CHAPTER 1

INTRODUCTION

I. INTRODUCTION

Natural toxins are unique toxins which possess some common properties whether they are obtained from plants, microorganisms, or animals. One common characteristic is that they exert a pronounced effect on the metabolism and biological functions of the intoxicated animals with just a minute quantity. Since ancient times man has pondered over the physiological effects of various toxins and venoms. How do these natural toxins work? Each toxin has its unique mode of action and its own characteristic structure, a property shared by drugs. Therefore many of these natural toxins in minute quantities are potentially good drugs.

During the early decades of the twentieth century, a research pioneer of U.S. Department of Agriculture, C.D. Marsh, studied the poisonous plants and their effects on animals (Keeler and TU, 1983). Subsequently, other investigators - botanists, chemists, geneticists, nutritionists, pathologists, physiologists and veterinarians followed the Marsh lead, preserved in the research, and solved many problems. Research programs, state, federal and in other nations, have steadily and significantly increased the information specific to plant poisonings. Early investigators have studied some species of toxic plants, categories of toxic agents, and species of susceptible animals. Such findings gradually concentrated scientific attention on specific aspects of the problems. As a result, chemists found specific culpable compounds and determined their pharmacological actions. Other investigators found target systems, organs, tissues, and cells, as well as the nature of changes produced in affected animals. A point of great interest is that some edible plants produce taratogenic compounds that are generally nontoxic to consuming maternal animals but are severely toxic to embryos in critically specific stages of development. Such poisoned embroys may die early or develop fetal deformities. These discoveries now constitute a base for abating and preventing injuries and deaths of food animals. But one must not be deluded by past achievements that all problems are solved. Among the numerous aspects requiring further study are determination of the full extent of plant, interactions in the production of toxins, the development of materials and technologies for controlling and possibly eradicating some unwanted species of plants and animals.

The study of each field of toxins has been taking an independent pathway. Scientists in a specific toxin field are often unaware of the activity in other toxin fields. It is thus desirable to have a primary source of information on all natural toxins so that scientists in a specific discipline of toxin research can easily obtain useful information from other toxin researchers.

Following paragraphs have provided information on previous studies on chemistry of the plant toxins, source, gross and histopathologic effects, and mechanism of action.

BRIEF ACCOUNT OF THE LITERATURE ON SOURCES, CHEMISTRY, GROSS AND HISTOPATHOLOGIC EFFECTS AND MECHANISM OF ACTION OF PLANT TOXINS USED IN CONTROL OF UNDESIRABLE FISHES

A plant toxin may broadly be defined as any compound present in plants which, under natural (but possibly abnormal) circumstances of production or consumption (or exposure) causes or is capable of causing an injurious consequence in one or more individual animals. The bulk of the literature of plant toxins, is based on postulated toxicity on the basis of known biochemical characteristics of the compounds under discussion and of known physiology of animal systems.

The present review is concerned with the impact of plant toxins in general on animal life in fresh water, particularly on fresh water fish which live in lakes, ponds, rivers, streams, irrigation canals and other water bodies. The review includes on the one hand the great amount of information gained in the chemical control of undesirable predators and trash fish as well as it also gives an access to a varied wealth of information on chemistry, source, histopathological effects and mechanism of action of plant toxins.

A. THE CHEMICAL CONTROL OF UNDESIRABLE FISH - PISCICIDES

Certain species of fresh water fish might be undesirable because they compete with game fish or food fish. These fishes are labelled as "trash fishes." These are quite apart from the predacious species which actually feed on food fish. In the field of applied biology, there has been a great intensification in the development and practical use of piscicides in the last twenty five years, particularly in the U.S.A. and the U.S.S.R. The development is particularly concentrated on manufacturing a wide range of new synthetic piscicides which possess unique and highly desirable selective properties. So that the selective piscicide which kill particular species of undesirable fish at concentrations which do not have an adverse effect on the desirable food fish. The outstanding example of this is the successful development of selective chemicals for controlling the sea lamprey (Petromyzon marinus) in the Great Lakes of North America by destroying the larval stages which inhabit the streams and rivers flowing into the lakes. Secondly, there is the use of piscicides to eliminate entire population of trash fish from lakes which, after a suitable interval can then be re-stocked with food fish.

1. Copper Sulphate :

One of the first compounds to be used in fish control was copper sulphate which proved useful in certain soft-water areas. At this point it is worth noting that copper sulphate was also the first chemical to be used on a large scale for the control of the aquatic snail, the intermediate host of human bilharziasis (Muirhead-Thomson, 1971).

2. Rotenone :

The first piscicide to be used extensively in fishery management was rotenone which has been used in the United States since 1934. In some instances as much as 105 tons of rotenone have been used in a single fish eradication experiment (Hopper, 1960).

Rotenone is an interesting naturally occurring toxin used as an insecticide, piscicide and parasitic control agent in United States and other Western countries (Gosalvez and Diaz-Gil, 1978). The compound was originally classed as an oddity used by natives of South America to poison small streams or pools, thereupon allowing harvesting of the floating killed fish for food. The natives recognized that rotenone was highly selective in toxicity. It killed fish, but they could safely eat the fish (Schery, 1952).

But Rotenone has a teratogenic property also. Rao and Chauhan (1971) found that the compound caused a variety of defects during development of chick embryos. Rotenone is a known inhibitor of the mitochondrial respiratory chain between flavoprotein and cytochromes. Khera et al. (1981) reported the defects due to high oral doses of rotenone in pregnant rats.

