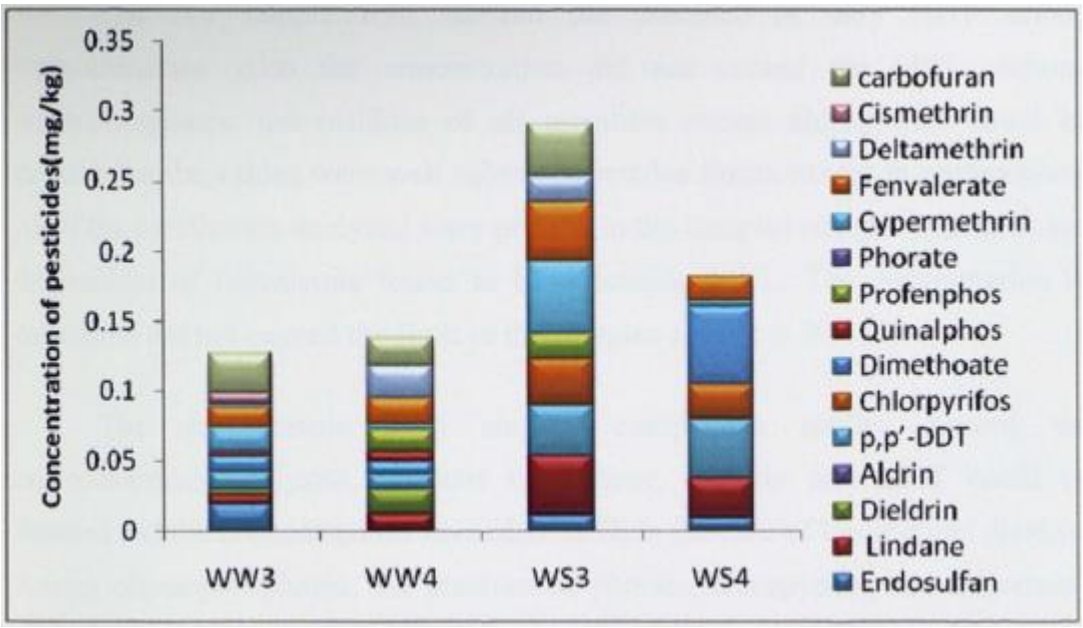


Figure 4.2: Pesticide residues obtained from the water samples (mgIL) collected from Kasargod WS3, WS4, WW3 and WW4 denote samples collected from Kodenkari stream, Shiriya river and from wells of Periya and Cheemeni areas in Kasargod were analyzed for pesticide residues by Gas Chromatography

Pesticide residues in rice samples from Trivandrum

Rice samples for analysis were collected from Connemara market (RMI) and Chalai market (RM2) and also from paddy fields from Palode (RFI) and Kallara (RF2). The samples are analyzed by gas chromatography equipped with ECD and NPD detectors. The concentration of individual pesticides in rice samples collected from different locations of Trivandrum is given in Table N.S and the values are compared in the figure 4.3.

In rice sample RFI, the residues of organochlorines found were that of endosulfan and lindane and the concentration of the residues was found to be below MRL. Among organophosphates, profenophos and quinalphos could be detected but the values were well below the residue limit. All of the pyrethroids except cismethrin analyzed were present in the samples but the residual concentration of each of the member never found to be exceeding MRL. The concentration of carbofuran also did not exceed the limit in the samples.

The nee sample RF2 showed the presence of only DDT among organochlorines. Also the concentration did not exceed the MRL. Among organophosphates, the residues of all members except chlorpyrifos could be detected but the values were well below the residue limits except in profenophos. All of the pyrethroids analyzed were present in the samples except cismethrin and the residues of fenvalerate found to be exceeding MRL. The concentration of carbofuran did not exceed the limit in the samples similar to RFl. The rice sample RM 1 showed comparable results. Among the organochlorines analyzed, residues of lindane, dieldrin and DDT could be detected and the concentrations exceeded MRL in the case of lindane and dieldrin. Among organophosphates, the residues of phorate, chlorpyrifos and dimethoate could be detected and the concentrations were well below the MRL except in the case of phorate. In the case of pyrethroids, all of them were present in the samples except cismethrin and deltamethrin. The residual concentration of fenvalerate was found to be above MRL. The concentration of carbofuran also exceeded the limit in the samples. In the rice sample RM2, residues of endosulfan, lindane, dieldrin and ppDDT could be detected among organochlorine pesticides, but the value were below the residue limit except of dieldrin. Among organophosphates, phorate, dimethoate and quinalphos were found to be present in which phorate concentration only was found to be above MRL. All of the pyrethroids analyzed were present in the samples except deltamethrin and cismethrin and the concentration of fenvalerate exceeded the limit in the samples. The carbamate, carbofuran could be detected in the samples and the amount was found to be above the MRL. It is evident from the table that none of the samples were found free from the contamination of insecticide residues. The most commonly detected residues were those of pp-DDT, cypermethrin, fenvalerate, carbofuran and phorate. It was also found that the residues of aldrin and cismethrin could not be detected in any of the samples. The result also shows that the maximum mean value for residual concentration was found in RMI samples followed by RM2, and then by RFI which indicates that samples collected from market areas show comparatively more contamination than the samples from the fields even though some residues are evaporated and lost during storage. This indicates high application of pesticides during storage.

Table 4.5: Pesticide residues (mg/Kg) present in the Rice samples collected from Trivandrum

Pesticides	RF1	RF2	RM1	RM2
Endosulfan	0.008±0.01	0	0	0.01±0.005
Lindane	0	0	0.018*±0.008	0.01±0.003
Dieldrin	0	0	0.013*±0.021	0.019*±0.009
Aldrin	0	0	0	0
p,p'-DDT	0.01±0.021	0.011±0.004	0.043±0.034	0.034±0.005
Chlorpyrifos	0	0	0.037±0.051	0
Dimethoate	0	0.003±0.003	0.01±0.002	0.012±0.003
Quinalphos	0.01±0.014	0	0.06±0.006	0.009±0.007
Profenphos	0.011±0.013	0.017*±0.011	0	0
Phorate	0	0.005±0.005	0.019*±0.02	0.024*±0.011
Cypermethrin	0.018±0.008	0.012±0.004	0.048±0.01	0.042±0.019
Fenvalerate	0.01±0.004	0.017*±0.032	0.022*±0.05	0.034*±0.016
Deltamethrin	0.032±0.005	0.011±0.021	0	0
Cismethrin	0	0	0	0
Carbofuran	0.008±0.009	0.007±0.06	0.02*±0.011	0.025*±0.018

NB:RMl, RM2, RFl and RF2 denote rice samples collected from Connemara market, Chalai market and also from paddy fields from Palode and Kallara. *values above corresponding MRL. The MRLs (mg/kg) for endosulfan: 0.02, lindane: 0.02, dieldrin; 0.01, aldrin: 0.01, DDT: 0.05, chlorpyrifos: 0.5, dimethoate: 0.02, quinalphos: 0.05, profenophos: 0.02, phorate: 0.02, cypermethrin: 0.5, fenvalerate 0.02, deltamethrin 0.05 cismethrin: 0.05 and carbofuran: 0.02.

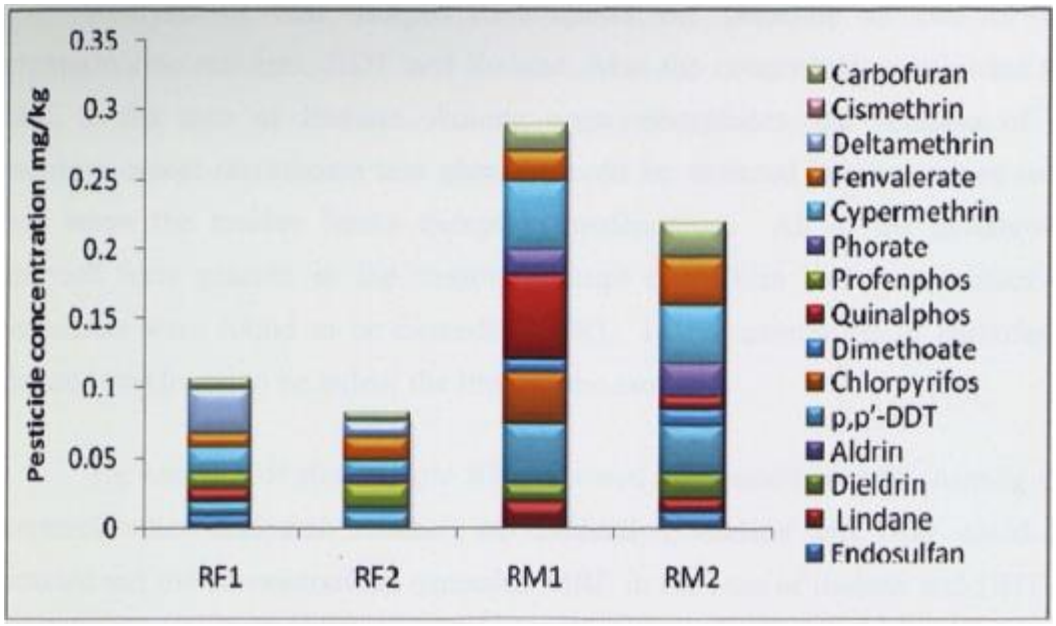


Figure 4.3: Pesticide residues (mgIKg) obtained from the Rice samples collected from Trivandrum.

RMI, RM2, RFI and RF2 denote rice samples collected from Connemara market, Chalai market and also from paddy fields from Palode and Ka1lara were analyzed for pesticide residues by Gas Chromatography.

Pesticide residues in rice samples from Kasargod

Rice samples for analysis were collected from Kasargod; from Periya (RM3) and Cheemeni markets (RM4) and RF3 and RF4 also from paddy fields from the same areas. The concentrations of individual pesticides in rice samples collected from different locations of Kasargod are given in Table 4.6 and the values are compared in figure 4.4.

In rice sample RF3, the residues of organochlorines found were that of endosulfan and DDT and the concentrations of the residues were found to be below MRL. Among organophosphates, chlorpyrifos and quinalphos could be detected but the values were well below the residue limit. All of the pyrethroids except cismethrin analyzed were present in the samples but the residual concentration of each of the member never found to be exceeded MRL. The concentration of carbofuran detected also was found to be below the limit in the samples.

Analysis of rice sample RF4 shows the presence of two of the organochlorine residues, DDT and lindane. Also the concentration exceeded the MRL in the case of lindane. Among or.ganophosphates, the residues of all members except dimethoate and phorate could be detected but the values were well below the residue limits except in profenophos. All of the pyrethroids analyzed were present in the samples except cismethrin and the residues of fenvalerate were found to be exceeding MRL. The concentration of carbofuran detected was found to be below the limit in the samples. The analysis of rice sample RM3 showed comparable results. Among the organochlorines analyzed, residues of endosulfan, lindane and DDT could be detected and the concentrations exceeded MRL in the case of lindane and DDT in the samples. Among organophosphates, the residues of all members except dimethoate and phorate could be detected but the values were well below the residue limits except in profenophos. In the case of pyrethroids, all of them were present in the samples except cismethrin and deltamethrin. The residual concentration of fenvalerate was found to be above MRL. The concentration of carbofuran also found to be exceeding the maximum limit in the samples.

In rice sample RM4, residues of endosulfan, lindane and pp-DDT could be detected among organochlorine pesticides, but the values were below the residue limit except for DDT. Among organophosphates, the residues of all members except dimethoate and phorate could be detected but the values were well below the residue limits except in profenophos. All of the pyrethroids analyzed were present in the samples except cismethrin and the concentration of fenvalerate and deltamethrin exceeded the limit in the samples. Carbofuran could be found out in the samples and the amount was found to be above the MRL similar to RM3. It is evident from the table that none of the samples were found free from the contamination of insecticide residues. The most commonly detected residues were those of pp-DDT, lindane, cypermethrin, fenvalerate, deltamethrin and carbofuran. It was also found that the residues of aldrin, dieldrin, phorate and cismethrin could not be detected in any of the samples. The result also gives an idea about the maximum mean value for residual concentration in RM4 samples followed by RM3, and then by RF3 which indicates that samples collected from market areas show comparatively more contamination than the samples from the fields even though some residues are evaporated and lost during the storage. This also provides an evidence for the high application of pesticides during storage.

Table 4.6: Pesticide residues (mg/Kg) present in the Rice samples collected from Kasargod

Pesticides	RF3	RF4	RM3	RM4
Endosulfan	0.003±0.015	0	0.005±0.05	0.011±0.008
Lindane	0	0.015*±0.007	0.039*±0.009	0.014±0.005
Dieldrin	0	0	0	0
Aldrin	0	0	0	0
p,p'-DDT	0.028±0.008	0.008±0.006	0.053*±0.008	0.074*±0.004
Chlorpyrifos	0.005±0.006	0.01±0.005	0.03±0.005	0
Dimethoate	0	0	0.01±0.003	0.052*±0.002
Quinalphos	0.043±0.008	0.023±0.004	0	0
Profenphos	0	0.027*±0.009	0.032*±0.004	0.019*±0.004
Phorate	0	0	0	0
Cypermethrin	0.038±0.008	0.014±0.008	0.048±0.003	0.042±0.009
Fenvalerate	0.01±0.006	0.027*±0.006	0.038*±0.005	0.044*±0.008
Deltamethrin	0.032±0.008	0.011±0.004	0	0.058±0.006
Cismethrin	0	0	0	0
Carbofuran	0.012±0.005	0.005±0.004	0.018±0.002	0.025*±0.005

NB: RM3, RM4, RF3 and RF4 denote rice samples collected from Periya and Cheemeni markets and from paddy fields from the same areas. Values are the mean of three samples analyzed in duplicate collected from each location Values above corresponding MRL.

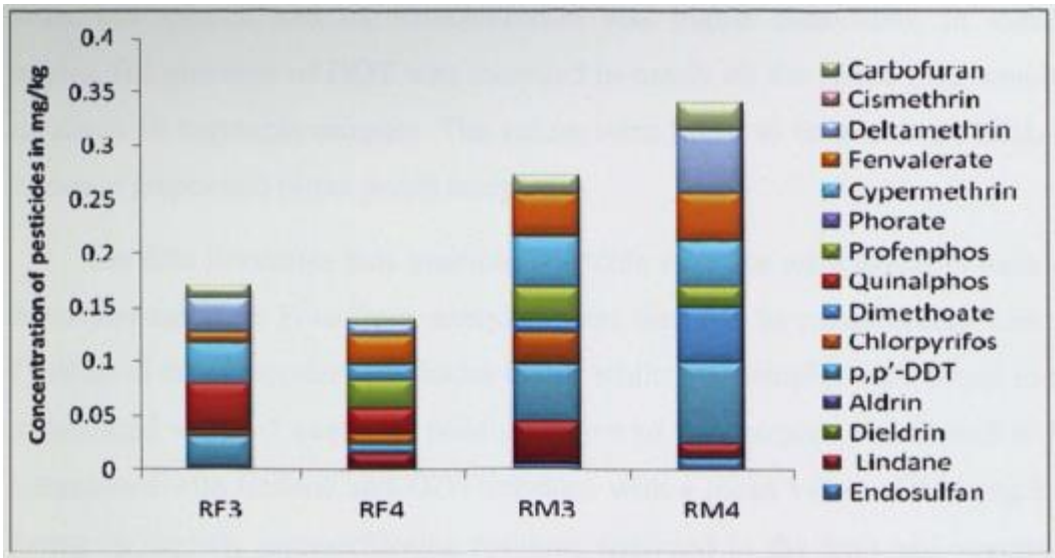


Figure 4.4: Pesticide residues (mglKg) obtained from the Rice samples collected from Kasargod. RM3, RM4, RF3 and RF4 denote rice samples collected from Periya and Cheemeni markets and also from paddy fields RF3 and RF4 respectively from the same areas were analyzed for pesticide residues by Gas Chromatography

Pesticide residues in fruits and vegetables collected from Trivandrum

The mean concentrations and range of pesticide residues (organochlorine, organophosphate and pyrethroid) found in fruits and vegetable samples from the local markets of Trivandrum city in Kerala are summarized in Tables 4.7, 8 and 9 and figures 4.5, 6 and 7.

Organochlorine pesticide residues in fruits and vegetables collected from Trivandrum

The mean concentrations and range of organochlorine pesticide residues found in fruits and vegetable samples are shown in Table 4.7 and Figure 4.7. Among the detected, Endosulfan was between 0.01 mglkg in carrot and 0.002 mgikg in cabbage and tomato. But the residual level was relatively lower than the pennitted levels in all the samples tested. The residues oflindane were present in almost all the samples in a range of 0.022 mglKg in carrot and 0.002 mglKg in orange. The quantity exceeded in MRL in samples of grapes, mango, tomato, bitter gourd and carrot. Similarly dieldrin was detected in the samples of grapes, mango, tomato, carrot and the concentrations were above MRL in all the positive samples. Aldrin residues could be detected only in three samples i.e. of banana, tomato and spinach and the

concentration was higher than MRL in tomato samples. The presence of DDT was recorded in nearly all the tested fruit samples· and almost all vegetable samples. The values were found to be above the MRL in the case of grapes and bitter-gourd samples.

The data illustrates that multiple pesticide residues were found in each of the samples analyzed. Four fruits analyzed were found to be contaminated with 1- 2residues of the component pesticides tested while five samples were found to be contaminated with 5-7 pesticide residues. Most of the samples were found to be contaminated with lindane and DDT residues with a mean value of 0.01 mg/Kg. Among the various organochlorine residues analyzed in the fruit and vegetable samples, lindane was found with the maximum concentration of 0.041mg/kg followed by that of pp DDT of 0.021mg/kg. It is clearly evident from the figure that each of the verities analyzed were contaminated with not less than two pesticide residues analyzed.

