CHAPTER-IV

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It has been pointed out earlier in the introductory chapter that natural toxins of plant origin have been used to clear the ponds by destroying weed fishes and other undesirable aquatic animals, in the pisciculture. In India it is gratifying to note that in recent years a growing interest is being taken to use indigenous variety of plants in fish nursery management, in developing fisheries technology.

It is significant to note that in India there are about 700 poisonous plant species belonging to over 90 families of flowering plants (Chopra et al. 1965) and there is no comprehensive review on these plants to show their toxic principle. Some of these plants are used as piscicides. Recently Vishwanathan and Joshi (1983) have published short but very important review on the toxic constituents of some Indian plants which stimulated us to search for natural piscicide from indigenous plant A. concinna, from Western Ghat. In their review they have classified the toxic substances into three categories, according to (i) its physiological manifestations e.g. as nerve and muscle poison, (ii) its chemical constitution e.g. alkaloid, glycoside or (iii) its botanical origin. But their survey has given information on the plants used in medicine or of their potential use in medicine, on edible plants and those used as adulterants in food, on toxic plants and allergens. In recent years due to the rising cost, the development of resistance and the pollution

caused by the chemical piscicides used in the control of undesirable fish species in the pisciculture, call for the discovery of the less expensive and less hazardous alternatives. With this view in mind, in few laboratories in India, the indigenous plants are explored to discover a plant product having potentiality to use as piscicide (Ramanujam and Ratha, 1980; Nanaware and Harold, 1987a,b,c,d,e; Nanaware and Bhosale, 1988). But the active principle of all these plants have not been fully discovered. The present investigation is the first of this type to show the nature of the natural piscicide.

Some work has been reported in this line, in North Eastern India (Ramanujam and Ratha, 1980a, 1980b and 1983), but there is no such study available on the plant toxins in this aspect, in the Western part of India. The Western Ghat region has many indigenous plant species with piscicidal potentialities. Therefore, as there is a vast scope for study on indigenous plants from Western Ghat of India, a common used indigenous variety of piscicidal plants, A. concinna is selected for the present investigation. The most of the earlier work was restricted to the plants belonging to the families Thymelaeaceae, Euphorbiaceae and Sapindaceae. But the plant selected for the present study belongs to the familyLeguminoceae.

The fish T. mossambica is locally and easily available fish. It creates number of problems in the nursery ponds in the local area. Due to these and previously described reasons in the second chapter of material and methods, this fish T. mossambica is selected for the present investigation.

Earlier investigators have used many plants for their study in pisci-culture, they have not been investigated in detail the chemical compositions

of these plants. But the present investigation provides the information on biochemical composition and phytochemical analysis of the indigenous piscicidal plant A. concinna. It has been brought to the notice, that the paucity of literature on the effects of plant toxins on vital organs of undesirable fishes in the eradication studies, the effects of the toxin extracted from the fruits of this plant has been observed on the important vital organs like buccal mass, gills, liver, kidney and intestine of T. mossambica also.

A. DISCUSSION ON SOLVENT EXTRACTION AND CHEMICAL COMPOSITION :

1) Solvent extraction :

Shade dried fruits without seeds, of A. concinna powdered and extracted using five different solvent petroleum ether, benzene, ethyl acetate, chloroform and ethanol. These solvents were used according to their increasing polarity. So the less polar components were moved in less polar solvent and more polar solutes in more polar solvents during the process of extraction. Among the five extracts (E_1, E_2, E_3, E_4 and E_5), the ethanol extract (E_5) contains piscicidal component. The active principle extracted from the fruits of A. concinna observed to be water soluble.

2) Chemical composition :

The chemical composition of fruits of A. cencinna shows more percentage of ethanol extracted components than other solvent extracts. At a comparative level also the percentage of ethanol extracted components is more than, the percentage of this component in the leaves of L. eriocephalus and in the fruits of S. laurifolius. This ethanol extracted component contains

a piscicidal active principle. In L. eriocephalus the percentage of this component is 3.25, in S. laurifolius it is 15.02 whereas in A. concinna it is 16.4324 indicating these fruits of A. concinna are rich source of piscicidal component. But inspection of doses required to 100% mortality of T. mossambica, the L. eriocephalus component was much more toxic than S. laurifolius and A. concinna. The dose for 100% mortality is 15 ppm of L. eriocephalus, 400 ppm of S. laurifolius while it is 350 ppm of A. concinna. Hence, it could be stated that the toxic component of plant A. concinna is less toxic than L. eriocephalus while it is more toxic than S. laurifolius.

