CHAPTER-I

Introduction

I. INTRODUCTION

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 following 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 control of undesirable predators and weed 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.

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 U.S.A. and 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.

A. 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. 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 piscidal 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 Cuttak 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. There-after a few of the researchers have undertaken the study on 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).

B. The survey of the work done 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). Milletia pachycarpa root powder is very effective at 2-6 ppm (Bhuyan, 1967; Ramanujan and Ratha, 1980). Bassia latifolia (Barrackpore, 1968; Chakraborty, 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 Wall 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).

C. The survey of natural Piscicides of plant origin :

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 utilise plant toxins produced in the country and by manufacturing them in a form suitable for administration in the developing fisheries technology.

1. Roteone :

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).

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 (Hooper, 1960).

Rotenone (Fig.1) 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).

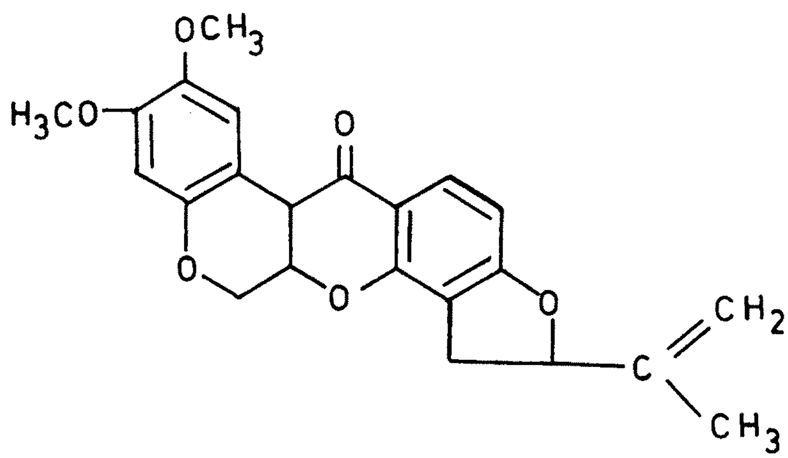


Fig. 1

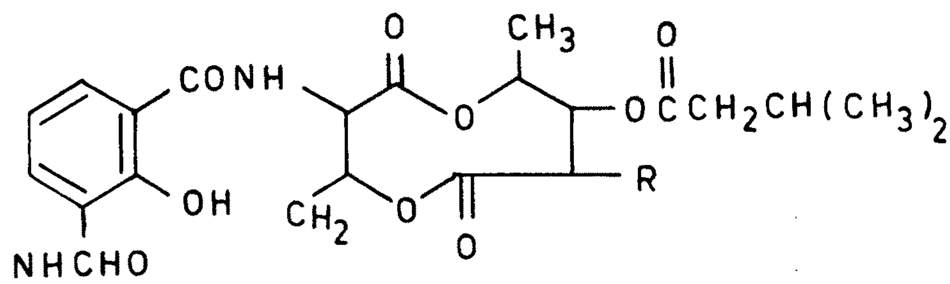
But Rotenone has a teratogenic property also. Rao and Chauhan (1971) found that the compound caused a variety of defects during development of chick embryos. Rotenone is a known inhibitor of the mitochondrial respiratory chain between flavoprotein and cytochromes. Khera et al. (1981) reported the defects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

2. Antimycin :

Antimycin A (Fig.2) 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 value (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.



Antimycin A_1; R = n - hexyl : $C_{28}H_{40}N_2O_9$

Antimycin A_3; R = n - butyl : $C_{26}H_{36}N_2O_9$

Fig. 2

3. Saponin :

Saponin from sugarcan jaggery (Gur) was found lathal to fish (Wealth of 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; Chakraborty et al., 1972) and Camdlia sipensis (Chowdhury, 1968) contain 7-8% saponin.

4. 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 Euphoribiaceae 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 factory (P)3 and Linimacrin, from Pimelea species, P.lingustrina and P.linifolia

5. Other toxins :

Other toxins like zugulone, 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.