Since Rotenone has not been used as a selective toxicant, it is of relatively high cost and due to its ability to dissolve rapidly in lakes and ponds. It's toxicity may be lost before the chemical has had time to become thoroughly dispersed throughout the waters of the lake (Muirhead-Thomson, 1971), its use in control of undesirable fishes has been reduced considerably.

3. Toxaphene :

The development of new synthetic insecticides disclosed valuable piscicidal properties. Toxaphene is a chlorinated camphene containing 67-69% chlorine. It is three times as toxic as rotenone and has the greatest toxicity to fish of any chlorinated hydrocarbon except endrin (Hooper and Grzenda, 1957; Fukano and Hooper, 1958 and Hooper, 1960). It also has a lower acute toxicity to humans than either endrin, dieldrin, aldrin or rotenone. It is now accepted as a general replacement for rotenone. In the state of Wisconsin over thirty lakes have been treated with this piscicide in the ten years from 1955-65 (Johnson *et al.*,1966).

The effective dose of toxaphene varies with the conditions and fish species and usually ranges from 0.02 to 0.1 ppm. One thing which is undesirable with the use of Toxaphene is its persistence and slow rate of detoxification in lakes. In some lakes it has remained toxic for periods up to four years also. The response of different species of fish does not vary. Hence this chemical cannot be used as a selective piscicide. Therefore, it was not recommended for gold fish control in California (Johnson, 1966).

4. Polychlorpynene (PCLP)

A compound very similar, but not identical to Toxaphene, called Polychlorpynene or PCLP. It has been used extensively for fish control in U.S.S.R. in recent years (Burmakin, 1968). In that country, smaller lakes in the temperate zone contain fish species as perch (Perca fluviatills), roach (Rutilus rutilus), ruff (Acerina cernua) which do not have any commercial value. Since 1957 attempts to change the composition of the fish and control of undesirable variety, were made and by the end of 1964 PCLP gave good results. Dose of PCLP (normally 0.05 ppm to 0.15 ppm) vary according to species, depth of water and temperature. Like Toxaphene PCLP also persists for longer time in lakes for a year or more. In the second stage, the toxicity of PCLP becomes weaker and conditions become favourable for mass development of invertebrates due to the lack of predation by fish. In the third stage when the water is no longer toxic, there remains no fish predators, no competition and sometimes no parasites. Therefore, there is a reduction in the standing crop of food organisms. In the fourth stage eutrophication of water takes place and the standing crop of food invertebrates increases once more (Burmakin, 1968).

5. Antimycin :

Antimycin A is an antibiotic produced by microscopic plants. It was discovered in 1945 by scientists in the Department of Plant Pathology at the University of Wisconsin, and was named Antimycin (antifungus) because of its powerful fungicidal properties. In 1963 Antimycin was reported to be very toxic to more than thirty fish species including most susceptible species like carp, pumpkinseeds and green sunfish (Walker et al., 1964). This was used for selective control of certain undesirable predatory fish, Micropterus salmoides (Powers and Bower, 1967). It is interesting to note that the large number of African food fish species (Tilapia mossambica) was also killed by Antimycin. The particular formulations of Antimycin used in the field trials in ponds and lakes are Fintrol-5, Fintrol-15 and Fintrol-30. These compounds are most effective in soft water and at lower pH values (Berger et al., 1969).

However, there are many physical difficulties in making exact measurements of area, depth and water volume of large ponds or in control operations.

6. Tri-fluoro methyl nitrophenol (TFM) :

This halogenated nitrophenol is selective in action on larvae of the sea lamprey (*Petromyzon marinus*) and fingerling rainbow trout (*Salmo gairdnerii*). It is most effective in soft acid waters. TFM application in field forms a striking contrast to methods currently used and it requires much high standards of accuracy (Baldwin, 1969).

Although the discovery of TFM has opened new possibilities and new horizons in scientific control of undesirable fish populations, it is not the complete and final solution to this problem. For field trials, large amount of TFM is required and the high cost of TFM has prompted a search for materials which might supplement it or replace it in a control programme.

7. Bayluscide or niclosamine :

Some investigators worked on chemicals to which TFM belongs (mononitrophenols containing halogens), whereas other group of investigators has revealed a new class of selective toxic piscicides in the form of substituted nitro salicylanilides, a group which contains one of the most widely used molluscicides, namely Bayluscide or niclosamine (Bayer, 1973). Bayluscide is basically used for controlling the aquatic snails. It had long been recognised that fish in Bayluscide treated ponds, were killed at molluscicidal dosage. But several years after piscicidal properties of Bayluscide were revealed (Howell *et al.*, 1964; Starkey and Howell, 1966 and Marking and Hogan, 1967).

Bayluscide has been shown to be toxic to at least eighteen species of freshwater fish.

8. Other Chemicals :

Besides above chemicals Aldrin ($C_{12}H_8Cl_6$) used to kill weed and predatory fishes (Chaudhuri, 1960), Dieldrin ($C_{12}H_8Cl_6O$) effectively killed fishes at 0.5 ppm, Endrin ($C_{12}H_8Cl_8O$) is the most poisonous chemical so far tried in India for eradication of fishes (Chaudhuri, 1960), organophosphates have been found successful for killing fish (Srivastava and Konar, 1965 and Konar, 1969a, 1969b).