B. DISCUSSION ON PHYTOCHEMICAL ANALYSIS :

1) Melting points :

The five extracts of fruits of A. concinna have melting points, ranging between 89-98°CThe melting point of ethanol extract (E_5) is 97°C to 98°C. It is 99°C in case of fruits of S. laurifolius (Bhosale, 1988), while it is 118-120°C in case of L. eriocephalus (Harold, 1987). The comparative account of melting points can not give any idea about the nature of the active piscicidal components in the extracts. To come to the conclusion by using melting points as the criteria for the identification of natural piscicides, more number of toxic components of plant origin must be analysed.

2) UV spectral analysis :

Intense peaks in between 220 to 425 nm were shown by UV spectra of five extracts E_1, E_2, E_3, E_4 and E_5 of fruits of A. concinna. Extract E_1 showed two λ maxima at 315 and 415 nm, extract E_2 showed two λ maxima

at 315 and 420 n m, extract E_3 showed only one maxima at 325 nm, extract E_4 showed two λ maxima at 280 and 425 nm and extract E_5 showed two λ maxima at 265 and 350 nm.

The spectra of extract E_1 and E_2 indicate the presence of ketone or enome and the spectra of extract E_4 indicates presence of benzoid compound.

3) NMR spectral analysis :

NMR of E_1 extract shows multiplet at δ0.6 ppm. indicating the presence of methyl groups. The presence of number of methylene groups indicated by encountering a broad multiplet at δ 1.5. Spectrum of extract E_2 shows singlet at δ 1.8 and unresolved multiplet at δ 1.3 and δ 3.7. Singlet at δ 1.8 indicates presence of methyl group and two sharp singlets centered at δ 1.3 ppm indicates presence of methylene group, ratio of methylene to methyl compounds in the same fraction is 3:4. A multiplet at δ 3.7 ppm may be due to the presence of tertiary methine. NMR spectra of E_3 and E_4 extracts show the alcoholic groups. NMR spectrum of E_5 extract shows peaks at δ 4.7 toδ 5.1, δ 7.1 and δ 9.5. The multiplet at δ 4.8 indicates presence of OH group. Two doublets at δ 4.7 to δ 5.1 show oliphinic trans-protons (coupling constant 18 Hz). Singlet at δ 7.1 indicates aromatic ring.

4) IR spectral analysis :

IR spectrum of E_1 extract showed stretching bands at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1560 cm^{-1}, 1580 cm^{-1}, 1710 cm^{-1}, 2900 cm^{-1}. The band at 1710 cm^{-1} indicates presence of keto group. The broad band at 2900 cm^{-1} indicates presence of OH group, the presence of ketone is also

observed. IR spectrum of E_2 extract showed streching bands at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1710 cm^{-1}, 2900 cm^{-1}. The band concentrated at 1710 cm^{-1} indicated presence of keto group and broad band at 3200 to 2500 cm^{-1} is due to the hydroxy group which is hydrogen bonded. IR spectrum of E_3 extract showed six streching bands at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1720 cm^{-1}, 1780 cm^{-1}, 2900 cm^{-1} and a broad band at 3500 to 3350 cm^{-1}, from this it can be concluded that this extract contains OH groups due to broad band, lactone due band at 1780 cm^{-1} and another ketone due to band at 1720 cm^{-1}. The IR spectrum of E_4 extract showed streching bands at 720 cm^{-1}, 1375 cm^{-1}, 1460 cm^{-1}, 1710 cm^{-1} and 2900 cm^{-1}. This extract showed presence of keto group due to band at 1710 cm^{-1}. The IR spectrum of ethanol extract also showed stretching broad band at 3500 cm^{-1} indicating the presence of -OH or hydroxyl group, a band at 3000-2900 cm^{-1} indicating (-C - H) and 1720-1700 cm^{-1} indicated the presence of ketonic group (C = 0) in the compound.

Thus UV, IR and NMR spectral results of the fruit extract of A. concinna are complimentary to each other indicating the possible presence of alcoholic and ketonic compounds in them.

The broad streching bands indicated the presence of impurities or complex nature of compounds. In next work by making further separation impurities can be removed and then new spectra can be obtained for confirmation of results.

