CHAPTER-I

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Due to increased demand of proteinous food, the importance and field of fish farming had been increased on a large scale. One has to face a number of problems like food, predation and heavy growth of undesirable fishes in the nursary ponds and lakes. Due to high reproductive rate, undesirable fish population in ponds or lakes becomes abnormal. These undesirable fishes are labelled as "Trash fishes." These fishes also feed on food-fish. They also compete with food-fishes and game-fishes. Rounsefele and Everthart (1953) have stated that when fish population becomes 'unbalanced' or 'undesirable' in an area, it may be necessary in the interest of good wild-life management and food fishes to control it or eliminate it. Such elimination frequently involves elimination of undesirable species. It clearly indicates that control of undesirable fishes has equal importance to the culturing of food-fishes. Such elimination is done either by netting or by poisoning.

A) CONTROL OF UNDESIRABLE FISHES BY SYNTHETIC CHEMICALS, PISCICIDES AND ANTIBIOTICS :

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 the wide range of new synthetic piscicides which possess unique and highly desirable selective properties. So that these selective piscicides will kill particular species of undesirable fish at particular concentrations which do not have an adverse effect on desirable food-fish. The outstanding example of this selective dose effect is 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 growing into the lakes. Secondly, there is the use of piscicides to eliminate entire population of trash fish from lakes which, after suitable interval, can then be restocked with food-fish.

Different synthetic chemicals such as copper sulphate, toxaphene, polychloropynene, antimycin, trifluromethyl nitrophenol (TFM), bayluscide or niclosamine are also used to control the fishes. But these chemicals also affect the total biomass.

Toxaphene has piscicidal properties but one thing is undesirable with the use of toxaphene, that is its persistance and slow rate of detoxification in lakes. In some lakes it has remained toxic for periods upto 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).

Polychloropynene or PCLP, a very similar compound but not identical to toxaphene, has been used extensively for fish control in U.S.S.R. in recent years (Burmakin, 1968). In that country, lakes in the temperate zone contain fish species as perch (Perca fluviatilis), reach (Ratilus rutilus), ruff (Acerina cernua) which do not have commercial value. Pat (1964) PCLP gave good results to control these undesirable fishes. Like toxaphene, PCLP also persists 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 water is no longer toxic, there are no fish predators, there is no competition and some times there are no parasites. Therefore, there is reduction in the standing crop of food organism. In the fourth stage eutrophication of lakes takes place and standing crop of food invertebrates increases once more (Burmakin, 1968).

Antimycin is an antibiotic produced by microscopic plants. It has been discovered by scientist in the Department of Plant Pathology, University of Wisconsin (1945). It is toxic to more than thirty fish species, from which most susceptible species is 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 use of antimycin.

Trifluromethyl nitrophenol (TFM) is selective in action on larvae of the sea - lamprey (Petromyzon marinus) and fingerlings of rainbow trout (Salmo gairdnerii) TFM application in field forms striking contrast to methods currently used and it requires much higher 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.

According to Bayer (1973) a molluscicide, namely, bayluscide or niclosamine which was basically used for controlling the aquatic snails, can be used for killing the fishes, at molluscicidal dosage. Bayluscide has been shown to be toxic to at least eighteen species of fresh water fish.

Besides above chemicals, aldrine ($C_{12}H_8Cl_6$) used to kill weed and predatory fishes (Chaudhuri, 1960), dieldrin ($C_{12}H_8Cl_6O$) effectively killed fishes at 0.5 ppm, endrin ($C_{11}H_6Cl_2O$) 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).

There are, mainly, two arguments against the use of synthetic chemicals and antibiotics 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 to the biomass. For these and for other reasons including their severe toxicity, prolonged residual effects on other biota including human beings, may remain stored in fish tissues, the synthetic chemical toxins are not preferred. These chemicals are very expensive also.

B) ALTERNATIVE WAYS FOR CONTROL OF UNDESIRABLE FISHES :

With the rapid increase in the pisciculture, it has become necessary to find out alternative piscicide. Rising costs, the development of resistance and pollution caused by synthetic chemical also call for the discovery of less expensive and hazardous alternative. One of the most preferable alternatives is the use of 'Natural toxins' as piscicides.