B. A Brief Survey of Piscicidal Plants and Their Use in Controlling Undesirable Fishes :

There are mainly two arguments against the use of pesticides or chemicals for controlling undesirable fishes. Firstly, these chemicals upset the balance of nature in fresh water bodies. Secondly these chemicals are misused so that the harm is caused. For these and for other reasons including their severe toxicity, prolonged residual effects on other biota including human beings many remain stored in fish tissues, the synthetic chemical toxins are not preferred. These chemicals are more costly also.

With the rapid increase in pisciculture, it has become necessary to find out alternative piscicides because of various above mentioned unavoidable reasons in using synthetic chemicals as piscicides. Therefore, natural toxins, mostly of plant origin, have been used for fish-nursery management, to clear the ponds from the weed fishes and other undesirable aquatic animals, prior to their use in pisciculture. In India, several ingredients prepared from a large number of indigenous plants have been successfully used to control trash fishes. There are more than hundred plants in India, containing piscicidal properties (Chopra et al., 1949; Chopra and Chopra, 1955, Chopra et al., 1958 Chopra et al., 1965). A list of well known and important Indian plants of piscicidal characters and the information regarding their parts used as suitable fish eradicant, their botanical names, and scientific work available, if any, has been provided in Table No.1.

India possesses one of the richest and certainly the most varied flora of any area of similar size on the surface of the globe. Roughly speaking, one-fifth of the Indian sub-continent is occupied by forests of different kinds. There are ever -green forests occupying the west coast of the Peninsula upto the ridges of the Western mountain chain, and the lower slopes of the Eastern Himalayas, with their magnificent and majestic trees, the 'sky scrapers' many centuries old. Many deciduous types are very valuable to the country. The Indian flora is also closely related to some other country. It is known that more than 2,000 plants have medicinal properties and some description have been enumerated in the literature of indigenous medicine. It is gratifying to note that in recent years a growing interest is being taken to use indigenous drugs for therapy. The second line of basic research has been in connection with the poisonous plants of Indian origin including the group of insecticides, insect repellent, etc. During the last two decades the research work on indigenous poisonous plants of piscicidal in nature has received considerable attention. A number of laboratories in India namely, Biochemical Adaptation Laboratory of North-Eastern Hill University, Shillong, Department of Zoology, S.G.T.B. Khalsa College, University of Delhi, Delhi, Central Inland Fisheries

Research Sub-station at Cuttack and Central Inland Fisheries Research Institute of Barrakpore have taken keen interest in studying piscicidal properties of Indian plants and their effects on fresh-water fish species. In 1975, Dr.V.G. Jhingran the then Director, Central Inland Fisheries Research Institute, Barrakpore, published a first standard and valuable book on "Fish and Fisheries of India" with full of consolidated information on the diverse facets of inland and Marine fish and fisheries of India. His review on fish poisons of plant origin was stimulative and encouraging for the investigators in this field. Thereafter a few of the researchers have undertaken the study of indigenous plants of piscicidal potentialities from the point of isolation of their active toxins, to testing their action on fish and making suitable preparation for field trials in the water systems.

The literature mentioned below is very valuable, as it contains not only information on the plants of piscicidal properties but it also gives chemical analysis of a preliminary nature, the chemistry and detailed examinations of active principles of plants, the pharmacological studies to determine the action of these active toxins, and their physiological effect on different varieties of fish species (Table No.1).

1. The Survey of the Research Work on Indigenous Piscicidal Plants :

The Council of Scientific and Industrial Research, New Delhi, in its publication had mentioned the use of sugarcane jaggery (Gur) as piscicide, since it contains upto 1 per cent of saponin (Wealth of India, 1962). In mainland China, the seed cake of tea (Camdlia sinensis) containing 7-8 % saponin, and croton seed are used (Hora and Pillay, 1962; Babu, 1965; Bhuyan, 1967 and Ramanujan and Ratha, 1980). An indigenous variety of derris, Derris trifoliata Lour var. Uliginosa (Roxb. ex-wild) is found to contain 1.2 - 2.2 % rotenone content (Das, 1969). Millettia pachycarpa root powder is very effective at 2-6 ppm (Bhuyan, 1967, Ramanujan and Ratha, 1980). Bassia latifolia (Barrackpore, 1968; Chakrawarthy, 1972), Madhuca indica Syn. Rassia latifolia (Chowdhury, 1968), Millettia piscidia (Das, 1969), Justica hayatai (Ohta et al., 1969), Randia dumetorum (Nandy and Chakraborty, 1976), Marchantia polymorpha (Kanasaki and Ohta, 1976) are also containing considerable important toxins which affect the fish life. In recent years also certain other plants (Eupatorum odoratum L., Myrica esculenta Buch. Ham., Polygonum hydropiper L. var. Flaccidum steward, Polygonum hydropiper L.var. Hydropiper, Potentia fulgens Well ex Lehm., Taxus buccata L., Xeromphis spinosa (Thunb.) Keay (= Randia dumetorum Poir), Zanthoxylum armatum DC. (= Z. alatum Roxb.) (Ramanujam and Ratha, 1980a; 1980b; 1983), Acorus calamus, Linn., Sapindus mukorossi Benth., Xeromphis spinosa Koeg (Virdi, 1982) and plants of Pimelea sp. (Family-Thymelaeceae) P.linifolia and P.ligustrina (Tyler et al., 1985) and other species of this family and Euphorbiaceae were found more effective (Hirota, et al., 1980).

