Chapter 1
INTRODUCTION
1. General Introduction

Pesticides are chemicals which are used for crop protection. Modern crop protection not only comprises the use of pesticides but also the introduction of insecticides, fungicides and herbicides. Since ancient times agriculture has been facing several catastrophes, which are increasing every day. Cramer (1) has published the most comprehensive and careful estimate of crop losses due to pests and pathogens. Severe crop losses reflected in the social and economic consequences. Chemical crop protection became established under the pressure of catastrophes. Intensification of agriculture also means intensification of attack by pests and pathogens. Mono-cultivation of plants which are often bred for the highest yields, offer environmental conditions which can lead to an explosive outbreak of pests. From all types of cultivation, cases are known where immediate measures are called for to protect the harvest. Chemical crop protection offers the possibility of preventing a catastrophe or of obtaining a rapid increase in the harvest, in so far as pests or pathogens are the limiting factors.

Planned increase of the yield per unit area requires additional measures. Varieties cultivated to give high yields are often particularly susceptible to pathogens, so that full yield can only be achieved with the aid of chemical crop protection. The yield achieved can only be assured when protective measures for both plant and the harvest itself are taken. Success depends on purposeful combination of agricultural methods, i.e., choice of variety, fertilization, use of insecticides, fungicides and herbicides. The strategic significance of crop protection mainly depends on these aspects.

Some of the early pesticides are inorganic compounds such as copper sulphate, lead arsenate, sodium fluoride and lime-sulphur mixtures and organic compounds such as pyrethrum and nicotine. As the control of pests has been found to be easy with the pesticides there has been an explosion and growing demand in the development of organic pesticides for food production. Some of these compounds have found use in the other fields such as human and veterinary medicine. For example, phosphonomycin, a herbicide and used as a growth regulator, finds application even as an antibiotic. However, care must be taken in practical use of these pesticides, as the use of these are associated with negative consequences, resulting from their toxicity and persistence in the environment.
1.2. Classification of pesticides

Pesticides can be broadly classified according to their function as insecticides, fungicides, herbicides and other specific pesticides which include rodenticides, molluscicides and nematocides. Based on the chemical composition and structure, pesticides can be classified into chlorinated hydrocarbons, chlorophenoxy acids, organic phosphates and carbamates. (3 - 7)

1.2.1 Chlorinated hydrocarbons

Diphenyl aliphatics

Chlorinated hydrocarbons are extensively used in agriculture and public health. These compounds are highly toxic and attacks the nervous system of the target organism. Some of the inherent disadvantages with these compounds are that they are more persistent in the environment when compared with other pesticides. Use of these compounds is restricted or banned nowadays, due to their toxic effects on various non target organisms.

\[
\begin{align*}
  &\text{Cl} \quad \begin{array}{c}
    \text{CH} \\
    \text{Cl}
  \end{array} \\
  \quad &\text{CCl}_3
\end{align*}
\]

**DDT**

The mechanism by which hydrocarbons exert their toxic effect is believed to be due to their dissolution in the fatty membrane surrounding nerve fibers resulting in the distortion of the membrane and interfering with the transport of sodium ion in and out of the nerve fiber. Metcalf (2) extensively reviewed, starting from the historical development, the role of DDT and other chlorohydrocarbons and their impact on various related parameters on environment. Because of their persistence in the environment, resistance that developed in several soil insects, and in some instances biomagnification in wildlife food chains, most agricultural uses of DDT and other related compounds of this category were cancelled.
Hexachlorocyclohexane (HCH)

Hexachlorocyclohexane is also known as benzene hexachloride. Five isomers of hexachlorocyclohexane, alpha, beta, gamma, delta and epsilon are known, among which only gamma isomer has insecticidal properties. Due to the reason, the gamma isomer is isolated in manufacture and marketed as odourless insecticide lindane. The effects of HCH resemble those of DDT, but occur much more rapidly, and results in a much higher rate of respiration in insects. The gamma isomer is a neurotoxicant whose effects are normally seen with in hours as increased activity, tremors and convulsions leading to prostration. It too, exhibits a negative temperature coefficient, but not as pronounced as that of DDT.

Cyclodienes

Cyclodienes are persistent insecticides and are stable in soil and relatively stable to UV light. They were used in large quantities as soil insecticides for the control of termites and soil borne insects whose larval stages feed on the roots of plants. Because of their persistence in the environment, resistance that developed in several soil insects, and in some instances biomagnification in wildlife food chains, most agricultural uses of cyclodienes were cancelled.

Unlike DDT and HCH, the cyclodienes have a positive temperature correlation. This group of insecticides acts on the inhibitory mechanism called the GABA (gamma-amino butyric acid) receptor. The receptor operates by increasing chloride ion
permeability of neurons. Cyclodiencs prevent chloride ions from entering the neurons, and thereby antagonize the calming effects of GABA.

**Pollychloro terpenes**

Only two polychloro terpenes were developed – toxaphene and strobane. Toxaphene was used in combination with DDT and later with methyl parathion. Toxaphene is a mixture of more than 177, 10 carbon polychlorinated derivatives. These materials persist in soils and disappear from the surfaces of plants in 3 – 4 weeks. This disappearance is attributed more to volatility rather than to photolysis or in plant metabolism. Toxaphene is easily metabolized by mammals and birds, and is not stored in body fat to the extent of DDT, HCH and cyclodiene.

Toxaphene and strobame act on the neurons, causing an imbalance in sodium and potassium ions.

![Toxaphene: A Mixture of Chlorinated Camphene](image)

1.a.2. Organophosphates

All organophosphates are derived from one of the phosphoric acids, and as a class are the most toxic of all the pesticides to vertebrates. The organic phosphates have two distinctive features: (i) They are much more toxic to vertebrates than other classes of insecticides, and (ii) most of them are chemically unstable or non-persistent and hence they are brought into agricultural use as substitutes for chloro organic pesticides.

The organophosphates work by tying up or inhibiting certain important enzymes of the nervous system, namely cholinesterase (ChE). The enzyme gets phosphorylated when it is attached irreversibly to phosphorous moiety of the insecticide, which results in the accumulation of acetylcholine (Ach) at the neuron/neuron junctions, causing rapid twitching of voluntary muscles and finally paralysis.

All organophosphates have various combination of O, C, S and N attached, resulting in six different subclasses: phosphates, phosphonates, phosphorothionates,
phosphonodithiocates, phosphorothiolates and phosphoramidates. Organic phosphates are divided into three groups - aliphatic, phenyl and heterocyclic derivatives.

The aliphatic organophosphates are carbon chain like structure. The first organophosphate to be used in agriculture, TEEP belonged to this group. Other examples are malathion, trichlorfon (Dylox(r)), monochlorophos (Azodrin (r)), dimethoate (Cyon (r)), oxydemetonmethyl (Metasystox (r)), dicrotophos (Bidrin (r)), disulfoton (Di-Syston(r)), dichlorvos (Vapona (r)), mevinphos (Phosdrin (r)) and acephate (Orthene (r)).

\[
\text{Malathion}
\]

The phenyl organic phosphates contain a phenyl ring with one of the ring hydrogens displaced by attachment to the phosphorous moiety and the other hydrogen replaced by Cl, NO₂, CH₃, CS or S. These compounds are generally more stable than aliphatics and hence their residues are long lasting. First ever phenyl organophosphorous pesticide was parathion (ethyl parathion), and subsequently, methyl parathion, profenophos (Curacron (r)), sulprofos (bolster (r)), isophenphos (Otenol (r)), Prfyn (r), fenitrothion (Sumithion (r)) and fampur (Cyflee (r), Warbex (r)) were introduced.

\[
\text{Ethyl-Parathion}
\]

Organophosphorous compounds with a heterocyclic ring have some advantages in specific purposes.
1.a.3. Organosulphur pesticides

Organosulphur pesticides are characterized by very low toxicity to insects and are used only as acaricides. They contain two phenyl rings, similar to DDT, with S in place of C as the central atom. These include tetr difon (Tedion (r)), propargite (Omite (r)) and ovex (Ovotron).