5) Atomic absorption spectrophotometric analysis :

Atomic absorption spectrophotometric results of many cations indicated that the fruits A. concinna bear a measurable amounts of divalent cations such as Ca^{++}, Cu^{++}, Mg^{++}, Fe^{++} and Zn^{++}. At a comparative level calcium concentration was more in all the five extracts of the fruits showing values 0.38 ppm (E_1 extract), 20.4 ppm (E_2 extract), 0.41 ppm (E_3 extract), 0.26 ppm (E_4 extract) and 0.33 ppm (E_5 extract). Among the five extracts the ethanol extract contained more Ca^{++} than the others. Cu^{++} concentration was observed as 0.07, 0.34 and 0.32 ppm in E_2, E_4 and E_5 extracts respectively. Cu^{++} was not found in the E_1 and E_3 extracts. Mg^{++} concentration was highest (1.05 ppm) in E_2 extract while it was in between 0.12 to 0.22 in E_1, E_3, E_4 and E_5 extracts. Fe^{++} concentration was found to be 0.09 in E_3 extract and 0.14 in E_5 extract, while it was not found in E_1, E_2 and E_4 extracts. Zn^{++} concentration was highest (0.281 ppm) in E_2 extract while it was only 0.077, 0.071, 0.094 and 0.109 ppm in E_1, E_3, E_4 and E_5 extracts. Co^{++}, Cd^{++} and Hg^{++} cations were not detected in all five extracts.

The importance of these cations from the present investigation can not be deduced. But these cations might be useful for the biochemical machinery of this plant.

These metallic ions might be forming chelation with the compounds extracted from the fruits which interfere in resolving the clear chemical structure and the functional groups in the compounds, as indicated by their IR, NMR and UV spectral patterns.

C. DISCUSSION ON WATER QUALITY ANALYSIS :

1. Temperature of water :

As temperature plays a vital role in determining the distribution, growth, reproduction, metabolism and behaviour of the aquatic organisms, it is one of the most important factors in the environment. Because it is one of the most easily measurable factors in the natural environment, and one which can be readily controlled in the laboratory, perhaps more is known about the reactions of fresh water animals to this one factor than to any other in the environment (Muirhead-Thomson, 1971).

Due recognition is given to this major role of water temperature in the various studies involving evaluation of pesticide impact, by carrying out all tests at controlled temperature, either at one constant temperature for all tests or at 2 or 3 constant temperature ranges in the laboratory. In trying to assess the effect of temperature on the impact of pesticide on fresh water life, it is important to recognize some of the many different facets which have to be taken into account. Differences in the temperature, or change in the temperature, can affect the general activity, metabolism and behaviour of fresh water forms. Temperature also influences the chemical and physical state of the pesticide. The actual rate of the uptake of the toxic chemical by the fresh water organism may be strongly influenced by prevailing temperature conditions. Some of the variations and vagoris of temperature effect on pesticide impact are well brought out in the extensive fish toxicity studies carried out in different laboratories (Applegate et al., 1961; Muirhead-Thomson, 1971). Most of these compounds show increasing

toxicity at higher temperature. Wtih some of these compounds susceptibility of fishes increases with the increase in the temperature (Macek et al.,1969).

In the present investigation, the toxicological experiments were performed nearly at uniform temperature, which avoids complications. This uniform temperature was nearer to the natural environment of the test fish. All the experiments were performed in the range of 24-27^{o}C. There was hardly difference of 3^{o}C in the experimental temperature. As far as the present studies are concerned, the effect of temperature on the impact of natural piscicide present in the fruit extract of A. concinna on fresh water fish T. mossambica was negligible. But to test influence of temperature on the chemical and physical state of the plant toxin, experiments increasing and decreasing temperature must be performed and to assess the relative increase in susceptibility of fishes to this plant toxin data on LC_{50} at different temperatures should be worked out. Such work is in progress in this laboratory.

2) pH of water :

The interrelation between pH and its role in determining toxicity of certain toxic chemicals is well brought out in studies or piscicide antimycin (Walker et al., 1964). As the mass of plant growth increases in the pools, pH of the water changes. Thus, pH acts as an indicator of the acid base shift. It appears that pH has no major effect in concern with the routine studies on fish toxicity. The general concept is understood by the terms 'hard' and 'soft' water is exemplified by the composition of the dilution water used in standard tests with insecticides in the U.S.A. (Henderson et al.

1960) and in molluscicide studies in South Africa (Meyling et al., 1962). A good example in which hardness of water may affect pesticide impact is provided by observations on the molluscicide, bayluscide, especially in view of the fact that studies were made at both laboratory and field levels in quite different context in different countries (Meyling et al., 1962). Bayluscide at all exposure periods was found to be more toxic to rainbow trout and to other test fish in soft water than in hard water. The greatest increase in toxicity was between medium hard water sugests degradation of bayluscide at high pH level and at higher alkalinities (Marking and Hogan, 1967).