'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 mere effect on the metabolism and biological functions of the intoxicated animals. Since ancient times man has been pondering over the physiological effects of various toxins and venoms. How do these natural toxins work ? Each toxin has its own characteristic structure, a property shared by drugs. Each toxin has also its unique mode of action. Therefore, many of these natural toxins in minute quantities are potentially good drugs. One of the preferable natural toxins is the 'Plant toxin' which can be used in large amount to control the undesirable fishes.

'Plant toxin' may broadly be defined as any compound present in plants which, under natural (but possibly abnormal) circumstances 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 animals' 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 on 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.

C) A BRIEF SURVEY OF PISCICIDAL PLANTS AND THEIR USE IN CONTROLLING UNDESIRABLE FISHES :

In India, several ingradients 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; 1958; 1965; Chopra and Chopra, 1955). 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 subcontinent is occupied by forest of different kinds. There are evergreen forests occupying the west coast of the peninsula upto the edges 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 3000 plants have medicinal properties and some descriptions have been enumerated in the literature of indigenous medicine. It is significant 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 Substation at Cuttak and Central Inland Fisheries Research Institute, Barakpore, 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', which is full of consolidated information on all aspects of Inland and Marine fish and fisheries of India. His review on fish poisons of plant origin was stimulating and encouraging for the investigators in this field. Thereafter 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 effects on different varieties of fish species (Table No.1).

D) A BRIEF SURVEY OF THE WORK DONE ON PISCICIDAL PLANTS :

The Council of Scientific Industrial Research (CSIR), New Delhi, in its publication has mentioned the use of sugarcane jaggery (Gur) as piscicide, because it contains an active piscicide, saponin up to 1 per cent (Wealth of India, 1962). In mainland China, the seed cake of tea (Camdlia sinensis) containing 7-8% saponin and croton seeds are also used as piscicides (Hora and Pillay, 1962; Babu, 1965; Bhuyan, 1967 and Ramanujam and Ratha, 1980). An indigenous variety of derris, Derris trifoliata Loar var. Uliginosa is found to contain 1.2 - 2.2% rotenone which kills fishes (Das, 1969), Millettia pachycarpa root powder is very effective at 2-6 ppm (Bhuyan, 1967; Ramanujan and Ratha, 1980), Bassia latifolia (Barrackpore, 1968; Chakraborthy, 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) also contain considerable important toxins which affect the fish life. In recent years certain other plants Eupatorum odoratum L., Myrica esculenta Buch. Ham., Polygonus hydropiper L. Var. Flaccidum steward, Polygonum hydropiper L. Var. Hydropiper, Potentia fulgens Wall ex Lehn. Taxus buccata L., Xeronphis 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 pimelia sp. (Family-Thymelaeceae) P. linifolia and P. ligustrina (Tyler et al., 1985) and other species of this family and Euphorbiaceae (Hirota et al., 1980), Laslosiphon eriocephalus Dec. (Harold, 1987) and Sapindus laurifolius (Bhosale, 1988) are found more effective.

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).

E) A SURVEY OF NATURAL PISCICIDES OF PLANT ORIGIN :

The previous work on plant toxins was mainly confined to the collection of general account of potentialities of piscicides in indigenous plants. Some investigators have 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 2-3 decades. The work in India in this line had 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.

1) Rotenone :

Geoffrey (1892) was the first scientist to isolate the active principle of Lonchocarpus nicou. Nagi (1912) isolated an identical compound from Derris cinensis, 'gyoto' grown in Formosa, called roten by the natives and was assigned the name "rotenone" because it was shown to be a ketone. A number of related compounds of rotenone have been isolated from plants of leguminosae and structure of these known as 'rotenoids' have been established. It was the first natural piscicide to be used extensively in fishery management. It has been used in 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 (Gassalvez 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). An indigenous variety, Derris trifolliats Lour, which occurs in the vicinity of Kakdwip (West Bengal, India) also contains 1-2% rotenone and is used to kill Mugil parsia, Channa punctatus, Tilapia mossambica (Das, 1969). Tephrosia and Lonchocarpus species (Leguminosae) also contain this toxin (Gosalvez et al., 1977).