1.a.4. Carbamates

The carbamate insecticides are derivatives of carbamic acid. The first successful carbamate insecticide introduced was carbaryl. Carbaryl has been used more than all the other carbamates combined. The distinctive qualities that made carbaryl popular are, 1) its very low mammalian oral and dermal toxicity and 2) exceptionally broad spectrum of insect control. Other carbamate insecticides are, methomyl (Lannate (r)), carbofuran (Furadan (r)), propoxur (baygon (r)), bendiocarb (Ficam (r)), carbosulfan (Advantage (r)), promecarb and fenoxycarb. Carbamates inhibit cholinesterase as organophosphates do, and they behave almost identical manner in biological systems. The difference being, some carbamates are potent inhibitors of acetylcholinesterase and also that the ChE inhibition by carbamates is reversible.
1.5 Formimidines

The formimidines comprise a small group of insecticides. The examples are chlorodimeform (Galceneron(r)), fermetenate (Cazzeol (r)) and amitraz (Mitac (r)). Their current value lies in the control of organophosphate and carbamate resistant pests. Poisoning mechanism of formimidines is different from other insecticides. Formimidines inhibit the enzyme monoamine oxidase, which results in the accumulation of these compounds, known as biogenic amines. Affected insects become quiescent.

1.6. Dinitrophenols

The dinitrophenol molecule has a broad range of toxicities and hence can act as herbicide, insecticide, ovicide and fungicide. However, because of the inherent toxicity of these compounds, they have all been withdrawn from the use as pesticides.

1.7 Organotins

The organotins are a group of acaricides which can also act as fungicides. Cyhexatin is one of the most selective acaricides known and has been used extensively against mites on deciduous fruits, citrus, green house crops and ornamentals.
The tin compounds inhibit oxidative phosphorylation at the site of dinitrophenol uncoupling, preventing the formation of high energy phosphate molecule ATP. These trialkyltinls also inhibit photophosphorylation in chloroplasts, and could therefore serve as algicidies.

1.4.8. Pyrethroids

Natural pyrethrum is not useful for agricultural purposes because of its cost and instability in sunlight. Many synthetic pyrethroids are now available, which do not have these shortcomings. The first generation pyrethroid, allethrin (Pyanin (r)) involves a very complex 22 step chemical reactions to reach the final product. The second generation include, tetramethrin (Neo-Pyanin (r)), resmethrin (Synthrin (r)), bioresmethrin (50X), Bioallethrin (r) and phenothrin (Sumithrin (r)). The third generation pyrethroids are fenvalerate (Pydrin (r)); discontinued, Tribute (r) & Bellmark (r) and Permethrin. This group of pyrethroids have exceptional photostability and insecticidal activity (0.1 lb ai/A). The fourth and current generation, have their effectiveness in the range of 0.01 to 0.05 lb ai/A. Recent addition to fourth generation pyrethroids are aceathrin and imiprothrin (Falle (r)).

The pyrethroids exhibit similar action to that of DDT, and are considered axonic poisons. There are two groups of pyrethroids. Type I, among other physiological responses, have a negative temperature coefficient, resembling DDT. Type II, in contrast have a positive temperature coefficient. Pyrethroids affect both the peripheral and central nervous system of the pests.
1.a.9. Nicotinoids

The nicotinoids are a new class of insecticides with a new mode of action. Imidacloprid was introduced recently and is used as an effective pesticide to a great extent. It is also a systemic insecticide, having good root-systemic characteristics and notable contact and stomach action. It is used for soil, seed or foliar treatment in cotton, rice, potatoes, vegetables, for the control of sucking insects, soil insects, whiteflies, termites, turf insects, with long residual control. The nicotinoids act on the central nervous system of insects, causing irreversible blockage of postsynaptic nicotinergic acetylcholine receptors.

**IMIDACLOPRID (Gaucho®, Provado®)**

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\begin{center}
\begin{tikzpicture}
\begin{scope}[scale=0.8]
\node (n1) at (0,0) {$\text{N}$};
\node (n2) at (1,0) {$\text{N}$};
\node (n3) at (1,-0.5) {$\text{Cl}$};
\node (n4) at (0,-0.5) {$\text{CH}_2$};
\node (n5) at (2,0) {$\text{OH}$};
\node (n6) at (2,-0.5) {$\text{CH}_3$};
\node (n7) at (3,0) {$\text{NO}_2$};
\end{scope}
\end{tikzpicture}
\end{center}
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1.a.10 Spinosyns

Spinosyns are the newest class of insecticides, represented by spinosad, a fermentation metabolite of the actinomycete *Saccharopolyspora spinosa*, a soil-inhabiting microorganisms. Spinosad is effective as a broad spectrum material for most caterpillar pests at the rate 18 to 40g/acre. It has both contact and stomach activity against lepidopteran larvae, leaf miners, thrips and termites, with long residual activity. Spinosad acts by disrupting binding of acetylcholine in nicotine acetylcholine receptors at the postsynaptic cell. (8)
1.a.11. Fiproles (Phenylpyrazoles)

The only insecticide belonging to this class is Fipronil, a systemic material with contact and stomach activity. It is used for the control of many soil and foliar insects and also for the seed treatment and a bait for cockroaches and termites. Fipronil is effective against insects resistant to pyrethroid, organophosphate and carbamate insecticides. Fipronil blocks the gamma-aminobutyric acid (GABA) regulated chloride channel in neurons, antagonizing the calming effects of GABA.

\[ \text{FIPRONIL (Frontline®, Icon®, Regent®)} \]

\[ \text{5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-3-4-}
\text{cyano-4-trifluoromethylsulphonylpyrazole} \]

1.a.12 Pyrroles

Chlorfenapyr is the only member of this unique chemical group. It is used as contact and stomach insecticide-miticide. It is useful for the control of whitefly, thrips, caterpillars and has ovicidal activity on some species. Chlorfenapyr is an uncoupler or inhibitor of phosphorylation which prevents the formation of ATP.

\[ \text{CHLORFENAPYR (Pirate®, Alert®)} \]

\[ 4\text{-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-}
\text{trifluoromethylpyrrole-3-carbonitrile} \]
1.13. Pyrazoles

The pyrazoles, represented by tebufenpyrad and fenpyroximate were designed primarily as non-systemic contact and stomach miticides. These compounds can control all stages of mites, give fast knockdown, inhibit molting of immature stages of mites and have long residual activity. Their mode of action is that of inhibiting mitochondrial electron transport at the NADH-CoQ reductase site, leading to the disruption of ATP formation.

**TEBUFENPYRAD (Pyranica®, Maaal®)**

\[
\begin{align*}
\text{C}_2\text{H}_5 & \quad \text{Cl} \\
\text{NC} & \quad \text{CH}_2\text{-NH-CH}_2\text{-} \\
\\text{CH}_3 & \quad \text{C(CH}_3)_3
\end{align*}
\]

4-chloro-N[4-(1,1-dimethylethyl)phenyl][methyl]-3-ethyl-1-methyl-1H-pyrazole-5-carboxamide

1.14. Pyridazinones

Pyridaben is the representative of this group, is effective against thrips, aphids, and provides exceptionally long residual control and rapid knock down at a broad range of temperatures. Pyridaben is a metabolic inhibitor that interrupts mitochondrial electron transport at Site 1.