Hardness and pH of the water influences, the toxicity of TFM to larval lampreys and to rainbow trouts. This chemical is most effective in soft acid waters in which minimum lethal concentration can be as low as 0.5 ppm. As pH, conductivity and alkalinity of the water increase, the doses requirement of TFM also increases to effect 100% kill of larval lampreys. In the hardest and most alkaline waters tested, the minimum lethal concentration for the larvae was 8.0 ppm. Changes in the toxicity of TFM to rainbow trout were comparable and the different toxic effects of this compound was retained regardless of its level of activity in any given water.

As in case of pH, the hardness of the water has no major effect on the toxicity to fish of chlorinated hydrocarbons with the exception of dipterex (Henderson et al., 1960). Since the hardness of the water definitely affects the water quality and consequently impact of the toxic compounds on the toxicity to fish of chlorinated hydrocarbons (Henderson et al., 1960). This also applies to most organophosphorous compounds with the exception of dipterex.

Impact of pesticides gets affected by the pH of the water itself. But according to Aquatic Life Advisory Committee (1955), pH be recognized as a poor criteria for the expression of the toxicity. According to them pH range of 5 to 9 was known to be non-toxic. Therefore, pH values be maintained between 6.5 and 8.5 to maintain the aquatic life.

It is observed in the present study that the pH of the water ranges from 6.9 to 7.3. The changes occurred in the per cent mortality seems not to be due to pH changes but due to the direct effect of the phytotoxin. More concentration of the plant piscicide produces the kill of T. mossambica. This action was observed to be similar to that of antimycin with regard to pH relationships. The concentration required to produce a complete kill of fingerling goldfish in 96 hours at $12^{o}C$, was 0.20 ppb at pH 5.0, 1.10 ppb at pH 8 and 60 ppb at pH 10. In the field it is suggested that an application of antimycin to soft water would be more effective, if treatment was made at daybreak. This would allow substantially greater exposure time before the rapid diurenal rise in pH begins to cause degradation of the antimycin (Burress et al., 1969).

Comparative study of the toxicity of endosulpan in freshwater fishes under different pH was done by Paul and Raut (1987). They observed with the increase of pH the lethal dose of the pesticide also increases gradually with exposure to 24, 48, 72 and 96 hours and concluded that a pH of water plays a role in the toxicity of endosulpan in fresh water. It is clear that a higher pH value demands more pesticides.

3) Hardness of water :

The action of toxic compounds on fresh water animals such as fish may be influenced by the quality of water with regard to such characters

as pH, alkalinity and hardness. In recognization of this, the majority of laboratories on the fish mortality, during the present investigation, toxicological experiments were performed at consistently uniformed conditions of the water quality maintaining the hardness of water in the range 40 to 55 ppm. Hence it can be conclusively proved that the fish mortality was due to the ethanol extract of fruits of A. concinna and was not due to the change in the hardness of the water. The ethanol extract of fruits of A. concinna did not change the hardness of water at measurable level.

4) DO (Dissolved oxygen) :

Dissolved oxygen is one of the most important factors limiting productivity in fresh waters (Srivastava, 1985) under a given set of conditions. When there is a lowered concentrations of oxygen in the water, fish begins to rise to surface or crowd near inlets. In extreme depletions of dissolved oxygen level, fish may die of asphyxia. According to the Aquatic Life Advisory Committee (1955) the dissolved oxygen content of warm water fish habitats shall be not less than 5 ppm. during at least 16 hours of any 24 hours period. DO of the water used for the present study ranged between 4.6-7.0 ppm. during the experimental procedures involving the ethanol extract of the fruits of A. concinna and there was no significant change during the treatment in DO, indicating that the ethanol extract of the fruits of A. concinna did not have any effect on the water quality and its toxicity may due to (1) Direct effect of the active principles in the plant toxin on the fish (2) Indirect effect where in the DO is made unavailable to the fish by toxin. Hence large haemorrhages and heavy secretion of mucus on the gills is formed, indicating that the plant toxin has an indirect effect on the film.

D) DISCUSSION ON PHYSIOLOGICAL RESPONSES :

1) Discussion on LC_{50} or LD_{50} :

Different concentrations of plant toxins are used to observe the responses of the fish T. mossambica. One of the better known uses of dose-response relationships deals with the determination of the LD_{50} or LC_{50} (lethal dose or lethal concentration). LD_{50} used in determination of biological resdponse, considered to be a 'yes-no' or 'binary' (on-off, present-absent, 0-1) response, in which it is determined. Whether the subject is dead or alive with no intermediate category. In otherwords, it is a point of separation.