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 the high oral doses of rotenone in pregnant rats.

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

2) Tigliane, Daphnane, Ingenane :

In recent years, tigliane, daphnane and ingenane plant derivatives have been tested for piscicidal activities. Gunasekera et al. (1979) obtained daphane ester from Cunuria spruceana. About 13 genera of Euphorbiaceae and seven genera of the Thymelaeaceae are known to contain tigliane and/or daphane derivatives (Hecker, 1978; Gunasekera et al., 1981). Recently, Tyler et al. (1981) have identified five biologically active dephane orthoesters, namely simple-ximacrin, linimacrin, simplex linimacrin 'd' and pimelea factor (P), from pimeles species, P. lingustrina and P. linofolia.

3) Saponin :

Saponin is also a natural toxin which can be used as piscicide. Saponin from the sugarcane jaggery (Gur) was found lethal 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 sppensis (Chowdhury 1968) contain 7-8% saponin.

4) Other active principles :

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.

F) A BRIEF SURVEY OF FISH SPECIES EMPLOYED IN RESEARCH ON INDIGENOUS PISCICIDAL PLANTS :

The different parts and their extracts prepared from some of the indigenous plants and active principles (toxins) isolated in pure form are properly used in fishery research. Investigators also carry out biological assays to test the toxicity of these toxins and suggest dose for different undesirable fish species for their eradication. The following fish species were employed in earlier investigations on plant piscicides :

A list of fish species employed in toxicological studies on piscicides of plant origin :

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) *Clarius batrachus* (Linnacus) - Chakraborty *et al.*, 1972.
9) *Colisa chuna* (Hamilton) - Chakraborty *et al.*, 1972.

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

11) Ctenopharyngoden 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 Vittatus (Bloch) - Virdi, 1972.

18) Noemacheilus botia (Hamilton) - Virdi, 1972.

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; Nanaware and Harold, 1987a-d; Bhosale, 1988.

G) A BRIEF SURVEY OF THE IMPACT OF PLANT TOXINS ON FISHES:

1) Behavioural responses :

Ramanujam and Ratha (1980a) performed toxicological 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, it showed violent and erratic movements, afterwards fish became sluggish and steady turning upside down and finally collapsed at the bottom of jar, in which they were kept. The opercular move ments increased at the initial phase and gradually decreased towards the lethal phase. They also observed that the dose required to kill the air breathing fishes was more as compared to that for gill-breathers. Bhuyan (1967 and 1969), Das (1969) for Chakraborty et al. (1972) have noted similar behavioural observations in their investigations. Recently, Harold (1987) and Bhosale (1988) have observed changed behaviour in swimming, in breathing and during feeding in T. mossambica after intoxication with plant extracts.

2) Anatomical and histopathological changes :

Very few investigations are available to show the anatomical and histomorphological changes in the different organs in the higher vertebrates due to plant toxins but not a single investigation has noted on such observations in fish species. Kiptoon et al. (1982) has noted very interesting observation in cattle poisoned by Lasiosiphon latifolium (Thymelaeceae). Feeding of this plant bull calves developed petechial hemorrhages of epicardium and submucosa of rumen, abdomen and intestine. Lymph nodes and spleen showed lymphocytic degeneration and cellular depletion in the follicles. There was also extensive liver fibrosis. Recently, Harold (1987) and Bhosale (1988) have observed histopathological changes in different target organs of T.mossambica due to the toxic action of plant toxins from L. eriocephalus and S.laurifolius, respectively.