Table 4.7: Organochlorine pesticide residues present in the fruits and vegetable samples (mg/ Kg) collected from Trivandrum market areas

Fruit/ Vegetable	Endosulfan	Lindane	Dieldrin	Aldrin	p,p'-DDT
Apple	0	0.007±0.005	0	0	0.002±0.05
Orange	0	0.002±0.008	0	0	0.008±0.004
Grapes	0	0.12*±0.002	0.027*±0.023	0	0.058*±0.003
Banana	0	0	0	0.008±0.017	0.024±0.015
Mango	0.008±0.01	0.012*±0.2	0.017*±0.018	0	0
Tomato	0.002±0.05	0.029*±0.02	0.023*±0.042	0.012*±0.04	0.012±0.06
Bittergourd	0	0.085*±0.02	0	0	0.047*±0.021
Spinach	0	0.008±0.02	0	0.008±0.021	0
Cabbage	0.003±0.028	0.004±0.02	0	0	0.016±0.04
Carrot	0.01±0.04	0.02*±0.03	0	0	0

N.B Values are the mean of three samples analyzed in duplicate collected from each locations. *values above corresponding MRL. The MRLs (mg/kg) for endosulfan: 0.02, lindane: 0.02, dieldrin; 0.01, aldrin: 0.01 and DDT: 0.05.

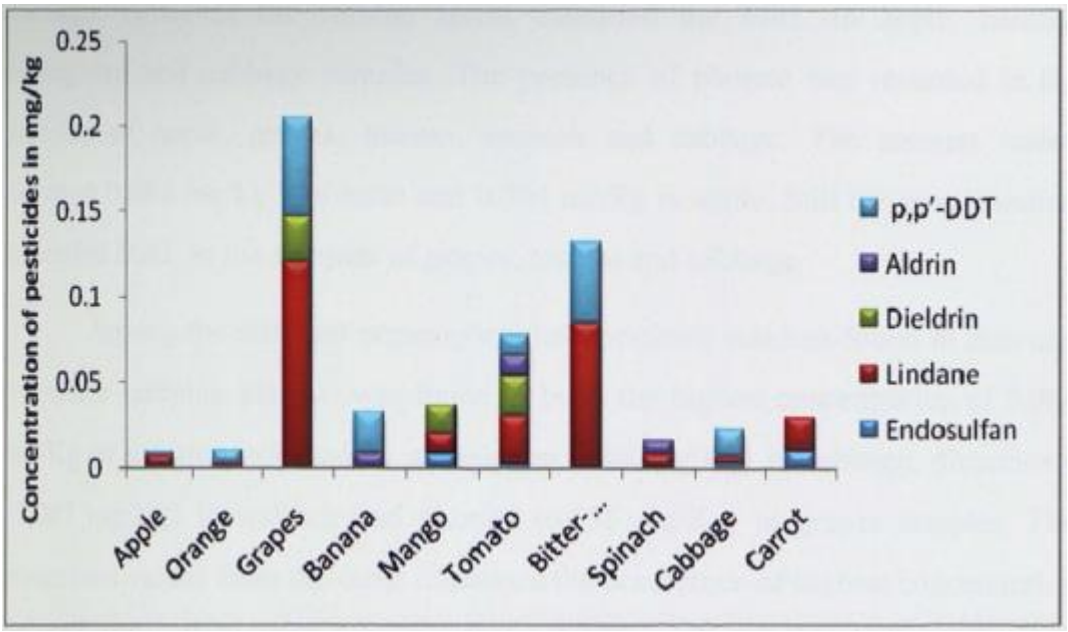


Fig. 4.5: Organochlorine pesticide residues obtained from the fruits and vegetable samples (mgt Kg) collected from Trivandrum market areas.

Organophosphate pesticide residues in fruits and vegetables collected from Trivandrum market areas

Analysis of five common organophosphate pesticides, chlorpyriphos, dimethoate, Profenophos, quinalphos and phorate which are widely applied in fruits and vegetables were carried out and the residue levels are shown in Table 4.8 and Figure 4.6. Among the residues, chlorpyrifos could be detected in orange and mango samples and in three of the vegetable samples including tomato, bitter-gourd and carrot in a considerable range. But the residual level was relatively lower than the pennitted levels in all the samples. The residues of dimethoate were found in samples of apple, orange and grapes in negligible amounts. The quantities were found to be above the pennitted level except in grape samples. Residues of dimethoate also could be detected in all of the vegetable samples except that of bitter-gourd and found to be above MRL in all the samples. Among fruit samples, profenofos was found to be present only in grapes in a negligible concentration but present in the samples of tomato and spinach. MRL was exceeded only in the samples ofspinach. Quinalphos could be detected in the samples of apple, orange, mango, banana, bitter gourd, carrot and cabbage. However the residue levels exceeded the MRL in apple, banana, bittergourd and cabbage samples. The

presence of phorate was recorded in the samples of apple, grapes, tomato, spinach and cabbage. The amount varied between 0.084 mg/kg in tomato and 0.004 mg/kg in apple. Still the concentration exceeded MRL in the samples of grapes, tomato and cabbage.

Among the different organophosphate pesticide residues found in fruit and vegetable samples, phorate was found to be in the highest concentration of 0.084 mg/Kg in tomato, followed by quinalphos (0.08 mg/Kg) in cabbage, dimethoate (0.047 mg/Kg) in spinach and phorate (0.035 mg/Kg) in grapes samples. The mean sum values from the table illustrates the occurrence of highest concentration of phorate in all the tested commodities (0.028 mg/Kg) followed by quinalphos (0.024 mg/Kg) chlorpyrifos (0.019 mg/kg) and dimethoate (0.015 mg/Kg). However the organophosphates present in nearly every samples are dimethoate and quinalphos.

Table 4.8 Organophosphate pesticide residues analyzed from the fruits and vegetable samples (mg/Kg) collected from Trivandrum market areas

Pesticides	Chlorpyrifos	Dimethoate	Profenphos	Quinalphos	Phorate
Apple	0	0.017*±0.05	0	0.016±0.03	0.004±0.03
Orange	0.012±0.003	0.021*±0.04	0	0.009±0.04	0
Grapes	0	0.008±0.025	0.003±0.002	0	0.035*±0.28
Banana	0	0	0	0.018±0.03	0
Mango	0.06±0.031	0	0	0.012±0.05	0
Tomato	0.029±0.002	0.018*±0.04	0.008±0.003	0	0.084*±0.006
Bitter-gourd	0.013±0.004	0	0	0.026*±0.07	0
Spinach	0	0.047*±0.05	0.028*±0.014	0	0.015*±0.04
Cabbage	0	0.013*±0.09	0	0.08*±0.07	0.042*±0.028
Carrot	0.008±0.06	0	0	0.004±0.08	0

N.B Values are the mean of three samples analyzed in duplicate collected from each locations. *values above corresponding MRL. The MRLs (mg/kg) for chlorpyrifos: 0.5, dimethoate: 0.02, quinalphos: 0.05, profenophos: 0.02, phorate: 0.02.

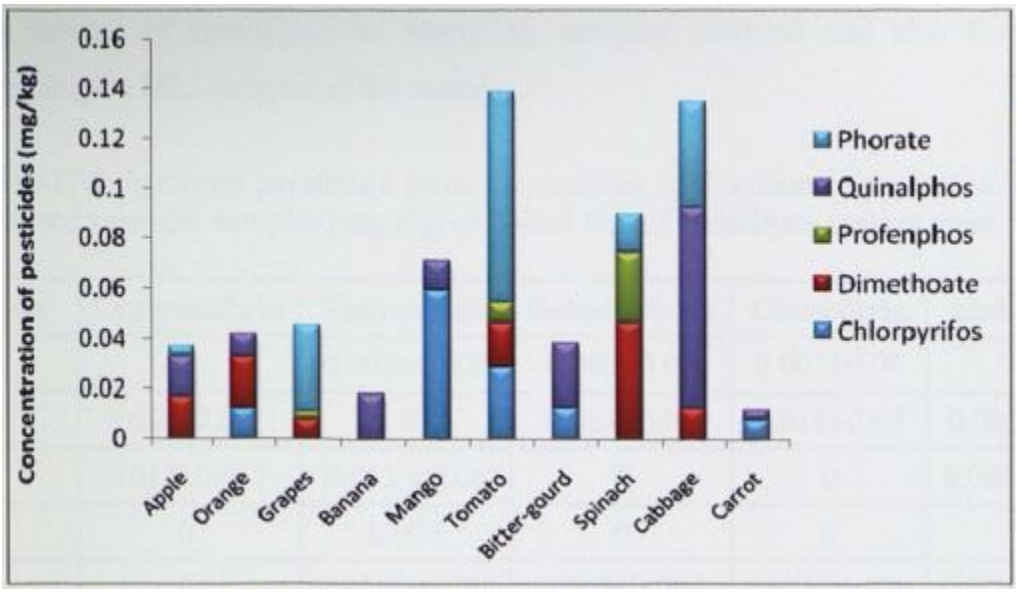


Fig. 4.6; Organophosphate pesticide residues obtained from the fruits and vegetable samples (mgIKg) collected from Trivandrum market areas

Synthetic pyrethroid pesticide residues in fruits and vegetables collected from Trivandrum market areas

The levels of four pyrethroid residues and carbofuran in fruits and vegetable samples are illustrated in Table IV.9 and Figure 4.7. The table shows that, cypennethrin could be detected in samples of orange and grapes but found undetected in the vegetable samples except in tomato. Still the values never exceeded the MRL in any of the samples. The residual levels of fenvalerate detected in more or less every sample tested except in orange, bitter gourd and cabbage. Furthermore the concentration exceeded the MRL in grapes, mango, and tomato and carrot samples. Deltamethrin was found out in nearly all the samples including apple, orange, mango, tomato, spinach and carrot. Nevertheless the concentrations were negligible and found exceeding the permissible limit only in carrot samples. Another pyrethroid pesticide cismethrin was seen in three of the fruit samples including apple, orange and mango and among the vegetable samples tested, only the cabbage showed the presence of the residue. However the quantities in all the commodities were well below the MRL. The mean sum values illustrate the occurrence of highest concentration of fenvalerate in all the tested commodities (0.018 mg/Kg) followed by deltamethrin (0.016 mg/Kg), cypermethrin (0.014

mg/Kg) and cismethrin (0.008 mg/Kg). The table also shows the presence of carbofuran in nearly all samples analyzed and also found exceeding the MRL in most of the samples.

Table 4.9: Synthetic pyrethroid pesticide residues and carbamate present in the fruits and vegetable samples (mgIKg) collected from Trivandrum market areas

Pesticides	Cypermethrin	Fenvalerate	Deltamethrin	Cismethrin	carbofuran
Apple	0	0.002±0.003	0.003±0.07	0.005±0.04	0
Orange	0.015±0.021	0	0.024±0.08	0.018±0.08	0.005±0.034
Grapes	0.012±0.037	0.015*±0.06	0	0	0.063*±0.028
Banana	0	0.005	0	0	0
Mango	0	0.025*±0.002	0.009±0.003	0.018±0.028	0.014*±0.004
Tomato	0.023±0.025	0.057*±0.004	0.023±0.06	0	0.018*±0.003
Bitter-gourd	0	0	0	0	0.013±0.009
Spinach	0.038±0.02	0.009±0.004	0.034±0.004	0	0.018*±0.015
Cabbage	0	0	0	0.012±0.019	0
Carrot	0.029±0.031	0.021*±0.003	0.046±0.005	0	0.018*±0.013

N.B Values are the mean of three samples analyzed in duplicate collected from each locations. *values above corresponding MRL. The MRLs (mglkg) for 0.02, cypennethrin: 0.5, fenvalerate 0.02, deltamethrin O.OS cismethrin: O.OS and carbofuran: 0.02.

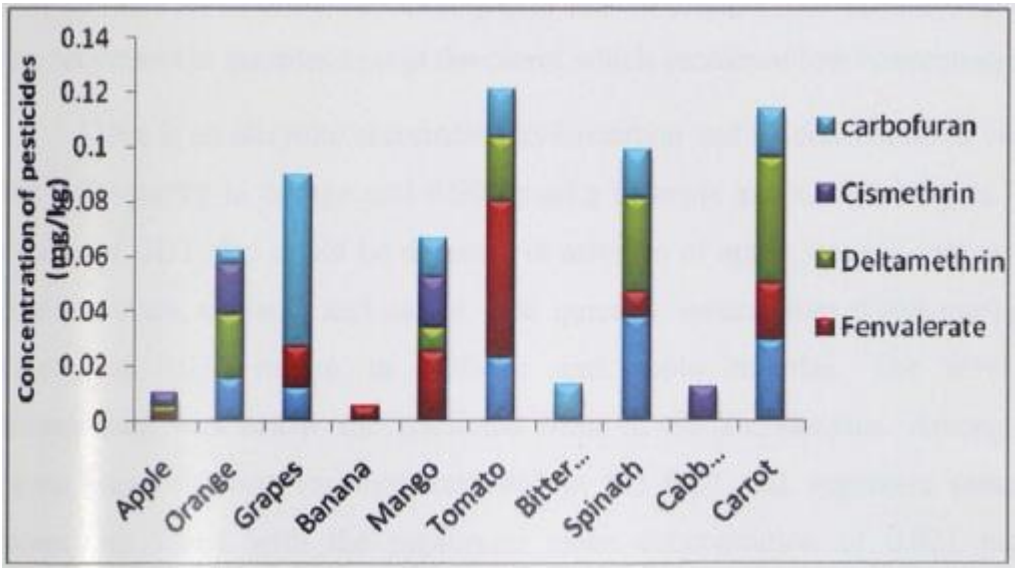


Fig. 4.7: Synthetic pyrethroid pesticide residues obtained from the fruits and vegetable samples collected from Trivandrum (mglKg)

Pesticide residues in fruits and vegetables collected from Kasargod

The mean concentrations and range of pesticide residues (organochlorine, organophosphate and pyrethroid) found in fruits and vegetable samples from the local markets of Kasargod in Kerala are summarized in Tables 4.10, 11 and 12 and Figures 4.8, 9 and 10.

Organochlorine pesticide residues in fruits and vegetables collected from Kasargod

The mean concentrations and range of organochlorine pesticide residues found in fruits and vegetable samples are shown in Table 4.10 and figure 4. 8. The residues of Endosulfan could be detected in grapes, mango and all the vegetable samples. The range was found between 0.006 mg/kg in spinach and 0.022 mg/kg in tomato samples. But the residual level was within the permitted level in all the samples tested. The residues of lindane were found to be present in the samples in a range of 0.058mg/kg in tomato and 0.003 mg/kg in cabbage. The quantity exceeded MRL in samples of apple, orange, tomato and carrot. Dieldrin concentration in fruits and vegetable samples was little high which varied between 0.014 mg/kg in apples and 0.005 mg/kg in orange. The residues were found in all the fruit verities except grapes and banana. Also the level of contamination was above the limit in all fruits detected except that in orange. The dieldrin residues were not present in samples except the carrot which contained low concentration. Aldrin is an alicyclic chlorinated hydrocarbon and its concentration varied from 0.004 mg/kg in orange and 0.008 mg/kg in apple and carrot samples. The presence of DDT also could be detected in samples of apple, orange, grapes and tomato, spinach, cabbage and carrot. The quantity varied from 0.008 mg/kg in tomato and 0.032 mg/kg in cabbage and apple samples. The level of contamination was below the restricted limit m all the samples. Among the various organochlorine residues analyzed in the fruit and vegetable samples, lindane was found with the maximum mean concentration of 0.021 mg/kg followed by that of pp-DDT of 0.012 mg/kg. It is clearly evident from the figure that nearly all the verities analyzed were contaminated more than two residues.

Table 4.10 Organochlorine pesticide residues (mgIKg) present in the fruits and vegetable samples collected from Kasargod

Pesticides	Endosulfan	Lindane	Dieldrin	Aldrin	p,p'- DDT
Apple	0	0.017*±0.02	0.014*±0.01	0.008±0.04	0.032±0.04
Orange	0	0.04*±0.03	0.005±0.03	0.004±0.0	0.021±0.02
Grapes	0.018*±0.04	0	0	0	0.017±0.06
Banana	0	0	0	0	0
Mango	0.013±0.01	0.004±0.02	0.013*±0.04	0	0
Tomato	0.022*±0.02	0.058*±0.04	0.009±0.02	0	0.008±0.01
Bittergourd	0	0	0	0	0
Spinach	0.006±0.07	0	0	0.003±0.0	0.009±0.03
Cabbage	0.009±0.03	0.003±0.06	0	0	0.032*±0.05
Carrot	0	0.028*±0.07	0.008±0.01	0.008±0.0	0.012±0.03
∑Meanlevel	0.008	0.021	0.006	0.003	0.012

N,B Values are the mean of three samples analyzed in duplicate collected from each locations. *values above corresponding MRL. The MRLs (mglkg) for endosulfan: 0.02, lindane: 0.02, dieldrin; 0.01, aldrin: 0.01 and DDT: 0.05.