Lethal concentration for 50% mortality (LC_{50}) can be estimated by three methods. (i) Simple graphical method in which linear response (per cent mortality) is plotted against the linear dose. (ii) Use of semilog paper method in which the dose-response (per cent mortality) is plotted on semilog paper against log dose because the response is more linear with the Logarithm of the dose than with the arithmatic value of the dose. (iii) This method is a statistical method in which logarithmic-probit values are used for the calculation of the LC_{50} value.

For the present study third statistical method is used for the calculation of the LC_{50} value. The equation of line or regression i.e. $Y = a + bX$ is used to estimate the LC_{50} value. By plotting graph of probit values of per cent mortality against log concentration, the LC_{50} value was also estimated.

It is observed in studies on fish toxicity that a slight degree of variability is normally encountered between different individuals of the same batch or the same species. Usually, there is the odd specimen that succumbs

to the effect of the toxicant before the main batch or the odd individual that manages to survive a little time longer when all others are dead. This natural variability is allowed in routine tests by baring results on not less than 20 fish exposed at each concentration (Muirhead-Thomson, 1976).

The per cent mortality was increased with increase in concentration and 100% mortality (LC_{100}) was reached within 9 hours with 400 ppm concen tration of ethanol extract E_5, and hence it was thought that the ethanol extract of fruits of A. concinna is most suitable for eradication of undesirable fishes. The lethal threshold concentration (LTC) for T. mossambica was found to be 200 ppm for the ethanol extract E_5 (Table No.8).

At lower concentrations (200, 225 and 250 ppm) of the E_5 extract intoxication, survived upto 96 hours. This might be due to the 'phenomenon of tolerance'. Such a type of tolerance phenomenon was observed in case of detergents (Degens et al., 1950), hydrogen ions and ammonium (Lloyd and Orr, 1969), cyanide (Neil, 1987) and zinc (Edward and Brown, 1964) and for Zanthoxylum aramatum fruit extract (Ramanujam and Ratha, 1983). OR this might be due to the physiological resistance to particular type of active principles of the fruit extract. Such type of physiological resistance was shown by mosquito fish (Gambusia affinis) towards chemicals such as DDT, BHC and dieldrin (Boyd and Fergusson, 1964) and by chichilid fish (T. mossambica) towards leaf phytotoxin of L. eriocephalus (Harold, 1987) and fruit phytotoxin of S. laurifolius (Bhosale, 1988).

From the above discussion the unescapable conclusion is that the ethanol extract of the fruits of A. concinna contains potent piscicide which can be used as a selective eradicant of undesirable fishes.

The LC_{50} values for 12, 24, 48, 72 and 96 hours are 245.206, 241.00, 210.481, 145.115 and 140 ppm respectively.

2) Behavioural responses :

It is observed that the behavioural responses of T. mossambica to each extract E_1, E_2, E_3 and E_4 were not distinct. But the responses to the ethanol extract (E_5) of the fruits of A. concinna were prominent and could be easily noticed. The behavioural responses were dependent on the concentration. At lower concentration (200, 225 and 250 ppm) the fish did not respond much but at higher concentrations (275, 300, 350 and 400 ppm) the behavioural responses were noticeable. Such concentration dependent behavioural responses were observed in phenol intoxication in the carp, Cyprinus carpio (Lukyanov et al., 1984) and in Channa orientalis (Nanaware and Mane, 1987). These responses were shown to be linked to the disturbances in the holinergic system of the brain. In the present investigation such relationship has not been studied but the studies possibly on the cholinergic system of the brain would possibly give an answer for behavioural responses of T. mossambica to the high concentrations of ethanol extracts of the plant toxin. Ramanujam and Ratha (1980a) have observed effect of ten different plants on behavioural responses of fishes Danio dangila and Heteropneustes fossilis and have shown that the dose required for air breathing fishes was more as compared to those for gill breathers. The observations of the present study are contradictory to their observations since T. mossambica is a gill breather even it requires more concentrations of this particular plant toxin to change the normal behaviour of the fish. But

the other observations like active movement in the initial phase of toxin treatment, change in colour, erratic movements, swelling of eye balls, keeping mouth open, turning upside down and finally collapsing to the bottom of the jar were almost similar to those observed by Ramanujam and Ratha (1980a) in Heteropneustes fossilis and D. dangila by Nanaware and Harold (1987) and Bhosale (1988), in T. mossambica.