D. The Survey of fish species used in research work on indigenous plant piscicides :

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) - Chakraborty et al., 1972.
2) Barilius bendelisis (Hamilton) - Virdi, 1982.
3) Catla catla (Hamilton) - Chakraborty et al., 1972
4) Channa gachua (Hamilton) - Bhatia, 1970.
5) Channa punctatus (Bloch) - Das, 1969; Virdi, 1982.
6) Channa striatus (Bloch) - Chakraborty et al., 1972.
7) Cirrhinus mrigala (Hamilton) - Chakraborty et al., 1972.
8) Clarias batrachus (Linnaeus) - Chakraborty et al., 1972.
9) Colisa chuna (Hamilton) - Chakraborty et al., 1972.
10) Colisa fasciata (Bloch) - Bhatia, 1970.
11) Ctenopharyngodon idella (Valenciennes) - Chakraborty et al., 1972.
12) Cyprinus carpio Var. Communis - Bhatia, 1970.
13) Heteropneustes fossilis (Bloch) - Ramanujam and Ratha, 1980; Virdi,1982
14) Hypophthalmichthys molitrix (Valenciennes) - Chakraborty, et al.,1972.
15) Labeo rohita (Hamilton) - Chakraborty et al., 1972.
16) Mugil parsia (Hamilton) - Chakraborty et al., 1972.
17) Mystus vittatus (Bloch) - Virdi, 1982.
18) Noemacheilus botia (Hamilton) - Virdi, 1982.
19) Notopterus notopterus (Pallas) - Chakraborty et al., 1972.
20) Punctius ticto (Hamilton) - Virdi, 1982.
21) Oryzias melastigmus (McClelland) - Hirota et al., 1980.
22) Tilapia mossambica Peters - Das, 1969; Chakraborty et al., 1972, Harold, 1987.

E The Survey of the impact of plant piscicides on fishes :

1. Behavioural Responses :

Ramanujam and Ratha (1980a) performed toxicity studies on common fresh water fishes, *Puntius shalynius* (Yazdani and Taludkav), *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. Anatomical and Histopathological Changes :

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) have noted very interesting observations 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

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 et al., 1982). Many toxins are known to have a direct effect on the haematologic parameters (Svobodova, 1971; 1975; Anonymous, 1966). Chakraborty 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 et al.,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.

F. 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 et al., 1982.) are some of the examples of the family Thymelaeaceae. Therefore, it is also hoped that the plants of these and other 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: when the ponds stocked in the combination of Tilapia, Rohu and common carp (Calcutta, 1954); 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)· 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.

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 due to 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 fruits of Sapindus laurifolius Vahl (Sapindaceae family) for the search of toxin, and effects of the crude powder and of the extracts on the gills, liver, kidney, intestine and buccal epithelium of fish, Tilapia mossambica. In the present investigation, it was proposed to carry out detailed -

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

2) Analysis of the different solvent extracted components by UV, NMR, IR, spectra.

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

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

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

6) Study of the effects of crude powder and extracts on the secretion of mucosubstances in the gills, oral mucosa, intestine, liver and kidneys of T. mossambica, by employing mainly histochemical techniques.

7) Study of the lethal effects of fruit powder and its extracts on T. mossambica.

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 Sapindus laurifolius belongs to a family Sapindaceae which is common in the Western Ghat Region of Maharashtra. The fruits are easily available 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, Sapindus laurifolius Vahl, the histology of the gills, liver, kidney, intestine &oral 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, intestine liver, kidney and buccal epithelium of T. mossambica. In fourth chapter the observations have been discussed in the light of existing information. The chapter fifth gives certain ideas which have been arrived at, the conclusion and the general summary of the study. This chapter is followed by the bibliography.

TABLE No.1

The list of Piscicidal plants, their botanical names, active principles, effective doses, duration of fish species and the references in the literature.

No.	Name of Plant	Part of plant used	Active principle	minimum effective dose.ppm.	Time taken for 100% Mortality, Hr.	Fish species used	References
1.	Croton tiglium	Seed powder		3 to 5 ppm			Babu, 1965 & Bhuyan, 1967.
2.	Bassia latifolia	Mahua oil	Saponin (4-6 %)	75 ppm (in pond) 60 ppm (Laboratory)		Cirrhinus mrigala, Puntius ticto, Cyprinus carpio, Colisa fasciata, Channa gachua.	Barrackpore, 1968 and Bhatia, 1970.
3.	Barringtonia acutangula	Seed (Powder)	2 Saponin types*	20 ppm	48 hr.	Wide variety of fish	Barrackpore, 1968
4.	Randia dumetorum	Fruit (Unripe powder)	- ,, -	12 ppm			
5.	Walsura piscidia	Bark (Powder)	- ,, -	10 ppm		Tilapia, Murrels	Chakraborthy, et al., 1972
6.	Camdlia sipensis	Seed (Tea cake)	Saponin (7-8 %)				Chowdhury, 1968.
7.	Nicotina tobacum	Leaves	Nicotin				Konar, 1969
8.	Millettia pachycarpa	Root (Powder)		2-6 ppm			Bhuyan, 1968

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Table No.1 contd....