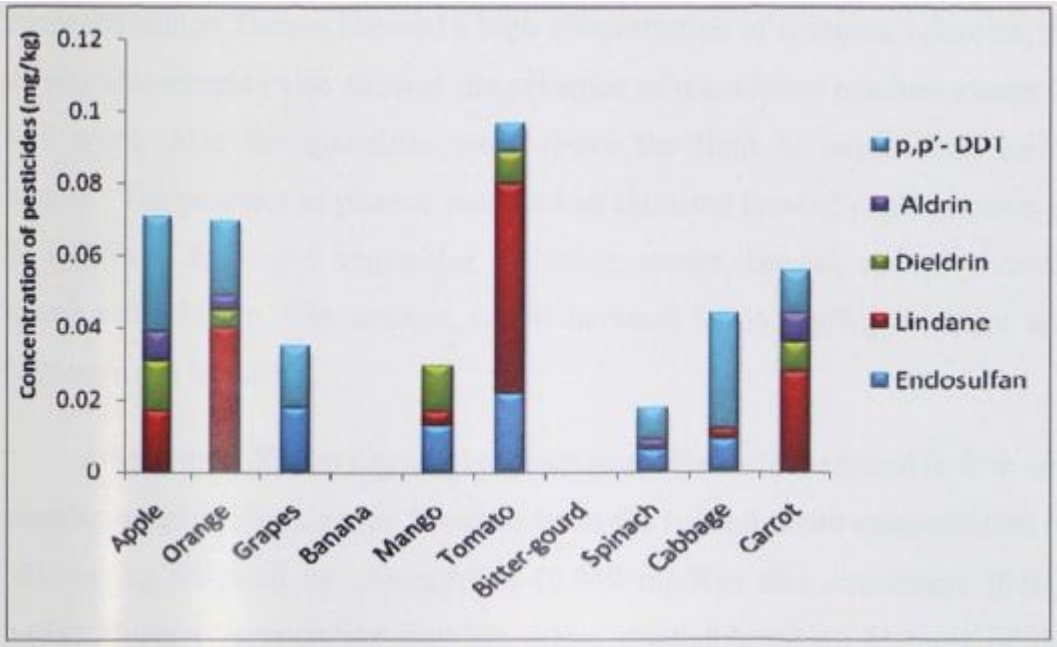


Fig. 4.8: Organochlorine pesticide residues (mgIKg) obtained from the fruits and vegetable samples collected from Kasargod

Organophosphate pesticide residues in fruits and vegetables collected from Kasargod

Analysis of four common organophosphate pesticides, chlorpyriphos, dimethoate, profenofos, quinalphos and phorate which are widely applied in fruits and vegetables were carried out and the residue levels are illustrated in Table 4.11 and Figure 4.9. Chlorpyrifos was detected in grapes and mango samples and found to be present in all of the vegetable samples except carrot in a considerable range. The detection range varied between 0.008 mg/kg in cabbage and 0.049 mg/kg in tomato samples. But the residual level was relatively lower than the permitted levels in all the samples except in tomato. Dimethoate residues were quantified in the samples. But except grapes, spinach and carrot, no other samples were found to be contained the residues. The range was found to be between 0.003 mg/kg in spinach and 0.049 mg/kg in grape samples. However, the residue level was above the limit in grapes and carrot. Profenophos was found to be detected in all the fruit samples except in mangoes. But the contamination level was within the limit in all the samples. The vegetable samples analyzed did not show detectable concentration of the residues except in tomatoes.

Quinalphos could be detected in all the fruit samples tested except in orange and mango. Grapes showed a high concentration of residues. Likewise, all the vegetable samples also showed the presence of quinalphos residues except in bitter gourd. Also the quantities were above the limit in tomato and carrot samples. The presence of phorate recorded an alarming level of contamination in the samples of fruits and vegetables including orange, banana, mango, tomato, spinach and cabbage. The amount varied between 0.106 mg/Kg in carrot and 0.006 mg/Kg in banana. Among the different organophosphate pesticide residues found in fruit and vegetable samples, phorate was found to be in the highest mean concentration of 0.033 mg/Kg followed by chlorpyrifos (0.018 mg/Kg) and dimethoate (0.018 mg/Kg). However quinalphos was the major organophosphate in most of the samples, followed by phorate and chlorpyrifos.

Table 4.11 Organophosphate pesticide residues (mglkg) obtained from the fruits and vegetable samples collected from Kasargod

Pesticides	Chlorpyrifos	Dimethoate	Profenphos	Quinalphos	Phorate
Apple	0	0	0.013±0.005	0.004±0.02	0
Orange	0	0	0.019±0.018	0	0.015*±0.005
Grapes	0.043±0.014	0.049*±0.04	0.031±0.005	0.038*±0.04	0
Banana	0	0	0.007±0.016	0.008±0.05	0.006±0.029
Mango	0.02±0.016	0	0	0	0.04*±0.023
Tomato	0.049*±0.04	0	0.016±0.018	0.02*±0.006	0.008±0.014
Bitter-gourd	0.027±0.018	0	0	0	0
Spinach	0.018±0.011	0.003±0.003	0	0.003±0.012	0.018*±0.003
Cabbage	0.008±0.014	0	0	0.006±0.022	0
Carrot	0	0.04*±0.016	0	0.013±0.005	0.11*±0.004
∑Mean level	0.018	0.018	0.011	0.012	0.033

N.B Values are the mean of three samples analyzed in duplicate collected from each locations. *values above corresponding MRL. The MRLs (mglkg) for chlorpy.nfos: 0.5, dimethoate: 0.02, quinalphos: O.OS, profenophos: 0.02 and phorate: 0.02.

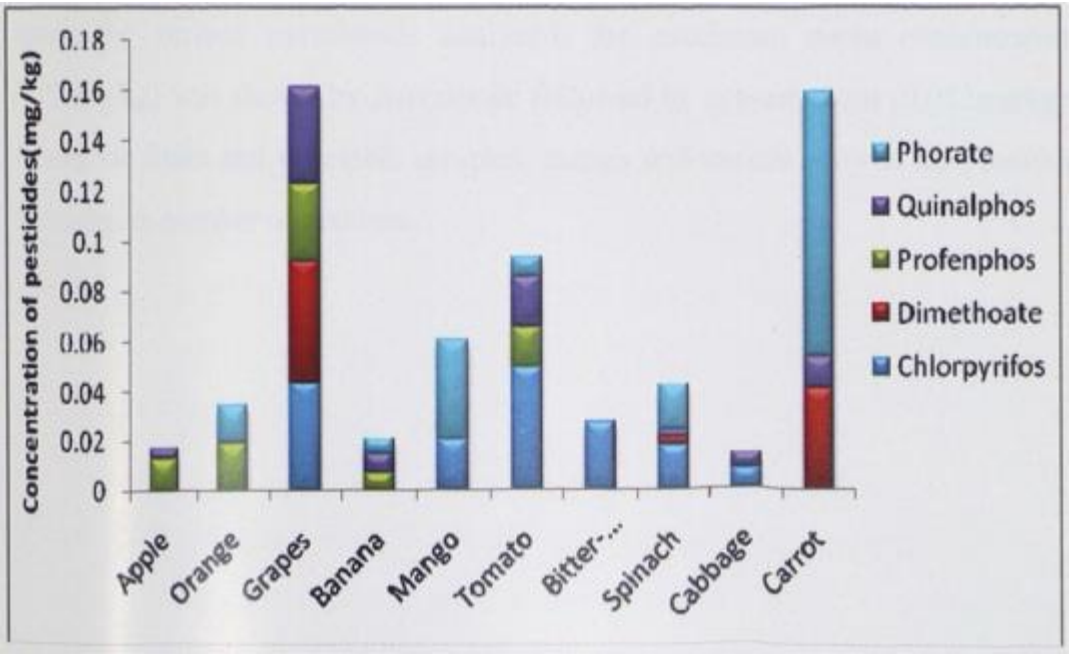


Fig. 4.9: Organophosphate pesticide residues (mgIKg) obtained from the fruits and vegetable samples collected from Kasargod

Synthetic pyrethroid pesticide residues in fruits and vegetables collected from Kasargod

The levels of four pyrethroid and carbamate residues in fruits and vegetable samples are illustrated in Table 4.12 and Figure 4.10. The results showed that, cypermethrin detected in all commodities were between 0.007 mg/kg in grapes and 0.028 mg/kg in banana. Still the values never exceeded the MRL in any samples. The residual level of fenvalerate was detected in more or less every sample except in banana, bitter gourd and carrot. Furthermore the concentration exceeded the MRL in apple, grapes, mangoes, and tomato and spinach samples. The residues of deltamethrin could not be detected in any of the samples except in tomato and carrot. Also the residual concentration was comparatively low. Another pyrethroid cismethrin also was present in negligible concentrations in the samples of mango and cabbage. The carbamate pesticide carbofuran could be detected in nearly all samples except grapes, cabbage and carrot. The level of contamination also was found to be high in all fruit samples. The range varied between 0.003 mg/kg in spinach and 0.037 mg/kg in mango samples. The' values were above MRL in banana and mango samples. The results illustrated that among the various pyrethroids analyzed; the maximum mean concentration (0.022mg/kg) was shown by

fenvalerate followed by cypermethrin (0.012mg/kg). Among the fruits and vegetable samples, mango and tomato showed the presence of maximum number of residues.

Table 4.12: Synthetic pyrethroid and carbamate pesticide residues obtained from the fruits and vegetable samples (mglkg) collected from Kasargod

Fruit/ Vegetable	Cypermethrin	Fenvalerate	Deltamethrin	Cismethrin	carbofuran
Apple	0	0.029*±0.004	0	0	0.005±0.01
Orange	0	0.003±0.013	0	0	0.012±0.018
Grapes	0.007±0.16	0.053*±0.016	0	0	0
Banana	0.028±0.002	0	0	0	0.018*±0.003
Mango	0.021±0.006	0.057*±0.018	0	0.005±0.03	0.037*±0.004
Tomato	0.024±0.005	0.028*±0.006	0.003±0.04	0	0.004±0.006
Bitter-gourd	0	0	0	0	0.008±0.018
Spinach	0	0.026*±0.004	0	0	0.003±0.015
Cabbage	0.024±0.02	0.009±0.008	0	0.002±0.04	0
Carrot	0.014±0.06	0	0.013±0.0	0	0
ΣMean level	0.012	0.022	0.004	0.002	0.011

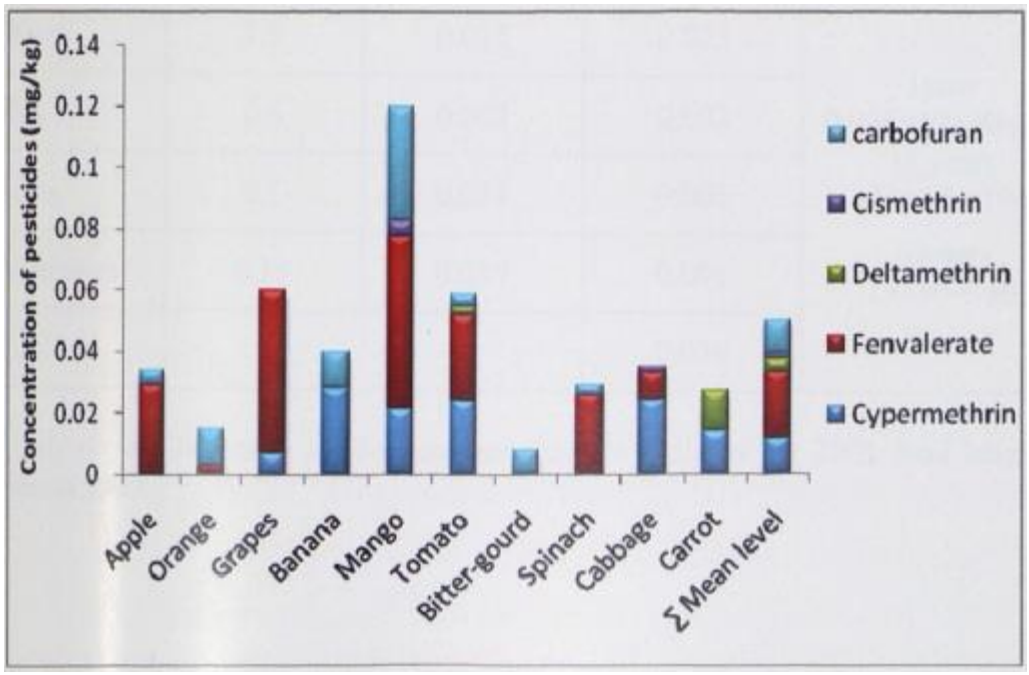


Figure 4.10: Synthetic pyrethroid pesticide residues obtained from the fruits and vegetable samples (mgIKg) collected from Kasargod

Calculation of total residues that can be accumulated from all the food varieties

From the above results, it could be possible to calculate the total residues that can be accumulated in humans who are consuming water, rice, fruits and vegetables. The average daily intake of food items based on the guidelines of FAO was used to calculate the total residues. Accordingly the concentration of each pest icides accumulated from all the varities analyzed per day is calculated as given below. The pesticides are selected as one from each of the main groups viz. endosulfan (organochlorines), phorate (organophosphates) and fenvalerate (synthetic pyrethroids) and the respective concentrations accumulated are estimated and shown in tables Table 4.13, 14 and 15.

Table 4.13: Estimation · of concentration of endosulfan that can be accumulated in humans and animals after consumption of the food types analyzed

Food Item	Daily Consumption*(x)L or Kg /day	Concentration of residue (y) mg/Kg or L	Concentration of residue per day (x×y) mg/Kg orL	Concentration of residues accumulated (mg/Kg) in
Water	1.5	0.015	0.023	1year 0.036×12×30 =13 15 years =13×15= 195 30years 13×30=390
Rice	0.6	0.003	0.002	
Fruits	0.1	0.031	0.005	
Vegetables	0.15	0.037	0.006	
Total			0.036	

Table 4.14; Estimation of phorate concentration that can be accumulated in humans after consumption of the food types analyzed

Food Item	Daily Consumption*(x)L or Kg /day	Concentration of residue (y) mg/Kg or L	Concentration of residue per day (x×y) mg/Kg orL	Concentration of residues accumulated (mg/Kg) in
Water	1.5	0.035	0.053	1year 0.088×12×30= 31.7 15 years 31.7 ×15= 475.5 30 years 13 ×30= 951
Rice	0.6	0.015	0.009	
Fruits	0.1	0.061	0.006	
Vegetables	0.15	0.132	0.02	
Total			0.088	

Table 4.15 Estimation of fenvalerate concentration that can be accumulated in humans after consumption of the food types analyzed

Food Item	Daily Consumption*(x)L or Kg /day	Concentration of residue (y) mg/Kg or L	Concentration of residue per day (x×y) mg/Kg orL	Concentration of residues accumulated (mg/Kg) in
Water	1.5	0.114	0.171	1year (0.4×12×30)= 144
Rice	0.6	0.119	0.077	
Fruits	0.1	0.142	0.014	15 years 144×15 = 2160
Vegetables	0.15	0.087	0.131	
Total			0.393	30 years 13×30 =4320

4.4 DISCUSSION

The present work provides the data on pesticide residue levels in ground water, rice samples, fruits and vegetables, collected from different locations of Trivandrum and Kasargod which are the southern and northern most districts of Kerala. Pesticide use has no doubt increased the agricultural production in general but persistent residues of these chemicals have tremendous harmful impact on the environment and also on human health. A considerable attention has been focused on the threat to human life coming from the dietary food, drinking water, and the residential risk caused by the presence of pesticide residues. The recovery studies demonstrate that the method has relatively good reproducibility. It is noted that in all the cases at lower fortification level, 0.1 mg/kg the percent recovery decreases. It might be because at lower residue concentrations, chances of error are usually enhanced but the results are within the range of permissible error. Pesticide residue analytical method should be so sensitive that at least the lower permissible levels can be quantitatively determined (Khan et al., 2007b). The present results are in full agreement with this recommendation. Based on these results, the method has been proven to be efficient and thus suitable for routine monitoring of pesticide residues in water and other food verities. The outcome of the study indicated that organochlorine pesticide residues was present in 36.2%, organophosphate residues in 24.7% while synthetic pyrethroid residues were present in 39.1 % in water samples studied. The high occurrence of pyrethroid insecticides residues in the samples is also an indication of alteration in usage pattern of insecticides in the area where shift has taken place from organochlorine pesticides to the easily

degradable groups of these insecticides in the last decade. Moreover the study revealed that among water samples, 66.8% of the samples showed contamination out of which 21.3% of the samples gave results with levels of pesticide residues above the MRL, while 45.5% of the samples gave results with levels of pesticide residues below the MRL. Only 33.2% of samples analyzed did not contain traceable level of the monitored pesticides. This report was in agreement with that of Reddy (2012) who reported his findings of pesticide residue levels in Hussain sagar and Mir Alam lakes situated in the heart of the Hyderabad city during 2005 to 2007. He could detect the residues of HCH, DDT, Endosulfan, heptachlor, Dicofol, methyl parathion, Malathion and chlorpyriphos. Almost 30% of the samples were above the Maximum Residues Levels.

Study also showed that compared to the well water, the residues of organophosphates and pyrethroids were found to be detected more in the samples from the stream and lake. This indicates drifting of pesticides, a consequence of pesticide application on crops and soil. Concern about pesticides in groundwater is especially acute in rural agricultural areas, where more than 80 percent of the population relies on groundwater for their drinking water, although application rates and the variety of pesticides used may be greater in urban areas. There are already some reports of groundwater contamination in India i.e. Haryana:-all samples of water from tube wells in agricultural areas were found to be contaminated with endosulfan, with 83% of samples exceeding the EU maximum permissible level of 0.1 µg/L, with levels up to 0.405 µg/L (Kumari et al., 2008); Hyderabad City-all the samples of domestic well water contained endosulfan, at levels up to 11.23 µg/L, assumed to derive from agriculture near the city (Shukla et al., 2006); and Thiruvallur area of Tamp Nadu where endosulfan is used on rice-total endosulfan in open well and bore wells (J ayashree & Vasudevan 2007a), Punjab- (Tariq et al., 2004), in a number of rivers- (Selvakumar et al., 2005), including water from the Yamuna River at Delhi, India (Aleem and Malik, 2005); streams, ponds and canals of the northern Indo-Gangetic alluvial plains (Singh et al., 2007b) and water of the Gomti River, (Malik et al., 2009). Results of the present study were also in good agreement with the data in the previous investigations from other parts of the world (Tariq et al., 2006, 2004; Ahad et al., 2001; Ahad et al., 2000; Jabber, et al., 1993) which discussed possible reasons of pesticides reaching in aquatic environment through the direct runoff, leaching, careless disposal of empty containers and, equipments washing.