Several investigators have observed that there is a good deal of variation in the active principles in the different parts of these plants and in different seasons in the same part of the plant. Even the same part and at the same time of the year shows remarkable variations in the contents of its active principles. For example, the young and old leaves of a plant and unopened and opened flowers differ materially despite the fact that they are collected from the same plant and during the same season (Chopra and Chopra, 1955).

2. The Survey of the Research Work on Active Principles (Toxins) in Piscicidal Plants :

The earlier studies on plant toxins were mainly confined to collection of a general account of potentialities of piscicides in indigenous plants. Some investigators had undertaken chemical analysis of a preliminary nature of these indigenous plants. Recent studies are intimately related to chemistry and detailed examinations of active principles of plants. The following is the scientific work on this subject in the last 2 to 3 decades. The work in India in this line has started with the main objective of making India self-sufficient and self-supporting by enabling her to utilize plant toxins produced in the country and by manufacturing them in a form suitable for administration in the developing fisheries technology.

a) Rotenone :

It is a naturally occurring toxin. The natives of South America used it to poison small streams or pools for harvesting floating killed fish for food (Schery, 1952). An indigenous variety, *Derris trifoliata* Lour, which occurs in the vicinity of Kakdwip (West Bengal, India) also contain 1-2 % rotenone and used to kill *Mugil parsia*, *Channa punctatus*, *Tilapia mossambica* (Das, 1969). Tephrosia and Lonchocarpus species (Leguminosae) also contain this toxin (Gosalves *et al.*, 1977).

b) Saponin :

Saponin from sugarcane jaggery (Gur) was found lethal to fish (Wealth or India, 1962). Mahua cake (*Bassia latifolia*) is known to contain about 4-5 % saponin called Mowrin (Bhatia, 1970). A wide range of saponins are known in plants (Tschesche and Wuff, 1973). Tomato and potato leaves contain saponins which are alkaloids called as tomatine and solanine respectively (Schlosser, 1975). Similarly seeds of *Barringtonia acutangula* (Barrackpore, 1968; 1969; Chakraworty *et al.*, 1972) and *Camdlia sipensis* (Chowdhury, 1968) contain 7-8 % saponin.

c) Tigliane, Daphnane, Ingenane :

In recent years, tigliane, daphnane and ingenane plant derivatives have been tested for piscicidal activities. Gunasekera *et al.* (1979) obtained a daphnane ester from *Cunuria spruceana* Baill. About 13 genera of Euphorbiaceae and seven genera of the Thymelaeaceae are known to contain tigliane and/or daphnane derivatives (Hecker, 1978; Gunasekera *et al.*,1981). These are used to poison killi fish, *Oryzias latipes* (Hirota *et al.*,1980). Recently, Tyler *et al.* (1985) have identified five biologically active dephnane orthoesters, namely simpleximacrin, simplex linimacrin d, pimelea factor (P) 3 and Linimacrin, from *Pimelea* species, *P. lingustrina* and *P. linifolia*.

d) Other Active Principles :

Other toxins like jugulone, pyrethrin pyrethroids alkaloids, glycosides and essential oils are also used as piscicides (Mauck and Olson, 1976).

The details of these active principles of indigenous plants along with their references in the literature have been summarised in Table No.1.

C. THE BRIEF SURVEY OF THE FISH SPECIES USED IN RESEARCH WORK ON INDIGENOUS PISCICIDAL PLANTS

The different parts of the indigenous plants and their extracts prepared from these plants or purified active constituents (toxins) isolated are properly used in fishery research. Investigators also carry out biological assays, test the toxicity of these toxins and suggest doses for different undesirable fish species for their eradication. Here also the survey assists a great deal in assessing the value of the responses showed by different fish species to toxins under investigation and their practical application to the field trials in the fresh and marine water systems. The following are the fish species used in earlier investigations. This information has also been included in the Table No.1.

1) Anabas testudineus (Bloch) - Chakraworty et al., 1972.

2) Barilius bendelisis (Hamilton) - Virdi, 1982.

3) Catla catla (Hamilton) - Chakraworty et al., 1972.

4) Channa gachua (Hamilton) - Bhatia, 1970.

5) Channa punctatus (Bloch) - Das, 1969; Virdi, 1982.

6) Channa striatus (Bloch) - Chakraworty et al., 1972.

7) Cirrhinus mrigala (Hamilton) - Chakraworty et al., 1972.

8) Clarias batrachus (Linnaeus) - Chakraworty et al., 1972.

9) Colisa chuna (Hamilton) - Chakraworty et al., 1972.

10) Colisa fasciata (Bloch) - Bhatia, 1970.

11) Ctenopharyngodon idella (Valenciennes) - Chakraworty et al.,1972.

12) Cyprinus carpio Var. Communis - Bhatia, 1970.

13) Heteropneustes fossilis (Bloch) - Ramanujam and Ratha, 1980; Virdi, 1982.

14) Hypophthalmichtys molitrix (Valenciennes) - Chakraworty, et al.,1972.

15) Labeo rohita (Hamilton) - Chakraworty et al., 1972.

16) Mugil parsia (Hamilton) - Chakraworty et al., 1972.

17) Mystus vittatus vittatus (Bloch) - Virdi, 1982.