3) Biochemical and physiological changes :

a) Haemolysis :

R.B.Cs. get affected by the plant toxins and haemolysis takes place. The crude powder of Gymnodinium breeve develops syndrome, although the death of grey mullet, Mugil cephalus has not been assessed. But partially purified extracts induce haemolysis of mullet cells suggesting neurotoxic properties in the extracts (Kim *et al.*, 1974). In cattle poisoned by *Gnidia latifolia* synonym *Lasiosiphon 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 haematological parameters (Svobodova, 1971, 1975; Anonymous, 1966). Chakraborty *et al.* (1972) worked on *Barringtonia acutangula* (L.) and found that it contained two 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 lethal action on an airbreathing (*H. 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 atleast 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 *Xeramphis 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* :

Crotan tiglium seeds inhibited intake of oxygen by the treated fish which leads to death (Babu, 1965). According to Virdi (1982) a thick layer of mucus was observed forming a coating over the body of the fish and also in the gill chamber during the treatment with extracts of different parts of Acorus calamus Linn., Sapindus mukorossi Benth. and Xeronphis spinosa Koey. This mucus 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 subcellular 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) Effect on muscular system :

The plant toxins saponin, alkaloids, glycosides and essential oils are present in many of the piscicidal plants. These plant toxins 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 :

L. 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 H. fossilis and P. 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, 1971, 1975; Ramanujam and Ratha, 1980) affected by piscicidal plant extracts.

f) Analysis of the problem and plan of present work :

In India more than one hundred plants have been reported to contain piscicidal components (Chopra et al., 1949) and a few of these aforedescribed plants have been studied to establish their piscicidal potentialities, C. tiglium (Babu, 1965; Bhuyan, 1967); Milletia pachycarpa (Bhuyan, 1968); Justica hayatai (Ohta et al., 1969); B. acutanguls (Chakraborty et al., 1972), Randia dumetorum (Nandy and Chakraborty et al., 1976); Marchantia polymorpha (Kanasaki and Ohta, 1976), Z. armatum (Ramanujam and Ratha, 1980a; 1980b; 1983) and S. mukorossi and X. spinosa (Virdi, 1982). 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 properties. 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 investigation, 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 belong to family Euphorbiaceae. P. linifolia and P. ligustrina (Tyler et al., 1985), P. brevishyla, P. ciliate (Rye, 1984) Daphnae gerkwa (Noko et al., 1983), L. latifolius (Kiptoon, 1982) and L. eriocephalus (Harold, 1987) are some of the examples of the family Thymelaeceae. Bhosale (1988) selected plant, S. laurifolius of family sapindaceae. But the plants of other families from the Western Ghat region of India have not been used in studies on the control of undesirable fish varieties from nursery ponds. Therefore, indigenous plant, Acacia concinna belonging to Leguminose family was selected for the present investigation.

The fish T. mossambica among all the varieties of fishes in pisci 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 cms. even 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 a very euryhyline and can tolerate high salinities. Many reports are found on its ability to grow and even breed in sea water (Popper and Lichatowich, 1975). Potts et al. (1967) have shown that young T. mossambicus can even live in 200% sea water. Thus, due to high prolificity at an early age and at small size, the pond is soon filled with 'wild' spawned fry, all competing for the food in the pond. This fish was introduced in Maharashtra State through Ceylon (Gazetteer of India Maharashtra State Fauna, 1974). T. mossambica is reported to be a herbivore (Vass and Hofestede, 1952). As against this an 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 being more destructive than longer one (Calcutta,1954). The above said voracious nature of this fish 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, the 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.

Rohu and common carp affect the production of Tilapia to the extent of only 30% as compared to controls when these species were stocked together in ponds. Whereas presence of Tilapia adversely affected the productivity of common carp to be 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 the growth and production of carps and its depredation on carp fry (Jhingran, 1668).

Therefore, it is strongly recommended that this species is not to be introduced into commercial fish farms, and to control this species in nursary ponds 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 excretary systems of the fishes. The digestive system and excretary systems are also playing important role during natural piscicidal intoxication. Because, some substances which are absorbed, 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 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, intestine, buccal mass 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 the 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 the plant Acacia concinna (leguminasae family) for the search of toxin, and study of effect of the crude powder and of the extracts on behaviour and target organs like gills, liver, kidney, intestine and buccal mass of the fish T. mossambica.