As part of a multicentre study conducted by the Indian Council of Medical Research, 2000 samples of rice were collected from rural and urban areas of 13 states of India. The samples were analyzed for residues of DDT and isomers of HCH by GC and detected in about 58 and 73% of the samples analysed. Concentrations exceeded MRL of 0.05 mg/Kg for each isomer in rice fixed by the Ministry of Health and Family Welfare of the Indian Government. There is no statutory limit fixed in India for DDT residues in cereals. Its maximum residue limit of 0.1 mg/ Kg in cereals recommended by Codex was exceeded in about 2% of the samples examined (Toteja et al., 2003).

Rice sample analysis showed that the market samples contain more residues compared to the samples collected from the fields. This condition was almost similar in two districts which indicates application of pesticides during storage to avoid pest attack. This also points towards another possibility that major part of rice in Kerala is imported and the presence of residues in shops may be due to the excess application of pesticides by the rice lobby. The study could evaluate that in the case of rice samples, 75% of the samples showed contamination out of which

28% of the samples gave results with levels of pesticide residues above the MRL, while 4 7% of the samples gave results with levels of pesticide residues below the MRL. Only 24.6% of samples analyzed did not contain traceable level of the monitored pesticides. As there is no report available for the data of rice analysis in Kerala, this work emphasizes the vital necessity for strict rules and continuous monitoring of the domestic and imported rice. A report from a multicentre study conducted by the Indian Council of Medical Research, shows that 2000 samples of rice were collected from rural and urban areas of 13 states of India. The samples were analyzed for residues of DDT and isomers of HCH by GC and detected in about 58 and 73% of the samples analysed. Concentrations exceeded MRL of 0.05 mg/Kg for each isomer in rice fixed by the Ministry of Health and Family Welfare of the Indian Government. There is no statutory limit fixed in India for DDT residues in cereals. Its maximum residue limit of 0.1 mg/ Kg in cereals recommended by Codex was exceeded in about 2% of the samples examined (Toteja et al., 2003).

In another study conducted by the ICMR, 1712 samples of wheat grain/flour were collected from urban and rural areas in 11 states of India. These samples were analysed for residues of DDT and different isomers of HCH by GLC. Residues of DDT were detected in 59.4% of 1080 samples of

wheat grain and in 78.2% of 632 samples of wheat flour. Different isomers of HCH were present in about 45-80% of the samples of wheat grain/flour. Residue levels of yHCH exceeded the MRLs in most of the samples of wheat flour (Toteja et al., 2006). In the case of fruits and vegetables, the data indicated that, organochlorine pesticide residues were present in 30.2%, organophosphate residues in 24.7% while synthetic pyrethroid residues were present in 41.1 % of the samples. Aldrin is an alicyclic chlorinated hydrocarbon and is rapidly converted to the epoxide from dieldrin (GESAMP, 1993). The presence of an average of 0.004 mg/kg in orange and 0.014 mg/kg in tomato respectively recorded in the analyzed samples declare that, there may be conversion of aldrin to dieldrin by an epoxidation in biological systems (Rumsey and Bond, 1974) and, therefore dieldrin is expected to be found in relatively higher levels than aldrin. Lindane is a reasonably stable compound and only under alkaline condition decomposes to yield trichlorobenzene. This result is comparable with mean concentration level of 0.002 mg/kg and 0.004 mg/kg reported in Nigeria and India markets respectively (Bhanti and Taneja, 2007; Adeyeye and Osibanjo, 1999). This further suggested that lindane is extensively used in agricultural sector on fruit and. vegetable cultivation.

The result also showed that of fruits and vegetables, 61 % of the samples showed contamination out of which 18.7% of the samples gave results with levels of pesticide residues above the MRL, while 42.3% of the samples gave results with levels of pesticide residues below the MRL. Only 39% of samples analyzed did not contain traceable level of the monitored pesticides. The present findings are in good agreement with various independent surveys conducted in India, Pakistan and Egypt (Seyed and Somashekar, 2010; Singh et al., 2008a; Gyana et al., 2007; Mukeherjee et al., 2007; Kumari et al.; 2006; Shahi et al., 2005; Parveen et al., 2004; Dogehim et al., 2001; Reddy et al., 1998, Ahuja et al., 1998; Mukherjee and Gopal 1996; Masud, 1992). Many reports are available from even developed countries on the monitoring of pesticide residues in fruits and vegetables detected above MRLs (Zawiyah et al., 2007; Cesnik et al.,· 2006; Blasco, et al., 2005; Dogheim et al., 1999; Roy, 1997; Kawamura et al., 1986; Atuma, 1985). Other international reports also support the result of present study (Ju-Mei Chang, 2005; Osibanio and Adeyeye, 1995). A similar study was also carried out in Danish market which indicated residues of pyrethroid insecticides in 54%ofthe samples of fruit and 13% of vegetables (Andersen and Paulsen, 2001). The results of the present investigation further support the findings of the study carried out in India by Kumar et al., (2006) with residues of cypermethrin and fenvalerate

ranging 0.045-0.064 mg/kg, 0.046-0.067 mg/kg respectively in grapes. The current study thus illustrate that pesticide residues were found in nearly all of the samples of water, rice, fruits and vegetable samples monitored from different locations of Thiruvananthapuram and Kasargod. Overall data reveal that about 70% of the samples showed contamination of which about 25% of the samples gave results with levels of pesticide residues above the MRL. Based on observations made in these studies, it is proposed that more extensive investigation covering all the food items and water in all the districts of Kerala be carried out to find the exact level of pesticide contamination.

CHAPTER-5

MOLECULAR MECHANISMS OF PESTICIDES RESIDUES INDUCED LIVER HEPATOTOXICITY ISOLATED

Exposure to too many environmental stressors such as pesticides is considered to have harmful health effects and contribute significantly to most of public health diseases. A number of physiological functions have clearly been shown to change hepatotoxicity of the pesticide. Moreover, there are evidence that the risk of cancer and neurodegenerative diseases increases with the exposure of pesticides. Recent reports also reveal pesticide ability to act as an endocrine disruptor and contribute to various adverse effects associated with hepatotoxicity to reproduction and development (Colborn, 2006; Eskenazi et al., 1999). A key element for the toxicology research is the identification and understanding of fundamental biological processes which change or are regulated by environmental factors, and stimulate the beginning of disease processes or a significant change in the course of the disease. Through multiple mechanisms involving communication routes and processes for signal transduction cells respond and adapt to environmental signals such as toxicants or stressors. A number of receptors sense the presence of foreign compounds in the cell and cause a cascade of events that cause these compounds to be neutralised and eliminated. In many cases, however, the metabolism of xenobiotic substances can lead to toxic metabolites or to reactive oxygen (ROS) species and cause cell-damaging oxidotic stress.

HEPATOTOXICITY

Hepatotoxicity is the inherent ability of a pesticide to cause injury or death, indicating how poisonous the chemical is. Acute hepatotoxicity is the ability of a substance to cause harm as the result of a single dose or exposure to a chemical. Chronic hepatotoxicity is the ability of a substance to cause injury as the result of repeated doses or exposures over time. The designation given to a pesticide indicating its relative level of hepatotoxicity is called the lethal dose, or LD50 value. This value identifies the dosage necessary to kill 50 percent of a test population.

Hepatotoxicity

Liver is a target organ and primary site of detoxification and is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms. Liver plays an important role in metabolism to maintain energy level and structural stability of body (Guyton and Hall, 2002). It is also the site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce hepatotoxicity (Hodgson, 2004). However, this will damage the liver cells and generate hepatotoxicity. Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and acid phosphatase (ACP) are hepatotoxicity markers and an alteration in their activities in liver may be an indicator of liver damage or disease. Aspartate transaminase is the mito-chondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys. Alanine transaminase is a cytosolic enzyme, which is more specific for the liver.

According to the results obtained from the previous chapter and other reports available, it is understood that nowadays the organochlorine pesticides are replaced by organophosphate pesticides and mostly by pyrethroids. From the literature it is also clear that endosulfan was one of the most widely used pesticides with alarming levels of hepatotoxicity issues and occurrence in various parts of India including Kerala and has been found deteriorating the environment even after it is banned from most of the countries. Hence we selected endosulfan, phorate and fenvalerate representing each of the three main classes of insecticides to study the various toxic effects induced by the pesticides in mammalian system.

Endosulfan

Endosulfan (6, 7, 8, 9, 10, 10- hexachloro- 1, 5, Sa, 6, 9, 9a- hexa hydro- 6, 9-methano-2, 4, 3-benza dioxa- thiepin-3-oxide) (Fig. V.1) is a polycyclic chlorinated hydrocarbon insecticide. Although classified as a yellow label (highly toxic) pesticide by the Central Insecticides Board, India is one of the largest producers and the largest consumer of Endosulfan in the world. Endosulfan is acutely neurotoxic to both insects and mammals, including humans. The United States Environmental Protection Agency (US-EPA) classifies it as Category I: "Highly Acutely Toxic" based on a LDso value of 30 mg/kg for female rats, while the World Health Organization classifies it as Class II "Moderately Hazardous" based on a rat LDso of 80 mg/kg.

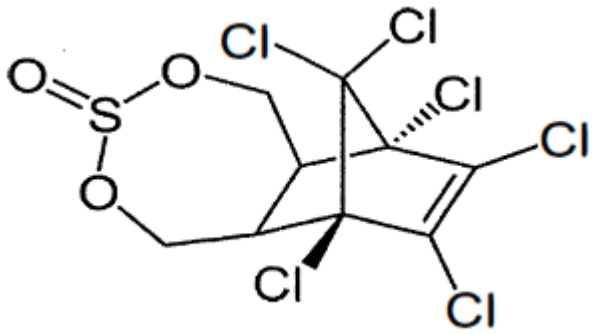


Figure 5.1; Structure of Endosulfan

Endosulfan is widely used in most of the plantation crops in India. Hepatotoxicity of endosulfan and health issues due to its bioaccumulation came under media attention when health issues precipitated in the Kasargod district (of Kerala state) was publicised and consequently, the pesticide was banned in the state of Kerala. Endosulfan became a highly controversial agrichemical due to its acute hepatotoxicity, potential for bioaccumulation, and role as an endocrine disruptor. Endosulfan is readily metabolised in animals by microsomal enzymes, initially to endosulfan sulphate and endosulfan diol; and excreted in urine and faeces. It has been found distributed to adipose, liver, kidney, heart, spleen, testes, epididymis, prostate, seminal vesicle, milk and muscle.

Phorate

Phorate is phosphorodithioic acid 0, 0-diethyl S-[(ethylthio) methyl] ester (Fig. 5.2); (Thimet; Timet). Phorate is systemic and broad spectrum organo phosphorus (OP) insecticide, commonly used in agriculture to control sucking and chewing pests. It is also used in pine forests and on root and field crops, including corn, cotton, coffee, and some ornamental plants and bulbs (Abhilash and Singh, 2009; Gan and Jans, 2007).

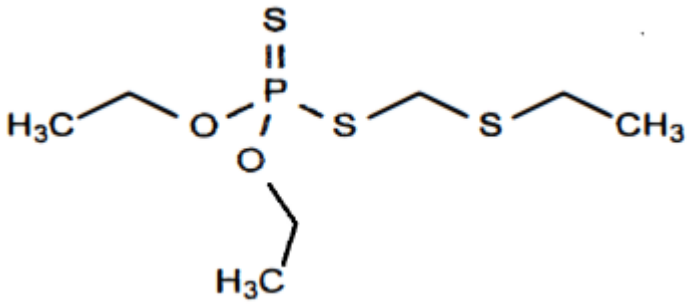


Figure 5.2 Structure of phorate

Phorate is bio transformed by oxidation of the thioether moiety to the corresponding sulphoxide and sulphone and by desulphuration of the P=S moiety to P =O, producing a phosphorothiolate ester. Phorate has been classified as a Class I, high-risk toxic OP compound with an LD50 of 1.1 to 3. 7 mg/kg body weight for rats. It is well known to inhibit acetylcholinesterase activity by phosphorylating the serine hydroxyl group in the substrate binding domain, which results in accumulation of acetylcholine and induces neurotoxicity (Fulton and Key, 2001; Oruc and Usta, 2007; Vandana and Zaman,1997).

Fenvalerate

Fenvalerate [cyano (3-phenoxyphenyl) methyl 4-chloro-a/pha-(lmethylethyl) benzene acetate] (Figure 5.3), a synthetic type II pyrethroid insecticide is a mixture of four optical isomers:- 22% of [2S, aS] isomer; 28% of [2S, uR] isomer; 22% of [2R, aR] isomer; and 28% of [2R, aS] isomer. Liver is the predominant site of fenvalerate metabolism through hydrolysis by hepatic microsomal esterases (Ghiasuddin and Soderlund, 1984) to yield fenvaleric acid (Kaneko et al., 1981; Okava et al., 1979). Fenvalerate displays moderate hepatotoxicity in

mammals (Ecobichon, 1996); oral LD50 values from 100 to over 3000mg/kg were found for several rodent species (UN-WHO, 1990).

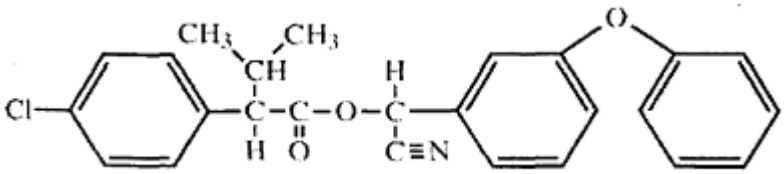


Fig.5.3 Structure of fenvalerate

Synthetic pyrethroids have emerged as a new class of agricultural pesticides and have found wide use over organochlorine and organophosphate pesticides. Pyrethroid pesticides (cypermethrin and fenvalerate) show high hepatotoxicity to a wide range of insects, including resistant strains (Elliot et al., 1978) low hepatotoxicity to mammals and birds (Parker et al., 1984) and rapid biodegradability (Leahey, 1979). However, the liberal use of pyrethroids increases the risk of intoxication to non-target organisms such as birds, animals and organisms

present in soil and water. Though pyrethroids have been shown to be rapidly metabolised in mammals, their role in producing oxidative stress has not been examined. For evaluating the hepatototoxic potential, the data from the analysis is used to calculate the total residues that can be accumulated due to the consumption of daily food. Hence three pesticides are selected from each class of insecticides, which are found in almost all samples, and their total residual concentrations are calculated. From the total residual concentration per day, the values could be calculated for one year and a life time as given in previous chapter. The hepatotoxic potential of the pesticides can be evaluated from these values by giving corresponding concentrations to rats orally and checking various liver hepatotoxicity parameters.

Materials and Methods

Dose determination for animal studies

The residual concentration that can be accumulated per day is calculated and the data is used to calculate the residues that can be accumulated for 1 year, 15 years and in 30 years. The

corresponding values for animals are found out and fixed as the dose for treatment of animals for various studies.

Endosulfan

Amount of residues that can be accumulated in 1 year = 13mg/kg

Corresponding value for animals (b.w. 0.250) = (13 ×0.250)/60

= **0.054 mg/kg**

Amount of residues accumulated in 15 years = 0.054× 15

= **0.81 mg/kg**

Amount of residues accumulated in 30 years = 0.054 ×50

= **1.5 mg/kg**

Phorate

Amount of residues that can be accumulated in 1 year = 31.7 mg/kg

Corresponding value for animals (b.wt. 0.250) = (31.7 ×0.25)/60

= **0.13 mg/kg**

Amount of residues accumulated in 15 years = 0.13 × 15

= **1.95 mg/kg**

Amount of residues accumulated in 30 years = 0.13×30

= **3.9 mg/kg**

Fenvalerate

Amount of residues that can be accumulated in 1 year = 144 mg/kg

Corresponding value for animals (b.wt. 0.250) = (144× 0.250)/60

= **0.6 mg/kg**

Amount of residues accumulated in 15 years = 0.6× 15

= **9 mg/kg**

Amount of residues accumulated in 30 years = 0.13 × 30

= **18 mg/kg**

The doses are fixed based on the above values and accordingly animals are grouped as follows.

Groups	**Pesticide concentration (mg/kg) (in groundnut oil)**
C	-
E1	0.05 (Endosulfan)
E2	0.1 (")
E3	1.5 (")
P1	0.1 (Phorate)
P2	2.0 (")
P3	4.0 (")
F1	0.5 (Fenvalerate)
F2	10.0 (")
F3	20.0 (")

The rats were treated with the doses of selected pesticides to study the cellular antioxidant status, specific enzyme markers and tissue histopathology in the liver tissues. Various tissues are analysed for determining the level of distribution and accumulation of residues in various organs.

RESULTS

Biochemical profile

Effect of Endosulfan, Phorate and Fenvalerate on blood and liver biochemical parameters is summarized below.

Effect of Endosulfan, Phorate and Fenvalerate on Cytochrome P450 enzyme system

The activity of Cytochrome P450 enzyme was estimated in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure VA. The data shows that following oral administration of the pesticides, there was a significant induction of hepatic P450 content and lipid peroxidation. The rats fed with endosulfan showed increase in the enzyme in which the increase was maximum in E3 groups. Similarly the enzyme levels were found to be increased in dose dependent manner in phorate and fenvalerate treated rats.