**PYRIDABEN (Nexter®, Sanmite®)**

\[
\begin{align*}
\text{O} & \quad \text{Cl} \\
\text{N-CH}_2\text{-CH}_2\text{-} & \quad \text{CH}_3 \\
\text{S-CH}_2\text{-CH}_2\text{-} & \quad \text{C(CH}_3)_3
\end{align*}
\]

2-(tert-butyl)-5-(4-tert-butylbenzylthio)-4-chloropyridazin-3(2H)-one

1.15. Quinazolines

The quinazoline group consist of only one insecticide, fenazaquin, a contact and stomach miticide, and has a biological activity similar to pyridazinones.
Benzoylureas are an entirely different class of insecticides that act as insect growth regulators, by interfering with chitin synthesis and are taken up more by ingestion than by contact. Their greatest value is in the control of caterpillars and beetle larvae. Benzoylurea class of pesticides, for example, diflubenzuron, are useful when the pests develop resistance to other pesticides, including pyrethroids. The benzoylureas act on the larval stage of most insects by inhibiting or blocking the synthesis of chitin. Typical effects on developing larvae are the rupture of malformed cuticle or death by starvation. Adult female boll weevils exposed to diflubenzuron lay eggs that do not hatch. Mosquito larvae control can be achieved with as little as 1.0g of diflubenzuron per acre of surface water.

**DIFLUBENZURON (Dimilin®)**

Botanical insecticides are natural products, toxicants derived from plants. Tobacco, pyrethrum, derris, hellebore, quassia, camphor and turpentine were the important plant products used before the organized search for the insecticides began. Use of botanical insecticides though climbed a peak for some time, has steadily declined, and pyrethrum is now the only botanical of significance still in use. Nicotine, extracted by
several methods from tobacco, rotenone produced in the roots of two genera of the 
legume family: *Derris* and *Lanneocarpus*, limonone or *d*-limonene extracted from the 
citrus peel and the oil extracted from the neem seeds are the other natural products that 
are useful as pesticides/insecticides.

\[
\text{CH}_3
\]

\[
\text{H}_3\text{C} \quad \text{C} \quad \text{CH}_2
\]

Limonene

1.a.18. Synergists (or activators)

Synergists by themselves are considered toxic or insecticidal. They are the 
material used with insecticides to enhance the activity. Synergists, such as piperonyl 
butoxide contain the methylenedioxyphenyl moiety, a molecule found in sesame oil. The 
synergists inhibit cytochrome P-450 dependent polysubstrate monooxygenases (PSMO), 
enzymes produced by microsomes, the subcellular units found in the level of mammals 
and in some insect tissues. PSMO bind the enzymes that degrade selected foreign 
substances, such as pyrethrum, allethrin, resmethrin or any other synergized compound. 
Synergists simply bind the oxidative enzymes and prevent them from degrading the 
toxicant.

\[
\text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O}
\]

Piperonyl Butoxide

1.a.19. Antibiotics

In this category are included the *avermectins*, which are insecticidal and 
antihelminthic agents that have been isolated from fermentation products of *Streptomyces 
avermitilis*, a member of the actinomycete family. *Abamectin* is the common name 
assigned to the avermectins, a mixture containing 80% avermectin B1a and 20% B1b,
homologs that have about equal biological activity. Clinch (t) is a fire ant bait, and Avid(t) is applied as a miticide/insecticide. Abamectin has certain local systemic qualities, permitting it to kill mites on a leaf underside when only the upper surface is treated. Shortly after exposure, larvae stop feeding and become irreversibly paralysed and die after 3–4 days. Rapid photo-degradation of abamectin occurs on the leaf surface. Avermectins block the neurotransmitter gamma aminobutyric acid (GABA) at the neuromuscular junction in insects and mites.

1.a.20. Fumigants

The fumigants are small, volatile organic molecules. They are usually heavier than air and may contain one or more of the halogens, Cl, Br or F. They are used to kill insects, insect eggs, nematodes and certain microorganisms in warehouses, grain elevators, soils and greenhouses and packaged products. Methyl bromide is the most used of the fumigants, and others being ethylene dichloride, hydrogen cyanide, sulfoxyl chloride, chloroethane, ethylene oxide and the familiar home use moth repellents naphthalene crystals and paradichlorobenzene crystals. Phosphine gas has replaced methyl bromide in few applications.

Fumigants are narcotics and are lipid soluble, have common symptomology; their effects are reversible; and their activity is altered very little by structural changes in their molecules. Liposolubility is an important factor in their action, since these narcotics lodge in lipid containing tissues found throughout the insect body, including the nervous system.

1.a.21. Insect repellents

Before a systematic approach to insect olfaction and behaviour was developed, it was assumed that substances repugnant to man would also be repellent to annoying
insects. Earlier repellents included smoke, plants hung in dwellings or rubbed on the skin, oils, pitches, tars and various earths applied to the body. Among the recent repellents only Diethyl toluamide (DEET) survived, and is used for biting flies and mosquitoes.

\[
\begin{align*}
\text{DEET-} & N,N\text{-diethyl-m-toluamide} \\
\end{align*}
\]

1.a.22. Inorganics

Inorganic insecticides are those that do not contain carbon. They are stable chemicals, do not evaporate and are usually water soluble. Inorganic compounds used as insecticides are sulphur, mercury, boron, thallium, arsenic, antimony, selenium and fluoride.

Arsenicals include copper arsenate, Paris green, lead arsenate and calcium arsenate. The arsenicals uncouple oxidative phosphorylation, inhibit certain enzymes that contain sulfhydryl (-SH) groups, and coagulate protein.

The inorganic fluorides include, sodium fluoride, barium fluosilicate, sodium silicofluoride and cryolite. The fluoride ion inhibits many enzymes that contain iron, calcium and magnesium.

Boric acid is used against cockroaches and other crawling household pests. Being a salt which is nonvolatile it will remain effective as long as it is kept dry and in adequate concentration. It has the longest residual activity. Sodium borate resembles boric acid in its action.

Silica gels or silica aero gels, light, white, fluffy silicate dusts are used for household pest control. The silica aero gels kill insects by absorbing waxes from the insect cuticle, permitting the continuous loss of water from the insect body. Silica preparations are generally fortified with pyrethrum and synergists.

1.a.23. Miscellaneous compounds

New molecular structures are being synthesized routinely by manufacturers with a view to develop insecticides with new modes of action. Two such compounds are
pyriproxyfen (Knack(r)), classified as pyridine IGR and buprofezin (Applaud (r)). Clofentazine belongs to the unique group, the tetrazines, used as an acaricide/ovicide. Sodium tetrathiocarbonate is employed as the water application and irrigated into the soil. It breaks down in the soil to form carbon disulphide, which acts rapidly and decomposes quickly. Cladosan (r) is a naturally occurring product derived from crab and shrimp shells and used as a nematicide.

![Buprofezin](image)

CLOFENTEZINE (Apollo®, Acaristop®)

3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine

CLOFENTEZINE (Apollo®, Acaristop®)

3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine

1.b. The drawbacks of chemical control of pests

When DDT was first discovered, it was felt that all insect pest problems were solved. This presumption was based on the chemical’s toxicity and its greatest stability. It was very soon realized that not only DDT, but many others like dieldrin and other cyclodienes and organophosphates have lost effectiveness against many pests, due to insects showing marked resistance to these chemicals.

Positive and negative aspects of pesticides

It is universally accepted that pests have become indispensable for higher yield from crops, protecting forests and horticulture, ensuring public and animal health, safekeeping of harvest in stores and warehouses and house hold articles from damage caused by weeds, insect pests and diseases. However, indiscriminate use of pesticides and not adhering to the recommendations with respect to dosage and period of application of the insecticides are supposed to give negative results. Crop damage,
damage to non-target organisms, resistance of pests to pesticides and residuals left over in the produce beyond tolerance limits are some of the factors which require careful attention. Other factors include, persistence of pesticides in the environment, secondary effects of pesticides on various environments and consequential toxicity. These factors are apparent from the fact that some of the pesticides considered to be safe and effective are removed from application by Law.