18) Noemacheilus botia (Hamilton) - Virdi, 1982.

19) Notopterus notopterus (Pallas) - Chakraworty et al., 1972.

20) Punctius ticto (Hamilton) - Virdi, 1982.

21) Oryzias melastigmus (McClelland) - Hirota et al., 1980.

22) Tilapia mossambica Peters - Das, 1969; Chakraworthy et al., 1972.

D. A Brief Survey of the Effects of Piscicides Chemicals and Indigenous Plant Toxins on Behaviour of Fish

1. Behavioural Responses to Piscicides

i) The effects of chemicals on behaviuour of fish :

The changes in the general behaviour i.e. locomotor activity and angular orientation of movements were observed when the sea cat fish, Arius felis was exposed to sublethal copper dose (0.005, 0.01, 0.05, 0.1 or 0.2 mg/l) for 72 hrs. (Steele, 1983). Fish reaction and behaviour when exposed to Malathion for Tilapia, Sarotherodon mossambicus and Sepat siam Trichogaster pectoralis was noted (Mohsin et al., 1984). The carp, Cyprinus carplo in phenol intoxication for about 72 hrs, moved slower. The degree of speed inhibition depended on the toxicant concentration and time of fish exposure to the toxic environment. These behavioural responses were shown to be linked to the disturbances in the cholinergic system of the brain (Lukyanov et al., 1984).

Chromium metal induced marked changes in teleost fish, Nuria denricus in swimming and feeding (Abbasi and Soni, 1984).

ii) The effects of plant toxins on behaviour of fish:

Ramanujam and Ratha (1980a) performed toxicity studies on common fresh water fishes, Puntius shalynius (Yazdani and Talukdav), Danio dangila (Hamilton) and Heteropneustes fossilis (Bloch) using ten different plants of piscicidal potentialities. In the initial phase of treatment of the toxin, the fish was more active, then showed erratic movements, turned upside down and finally collapsed at the bottom of the jar, where they were kept. The gill movements increased at the initial phase and gradually decreased towards the lethal phase. They also observed that the dose required for air-breathing fishes was more compared to that for gill-breathers. Bhuyan (1967 and 1969), Das (1969) and Chakraborty et al. (1972) also have noted similar behavioural observations in their investigations.

2. Morphological and Histological Changes in Fishes due to Piscicides

i) Effects due to chemicals :

Liver & Gill histology was greatly altered in carps subjected to DDT, Lindane and α-HCH (Hexochlorocyclohexane) (Studnicka et al.,1983). Rojik et al. (1983) electronmicroscopically studied the effects of copper sulfate and zinc chloride on the histological structure of liver, kidney and gill of 3 fish species with different feeding habits. The cytoplasm of the respiratory cells of the gill became electron transparent and cytoplasmic organelles disappeared totally, pillar cells and pericytes remained intact. Effect of Emisan-6 in Anabas scandens on the morphology of fish erythrocytes was intense. Typical, vacuolization with dotted matrix initially was marked. In intense exposure, the erythrocyte membrane disappears and R.B.Cs shrink and disintegrate causing complete hemolysis (Panigrahi et al., 1984). Chromium poisoning caused erosion of fin-rays of fish Nuria denricus

(Abbasi and Soni, 1984). Similar histological changes have been observed in gills of different fish species by different investigators. In Channa gachua endosulfan induced separation of gill epithelium from basement membrane, fusion of adjascent gill lamellae erosion at the distal end of gill filaments and loss of cell membrane (Dalela et al., 1979). Treatment with a dye methylene blue initially showed swelling and thickening of gill lamella and in interlamellar filaments and then the epithelium became necrotized and sloughed off (Ahmed and Ghufran 1984). Liver and gonads were greatly affected due to Aroclor, 1254 poisoning showing vacuolation of hepatocytes of Slamo garidneri, fragmentation of developing oocytes in ovary but changes were not observed in testis. EM study showed enlargement of rough endoplasmic reticulum of hepatocytes and damage to spermatozoa and proliferation of the smooth endoplasmic reticulum of the oocytes (Sivarajah et al., 1978). Similar effects with cadmium treatment to Garra mullya (sykes) on liver (Wani & Latey, 1983) and with mercury toxicity to Sarotherodon mossambicus on liver and intestine, where proliferation of fibroblasts in hepatocytes and rupture of intestinal villi, increase in goblet cell population were prominantly observed (Naidu et al., 1983).

ii) Effects due to plant toxins :

Very few investigations are available on the studies of plant toxins with respect to the histological and morphological observations, and not a single investigation has noted on such observations on fish species. Kiptoon et al. (1982) has noted very interesting observation in cattle poisoned by Lasisiphon latifolium (Thymelaeaceae). Feeding of this plant, by bull calves developed lymphocytopenia. Acutely intoxicated calves developed petechial hemorrhages of epicardium and submucosa of rumen, abomasum and intestine. Lymph nodes and spleen showed lymphocytic degeneration with cellular depletion in the follicles. There were extensive liver fibrosis.