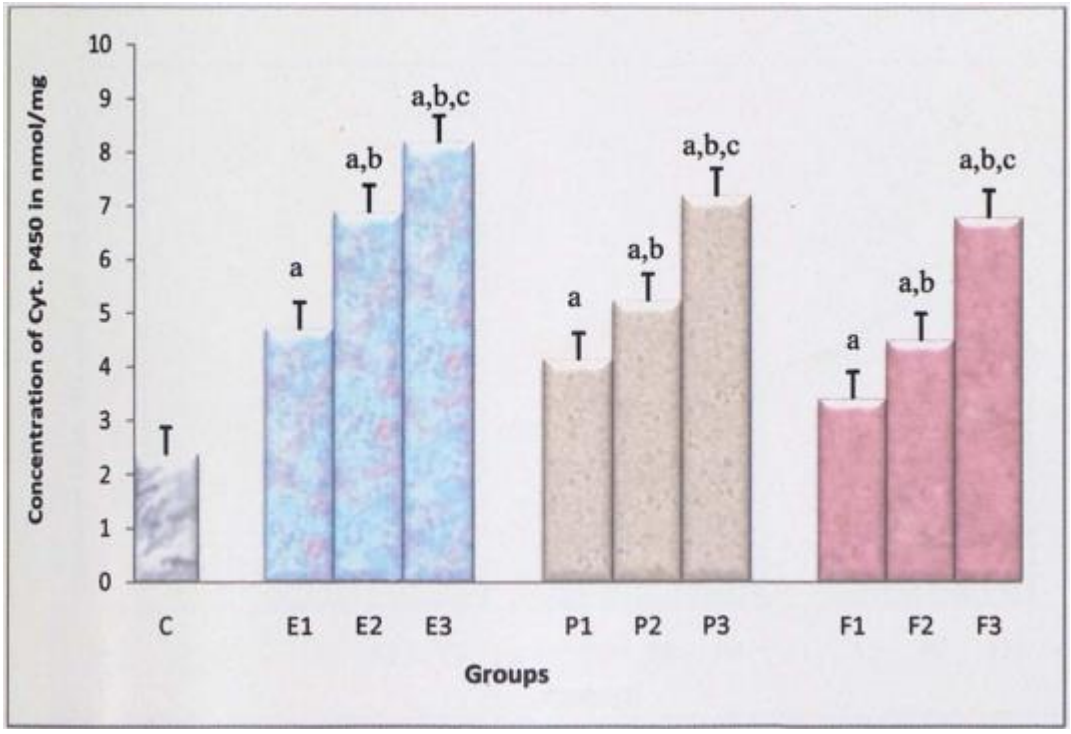
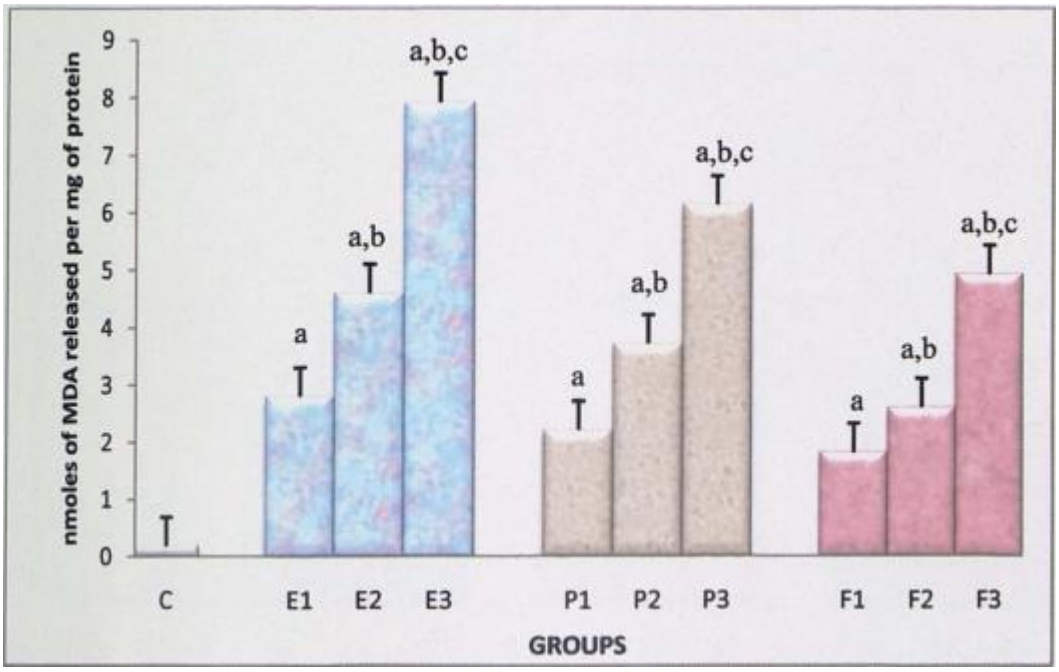


Fig. 5.4: Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on cytochrome P450 content in rat liver following daily oral administration of 90 days

Effect of Endosulfan, Phorate and Fenvalerate treatment on the concentration of Malondialdehyde (MDA)

The malondialdehyde levels were calculated in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure V.5. The figure shows that malondialdehyde level was significantly increased in all the pesticide treated groups compared to control. Endosulfan treated group showed the maximum variation evidenced by the highest concentration of MDA detected in E3 group. The result confirmed the ability of organochlorines to mediate the oxidative stress by enhancing the lipid peroxidation. The phorate and fenvalerate treated groups also showed a regular concentration dependent increase in the formation of MDA.



Fig, 5.5 Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on lipid peroxidation in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the concentration of Reactive Oxygen Species (ROS)

The Reactive Oxygen Species levels were calculated in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure 5.6. The figure illustrates that ROS level was significantly increased in all the pesticide treated groups when compared to control group. Hepatic level of ROS was enhanced in the endosulfan treated rats by 20, 23 and 27% in E1, E2 and E3 groups respectively when compared to vehicle controls. Phorate treated rats also showed an increase by 25%,33% and 30% in PI, P2 and P3 groups respectively. Similarly the level of ROS was enhanced in the fenvalerate treated rats by 19%, 22% and 27% in F1, F2 and F3 groups respectively.

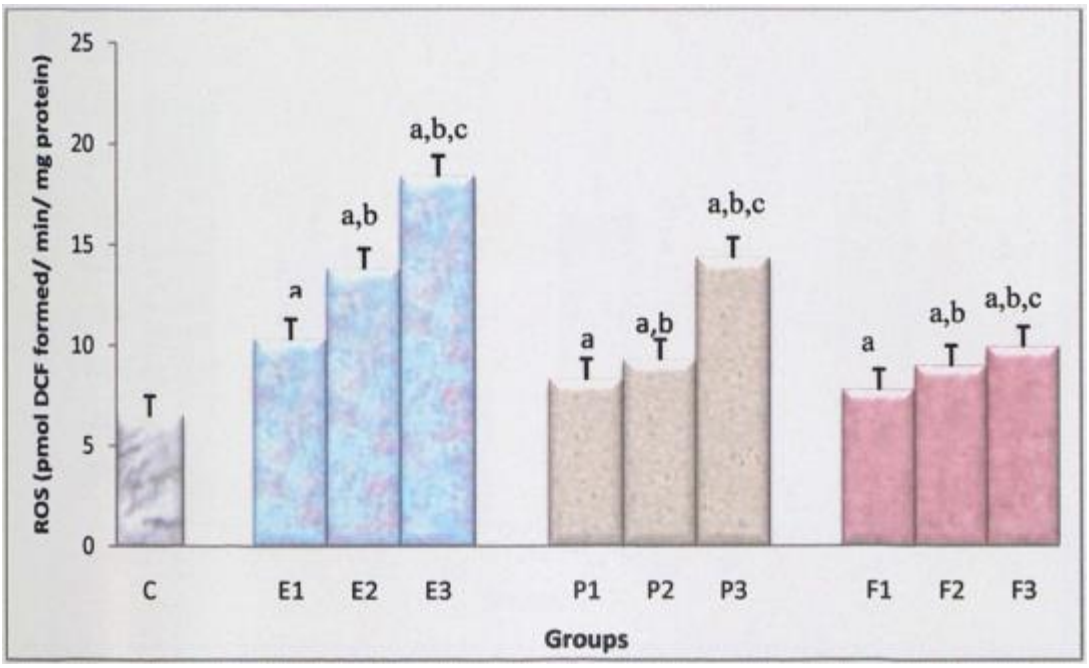


Fig. 5.6 Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on ROS in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Superoxide dismutase (SOD)

The activity of superoxide dismutase was calculated in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure 5.7. As shown in figure, SOD activity was recorded to be decreased in treated groups when compared with control group. The reduction was found to be in a dose dependent manner for each group. However the endosulfan treated group showed the maximum reduction in the activity and the fenvalerate treated group showed the minimum.

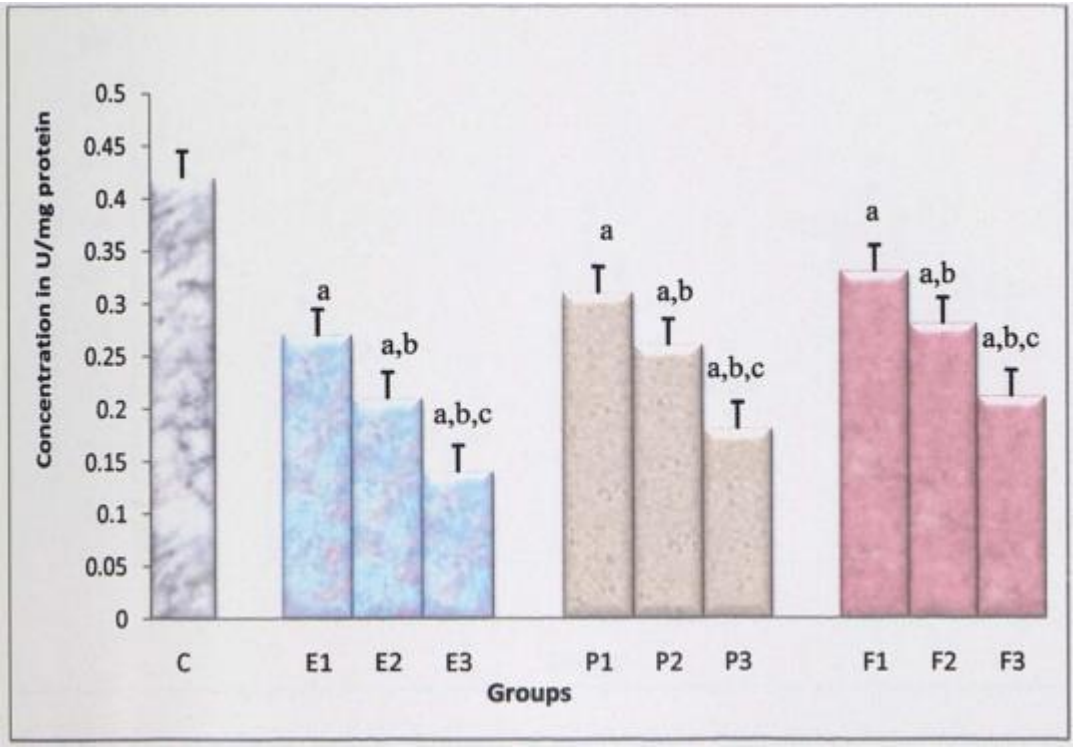


Fig. 5.7 Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on Superoxide dismutase in rat liver following dally oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Catalase (CAT)

The catalase levels were calculated in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure 5.8. As shown in figure, catalase activity was recorded to be decreased in treated groups when compared with control group. The reduction was found to be in a dose dependent manner for each group. However the endosulfan treated group showed the maximum reduction in the activity in the E3 group. Similarly phorate and fenvalerate treated groups also showed a regular decrease in the activity.

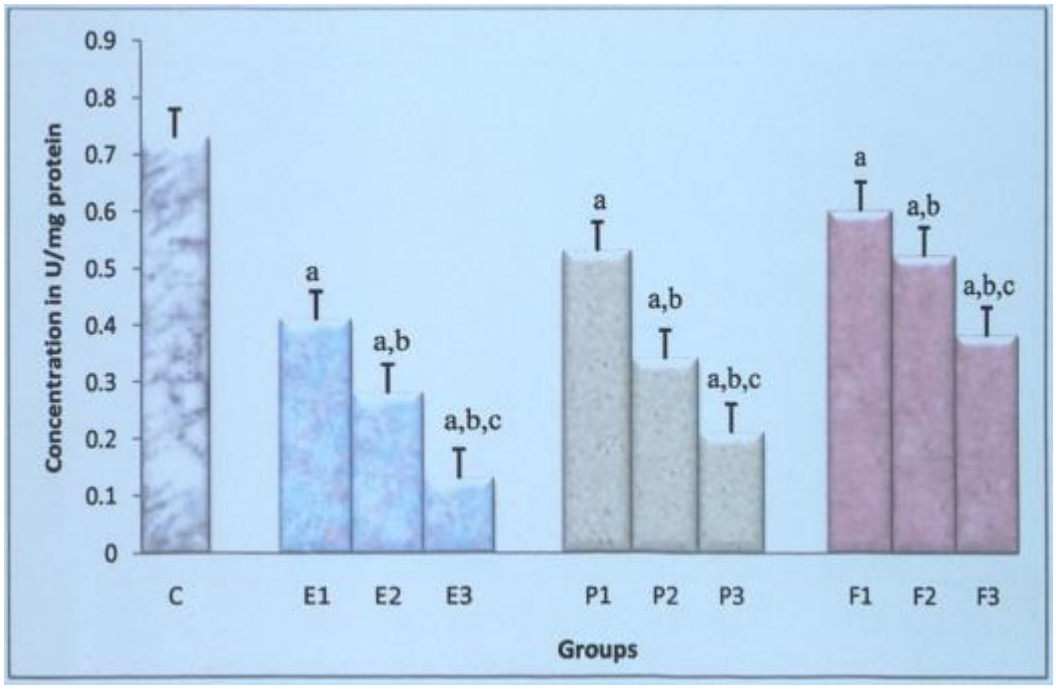


Fig. 5.8; Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on catalase in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the concentration of Glutathion (GSH)

The glutathione level was measured in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure 5.9. The glutathione (GSH) content was significantly decreased in plasma of rats treated in all of the pesticides. The endosulfan treated groups showed a maximum reduction in the GSH level and it was foud to be dose dependent. A regular decrease was also seen in the phorate treated groups but the effect was less compared to the endosulfan treated groups. The decrease was less evident in fenvalerate treated rats, compared to the other two groups.

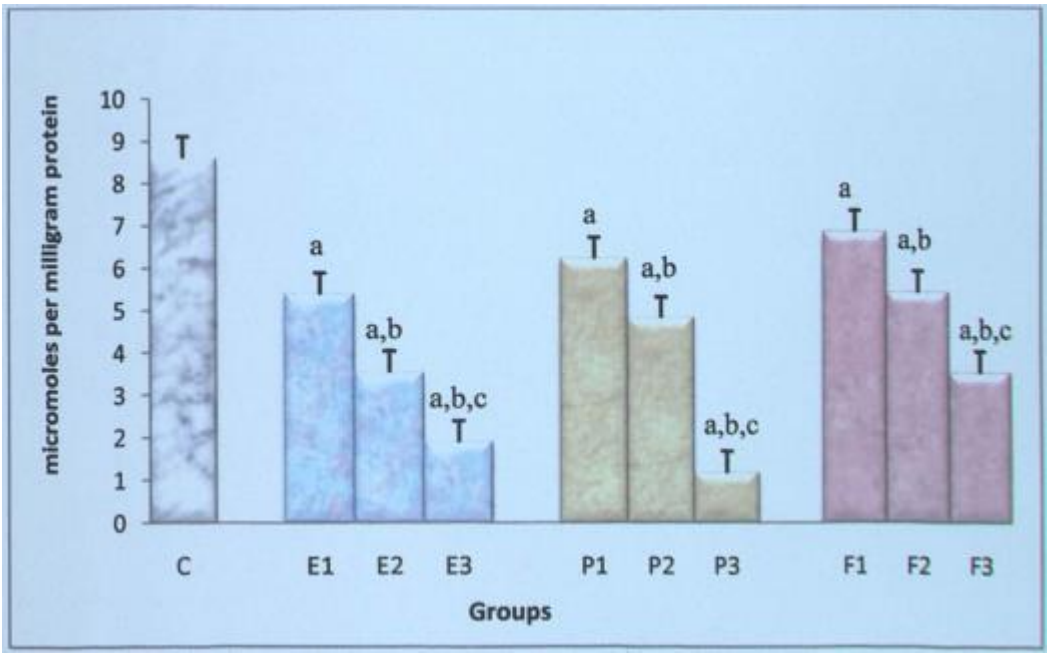


Fig. 5.9; Effect of various concentrations of Endosulfan, Phorate and glutathione in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerafe on the activity of Glutathion S Transferase (GST)

Figure 5.10 shows the GST levels estimated in Endosulfan, Phorate and Fenvalerate treated rats. The figure illustrates the negative impact of the residues on the enzyme activity. The enzyme activity was found to be decreased according to the dose increased in all the pesticide treated groups compared to the comol. The phorate and fenvalerate treated groups showed a lesser but regular impact with respect to the concentration.