Resistence of pests to pesticides

The development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to majority of individuals in a normal population of the same species. One public health insect, the house fly, was known to be resistant in some populations but gradually increased to 5 – 11 species to one or more of the insecticides, and further the number has increased to 102 species; 55 to DDT, 84 to chlordrin and 17 to organophosphorous compounds, as time progressed (9, 10 ). Some insects have developed resistance to all three kinds of pesticides. Development of resistance has led to great difficulties in both vector control and agriculture. Replacement of one pesticide by the other can yield only a temporary solution to the problem as explained by Brown and Pal (11). Resistance to organophosphates and carbamates involves the hydrolysis of the pesticide by insects and detoxification by non-specific esterases. Resistance makes it difficult (12). Resistance, on the other hand prompts the usage of higher dosage of pesticides and results in the accumulation of toxic chemicals in the environment. Finally, it can be said that it is highly unlikely that insects can be controlled exclusively with synthetic insecticides.

1.b.1. Effects of pests on agricultural ecosystem

A pest in a crop is only a part of a complicated ecosystem. The whole entity of the crop forms an integrated community with interaction between various species which reduce the possibility of any one of them increasing explosively. Even the pest species will be regulated by natural enemies to some extent. Further, there are several potential pests which have never caused important damage because their populations have always been kept low by density dependent mechanisms.

It is always impossible to predict all the consequences of the application of pests to such an ecosystem. Pesticides are always applied to the ecosystem, and not merely to
pests (13). It is sure that there will be a big fall in the number of target pest, but number of other organism will also be destroyed, among which will be some of the species which will help reduce the population density of pests. Parasites and predators of insect and mite pests are more easily killed by pesticides. The use of insecticides is started for treatment of symptoms of trouble, and likely to be ended up with having little effect on the cause. The longer the usage of the pesticides, the harder it will become to stop as it could take several seasons for the natural enemies to re-establish themselves, if the treatment covers a wide area. The development of resistance aggravates the problem as the amount of pesticide used must be continuously increased until a intolerable limit is reached (15,16).

1.b.2. Toxicity of pesticides to non-target organisms

A review of the ecological effects of pesticides on non-target organisms has been brought about by Pimental (14). DDT has been reported to be highly toxic to aquatic environment and the residue burden in human beings was implicated as one of the causes for increased incidence of many diseases such as cancer, still births, cardiac arrest and infertility. Unregulated and improper use of pesticides could pose health hazard to people involved in the manufacture, handling and application in the fields. Butler (15) showed that chlorinated hydrocarbons interfere with the photosynthesis in algae and oyster growth is highly affected by the presence of even low concentrations of DDT. The chlorinated hydrocarbons accumulate in the reproductive organs of fish and cause adverse effects on reproduction (16-20). The presence of DDT and dieldrin in aquatic environment causes lethal and sublethal effects to a wide range of species. The sublethal effects cause brain damage and the residuals are capable of interfering with calcium metabolism of birds. The essential carbamie anhydride enzyme, which essential for egg formation, is inhibited in brown pelican, Bermuda petal, bald eagle and results in the decrease of reproduction rate (21-23).

There are evidences to indicate that organophosphate pesticides, diazinon, parathion and phosphamidon are more toxic to birds than chlorinated hydrocarbons (24).

The carbamates insecticides exhibit a wide range of mammalian toxicities. Carbaryl and natum are less toxic to fish as they degrade very rapidly in water due to hydrolysis. Carbofuran is found to be highly toxic to mammals, (25) whereas Bendiocarb
and zectran are found to be toxic to pheasant, black birds, mallard ducks and house sparrows (26).

There are no reports of chronic toxicity of chlorinated insecticide to man, but there are some indications for the existence. It is observed that pesticides induce high levels of liver enzymes in wide range of vertebrates including monkey. It seems very likely that such induction will be observed in man as well.

1.b.3. Possible effects on mankind

Study of the effect of pesticide pollution on non-target species, particularly on mankind, especially the danger of residues in the food is important. Thus there is control over the use of pesticides and these are reviewed very frequently. An indication of the possible long term dangers of the pesticides can be obtained from a study of people who are exposed to them as part of their work either during manufacture or during application. Further, the problem of food contamination and environmental pollution is complicated by the presence of residues of more than one kind of chemical. As the number of possible combinations becomes large, chance of harmful effects increases through synergic action. It is obvious that any insecticide or pesticide is undesirable in human tissue. However, the possible disadvantages in their use must be balanced against known advantages. The ill effects of pesticides have been summarized by several authors (27,28).

1.b.4. Effects of pesticides on general environment

The primary concern about the pesticide is for their possible effects on health and precautions to be taken to avoid both acute and chronic poisoning. Apart from these aspects there may be greater danger to many other systems of our environment.

The fate of pesticides after internal application, or after contamination by drift, depend on several factors, namely soil type, moisture and temperature, wind or air movement, cropping and cultivation, the method of application to the soil and soil microorganisms (29). Newsom (30) in his review of the consequences of insecticide use on non-target organisms reports that rates of application usually recommended do not lead to the accumulation of large amounts of residues in soils, and in most cases the amounts would not exceed the quantities applied in one year. Edward et al., (31) have studied the effect of various soil pollutants on the small soil-dwelling invertebrates. One
of the consequences of insecticide treatment is the increase in the number of some species of mites, pauropods. Fungicides containing heavy metals such as mercury or copper may contaminate the soil.

1.6.5. Minimizing persistent pesticides in the environment

If alternative methods of pest control are not found and the existing pesticides are continued to be used at the present rates, further accumulations in the physical and biological environments will continue. Many pesticides and insecticides have been developed, some of them are less persistent. There has been a continuous search for such chemicals, since restrictions are placed on the application of DDT, aldrin and dieldrin. New organophosphates and carbamates were developed, with a persistence of less than one year. Apart from being advantageous from the point of view of persistence, they have the additional advantage in that they do not accumulate in animal and plant tissues as do organochlorines do. However, many of them have higher mammalian toxicities and are more hazardous to the pesticide operator. A few pests such as chafer and rootworms are difficult to control with non-persistent pesticides. The main problem of finding alternatives to persistent pesticides is the cost factor. Metcalf (32) suggested the replacement of most persistent organochlorines with biodegradable analogues.

Many problems arise because of the careless use of the persistent pesticides. Efficient methods for the application, such as spraying and incorporation in the soil where actually required will reduce the quantity of pesticide applied. Adsorption of inert granules and enclosing in microcapsules will increase the life of the pesticide in the soil by controlling the rate of release of the pesticide.

1.6.6. Removal of pesticides

Once insecticides get thoroughly incorporated into soil, they are adsorbed onto soil fractions and persist much longer than if they merely lie on the soil surface. Cultivating practices should, hence hold the key for persistence period control. Lichtenstein (33) explored the possibility of inactivating residues of organochlorine in soil by absorbing them on activated carbon.

Microorganisms are very important in breaking down persistent insecticides in soil. Particular microorganisms cause much more insecticide degradation than others.
Guenzi and Beadle (34) showed that DDT broke down to DDD much more rapidly under an aerobic condition and even faster if 1,000 ppm ground alpha foliage was added to soil. Kearney, (35) Yoshida and Castro (36) Tsukano and Kobayashi (37) reported that DDT and γ-BHC broke down much more rapidly in flooded than in non-flooded rice fields. A non-toxic chemical that causes DDT to decompose several weeks after the application was developed. The resulting compound was only one tenth as toxic as DDT.

If the drinking water gets contaminated, the pesticides are to be removed. Robbeck (38) tested the effectiveness of various filtering methods for the removal of organochlorine pesticides and found that conventional treatment with coagulation, followed by sand filtration, varied in effectiveness. Softening with lime and soda ash with an iron salt as coagulant did not improve the results. Treatment of permanganate or chlorine had no effect on the insecticides. At large and impracticable concentrations, ozone decreased organochlorine insecticides in water. The addition of activated carbon greatly decreased the amounts of insecticides in water, but the amount of carbon required is very large (39). Kearney (40) suggested irradiation with UV or γ-radiation to effectively destroy many pesticides, particularly in dilute aqueous solutions.