3. Biochemical and Physiological Changes in Fishes due to Piscicides

i) Effects due to chemicals :

First and foremost effects of the chemicals to the fish is haemolysis which has been observed in Anabas scandens due to Emisan-6 poisoning (Panigrahi et al., 1984), plant toxin first causes paralysis or haemolysis (Chopra et al., 1958). Similarly various inorganic and organic chemical constituents and enzymes of blood and other tissues are greatly affected. Acetylcholinestrase (AchE) activity of muscle, gill, liver and brain due to methyl parathion intoxication in the Tilapia mossambica was decreased in all tissues whereas acetylcholine was increased correspondingly, indicating disruption of nerve impulse conduction (Rao and Rao, 1984). Lukyyanov et al. (1984) observed acetylcholinesterase in retina and tectum of Cyprinus carpio in phenol intoxication. The presence of phenol in water caused certain changes in fish optomotor reaction. It moved slower and with retarded speed. It was due to great decrease of acetylcholine esterase in these tissues. These reactions were found to be linked to the disturbances in the cholinergic systems of the brain.

Aerobic oxidation of nutrients was adversely affected in quinalphos treatment to C. punctatus. In blood, plasma protein, glucose, lactic acid, Hb decreased, whereas glycogen in liver and muscles increased but lactic acid was decreased. Lactate dehydrogenase decreased in liver, kidney, muscle, intestine, brain and gills. Pyruvate dehydrogenase of liver, kidney and muscle decreased but was elevated in intestine and brain. In intestine succinate dehydrogenase activity elevated but in liver, kideny muscle brain gills it was significantly reduced (Hilmy et al., 1983). Alteration in levels of Hb, plasma proteins, glucose, lactic acid in blood, glycogen and lactic acid in liver and white skeletal muscles were observed in C. punctatus exposed to quinalphos by Sastry and Siddiqui (1984) also. The variance ratios of cations and anions were consistently more concentrated in the serum of DDT and Endrin exposed fish, Anguilla vulgaris and Mugil cephalus In these fishes serum cholesterol was sharply elevated in response to these pesticides (Hilmy et al.,1983). During 24 hrs. sublethal exposure to nitrite, methemoglobin in blood of fish, Ictalurus punctatus and Tilapia aurea was increased (Palachek and Tomasso, 1984). In fresh water cat fish, Heteropneustes fossilis, alterations in some enzymes and metabolites were observed in cadmium intoxication. Liver and muscle glycogen was depleted in 15 and 30 days but 60 days treatment showed 2 times increase in muscle glycogen. Lactic acid fell significantly after 15 and 60 days but elevated after 30 days. Acid phosphatase activity was inhibited in liver, ovary, and gill, but it was increased in kidney and intestine Alkaline phosphatase was decreased in liver, kidney, intestine but it elevated in ovary and muscles. Hexokinase of kidney and ovary was inhibited but increased in intestine. It was increased in liver, gill and muscle after 15 and 30 days but inhibited after 60 days of exposure. Xanthine oxidase decreased in liver and muscle and was elevated in kidney, intestine, ovary, gill. Glutamate dehydrogenase fell significantly in intestine, ovary and gills on the other hand in the liver, kidney, and muscles, it was elevated (Sastry and Subhadra, 1985).

ii) Effects due to Plant Toxins

a) Haemolysis :

Piscicides of plant origin will affect the R.B.Cs. and haemolysis will occur. The crude Gymnodinium breeve toxin develops syndrome,although the death of grey mullet, Mugil cephalus has not been assessed. But partially purified extracts induces haemolysis of mullet cells suggesting neurotoxic properties in the extracts (Kim et al., 1974). In cattle poisoned by Gnidia latifolia synonym Lasisiphon latifolium (Thymelaeaceae) lymphocytopenia results. In acutely intoxicated bull calves haemorrhages of the epicardium and submucosa of the rumen, abomassum and intestine take place. In chronic cases, lymph nodes and spleen showed lymphocytic degeneration with cellular depletion in the follicles (Kiptoon, 1982). Many toxins are known to have a direct effect on the haematologic parameters (Svobodova, 1971; 1975; Anonymous, 1966). Chakraworty et al. (1972) worked on Barringtonia acutangula (L.) and found that it contained 2 saponins possessing strong haemolytic properties.

b) Effect on Nervous System :

The Icthyotoxicity of Gymnodinium breeve caused neurotoxic effects in Mullet (Kim et al.,1974). The fruit extract of Zanthoxylum armatum caused the lethal action on an air breathing (Heteropneustes fossilis) and a gill breathing (Puntius shalynus) fish due to the inhibition of acetyl-cholinesterase activity in the brain indicating that the piscicidal component of this fruit was at least a neurotoxin (Ramanujam and Ratha, 1983). Similarly, many components of the piscicidal plants act on the nervous system causing paralysis (Chopra et al.,1958). Toxins of Acorus calamus L., Sapindus mukorossi Benth. and Xeromphis spinosa koey were proved to be the most toxic for fish eradication. The results indicated that their toxin affected the nervous system of the fish (Virdi, 1982).

c) Effect on respiration :

Babu (1965) described that Crotan tinglium seeds inhibited intake of oxygen uptake by the treated fish which leads to death. According to Virdi (1982) a thick layer of mucous was observed forming a coating over the body of the fish and also in the gill chamber during the treatment with extracts of different plant part of Acorus calamus Linn., Sapindus mukorossi Benth. and Xeromphis spinosa Koey. This mucous over the respiratory surface seems to be responsible for the obstruction in the exchange of gases. Lower rate of gaseous exchange compels the fish to swim at the surface of water more frequently for gulping atmospheric air, and finally when the obstruction continues, the fish succumbs. A. calamus gradually decreased the rate of respiration. At the sub-cellular level, plant toxin is known to block the NAD-dehydrogenase segment of the mitochondrial respiratory chain, resulting in reduced oxygen uptake by fishes (Horgan et al., 1968; Lindhal and Oberg, 1960).