In the present investigation it was proposed to carry out work in the following manner :

1) Analysis of the fruit powder of the indigenous plant A. concinna from Western Ghat area of South India employing mainly solvent fractionation methods.

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

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

4) Study of the effects of ethanol extract fraction on the behaviour of T. mossambica.

5) Study of the lethal effects of the ethanol extract to observe % mortality of fish T. mossambica and to calculate LC_{50} value.

6) Study of the effect of ethanol extract on histology of gills, liver, kidney, intestine and buccal mass of T. mossambica by employing mainly histological techniques.

7) Study of the ethanol extract on the secretion of mucosubstances in the gills, liver, kidney, intestine and buccal mass of the fish 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 A. concinna selected for the present study belongs to the family Leguminasae, which is common in Western Ghat region of Maharashtra The fruits of this plant are easily available and easy to obtain locally. The fruits of this plant are known to contain the natural plant toxin which is piscicide in nature. Therefore, as this plant bears piscicidal properties and as it is easily available it was selected for the present study.

2) Chemical characterization of the plant toxin :

Some work is available on chemical characterization of plant toxins. However, none of the studies has yet been taken up for detailed spectrophotometrical analysis of plant piscicides and their efforts on fish life. Hence the present study is first of this type and has been undertaken to analyse the piscicide of indigenous plant, A. Concinna.

3) Choice of the animal :

The fish selected for the present study T. 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 experiments and can be maintained in the glass acquarium. The fish showed sudden high mortality to other plant toxins, so it was thought desirable to expose to the presumed toxicant in the selected plant in order to determine limits of tolerance and lethality. Hence this fish was found suitable for the present study.

4) Study on the behavioural responses to fish :

Normally synthetic pesticides, biocides and also natural toxins affect the behaviour of the animal. But such work on plant toxins is meagre. Hence the plant toxin from fruits of A. concinna was selected to study the behaviour, % survival and % mortality of the fish T. mossambica.

5) Study on mucosubstances :

During the treatment of plant toxins death of the fish takes place due to the secretion of thick mucus surrounding the body and also in the gill chamber. This mucus 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 secrete excess mucus and get clogged and damage the internal organs (Lagler, 1952; Harold, 1987 and Bhosale, 1988). Except these reports, no investigator has taken a keen interest in the muco-polysaccharides 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, intestine and buccal mass is yet to be carried out. There is need for such histochemical characterization of mucosubstances in these organs and also of finding out whether they differ from their muco-substance contents. Such studies are carried out before and after the treat-ment of plant toxins in the present investigation.

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 reflectioins of alterations in their concentrations. Such studies would be useful to understand the alterations in them in various organs in response to the plant toxin and in relation to the functional specialization of each organ and in the entire spectrum of events occurring in the physiology of each system. Hence it is believed that such study will be the 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 presents in detail the description of the plant and fish, the phytochemical methods employed for analysis of the fruit powder of indigenous plant A. concinna, methods employed for analysis of water, methods employed for analysis of behavioural responses of the fish and histochemical and biochemical techniques employed in the present study. The third chapter describes the observations on the phytochemical constituents of the fruit powder of the plant and histomorphological and histochemical localization and alterations in the mucosubstances of the gills, liver, kidney, intestine and buccal mass of T. mossambica during intoxication due to plant toxin. In the fourth chapter the observations have been discussed in the light of existing information. The fifth chapter gives certain ideas which have been arrived at, the conclusion and the general summary of the study; this chapter is followed by a 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 P.linifolia			Daphnane orthoesters 1)Simplex macrin 2)Simplex linimacrin 3)Factor P3 pimelar 4)Linimacrin C			Tyler et al., (1985.)

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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	Piiheallobium 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 (Thymelaeaceae)	Leaves		3 ppm	12 hrs.	Tilapia mossambica	Nanaware and Harold, 1987, a-e, Harold, 1987.
28	Saphindus laurifolius (Saphindaceae)	Fruits		200 ppm	12 hrs.	Tilapia mossambica	Bhosale, 1988.