Run off from agricultural land is an important source of pesticides in water and control of run off will be one way of decreasing contamination of water.

It is also important to minimize the pesticides in food for human beings. A considerable portion of the pesticides in food of plant origin can be removed by processing; washing by warm water and adding detergents to wash water, peeling or heating. Lessening the concentration of insecticides residues in the bodies of livestock is more difficult, but has been achieved for dieldrin by feeding cattle with charcoal. Certain drugs, barbiturates, have also increased the excretion of DDT from cattle (41).

1.b.7. Substitutes for pesticides

It is possible in some instances, to control pests without the use of pesticides adopting methods such as, timing of sowing dates, timing of harvesting, rotating crops, using additional cultivation to kill soil pests and the use of resistant crop varieties (42).

Biological control of pests has attracted attention for many years. However, there are several limitations on achieving the classical form of biological control of pests, which consists of introducing a hymenial biological agent into the territory of the pest.
One form of biological pest control that has been partly successful against a variety of pests is the breeding of varieties of crop plants that resist attack. The selection of such stocks is a slow process and the most resistant varieties are not high yielding.

Insects are very sensitive to odours (43) and a very small quantity of an attractant can be used to attract them to traps. The attractants are usually food or sex based, are effective to long distances, highly specific and attract a few closely related species and often only males. A phenolic attractant has helped to control chafers.

Use of chemosterilants on insects that are trapped by attractants and then releasing sterilized insects can also be adopted with success. Breeding insects and using radiation to sterilize insects are being investigated (44).

Biological pesticides, which depend on the dissemination of spores of pathogenic microorganisms, yielded numerous pathogens that are suitable for control of pests.

1.b.8. Establishment of tolerance limits

One of the most successful techniques in the prevention of the spread of pests have been the use of quarantine methods to prevent the entry of new pests into the areas previously free from attack. Such a step is possible only by legislation and restriction, and was successful in many instances.

Governments have formulated rulings as to amounts of poisonous chemicals permitted in foodstuffs. As the toxicity and persistence data of a particular pesticide becomes available, these regulations are constantly revised. However, there are wide divergence of opinion among pharmacologists and toxicologists as to the hazard of any pesticide, which will be reflected in the variation of tolerances set on various pesticides by different countries. Tolerances are expressed as the maximum dose level (ppm in the diet) that cause no deleterious effect in the most sensitive species of animal, average body weight of a man, a safety factor of 20 to 1,000 and the weight of the average individual that may consume residues. In setting such tolerance limits very wide safety margins, up to 100 times, are incorporated to account for the appearance of one pesticide in several foods or drinks, to allow for the interactions between combination of pesticides and/or drugs and greater sensitivity of children and old people.
If excessive, careless or unnecessary use of pesticides can be controlled, many of the problems that these chemicals cause can be mitigated. Ecological hazards can be minimized if governments insist on adequate research before a pesticide is introduced.

1.4.9. Dynamics of pesticides in the environment

To decide whether the continued use of persistent pesticides, which are in many ways beneficial, will lead to a long term contamination of the environment which may directly or indirectly endanger certain animals, plants or man, knowledge of the mechanism of transport, the routes of degradation, the ultimate fate of these pesticides and how long the hazards persist, is to be clearly understood (45).

Compartmental models of pesticide movement in the environment

Decisions on the continued use of persistent pesticides are required to evaluate the potential and long term hazards. The use of system analysis, using compartmental models, will be of help in these instances.

Robinson's model

Robinson defined an eco system as an area that contains living organisms (biotic) and non-living (abiotic) components, interacting to produce an exchange of materials and energy between the living and non-living components. The living organisms were classified into three groups: the producers, the consumers and the decomposers. The eco system was considered as a superset, with the tropical levels as sets within the superset, individual species forming subsets of the tropic levels and individual organisms as members of the subsets.

The total annual biomass of a tropic level was taken as

\[ \sum_{j} m_{jk} x_{jk} \]

And the total content of a particular pesticide in this tropical level was expressed as

\[ \sum_{jk} m_{jk} x_{jk} \times k \]

where \( m_{jk} \) is the body weight of the \( k \)-th member of the \( j \)-th species in the \( i \)-th tropic level and \( x_{jk} \) is the average concentration of the insecticide in the whole body of the individual.

At a given instance of time, the total amount of an insecticide in the ecosystem would be

\[ \left( \sum_{jk} m_{jk} x_{jk} + W \right) \]

where \( W \) is the mass of the insecticide in the non-living components.
soil, water and air. In a completely isolated ecosystem, the total mass of the insecticide would remain constant, although its distribution would vary unless the insecticide is degraded. The environment consists of numerous interrelated ecosystems. With the assumption that the total mass of an insecticide i-th tropic level could increase as a result of transfer from the (i-1)th level and may decrease either by degradation within the i-th level or by transfer to (i+1)th level. The total biomass in the i-th level could increase as a result of migration of organisms into the ecosystem and reproduction or growth of the organisms in that level. The chief factors responsible for the decrease in biomass would be, migration of organisms from the ecosystem, death of individuals and loss of weight by starvation. Thus, the ecosystem could be regarded as a box with transfer functions that define the ratio between the input and output of mass, energy or insecticide residue. Tropic levels would correspond to subdivisions within the box. The environment could be considered a series of such boxes. A model such as these, would present a sound basis for assessing the validity of inferences drawn from field observations of levels of residues and enable suitable sampling schemes to be devised. It was later felt (46) that the simplest of all models of pesticides through the environment would consist of a simple flow system between biotic and abiotic components with loss by degradation from both.

Conclusions which could be drawn from the model, were that it seems probable that the continued use of even the persistent pesticides could eventually result in a steady state, in which total annual loss by the various degradation processes would balance the annual rate of usage, although the pesticide is not evenly distributed between the major environmental compartments. Most of the points of Robinson (loc.cit.) very sound and demonstrated the potential of mathematical models, based on sound assumptions and adequate data.

The model proposed by Harrison and his colleagues

A model was proposed by Harrison (47) to summarize transport of DDT in an inland ecosystem. It is based on tropic levels which allow a simplified quantitative assessment of transfers through complex food webs. Mathematical formulations to account for the flow of DDT were calculated and developed a model that could be used to predict population changes attributable to DDT.

Randers and Meadows model
The model proposed by Randers and Meadows, (48) postulated that decision on continual usage of a persistent pesticide should be based on the considerations, such as, the actual benefits in health, comfort and agricultural productivity, total cost and disturbances of natural ecological balances incurred, distribution of benefits and costs over space and time and comparison of the requirements of the future generation with the earlier generation. The model was tested using the most optimistic and most pessimistic parameters. The general behaviour of the model was consistent based on which it was concluded that performance of the model justified decision making on the future use of a persistent pesticide.

Woodwell, Craig and Johnson's model

Woodwell (49) based their model the fate of the pesticide released into the atmosphere, associated health hazards and persistence of the pesticide. The values for the various parameters were derived from variety of sources. Mechanisms were assumed to account for the loss of pesticide from soil such as volatilization, removal with plant material, water runoff and chemical degradation. It was suggested that most of DDT produced has been degraded to innocuousness or sequestered in places where it is not available to biota. How and where these residues are held have still not been fully resolved.

Stewart (50) questioned the validity of some of the assumptions made in the model. Such controversy shows that however well a model is constructed, its effectiveness depends completely on adequate data and conclusions reached on in adequate data are of little use.

From the discussion of the different mathematical models, it is clear that adequate residue data in some parts of the environment and in biota is available. Much more information is still needed on residues in the atmosphere on a worldwide scale, amounts in soil in tropical areas and sites far from pesticide application, amounts in sea water, the vertical distribution of pesticides in the oceans and related aquatic organisms and degradation process of persistent pesticide.