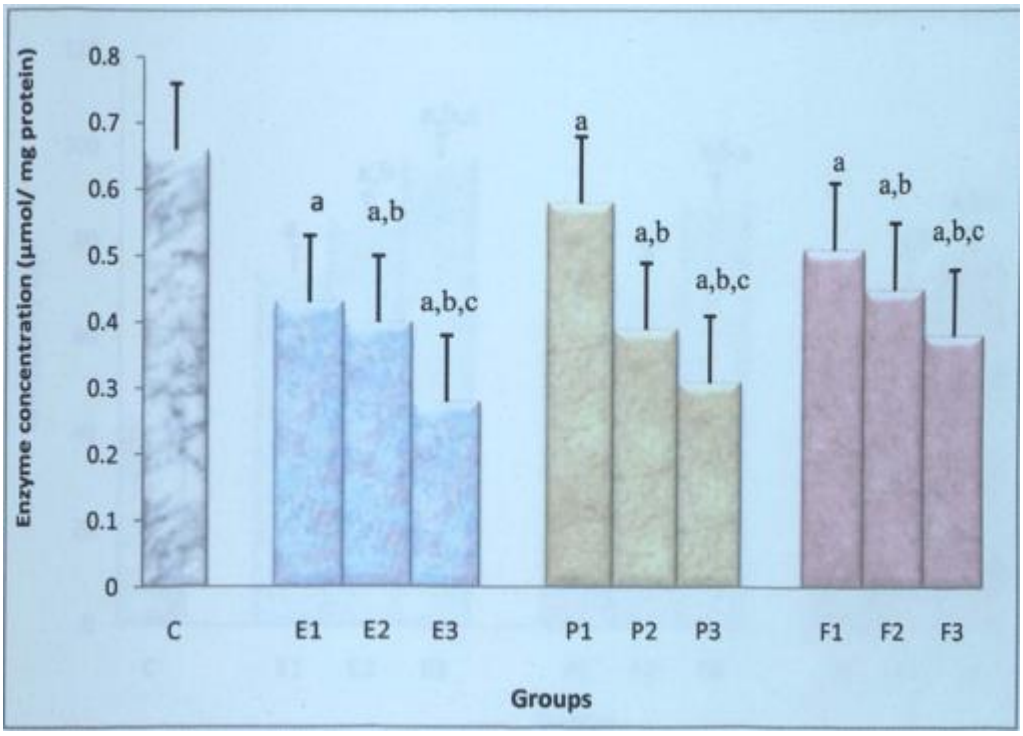


Fig. 5.10; Effect of various concentrations of Endosulfan, Phorate and Fenvalerate treatment on GST in rat liver following daily oral administration for 90 days.

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Lactate dehydrogenase (LDH)

Lactate dehydrogenase level in plasma was estimated in Endosulfan, Phorate and Fenvalerate treated rats. The treatment with the pesticides caused increase in LDH in all the groups but the enhancement was found to be maximwn for endosulfan treated group. Phorate and Fenvalerate treated groups also showed a dose dependent increase in the enzyme concentration similar to endosulfan but to a lesser extent. The results are shown in Figure 5.11.

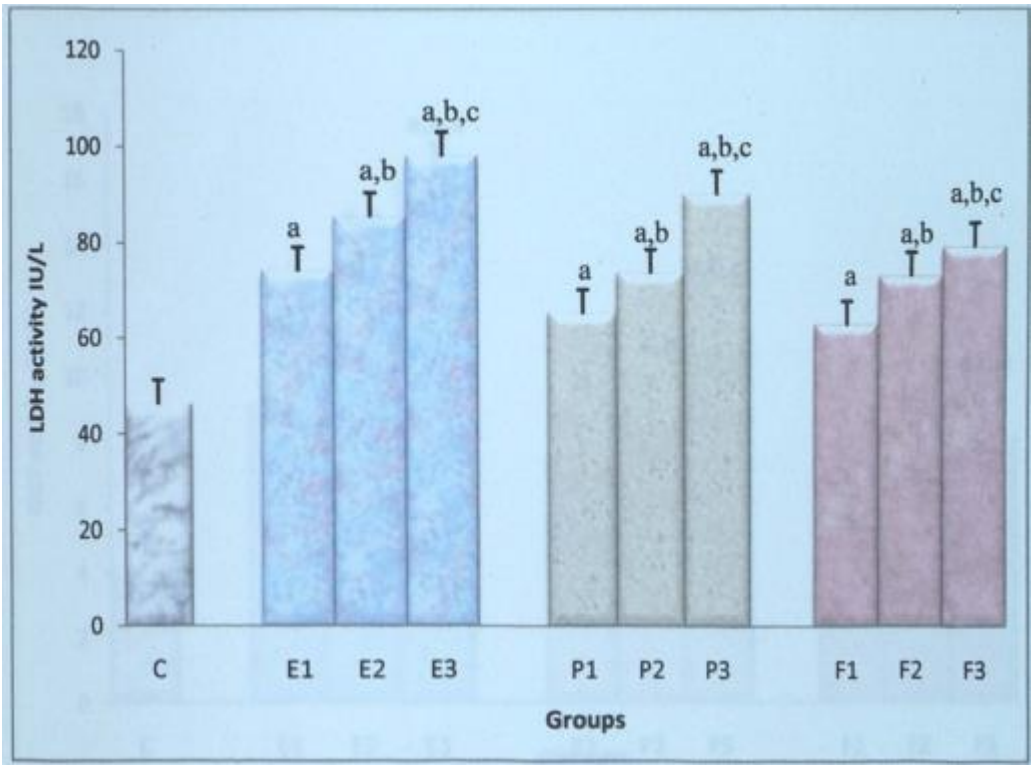


Fig. 5.11; Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on Lactate dehydrogenase in plasma following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of y- Glutamyl transferase (GGT)

The GGT levels were estimated in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure 5.12. The figure showed that the treatment with the pesticides caused significant increase in GGT level in all the groups and the enhancement was found maximum in E3. The phorate and fenvalerate treated groups also showed an increase in the enzyme activity dependent on the dose ofthe treatment.

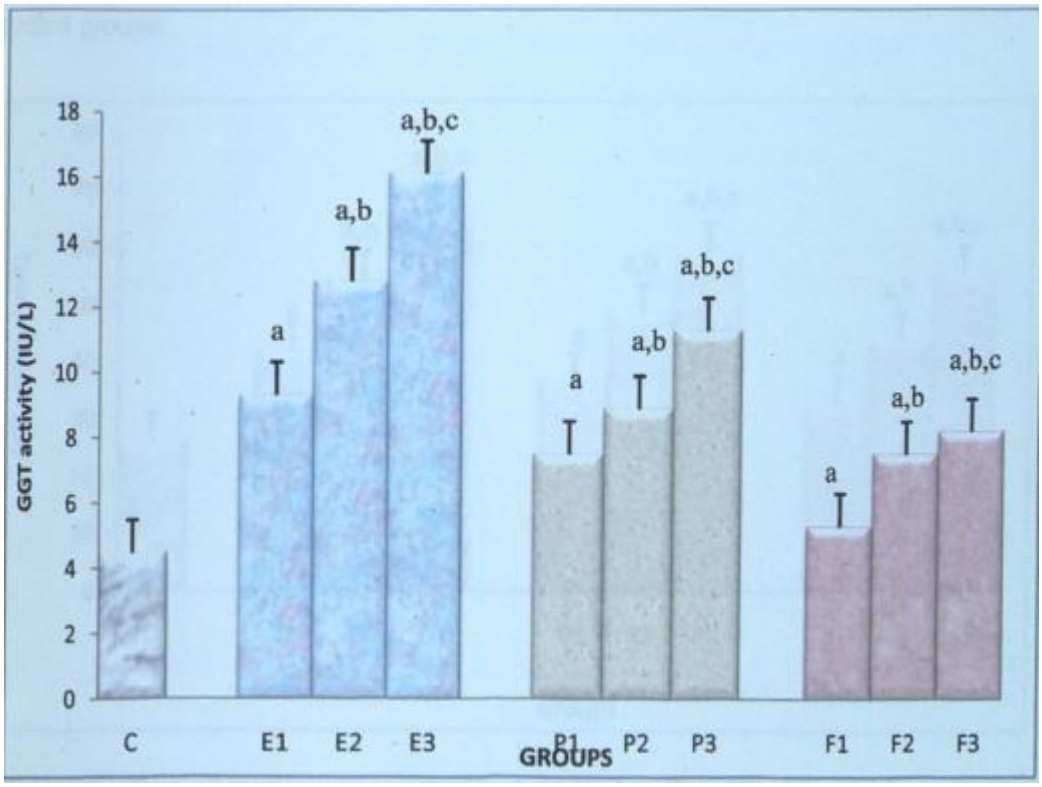


Fig. 5.l2 Effect of Endosulfan, Phorate and Fenvalerate on GGT in rat liver foUowing daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Acid phosphatase (ACP)

The acid phosphatase activity was measured in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure 5.l3. There was a significant increase in the activity of ACP in all the treated groups compared to the control group. ACP activity showed a very high increase in endosulfan treated groups with respect to the dose applied. Phorate treated groups also showed an increased ACP activity with respect to the dose. Fenvalerate was also found to be enhancing the enzyme activity but the effect was less compared to the other two treated groups.

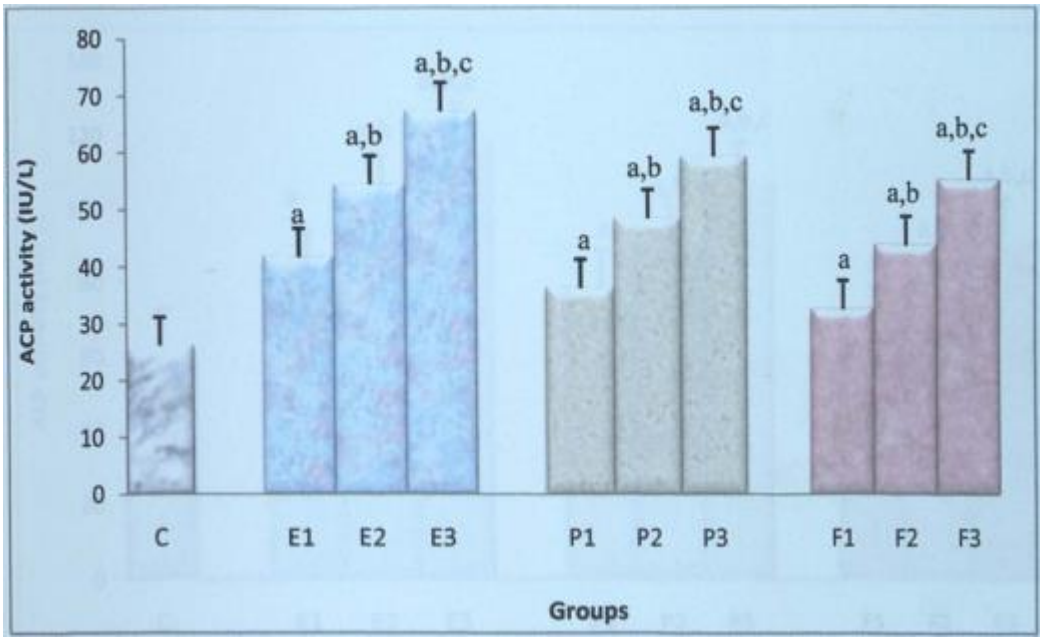


Fig.5.l3: Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on ACP in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Alkaline phosphatase (ALP)

Figure 5.14 shows the Alkaline phosphatase activity estimated in Endosulfan, Phorate and Fenvalerate treated rats. The results demonstrate that there was significant increase in the ALP activity in all the treated groups compared to the control group. ALP showed a very high increase in endosulfan treated groups with respect to the dose applied and the E3 group showed maximum activity. Phorate treated groups also showed an increased activity of ALP with respect to the dose applied. Fenvalerate also found to be enhancing the enzyme activity but the effect was less compared to the other two pesticides.

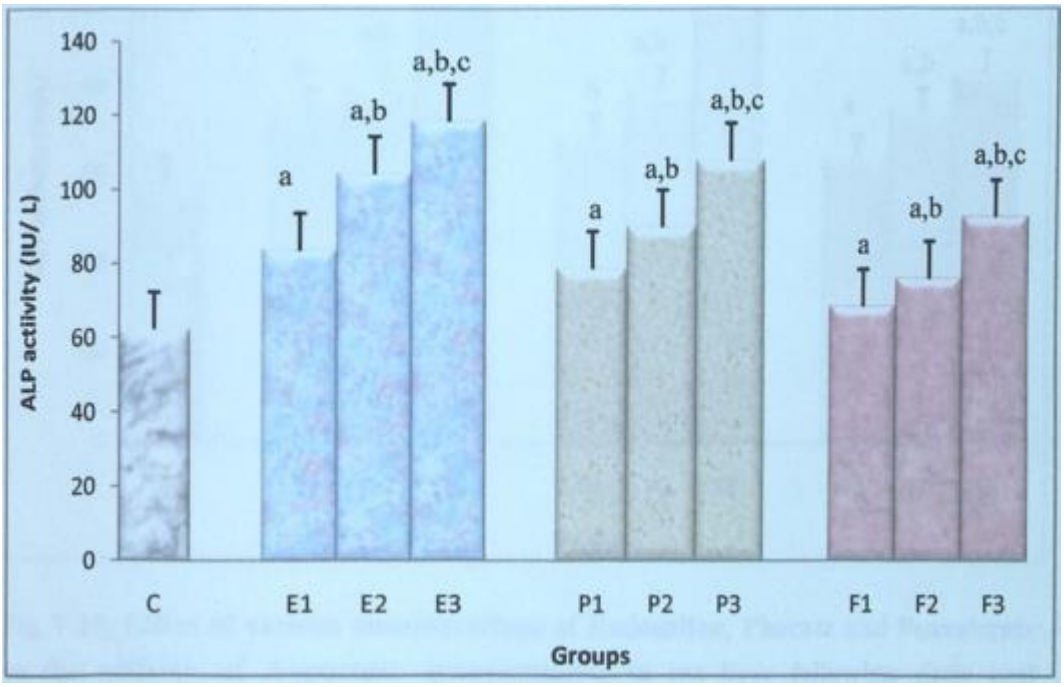


Fig.5.14 Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on the activity of alkaline phosphatase in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Aspartate transaminase (AST)

Figure 5.15 shows Aspartate transaminase levels measured in Endosulfan, Phorate and Fenvalerate treated rats. The figure shows that AST showed a regular increase in all the pesticide administered groups. AST was increased maximum in the endosulfan treated groups and the effect was found to be dose dependent. Phorate and fenvalerate treated groups also showed similar effects eventhough the effect was very less in fenvalerate groups.

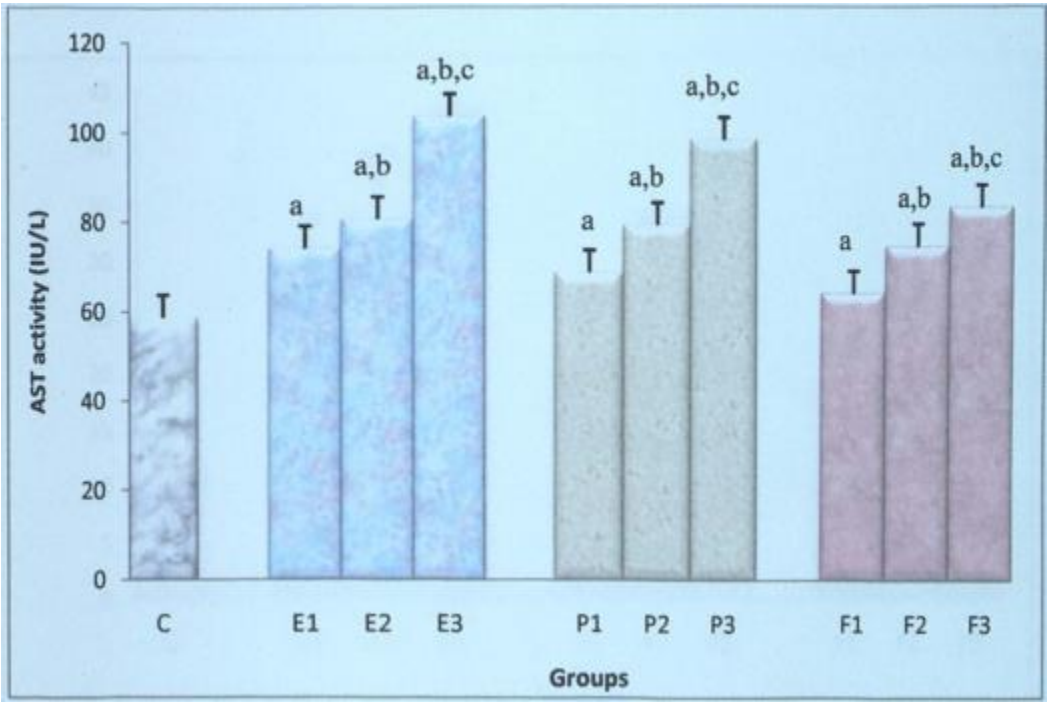


Fig. 5.l5; Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on the activity of Aspartate transaminase in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Aspartate transaminase (AST)

Figure 5.15 shows Aspartate transaminase levels measured in Endosulfan, Phorate and Fenvalerate treated rats. The figure shows that AST showed a regular increase in all the pesticide administered groups. AST was increased maximum in the endosulfan treated groups and the effect was found to be dose dependent. Phorate and fenvalerate treated groups also showed similar effects eventhough the effect was very less in fenvalerate groups.