No.	Name of Plant	Part of plant used	Active principle	minimum effective dose.ppm.	Time taken for 100% Mortality, Hr.	Fish species used	References
9.	Myrica esculenta Buch-Ham.	Bark		80-100 ppm	12-15 hr	Puntius shalynius, Danio dangila Heteropneustes fossilis	Ramanujam and Ratha (1980a, 1980b, 1983)
10.	Polygonum hydropiper L. var. hydropiper	Leaf		100-125 ppm	10-12 hr	,,	
11.	Potentilla fulgens Wal ex.Lehm	Root		150-200 ppm	8-10 hr	,,	
12.	Xeromphis spinosa (= Randia dumetorum) (Thumb.)Key	Fruit		120-140 ppm	10-12 hr	,,	Ramanujam and Ratha (1983)
13.	Zanthoxylum armatum DC. (= Z.alatum Roxb.)	Fruit (powder)	5 extracts	60 ppm (powder)	8-9 hr	Puntius shalynius, Heteropneustes fossilis (Bloch)	Ramanujam and Ratha (1980a)

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Table No.1 contd...

No.	Name of Plant	Part of plant used	Active principle	Minimum effective dose.ppm.	Time taken for 100% Mortality, Hr.	Fish species used	References
14	Acorus calamus	Root Rhizome, Leaves, Fruits		200 ppm	48 hr	Puntius ticto Ham., Barilium bendelisis Ham., Mystus vittatus Bloch, Nemacheilus rupecola M.Clelland, N.botes Ham. Heteropneustus fossilis Bloch, Channa punctatus Bloch.	Lal and Virdi, (1972), Virdi, (1982)
15.	Sapindus mukorussi	Root bark, Stem bark leaves, Green twig, Fruit pericarp, Seed, endosperm		200 ppm. (Except pericarp)	48 hr. Root bark, Stem bark, Fruit pericarp		
16.	Xeromphis spinosa (Thumb.) Keay.	Root bark, Stem bark, leaves, fruit.		200 ppm	48 hr. Root bark, Fruit pulp, Seeds.		
17.	Pimelea sp. (Thymelaeceae) P.ligastrina			Daphnane orthoesters 1)Simplex macrin			Tyler et al., (1985.)
	P.linifolia			2)Simplex linimacrin 3)Factor P3 pimelar 4)Linimacrin C			

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Table No.1 contd..

No.	Name of Plant	Part of plant used	Active principle	Minimum effective dose ppm.	Time taken for 100% Mortality, Hr.	Fish species used	References
18	Euphorbiaceae (Critonoideae Euphorbiaceae) Thymelaeaceae (Daphne, Gnidia, Lasiosiphon, Pimelea) Sunaptolepis, Aquilaria, Daphnopsis.		Tigliene, Daphnane Ingenane			Killi fish - Oryzias latipes	Hirota et al., 1980 Thorne, 1968.
19	Gymnodinium breeve					Mullet - Mugil cephalus	Kim et al., 1974.
20	Pliheallobium biramium	Bark					Chopra et al., 1965.
21	Derris trifoliata	Root	Rotenone				Das, 1969.
22	Diospyros cordifolia						Sharma and Simlot, 1971
23	Calotropis procera	Flowers					Sharma, 1980.

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Table No.1 contd...

No.	Name of Plant	Part of plant used	Active principle	Minimum effective dose ppm.	Time taken for 100% Mortality, Hr.	Fish species used	References
24	Aegle marmelos (Rutaceae)	Root					Joshi, 1986.
25	Butea monosperma (Fabaceae)	Stem					Joshi, 1986.
26	Euphorbia neriifolia (Euphorbiaceae)	Dendrons					Joshi, 1986.
27	Lasiosiphon eriocephalus	Leaves		3 ppm	12 hrs.	Tilapia mossambica	Nanaware and Harold, 1987, a-e, Harold, 1987.