As more data is accumulated from surveys, it should become clear quite soon whether the persistent pesticides are as ecologically hazardous as it has been supposed. In the meantime, the current restrictions and ban on the use of persistent pesticides should
be adequate to ensure that the environment does not become irrevocably polluted by these very persistent chemicals. However, continued attention and vigilance is necessary to ensure that environmental contamination by these and other possibly as yet unrecognized chemicals do not occur. The present ecological awareness of these problems should go far in ensuring that disastrous environmental pollution is avoided.

1.e. Analytical techniques employed for the determination of pesticides

A careful study of the discussions about the pesticides presented in the previous sections clearly lays stress on the importance of the knowledge of the quantity of the pesticide handled at various stages, from the production and formulation, application after proper dilutive dispersion and finally the residue of the pesticide itself or its degraded product in the produce.

Sensitive and reliable analytical techniques have to be employed for the evaluation of pesticides in formulations, residues and the degraded products. Any particular technique cannot be expected to be universally applicable. The choice of the technique depends on the pesticide being analyzed, quantity involved and the matrix. The methods generally applied for pesticide analysis include spectrophotometry, chromatography and electroanalytical techniques. A survey of literature pertaining to these techniques is presented in the next few sections.

1.e.1. Spectrophotometry

Spectrophotometric methods are simple, sensitive and inexpensive and the sample can be retrieved for further scrutiny by other analytical methods. These methods are sensitive up to submicrogram quantities. TLC coupled with IR, HPLC coupled with UV-Vis are employed for the separation, identification and quantitative analysis of pesticides.

1.e.2. Ultraviolet spectrophotometry

UV absorption methods have the advantage that they are rapid and sensitive. They have also some minor limitations, for example, the cleanup requirement is more complex. The selected solvent should not absorb radiant energy at the selected wavelength, a condition that may be difficult to meet as only few solvents are transparent to UV. UV methods coupled with GLC and HPLC are better suited for residue analysis.
Many methods for the analysis of all the major class of pesticides are reported in the literature. Blinn and Gunther (51) and Their (52) presented reviews of pesticide analysis by UV technique. Decker and Frank (53) analyzed the herbicide isopropalin, trifluralin and benifin was analysed by Gnaegi et al. (54) in industrial atmosphere. Dranovakaya et al., (55) reported the UV spectrophotometric analysis of naphthalene acetic acid from apples and potatoes.

Carbaryl in formulations was assayed by Kanazawa (56). Milner et al., (57) employed UV technique for the simultaneous estimation of esters of 2,4-D and 2,4,5-T. Gertig et al., (58) developed a method based on UV spectrophotometry for the evaluation of P,P'-DDT, P,P'-DDE and P,P'-TDE in formulations. Fenithion was determined by UV spectrophotometry (59). Handa (60) has described a method for the determination of carbofuran residue in water. Khalaf et al., (61) demonstrated a flow injection spectrophotometric method for the determination of propoxur with p-aminophenol.

1.e.3. Visible Spectrophotometry

Visible spectrophotometry and colorimetric methods are employed for the analysis of pesticide and its formulations and their quality control. Since a number of reagents can be employed in these methods confirmation of results by correlation is possible. As the pesticides themselves are colourless, colours are to be developed by employing appropriate reagents. Handa (62), during the analysis of bendiocarb by spectrophotometric analysis, developed colour by first hydrolyzing the pesticide with an alkali and treatment of the resulting phenol with nitric acid.

Appaiah (63) determined propoxur in vegetable and water systems by colorimetry. p-Nitrobenzene diazonium fluoroborate was used as the reagent for the determination of carbaryl residues on okra fruits. Averill and Norris (64) estimated parathion colorimetrically by reducing the pesticide into its corresponding amine. Urea type herbicides in agricultural samples were estimated by colorimetry by Singmaster (65). Bhagawat and Ramachandran (66) reported a colorimetric method for the assay of malathion and malaoxan. Chiranjeevi (67) reported a colorimetric method for the determination of malathion with methyl violet – B. Sastry (68) have determined carbaryl and propoxur in insecticide formulations, water and grains by spectrophotometric method. Garcia and Cruces (69) have demonstrated the application of differential
derivative spectrophotometric method for the analysis of carbaryl and its metabolites. Trace levels of aldicarb in water samples were analysed by an indirect method by Crando and Javaner (70). Naidu and Naidu (71) developed a simple spectrophotometric determination of carbofuran and propoxur. Salvador (72) presented a direct derivative spectrophotometric determination of carbaryl. 2-aminobenzophenone was utilized as a reagent for the determination of carbofuran and bendicarb (73). Mathew (74) described a simple spectrophotometric method for the determination of carbaryl in soil and insecticidal formulations. p-aminophenol was selected as a reagent for the determination of propoxur by Delignardia (75). Bhattacharjee and Gupta (76) described an extractive spectrophotometric method for the determination of phenol in water and biological samples. A sensitive spectrophotometric method was developed by Venkateswarlu and Seshiah (77,78) which employed 4-aminoantipyrine as a reagent for the determination of propoxur.

Naidu and Naidu (79) developed a simple, rapid and sensitive spectrophotometric method for the determination of Bendicarb in insecticide formulations, grains and water using p-aminobenzenesulphonamide. Carbaryl and its hydrolysis product, 1-naphthol in water were assayed by Khalaf (80). The same authors (81) determined ethiofencarb in water using p-aminophenol as a reagent. Raju and Gupta (82) proposed a spectrophotometric method for the determination of propoxur in grains and vegetable samples. p-aminobenzoic acid as a reagent. Raju (83) outlined a rapid and sensitive screening method for the determination of carbaryl and propoxur using p-nitroaniline. p-amino-acetophenone was also suggested as a reagent by these workers.

Various pesticides were determined in several samples of environmental samples using spectrophotometry (84-86). Several researchers reported simple and sensitive spectrophotometric method for the determination of carbaryl (87-88) and propoxur (89) in their formulations and environmental samples.

1.e.4. Infrared spectrophotometry

Infrared spectrophotometric technique has many inherent advantages. It is seldom necessary for the compound to be modified by chemical reaction for quantization which is often required in the colorimetric methods. Due to the uniqueness of IR absorption of molecules, these studies provides a definite indication that the material
determined is the species of interest. Infrared spectrophotometry is extensively used for the assay of technical grade pesticides, their formulations and pesticide residues. Blinn et al., (90) described a method for the determination of aldrin and dieldrin. IR spectrophotometric method was employed for the determination of endrin in water systems by Gershan (91). Separation and determination of carbaryl and its intermediates has been reported by Vasilev et al., (92). Mc Culley and Cook (93) have discussed the application of IR method with reference to the determination of organophosphate pesticides. Wurrey et al., (94) employed FTIR spectroscopy for the quantification of dioxins. Dowling et al., (95) reported a method for the determination of DDT isomers. Stepanyan and his coworkers (96) examined the IR spectra of aliphatic amine carbamates and formulated solutions for inverse spectroscopic problems. Marshall (97) reported IR procedures for the determination of various pesticide samples. Schnelder et al., (98) analyzed polychlorinated biphenyls in commercial mixtures by FTIR spectrometry.

1.e.5. Chromatography

Chromatography was suggested as a technique for the separation of complex mixtures by adsorption by Tswett (99). The method is based on the separation of a mixture of solutes brought about the dynamic distribution or partition of dissolved or dispersed materials between two immiscible phases, on of which is moving past the other. The chromatographic technique was revived by Kuhn and Lederer. These workers demonstrated the chromatographic technique by separating α and β isomers of carotene.

Chromatography is an analytical procedure in which the flow of liquid or gas facilitates the separation of substances by differential migration from narrow zones in a porous sorptive medium (100). The principle underlying the chromatographic technique is the redistribution of constituents of a mixture between two or more phases. The distribution takes place between a stationary phase and a mobile phase.