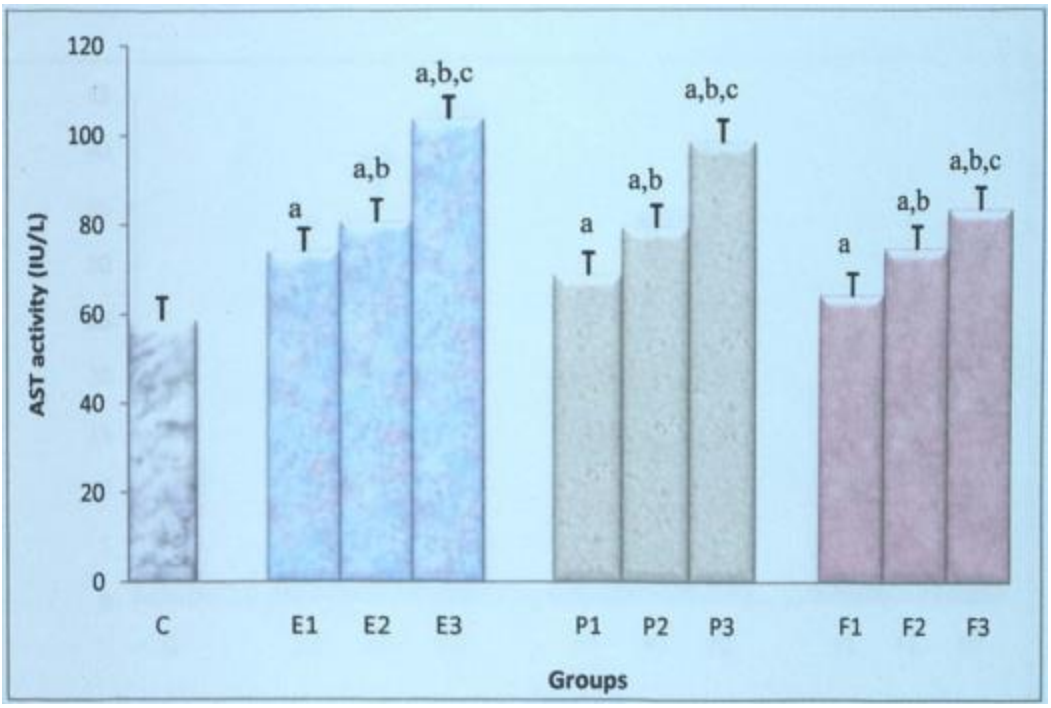


Fig. 5.15; Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on the activity of Aspartate transaminase in rat liver following daily oral administration for 90 days

Effect of Endosulfan, Phorate and Fenvalerate on the activity of Alanine transaminase (ALT)

The alanine transaminase levels were calculated in Endosulfan, Phorate and Fenvalerate treated rats and the results are shown in Figure 5.16. The figure shows that ALT activity showed a regular increase in all the pesticide administered groups. ALT was increased maximum in the endosulfan treated groups and the effect was found to be dose dependent. Phorate and fenvalerate treated groups also showed similar effects eventhough the effect was less in fenvalerate treated groups.

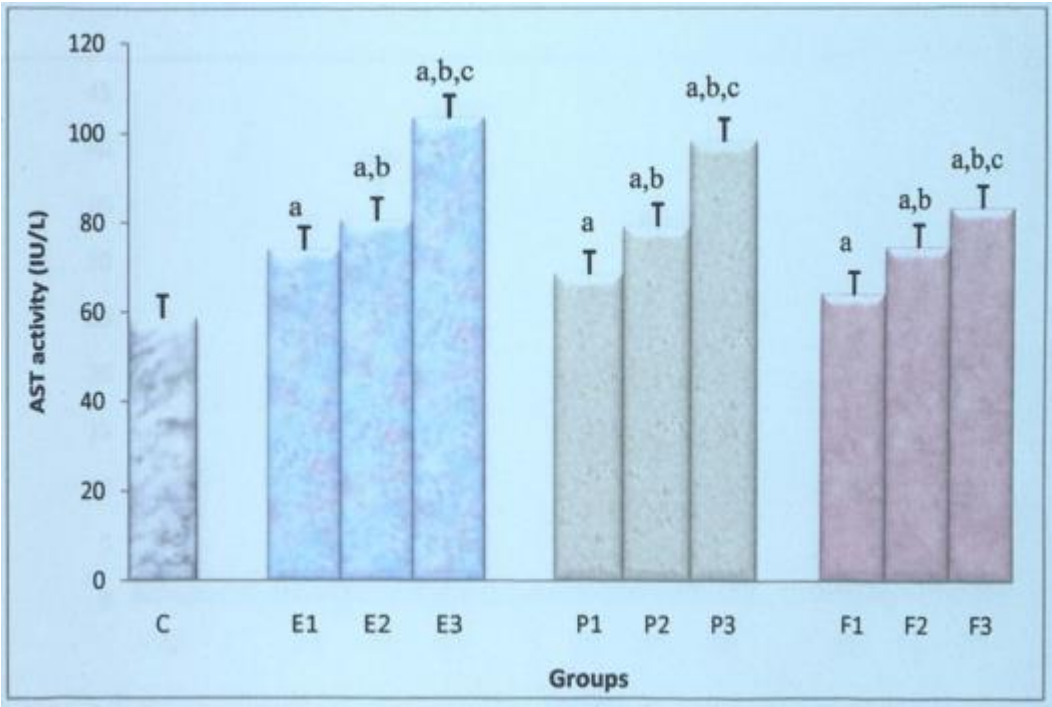


Fig. 5.l6; Effect of various concentrations of Endosulfan, Phorate and Fenvalerate on Alanine transaminase in rat liver following daily oral administration for 90 days

The concentration of the pesticides in rat tissues following oral administration of endosulfan, phorate and fenvalerate

The pesticide levels in liver, brain, kidney, lungs and heart in rats following consecutive daily oral administration for 90 days has been presented in Table 5.1. The distribution of endosulfan in the rat tissues was found to be as follows: liver > kidney lungs > Brain > heart. It is reflected in table that like other organochlorine pesticides, endosulfan also has a tendency to accumulate in these organs due to its lipophilic character. The distribution of phorate in the rat tissues was as follows: liver> kidney> lungs> heart> brain. Phorate was found accumulated most in liver and lowest in brain. Similarly the distribution of fenvalerate in the rat tissues was as follows: liver > kidney > lungs > heart. Fenvalerate also showed its maximum accumulation in liver and minimum in heart but the residue was found to be absent in brain.

Table 5.l; The tissue residual concentration in various organs following oral administration of endosulfan, phorate and fenvaterate for 90 days.

Substrates	Concentration (mg/Kg)		
	Endosulfan	Phorate	Fenvalerate
Liver	2.45±0.11	1.92±0.09	0.33
Kidney	1.28±0.05	0.71±0.14	0.21±0.42
Lungs	0.78±0.12	0.42±0.03	0.19±0.22
Brain	0.46±0.38	0.11±0.08	0
Heart	0.21±0.03	0.35±0.05	0.08±0. 09

HISTOPATHOLOGY

Histopathological investigations ofliver tissues in Endosulfan, Phorate and Fenvalerate treated rats are shown in Figure 5.l7, 18 and 19. The figures revealed mild to marked changes in liver upon administration of pesticides. Endosulfan treated rat liver showed the changes including leukocyte infiltrations, activated Kupffer cells, dilatation in blood sinusoids and extensive cytoplasmic vacuolization (Figure 5.17). All these features suggest hepatocellular damage.

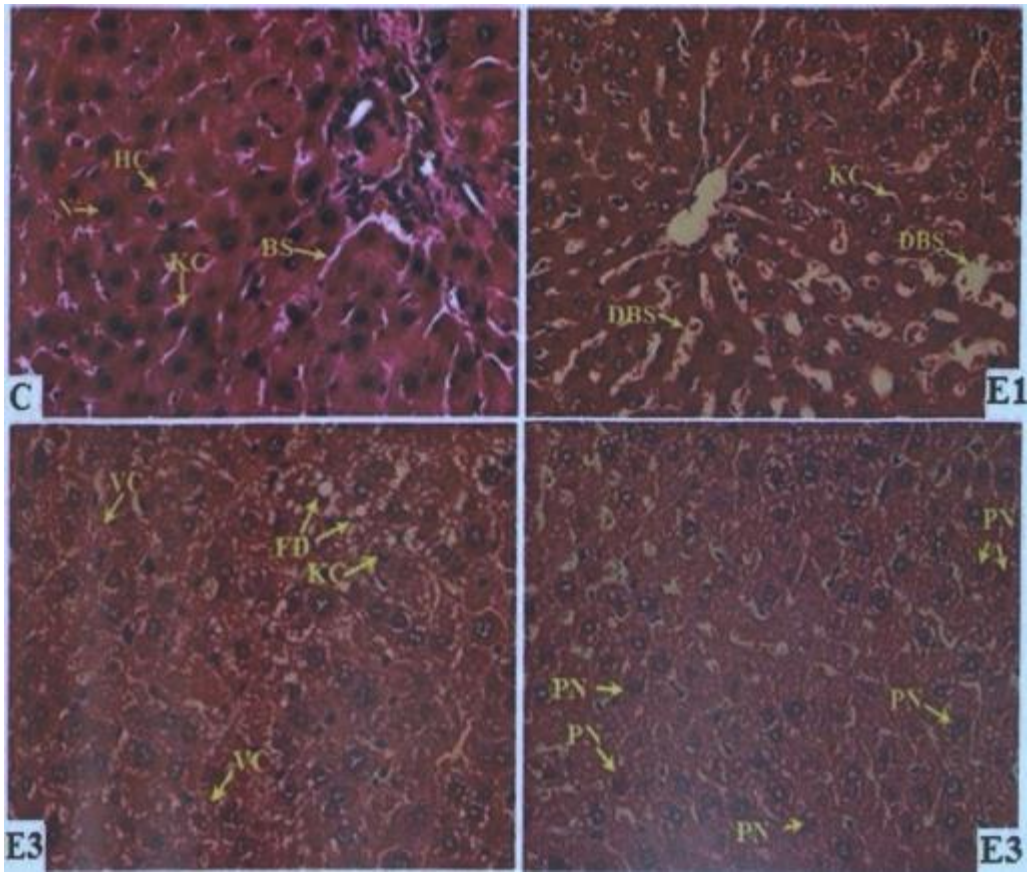


Fig.5.17; Photomicrograph of rat liver after consecutive daily oral administration of endosulfan at different doses for 90 days

C: liver sections of control group showing normal histological appearance ofthe liver, including central vein (CV), blood sinusoids (BS), hepatic cells (HC), Kupffer cell (KC) and centrally located nuclei (N). EI-E3: liver sections of endosulfan treated groups exhibiting appearance ofpyknotic nuclei (PN), fatty infiltrations (FD) and activated Kupffer cells (KC), dilatation in blood sinusoids (DBS) and cytoplasmic vacuoles (VC).

Phorate treated rat liver showed the changes including mild to moderate diffuse granular degeneration; macro vesicular fatty change and mild periportal multifocal lymphocytic/leucocytic infiltration (Figure 5.18). Also bile pigmentation and necrotic granulomas were visible. This result also confinned the hepatocellular damage occurred due to pesticide hepatotoxicity.

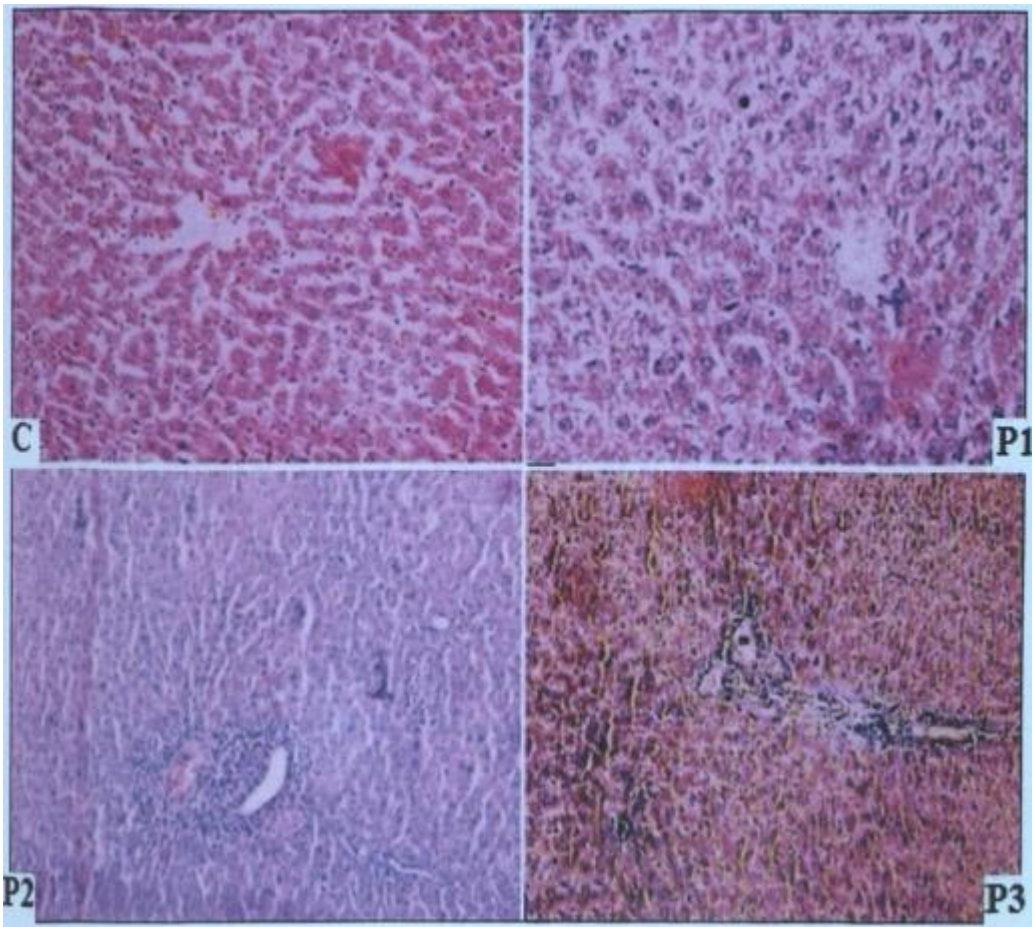


Fig.5.l8; Photomicrograph of rat liver after consecutive daily oral administration of phorate at different doses for 90 days.

C- Control rat liver, PI-P3: liver sections of phorate treated animals showing minimal diffuse granular degeneration; mild to moderate diffuse granular degeneration; macrovesicular fatty change and mild periportal multifocal lymphocytic/leucocytic infiltration. P3 shows necrosis, bile pigmentation and necrotic granulomas.

Fenvalerate treated rat liver sections showed the changes including leukocyte infiltrations, activated Kupffer cells, dilatation in blood sinusoids and extensive cytoplasmic vacuolization hepatocellular necro-infI.ammation, mild to severe multifocal fatty degenerations and necrosis (Figure 5.19). All these features suggest mild to severe hepatocellular damage.

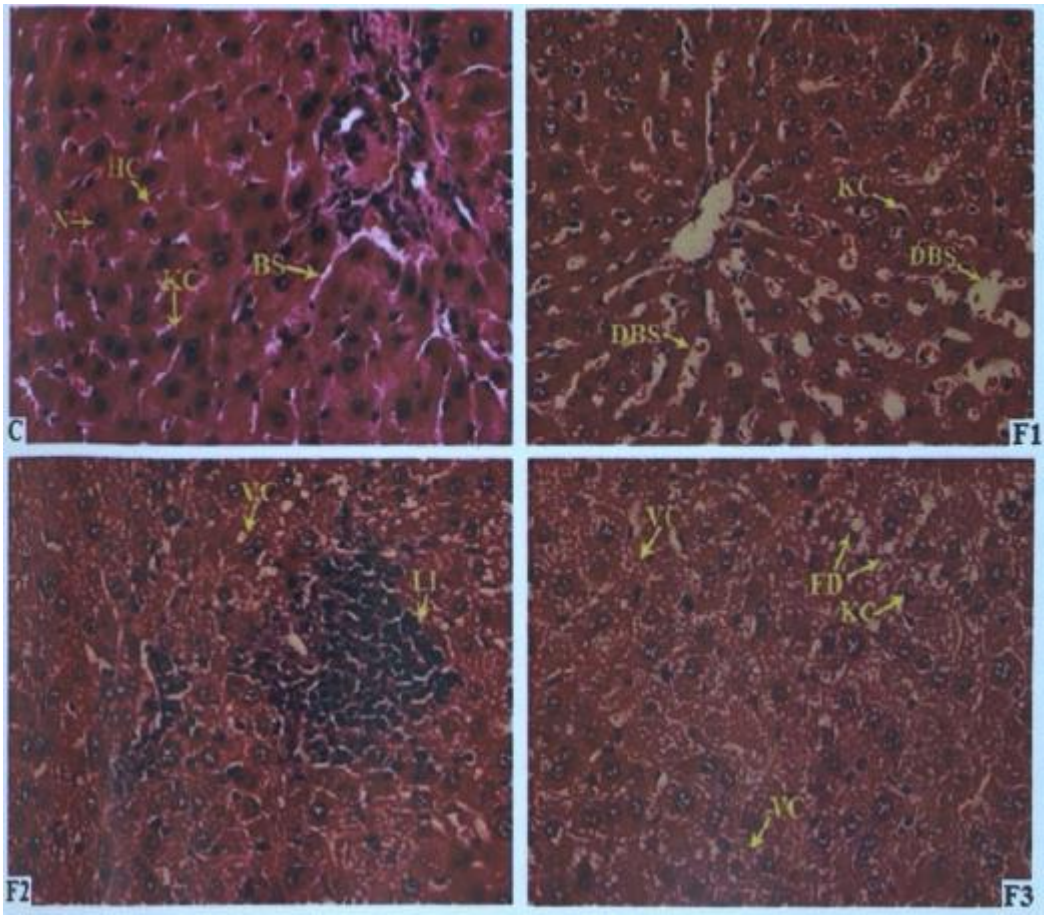


Fig.5.l9; Photomicrograph of rat liver after consecutive daily oral administration of fenvalerate different doses for 90 days

C: liver sections of control animals showing normal histological appearance ofthe liver, including central vein (CV), blood sinusoids (BS), hepatic cells (HC), Kupffer cell (KC) and centrally located nuclei (N). FI-F3: liver sections of fenvalerate treated rats (FI-O.05, F2-0.1 and F3-1.0mglKg) exhibiting appearance of pyknotic nuclei (PN), fatty infiltrations (FD), inflammatory leukocyte infiltrations (LI) and activated Kupffer cells (KC), dilatation in blood sinusoids (DBS) and cytoplasmic vacuoles (YC).