d) Effects on Muscular System :

Many of the piscicidal plants contain saponin, alkaloids, glycosides and essential oils. They mostly act on the muscle activity directly (Chopra et al., 1958). The direct effect of the toxin on the fish was inhibition of acetyl cholinestarase activity in the muscle (Ramanujam and Ratha, 1983).

e) Effects on enzymes and other chemical constituents :

Lasisiphon latifolius (Thymelaeaceae) toxication to cattle increased blood urea nitrogen and lactate dehydrogenase whereas aspartate amino transferase, creatine phosphokinase and alkaline phosphatase were decreased There was no change in serum protein concentration (Kiptoon, 1982). In fish, Heteropneustes fossilis and Puntius shalynus enzyme acetylcholinesterase of brain and muscle was greatly inhibited (Ramanujam and Ratha, 1983). It is also known that NAD-dehydrogenase, a mitochondrial enzyme (Horgan et al.,1968; Lindhal and Oberg, 1960) and haemoglobin concentration (Svobodova, 1975, 1971; Ramanujam and Ratha, 1980) affected by piscicidal plant extracts.

E. Analysis of the Problem and Plan of Present Work

In India, more than hundred plants have been reported to contain piscicidal components (Chopra et al.,1949) and a few of these afore described plants have been studied to establish their piscicidal potentialities, Croton tiglium (Babu, 1965; Bhuyan, 1967); Milletia pachycarpa (Bhuyan,1968); Justicia hayatai (Ohta et al.,1969); Barringtonia acutangula (Chakraborty et al., 1972); Randia dumetorum (Nandy and Chakraborty, 1976); Marchantia polymorpha (Kanasaki and Ohta, 1976), Zanthoxylum armatum (Ramanujam and Ratha, 1980a; 1980b; 1983) and Sapindus mukorossi and Xeromphis spinosa (Virdi, 1982) etc. Ramanujam and Ratha (1980a; 1980b and 1983) have reported that out of 150, more than 40 plants of piscicidal properties occur in North Eastern India. They have collected and identified the 10 plants having piscicidal effect. The Western Ghat region has many indigenous plant species with piscicidal potentialities. However, none of them has yet been taken up for detailed chemical and biological investigations, to the extent that another potential piscicide could be developed. Therefore there is a vast scope for study on indigenous plants from Western Ghat region of India.

Most of the plants used for investigation are belonging to family Thymelaeaceae and Euphorbiaceae. Pimela linifolia and Pimela ligustrina (Tyler et al., 1985), Pimela brevishyla, P. calicola, P. ciliate (Rye, 1984); Daphnae gerkwa (Noko et al.,1983); Lasisiphon latifolius (Kiptoon, 1982) are some of the examples of the family Thymelaeaceae. Therefore, it is also hoped that the plants of these families from the Western Ghat region of India would be of much use in studies on the control of undesirable fish varieties from nursery ponds.

The fish, Tilapia mossambica among all the varieties of fishes in culture has created a number of problems. The biggest drawback of this fish in ponds is its early reproduction. It attains sexual maturity at a length of 8-9 cm when about 2-3 months old (Chimits, 1955). The female produces 100-300 or more young fish per spawn and breeds throughout the year at intervals of about 30-40 days. It is very euryhyline and can tolerate high salinities. Many reports on its ability to grow and even breed in sea water (Popper and Lichatowich, 1975). Potts et al. (1967) has shown that young T.mossambicus can even live in 200 % sea water. Thus due to the high prolificity at an early age and small size, the pond is soon filled with 'wild' spawned fry, all competing for food in the pond. This fish was introduced in Maharashtra State through Ceylon (Gazetter of India, Maharashtra State Fauna, 1974). Tilapia mossambica Peter's is reported to be a herbivore

(Vass and Hofestede, 1952). The omnivorous feeding habit of the fish was mentioned by Panikar and Tampi (1954). Tilapia, when cultured with major carps in ponds was observed to feed on carp spawn (1-5 mm.long) even in the presence of its natural food items. The medium sized specimens of about 25 mm long of Tilapia being more destructive than longer one (Calcutta, 1954). The above said voracious nature of the fish species was confirmed at Cuttack during 1958-59. To determine the influence of Tilapia on the survival and growth of 15 days old fry of Rohu and common carp demonstrated that the total fish production in ponds stocked with Rohu and common carp fry was more than that of -

a) In ponds stocked with Tilapia alone or in the combination of Tilapia, Rohu and common carp (Calcutta, 1954).

b) The presence of Tilapia population appeared to have brought in a fall in the survival of Rohu fry by 17-22 %, and the common carp by 16% (Calcutta, 1954).

c) In case of Rohu as well as common carp, the presence of Tilapia seemed to have caused very poor growth. The average individual weight attained being only about half of that in controls and

d) In ponds where all these species were stocked together, the presence of Rohu and common carp adversely affected production of Tilapia to the extent of only 30 % as compared to controls. Whereas the presence of Tilapia adversely affected the productivity of common carp to the extent of 61 % and that of Rohu to about 64 %. Under Indian conditions the fish is unsuitable for culture along with Indian major carps. The fish adversely affects growth and production of carps and its depredation on carp fry (Jhingran, 1968).