Chromatographic techniques are classified on the basis of the basic phenomenon underlying the process. Partition chromatography is a technique for the separation of a mixture by partition between the moving solvent and a stationary liquid, which is held on a suitable solid support. When the solvent is a liquid, it is termed liquid – liquid
Adsorption chromatography is a technique in which small differences in the adsorption behavior of substances between a moving solvent, liquid or gas, and a stationary solid phase, are used to achieve the separation. When the moving phase is a liquid, it is called liquid–solid chromatography or adsorption column chromatography and when the moving phase is a gas it is called gas–solid chromatography (GSC).

Chromatographic techniques include column chromatography, paper chromatography, thin layer chromatography (TLC), gas chromatography (GC), and high performance liquid chromatography (HPLC). The last three techniques are widely employed in the assay of pesticides and pesticide residues.

Thin layer chromatography

Thin layer chromatography is widely employed for qualitative, quantitative and preparatory analysis. Kichner and Rice (101) employed TLC technique for the determination of pesticide residues. TLC technique involves the adsorption of the test compound on a thin layer of the adsorbent or stationary phase and the solubility of the compound in a solvent system or mobile phase. The mobile phase is allowed to migrate from the origin to a predetermined front. The test substance is then analyzed in terms of relative $R_f$ value. Determination of pesticides by TLC envisages detection limits ranging from nanogram to picogram level. The technique is also useful for semi quantitative and qualitative analysis of insecticides and can also be used for the clean up during the assay of insecticide formulations.

Abdel-Kader et al., (102) reported a TLC method for the determination of the residues of carbofuran and its two carbamate metabolites in fresh water, soil and plant tissue. The application of TLC technique for pesticide analysis was reviewed by Sharma (103) and Stahr (104). Herbicide residues were assayed in agricultural crops, foods, soil and water using silica gel TLC by Kovac and Tekel (105). Sharma and Koropchack (106) developed a quantitative procedure for the estimation of chlorophenoxy acid herbicides and their salts in water by TLC. Chiba et al., (107) evaluated the suitability of TLC for the determination of DDT, DDE, aldrin and dieldrin residues in soils. Askew and coworkers (108) described a general method for the determination of...
Gas chromatography

Gas chromatography (GC) can be employed for the separation of vaporized components utilizing their distribution between a stationary phase and a moving inert gas phase. Depending on the nature of the stationary phase, GC can be divided into two categories; gas solid chromatography (GSC) where a solid is used as the stationary phase, and the second is Gas liquid chromatography (GLC) where a thin layer of nonvolatile liquid distributed on a solid support is the stationary phase. In both the instances, the mobile phase is an inert gas, known as the carrier gas.

Brauckhoff and Their (110) determined 15 carbamate pesticides and 4 metabolites in fruits and vegetables using GC with an electron capture detector. Kiviinanta (111) used GC for the determination of 3000 samples of pesticide residues of food and environmental origin. Stan and Mueller (112) developed an automatic hot splitless, cold splitless and on column injection technique for capillary GC analysis of organophosphate pesticides. Bowman (113) used GC for the determination of organophosphate pesticides. Coburn et al., (114) analysed carbamate pesticide residues in natural water. Olsen et al., (115) assayed chlorophenoxy acid herbicides in water and soil using GC with electron capture detector. Oruska (116) utilized open tubular capillary column GC for pesticide residue analysis. BabioItka et al., (117) developed a method for the determination of ethyl carbamate in alcoholic beverages through capillary gas chromatography with mass spectrometry. Residue levels of ethyl carbamate in wines and spirits were evaluated by GC - mass spectrometry (118). Conacher et al., (119) and Lau et al., (120) employed a similar procedure for the assay of ethyl carbamate and confirmation was obtained from GC - mass spectrometry. Massoba (121) studied the application of GC - matrix isolation with the help of fourier transform infrared spectrometry to the determination of ethyl carbamate in food samples. Deleu and Copin (122) analyzed carbofuran and atropine residues by capillary GC - mass spectrometry. Pierce et al., (123) reported the determination of ethyl carbamate in distilled alcoholic beverages by GC with flame ionization or mass spectrometric detection. Hurtman and Rosen (124) determined ethyl
carbamate in commercial based sauces by GC - mass spectrometry. Lau et al., (125) have investigated the GC and mass spectrometric characteristics of 16 short chain (C1 - C5) alkyl carbamates. Hurst et al. (126) presented the methodology for the measurement of ethyl carbamate in blood by capillary GC - mass spectrometry using selected ion monitoring. GC - mass spectrometric determination of ethyl carbamate as the xanthydyl amide derivative in Italian aqua vitae samples was reported by Giarbetti et al., (127). Burguera and Burguera (128) used GC for the determination of organophosphorous insecticides by flow injection with a molecular cavity detector. Oller and Cranmer (129) employed GC coupled with electron capture detector for the analysis of chlorinated insecticides. Okumura and Nishikawa (130) reported a GC-MS technique for the analysis of organophosphorus pesticides in environmental samples in the range of sub ppb.

Climent and Miranda (131) discussed the the use of photo-degradation of carbamate pesticides with gas chromatography with mass spectrometry. Alberani et al (132) studied the pesticide residues and their metabolites in surface and underground water using gas chromatography. Santos Delgado and co-workers (133) discussed the stability of carbamate pesticides and analyzed by GC with flame ionization and nitrogen-phosphorus detection. Richter and co-workers (134) reported the screening and determined the pesticides in the soil using GC with subcritical water extraction. Reported a new analytical method for the determination of 34 multi-class residues of pesticides in drinking and natural (ground, surface and sea) waters by solid-phase micro-extraction coupled with gas chromatography-tandem mass spectrometry described by Arrebola et al (135).

High performance liquid chromatography (HPLC)

Gas liquid chromatography is useful for effecting separation and quantization of components in mixtures of low concentration. It fails in the case of compounds having low vapor pressure or unstable at elevated temperatures. Liquid chromatography has to be preferred in such instances employing solid absorbants of small particle size, 5 - 10\(\mu\)m and high pressure, 300 - 3000 psi for steady flow of solvent. Such a technique is called high performance liquid chromatography and is useful for nonvolatile solvents. Differential migration of individual components of the mixture through the liquid column forms the basis for the separations by HPLC. The differential migration results from the
equilibrium distribution of different components between the particles of the stationary phase and the flowing solvent. Differential migration is determined by the experimental variables that affect the distribution and the composition of the stationary and mobile phases and the separation temperature. The basic difference between liquid chromatography and the gas chromatography lies in that the former is based on the vapour pressure.

HPLC is used for the analysis of chlorinated hydrocarbons, organophosphates and carbamate residues, which cannot be monitored precisely by methods such as GLC and TLC. HPLC can be conveniently used for the separation, identification and estimation of non-polar homologues and mixtures of compounds of different functionality. HPLC has been extensively used for pesticide analysis of formulations and to some extent in residue analysis as a minimum sample preparation is required.

Frei et al., (136) analysed carbamate insecticides using fluorogenic labeling. Skelly et al., (137) described a HPLC multi residue method for the analysis of seven N-methyl carbamate insecticides. Separation and identification of various chlorinated insecticides was reported by Krause (138) and Horgan et al., (139). Organophosphates, camphor, diazinon, malathion, methyl parathion were quantified by HPLC technique (140). Olek et al., (141) determined carbamate pesticides by HPLC with electrochemical detection. Miles and Moya (142) determined carbamates, carbaryl oximes, carbanthionic acids and substituted amines by HPLC post column reaction fluorescence detector. Goewie and Hogendoom (143) described an automated HPLC method for the determination of carbaryl, carbofuran and propoxur in total diet sample extracts. Chiu et al., (144) compiled the thermodynamic spray ionization mass spectra of selected carbamate pesticides using both single and triple stage, quadruple mass spectrometers in conjunction with HPLC. Tena et al., (145) designed a technique for the sensitivity enhancement of HPLC by flow through sensor for pesticide mixtures.