DISCUSSION

As a consequence of the use of endosulfan, phorate, and fenvalerate in rats, we found a significant induction of hepatic CytP450 enzymes. These results indicate that P450 isoenzymes are likely to be induced by pesticides. Therapy may induce P450's enzyme system,

which is demonstrated in the study of Kaloyanova et al. in 1984, as an increased biotransformation into p=o analog. In all treated rats, the level of MDA was also raised depending on concentration. An important indicator of lipid peroxidization is a major oxidant produced in peroxidized multiple insulating fatty acids (Banerjee et af., 1998; Hazarika et al., 2003). Based on experimental findings, an increased microsomal oxidative capacity induced by the insecticide may be associated with lipid peroxidation within the liver. Lipid peroxidation produces a variety of lipid hydroperoxide products like malondialdehyde, four hydroperoxy-2-nonenale and 4-oxo-2-nonenale (4HNE). Those aldehyde products react with individual nucleotides and nucleophilic amino acids and thus mediate several hepatotoxic signaling effects (West and Marnett, 2006). An increased lipid peroxidative indicator, which was demonstrated by a significant increase in the thioBarbituric acid reactive substances detected in liver homegrown products, paralleled the improvement of liver microsome P450 content and radical production of oxygen. Higher cytochrome P450 levels would thus lead to high free production rates which, in the case of tummy, would lead to higher rates of peroxidation.

The study has also shown that in the pesticide treated groups reactive oxygen levels in comparison to controls are significantly increasing. Other cellular elements, like lipids, are known to be attacked, leaving reactive species that in turn can link to the DNA foundation. Nevertheless, these radicals may interact to form persistent regulators with cell macromolecules to inhibit the transcription of genes within those cels. Many xenobiotics have been identified to generate free radicals in the biological system (Kehrer, 1993). The cells have intrinsic ROS detoxification mechanisms produced both physiologically and pathologically. Reduced glutathione (GSH) is the main antioxidant molecule in the cell and is able to directly scavenge ROS such as -02-, -OH and -NO because of its high cytosolic concentration. The GSH peroxidase (GPX) and catalase of H202 is reduced to H20 (CAT). Unbalances in the production of ROS and antioxidant defence in the body have a series of important health effects, such as mitochondria, DNA and proteins, damages in which the cells swell up in the tumor and ultimately cell death resulting from apoptosis (Franco et AI, 2009; Ryter et al. 2007). The primary cause of lipid peroxidation, membrane fluidity change and damage to DNA, as well as cancerous processes is considered to be oxidative stress (Singh and Pandey, 1989; Bagchi et al., 1995; Naqvi and Hasan, 1992; Gupta and Shukla, 1998).

Damage to cells by oxidants is minimized by enzymes such as SOD, catalase and glutathione that are antioxidants. The activity of SOD and catalase in rats treated with pesticides has decreased in our study. In all three treatment groups, Glutathione levels have also been shown to decrease significantly. The reduction in SOD and CAT activities could probably be caused by increased reactive oxygen generation of species (Kale et aI., 1999). Greater ROS generation and less SOD, CAT and GSH activities mean that the antioxidant defense systems that overcome the influx of Ros do not exist (Verma and Srivastava, 2003; Verma et al., 2007). In addition, earlier studies have shown that cell redox homeostatic alterations have occurred in the production of ROS by induced OP pesticides and antioxidant enzyme depletion or impairment (Bagchi et aI. 1995; Muniz et AI. 2008), which is consistent with our findings. In addition, inhibitions of enzymes involved in free radicals may lead to accumulation of the lipid peroxidation of H202, DNA modulation, altered gene expression and cell death suggested (Fetoui et aI., 2009; Halliwell and Gutteridge, 1999). Several studies have shown that organochlorine (Bagchi, 1992, 1993), organophosphate and pyrethroids have been involved in toxicology of reactive oxygen species (Kale, 1999; Gultekin et aI., 2000; Karoz et ai., 2002; Oncu et aI., 2002; Gultekin et al., 2001; Bagchi 1995; Naqvi 1992; Gupta 1998; Manna, 2004).

The results indicated a significant reduction in glutathione transferase (GST) in pesticide therapy. The GST is a multi-component enzyme which has an important role in the detoxification of many xenobiotics and protects tissue against oxidative stress (Foumiere et al., 1992). GST is involved in the detoxification process by catalyzing a variety of electrophiles, including insecticides, which are easily disposed of by excretion, for less toxic conjugation. The decreased activity of GST shows the oxidative damage caused by the administration of pesticides. In the biological processes present in hepatic, phosphatases, mainly acid phosphatase (ACP), are important in detoxification, metabolism and energetic macromolecular biosynthesis. for different essential functions, they are critical enzymes. In both phosphatases the current study showed an increase in (ACP and ALP). Any interference with these enzymes would lead to biochemical damage and tissue and cellular function lesions (Khan et al., 200 I). A plasma or serum analysis of the activity of these fundamental liver function enzymes can be used to evaluate the integrity of tissues after exposure to certain toxicological substances (s). These enzymes are usually liver markers with a number of forms of disorders that affect the

functionality of the liver tissues that are above the homosexual limits (Uboh et al., 2007,2008,2009, 2010b).

This study has shown an increase in endosulfan, phorate and fenvalerate treated rats in both transaminases Aspartate transaminase (AST) and Alanine transaminase (ALT). Two of the most reliable hepatocellular injury or necrosis indicators are AST and ALT. Both are found in ALT, which is mostly present in the liver cytosol and are a more specific indicator of inflammation in the liver than the AST, also in conditions affecting other organs such than the heart, the skeletal muscles, the kidneys, the brain, the pancreas and the blood cells. The increase in AST and ALT suggests that the hepatocellular membrane has changed its permeability due to injury to the liver resulting in the release into the blood of soluble cytosol enzymes (Manna et aI., 2003). It was reported by Rahman et al. (2000), that increased phosphatases and transaminases in different tissues were due to increased plasma or cellular necrosis permeability, showing the stress conditions of the treated animals. The results of these reports were consistents with the report.

Results showed an increase in dehydrogenase activity in plasma of plagued rats. LDH activity is the switch to aerobic breathing from anaerobic glycolysis. The increased activity of LDH may result from a leakage in the enzyme and/or inhibition of the hepatocellular necrosis (Wang and Zhai, 1988). In groups treated with the pesticides the activities of gamma glutamyl transferase (GGT) have also been identified. GGT activity is considered an early indicator of human oxidative stress. The GGT induction can occur as an adaptation to protect cells against oxidative stress, allowing them to access more cystein and thereby increase intracellular glutathione. ALT, ALP and GGT enzyme activity levels represented brain, heart, liver, kidney and testis functional status. The serum content of the activity of the enzyme reflected the overall status of the animal in subjects such as toxins, for example. Infection or damage. The variation in enzyme activity is linked to cell damage intensity. Residue levels of pesticides in various tissues have been analyzed and quantified. Results show that the tissues in different concentrations accumulate residues. In endosulfan, phorate and fenvalerate treatment groups, the accumulation was maximal in the liver. Finally, with the exception of biochemical changes. Data from eosin and hematoxylin stained liver sections have also been verified. A clear inflammation in hepatocytes indicated that the frequency of hepatocellular damage

increased, with the increasing dose of pesticides in rats. The histopathological data also confirmed the serious toxicological effects in liver caused by these chemicals.

CHAPTER-6

CONCLUSION

The most important goal of Indian planning and policy remains farming development. Efficient pest management is one of the strategies for enhancing crop productivity as over 45% of annual food production loses due to infestations. In agriculture and urban landscapes, pesticides are often seen as quick, easy and cheap solutions to the management of weeds and insect pests. Pesticides also play an important role in maintaining control of numerous terrible diseases. These chemicals offer the best opportunity for those who juggle with risk-benefit equations because of the large benefits that people derive from pesticides. It is now clear, however, that exposure to chemical substances causes a range of human health problems both occupationally and ecologically. Contamination of pesticides poses significant environmental risks and non-target orgams that range from beneficial soils to insects, plants, fish and birds to microorganisms. Unlike common misunderstandings, the environment can be endlessly harmed even by herbicides. Weed killers can indeed be particularly difficult because they are used in relatively large quantities. Inherent difficulties exist with fully assessing the health risks of pesticides to human health. There are many human variables, for example: age, sex, race, socio-economic condition, diet, health status, etc., which all affect human exposure to pesticides. However, the effects of these variables are virtually unknown. The effects of low levels of pesticide exposure over the long term can be greatly influenced by concurrent exposure to other pesticides, as well as air, water, food and pharmaceuticals. Between 1976 and 2000, over 50,000 villagers were exposed to endosulphan, a persistent organic pesticide, in the district of Kasargod, Kerala, India, sprayed on the cashew plantings owned by the Indian Plaintiff Corporation. The weakening of rare diseases, like mental delays, cerebral paralysis, cancer, etc., affected more than 3000 people living near, upstream and downwind. The National Human Rights Commission (NHRC) ordered the National Institute of Employment Health (NIOH) to respond to the agitation of people and found that the symptoms were responsible for 'aerial exposure' to endosulfan.

In the area affected and in an uninfluenced area of Kasargod district, a health survey was conducted. In the affected area conditions have been found to be more severe, like congenital anomalies, a mental retardation, cancer and infertility. Kasargod is only one example of the Indian tragedy. Not only is India one of the largest pesticide users, it is also one of the biggest producers. The government in Kerala has banned its use indefinitely and has failed to cope

with the media war against endosulfan poisoning in Kasargod. However, the villagers and activists in Kerala fear that the ban can always be lifted. In various national and international forums Indian activists have tried to raise the issue of the endosulphan ban, but with little success. In Kerala, Karnataka, Andhra Pradesh, Punjab, and Assam, endosulfan is still in use in different parts of India. The tragedy of Kasargod has once again shown that India needs a pesticide policy that is pursued by health professionals rather than by the pesticide sector. In light of these situations, we have conceived our study to identify residual concentrations in the food commodities of the Trivandrum and Kasargod markets and water from the regions mentioned above. The results led us, by using rats as an animal model, to investigate the dangers in molecular animals of these chemicals.

Application of pesticides results in residues of agricultural products as well as contamination by leaching, percolation or rush of natural aquifers. Water samples were taken from Trivandrum and Kasargod, the southernmost and northernmost districts of Kerala, to evaluate the potential impact of the pesticides on aquatic ecosystems and drinking water supplies. In order to evaluate total food exposure, rice, fruit and vegetable samples were also collected from the above areas. The samples have been analyzed with ECDINPD detectors by gas chromatography. The analyzed matrices showed a wide range of pesticide residues and the residue levels found in many samples above the allowable FAO/WHO limits. The residues of pesticides can be found in both the areas of Trivandrum and Kasargod in water, rice, fruit and vegetables. There was more contamination in the district of Kasargod, but some of the pesticides were also highly concentrated in the district of Trivandrum. The study could also show the present status of exposure to hazardous pesticides by the local population of Kerala. 66.8% of samples showed contamination, of which 21.3% of samples showed results of pesticide residue levels above MRL, while 45.5% of the samples produced results with residue levels below the MRL. The monitored pesticides were not traceable at only 33.2% of the samples analysed. In addition the data show that the residues in water samples of the lake/stream are more than the residues from water samples of the well. This shows that pesticides are incorrectly applied and managed on crops near water sources.

75% of rice samples showed pesticide contamination, of which 28.5% of the samples reported results of pesticide residue above MRL, while 46% of the samples reported pesticide residue

below the MRL, with rice rice samples reported contamination above MRL. The level of pesticides monitored was only 24.6 per cent of the samples analyzed. In the same case as fruits and vegetables, 61% of the samples had been contaminated, with 18.7% of the samples having above-MRL pesticide residues levels, while 42.3% had below-MRL pesticide residue levels. The traceable levels of monitored pesticides were not present in only 39% of the samples analysed. The figures show that rice is the most contaminated type of food, followed by water and fruit and vegetables. The study shows also that organophosphates with pyrethroids and organochlorines are at their highest levels of contamination. Three commonly used pesticides from each of the principal insecticide VIZ groups, endosulfan (OC) phorate (organophosphates) and fenvalerate are selected for hepatotoxicity and molecular events induced by the pesticides (synthetic pyrethroids). The pesticide dose could be calculated on the basis of the data obtained from the analysis based on the residues that can be accumulated per year and in another 15 or 30 years. In order to study the toxicological effects of these pesticides, rats were given the corresponding concentrations (endosulfane, phorate and fenvalerate) of the pesticides selected. After 90 days of pesticide administration, biochemical parameters were observed and estimated in the liver and blood. In endosulfan, phorate and fenvalerate treated rats the levels of antioxidant enzymes SOD, CAT and GSH were decreased. The level of hepatotoxic liver markers like aminotransferases (ALT and AST) and phosphatases was increased by dose dependent (ACP and ALP). There was also a significant increase in other hepatotoxicity marker enzymes i.e. LDH and GGT activities. The cytochrome level P450 was also found to be increased and these pesticides could be turned into reactive mediums. The presence of an estimated high level of ROS has also confirmed this. Intracellular ROS generations are attributed to mitochondrial dysfunction and minor activities of the SOD, CAT, GSH and higher LPO antioxidant enzymes observed in rats exposed to pesticides in the liver. Histopathological changes also confirmed a significant structural damage to the liver tissue due to the pesticide administration.

In addition, by measuring DNA damages with the Comet test and alkaline elution method, the genotoxic potential of the selected pesticides was measured. The result shows that the administered pesticides can cause alterations in mammalian cells (ratts) in vivo, at least to a limited extent. The maximum damage to DNA has been caused by endosulfan. The potential inhibitory influence of these agents on the DnA repair system can be interpreted for the

genotoxic effect (DNA damage) of the pesticide treatments mentioned in this study. The damage caused by the pesticide application to the DNA is confirmed by measuring the DNA single-strands (DNA-SSB). Therefore, the mutagenic/genotoxic nature of these pesticides in rat liver is confirmed.

Molecular analyzes in rat liver have shown that apoptotic change can be caused by the pesticides under study. The intrinsic ROS generation was found to trigger pesticides, which caused oxidative damages to the entire cell. Our study showed that the in vivo use of pesticides can increase p53 expression significantly and suggests that the expression of p53 plays a key role in the apoptosis process. In the pesticide-induced apoptosis, therefore, both phosphorylation status and p5,53 expression levels can play a major role. Caspase 3 activity in rat liver was measured in order to measure the role of pesticides in the activation of the caspase pathways. Caspase 8 or 10, followed by caspase 3 activation is known to be activated on the cell surface via the death-receptor mediated activation. Caspase 9, which is considered one of the leading initiators, and case 3 as an effector caspase can be amplified with this pathway. The induction of caspase 3 therefore confirms the apoptotic induction of mitochondria by pesticides.

The high levels of expression of Bax, while the expressions of the Bel2 proteins decrease at the same time, also confirmed the role of pesticides. In the treated groups, cytochrome c has also been found to be high. In pesticide exposed cells, dose-dependent increases in the translocation of Bax's protein from cytosol to mitochondria and the cytochrome-c protein were observed. These findings confirm the induction of cytoplasmic p53 and its role in the permeability of the mitochondrial membrane in cells. The improvements in prapoptotic proteins have confirmed the effect on molecular molecular cell death of endosulfan, phorate and fenvalerate. Because of our experimental results, we proposed the possibility that cellular oxidative stress, DNA damages and intrinsic apoptosis pathway in the rat liver will be explained when exposed to pesticides. We proposed a hypothetical model pathway. The pesticides could trigger inherent ROS generation, which then caused oxidative damage, as shown in the pathway. An increase in ROS levels can lead to DNA damage that can increase the tumed p53 protein, elevate Bax and attenuate Bel2 protein, resulting in an improved Bel-2/Bax ratio. Changing the Bel-2/Bax ratio led to cytochromium c release. Finally, the

cytochrome c formed Apaf-l and caspase-9 to activate the apoptosome complex caspase-3. The molecular mechanism involved in hepatotoxicity of pesticides could explain this pathway.

Key findings of the study

- The study presents the dietary exposure assessment ofthe local population to the residues of fifteen commonly encountered pesticides under four pesticide groups, namely organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), carbamates, and pyrethroids.
- As expected, the residues of all four groups of pesticides analyzed were found at noticeable levels in water and food samples such as rice, vegetables and fruits. About 70% of the samples were found to be contaminated by pesticide residues in which around 25% of the samples showed values higher than the permitted limit. The study concluded that dietary exposures to the pesticide residues analysed would be likely to pose unacceptable health hazards.
- The toxicological data demonstrate that the pesticides endosulfan, phorate and fenvalerate could induce hepatotoxicity, dose dependently. The comet assay also confirmed that the pesticides have the potential to enhance genotoxicity.
- The apoptotic changes induced by the pesticides showed their potential to bring about apoptotic cell death.

We hope to increase awareness about the danger; for human beings, for wildlife and for the environment, there are pesticides. Based on the findings above, the results highlight the crucial need, in order to protect consumers from pesticides exposure, to continue surveying and monitoring programs for pesticide in all food commodities.

Recommendations

Integrated pest management (IPM)

The integrated pest management (IPM) approach, being promoted since 1985, is an eco-friendly strategy of pest containment by exploiting the role of natural agents/forces in harmony with other pest management tactics and with the sole aim to effect minimum disturbance to environment.

Organic farming

The key principles and practices of organic food production alms to encourage and enhance biological cycles within farming systems to maintain and increase long time fertility of soil, to minimize all forms of pollution caused by fertilizers and pesticides and to produce food of high quality in sufficient quantity. However, this process is costly, labor intensive, and in some cases ineffective.

Bio pesticides

There are numerous bio pesticides that can be grown and mixed by farmers with comparably no risk to health. These include larvaticides formulated using aloe extract; repellents using plants like lemongrass or coriander, or cow urine or buttermilk solutions; and insecticides using ginger and chilli. Farmers can even formulate botanical rodenticides using Neem, Gliricidia or the leaves and unripe fruit of papaya plants.

CHAPTER-7

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