It is therefore, strongly recommended that this species not to be introduced into commercial fish farms, and to control this species in nursary pond is a big problem in pisciculture.

The aforedescribed short but critical review of the existing information on the effects of indigenous plant toxins on the behavioural, histological, histochemical and biochemical changes in the fish organ systems and their physiology, reveals that only respiratory, circulatory and nervous systems have been studied to some extent, there is still a vast scope for further work on these systems and also on digestive and excretory systems of the fishes. The digestive system and the excretory systems are also playing important role during intoxication of the pollutants. Because, some substances are absorbed which damage the gill filaments directly. The filaments may actually be eroded or they become clogged by causing oversecretion of mucus. The swallowed toxins damage the internal organs, some are stored up by the fish until the accumulation becomes injurious. Still other toxins irritate the surface of the fish and bring on secondary infections of bacteria or mold. Thus, gills, liver, kidney, buccal epithelium and skin becomes the important target organs for such studies which have been neglected in the previous research work. Similarly mucus secretion studies have not been paid much attention.

Some of these above mentioned problems are being undertaken for detailed analysis in the Department of Zoology, toxicology section, Physiology laboratory, Shivaji University, Kolhapur. The present dissertation concerns with the phytochemical analysis of the leaves of Lasiosiphon eriocephalus (Thymeleaceae family) for the search of toxin, and effects of the crude powder and of the extracts on the gills, liver, kidney and buccal epithelium of fish, Tilapia mossambica. In the present investigation, it was proposed to carry out detailed -

1) Analysis of the indigenous plant Lasiosiphon eriocephalus from Western Ghat area of South India employing mainly solvent fractionation methods.

2) Analysis of the different extracted components (U.V., NMR, IR, M.P., TLC, etc.).

3) Study of the effects of crude powder on the behaviour of the fish, Tilapia mossambica.

4) Study of the effects of different extracted fractions on the behaviour of Tilapia mossambica.

5) Study of the effects of crude powder and extracts on histology of gills, buccal epithelium, liver and kidney of Tilapia mossambica, by employing mainly histological techniques.

6) Study of the effects of crude powder and extracts on histochemistry of mucosubstances particularly from their cellular localization, characterization and probable functional roles in the gills, buccal epithelium, liver and kidneys of T.mossambica, by employing mainly histochemical techniques.

The reasons that led us to take up this problem are summarised below :

1) Choice of the indigenous plant :

The plant selected for the present study Lasiosiphon eriocephalus belongs to a family Thymeleaceae which is common in the Western Ghat Region of Maharashtra. The leaves are available throughout the year and easy to obtain locally. Therefore, this plant satisfies all the requirements for an ideal study of the type mentioned above.

2) Chemical characterization of the plant toxin :

Only a limited work has been done in this field. However, none of the studies has yet been taken up for detailed chemical and biological investigation to the extent that another potential piscicide could be developed. The present study is first of this type.

3) Choice of the animal :

The fish selected for the present study, Tilapia mossambica is found in plenty in fresh water tanks, ponds and rivers surrounding Kolhapur city and is easily available. The size of the fish is small (8-9 cm) and easy to handle in bio-assay experiment and maintained in glass acquarium. The fish shows sudden high mortality to the plant toxins so the fish can be exposed to various dilutions of presumed toxicant in order to determine limits of tolerance and lethality. Hence it was best suited for the present study.

4) Mucosubstances :

The plant toxins cause death of the fish due to the secretion of thick mucous surrounding the body and also in the gill chamber during the treatment time. This mucous over the respiratory surface seems to be responsible for the obstruction in the exchange of gases (Virdi, 1982). Due to certain plant toxins gill filaments oversecrete the mucus and get clogged and damage the internal organs (Lagler, 1952). Except these reports, no investigator has taken a keen interest in the mucopolysaccharides in the different organs of fishes. Therefore some lacunae still remain in their understanding in the physiology of different organ systems of fishes.

A complete histochemical characterization of the mucosubstances in the gills, liver, kidney and buccal epithelium is yet to be carried out. There is a need for such histochemical characterisation of mucosubstances in these organs and also of finding out whether they differ from the mucosubstances localised in each organs. Such studies are carried out before and after the treatment of plant toxins.

For studies on mucosubstances, histochemical techniques are ideal, since they provide information on the cellular and tissue localization of the various mucosubstances and variations in the staining intensities can be taken as reflections of alterations in their concentrations. Such studies would be useful to understand the alterations in them in variousorgans in response to the plant toxicant and in relation to the functional specialization of each organ and in the entire spectrum of events occuring in the physiology of each system. Hence it is believed that such a study will be first of its type.

For the convenience of presentation and ease of understanding, the next part of the thesis is divided into four chapters. The second chapter describes in detail the description of the plant the phytochemical methods employed for analysis of the indigenous plant, Lasiosiphon eriocephalus, the histology of the gills, liver, kidney and buccal epithelium of the fish, Tilapia mossambica and histochemical and biochemical techniques employed in the present study. Chapter 3 describes the observations on the phyto chemical constituents of the plant and mucosubstances of the gills, liver, kidney and buccal epithelium of T.mossambica. In fourth chapter the observations, have been discussed in the light of existing information and certain ideas have been arrived at and the conclusions are put forward with a general summary which is in chapter V followed by bibliography.