Abad et al., (146) reported monoclonal enzyme immunoassay and HPLC with fluorescence detector for the analysis of carbaryl and carbofuran in cucumbers and strawberries. Parrilla et al., (147) developed reversed-phase liquid chromatographic column switching for the analysis of N-methyl carbamate pesticides and their metabolites in urine samples. Lopez-Blanco and co-workers (148) described solid-phase extraction
and solid-phase micro-extraction for the quantitative detection of carbofuran in water samples using HPLC-photodiode array detection. Orejuela and Silva (149) analysed some phenoxy type residues of N-methyl carbamate in fruit juices employing HPLC with peroxyxalate-chemiluminescence detection at ng levels. Matrix solid-phase dispersion microextraction and determination of pesticide residues by HPLC with UV detection reported by Valenzuela et al., (150). Wu and co-workers (151) monitored polar pesticides in water and wine samples by solid-phase extraction coupled with HPLC-MS. A liquid chromatographic method for the multi-residue analysis of carbamate pesticides in soils were described by Sanchez-Brunete et al., (152). Ichinoi et al., (153) developed automatic reversed-phase HPLC technique for the simultaneous determination of carbaryl and propanil in human serum and urine samples.

A sensitive and selective method for the determination of propoxur in eggs with liquid chromatography using diode array detection by Hamscher et al., (154). The determination of carbaryl and some organophosphorus pesticides in drinking water was studied by Driss and co-workers (155) using reversed-phase HPLC technique. Mickova et al., (156) discussed the correlation study of enzyme-linked immunosorbent assay and HPLC-MS for the monitoring of N-methylcarbamate pesticides in baby food. Consuelo et al., (157) developed HPLC technique for monitoring of multi-residue determination of N-methylcarbamate pesticides in fruit and vegetable samples.

1.c.6. Electroanalytical techniques

Electroanalytical techniques for the analysis of pesticides gained much importance in recent times. Polarography is the important electroanalytical method frequently employed in pesticide analysis.

Mendez et al., (158) employed polarographic technique for the simultaneous determination of parathion and paraoxon. Hitchman et al., (159) have reported the application of differential pulse polarography for the determination of bendiocarb in soil. Volumetric characterization of number of chlorinated hydrocarbons was reported by Farewell et al., (160). Heath et al. (161) used linear scan voltammetry for the analysis of parathion, paraoxan and p-nitrophenol. Booth and Fleet (162) reported the determination of some methyl carbanates in which sulphur atoms are absent. Parentizjoper et al., (163) reported electrochemical determination of carbaryl in natural water and soil samples.
Huerladiaz and Rodriguez (164) measured the solubility and solvatochromic constants of carbaryl in sea water and other electrolyte solutions. Fernando et al., (165) presented a technique for the rapid detection of anticholine acetyltransferase insecticides by reversible light addressable potentiometric biosensor. Walcarius (166-167) have been used in electromanipulation and electrocatalysis techniques with great promise, especially in improved the sensitivity and selectivity for the analysis of pesticides. Vaquez Diaz et al., (168) described differential pulse polarographic technique for the analysis of iron in carboxylic acids, waters, fruit juices and wines. Hye-Sung Lee et al., (169) described a cholinesterase-based flow injection biosensor technique for the determination of nine carboxylic pesticides over the concentration range of 10^{-11} M. Gupta et al., (170) developed nitric oxide medulate high energy phosphates in the brain regions of rats intoxicated with carbofuran and studied by the prevention through N-tert-butyl-alpha-phenylnitrone or vitamin E using biometric technique. Atroz et al., (171) developed a stripping voltammetric and polarographic techniques for the determination of anti-fungal ketoconazole on the mercury electrode. Pappas and co-workers (172) studied the effects of storage conditions and fruit processing on the degradation of parathion methyl in apples and lemons.

1.0. Scope of the present study

A survey of literature presented in the preceding sections reveals that spectrophotometric methods, electroanalytical and mass spectrophotometric techniques are frequently employed for the qualitative identification and quantitative determination of pesticides and their formulations. Most of these techniques require sophisticated and expensive analytical instruments and seldom does not meet the requirements of routine analysis. Routine analysis of pesticides and pesticide residues, frequently involves the analysis of very large number of samples. Analytical methods which are sensitive, selective and rapid and involving inexpensive equipment and lower cost of the analysis are best suited for this purpose.

The present work is designed to develop method based on the spectrophotometric measurements for the determination of the carboxylate pesticides, propoxur, carbofuran, carbofuran and carbaryl, in their insecticidal commercial formulations, fortified samples of water, grains and vegetables.
The carbamates mentioned have been selected as (i) the synthetic organic carbamates as water soluble insecticides gaining importance in recent times, (ii) these insecticides in insecticide formulations and their residues have been monitored only to a limited extent when compared to the other classes of pesticides and (iii) these insecticide metabolites are characterized by low persistent levels in environment.

Further, very few attempts have been made for the determination of these pesticides by spectrophotometry and colorimetry in aqueous media. The developed methods are based on the alkaline hydrolysis of the carbamates. The resulting phenols are coupled with reagents to produce coloured compounds which are monitored spectrophotometrically. The reagents $p$ - amino benzoic acid, 2-amino benzophenone, 1-amino-2-naphthol - 4 - sulphonic acid, 3 - methyl - 2 benzothio zolines hydrazone hydrochloride and $p$ - aminophenol were used for coupling. In these methods, the stability of the coloured product was dependent on time, temperature and pH. Further, the reported method suffers from sensitivity, selectivity and interference due to the other species present in commercial formulations of these insecticides and samples of water grains and vegetables of agriculture importance. Laborious clean up procedures are needed for these determinations.

In the present investigation, an attempt is made to overcome these shortcomings, 4-methoxyaniline, 2,4-dimethoxyaniline, 4-aminobenzaldehyde, 2,6-dibromo-4-methoxyaniline are selected as reagents. The hydrolyzed phenolic products of the pesticides were coupled with the reagents in presence of alkaline $K_3[Fe(CN)_6]$ to produce coloured cyanogen coupled product at pH 9.5. The coloured cyanogen product increases the sensitivity, selectivity and stability. The color forming reagents with carbamates were shown in table-1. The absorbance of the color derivatives of carbamates were measured using HITACHI U 3400 and shaimadzu model UV 240 spectrophotometer with 1-cm quartz cell. The pH measurements were studied using an ELICO digital pH meter.
### TABLE - 1

**Pesticides and Reagents**

<table>
<thead>
<tr>
<th>Name of the pesticide</th>
<th>Name of the Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur</td>
<td>4-methoxyaniline</td>
</tr>
<tr>
<td></td>
<td>2,4-dimethoxyaniline</td>
</tr>
<tr>
<td></td>
<td>4-aminobenzaldehyde</td>
</tr>
<tr>
<td></td>
<td>2,6-dibromo-4-methoxyaniline</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>4-methoxyaniline</td>
</tr>
<tr>
<td></td>
<td>2,4-dimethoxyaniline</td>
</tr>
<tr>
<td></td>
<td>4-aminobenzaldehyde</td>
</tr>
<tr>
<td></td>
<td>2,6-dibromo-4-methoxyaniline</td>
</tr>
<tr>
<td>Carbosulfan</td>
<td>4-methoxyaniline</td>
</tr>
<tr>
<td></td>
<td>2,4-dimethoxyaniline</td>
</tr>
<tr>
<td></td>
<td>4-aminobenzaldehyde</td>
</tr>
<tr>
<td></td>
<td>2,6-dibromo-4-methoxyaniline</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>4-methoxyaniline</td>
</tr>
<tr>
<td></td>
<td>2,4-dimethoxyaniline</td>
</tr>
<tr>
<td></td>
<td>4-aminobenzaldehyde</td>
</tr>
<tr>
<td></td>
<td>2,6-dibromo-4-methoxyaniline</td>
</tr>
</tbody>
</table>
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