Thus, from the observations it is seen that i) The fish T. mossambica showed no noticeable behavioural changes during the treatment of extracts E_1, E_2, E_3 and E_4. ii) The fish T. mossambica showed distinct behavioural changes during the treatment of E_5 or ethanol extract. iii) Changes induced by E_5 extract were dependent of concentration of extract and duration of treatment. iv) The behavioural changes showed by T. mossambica during the treatment of E_5 extract were identical to the responses of different other fishes to piscicidal chemicals and other plant toxins.

3) Discussion on histopathology and mucosubstance secretion :

i) Buccal mass.

a) discussion on histopathological alterations :

If the toxin is mixed into the water buccal mass is the first organ of the fish exposed to this toxin. Except few investigators (Nanaware and Harold, 1987; Harold, 1987 and Bhosale, 1988) this organ has not been studied in the toxicological investigations from the histological as well as histochemical point of view. In this investigation histochemical alterations in the buccal mass of fish T. mossambica due to toxins, has been studied.

histological observation has been recorded in the case of fish T. mossambica to show fungiform and filliform papillae in the buccal mass of this fish. Histologically, three types of goblet cells are observed in the epithelium of buccal mass. (i) First type goblet cells (ii) Second type goblet cells and (iii) Giant goblet cells. The number, size and the distribution of these cells had been affected during treatment of plant toxin from the fruits of A. concinna.

Population of first type goblet cells and size of second type and giant goblet cells had been increased during the low dose treatment. In the treatment of higher doses the number of these cells had been increased with the increased staining reactivity and the columnar form of first type goblet cells had been changed to the rounded form.

The thickening and the vacuolization had been observed prominently in the connective tissue during the toxin treatment. These histopathological alterations seemed to be dose dependent and probably providing more cells for mucin secretion and provides resistance to the toxin action. With the help of present investigation, it is not possible to ascertain the other functional relationships.

b) Discussion on mucosubstance alteration :

In response to plant toxin treatment, the whole epithelium of the buccal mass had filled with large quantities of mucosubstances. It is well known that the mucins in the buccal mass of the vertebrates perform several functions in binding food particles, providing lubrication for swallowing the food, create least friction along the gastrointestinal tract, provide antibacterial action for killing bacteria in the food and provide protectiion against

mechanical and chemical injury, etc. (Jørgensen, 1976). But their enormous production in the several mucin producing cell types, in all the possibilities seemed to be involved in the protection against injury caused by the plant toxin. Other functions of these mucosubstances are not known.

ii) Gills.

a) Discussion on histopathological alterations :

Histological structure of the gills in fishes greatly affects due to the chemical intoxication (Studnicka et al., 1983; Rajik et al., 1983). The piscicidal compounds whether of synthetic origin or of plant origin, the gills are the next organ which get damaged by these piscicides (Metelev et al., 1971). Among the chemicals, organic and inorganic elements involved for production of ill changes in the gill histology. It has been observed that the size and number of mucous secreting cells were increased in T. mossambica and R. daniconius in the sublethal doses of spent wash which contains both organic and inorganic substances causing production of large amount of mucus. This voluminous thick mucus inhibits normal respiratory process and fishes die. Gill histological alterations include loss of cells due to sea bloom (Shimada, 1983), due to hydrocarbons (Lopez, 1981), due to spent wash (Nikam, 1986). Loss of chloride cells due to other effluents in fathead minnows, Pimephales promelas (Leino and Maccormic, 1984), curling of secondary lamellae due to loss of pillar cells (Skidmore and Votell, 1972); Haniffa and Sundervadhanam, 1984 and Nikam, 1986), separation of gill epithelium from basement membrane, fusion of adjuscent gill lamellae, erosion at the distal end of gill filaments, loss of cell membrane in Channa gachua due to endosulfan (Dalela et al., 1979), swelling and thickening of gill lamellae, necrosis

and sloughing off in gill epithelium and interlamellar filaments (Ahmed and Ghufran, 1984) and similar alterations in the T. mossambica in phytotoxin intoxication (Harold, 1987; Bhosale, 1988).

Chemical substances are used in lot to study the histological changes, but a few invesdtigation is available on the plant toxin to show such changes. Kiptoon et al. (1982) studied the histology after the treatment of plant toxin to gastrointestinal tract and liver. Only Harold (1987) and Bhosale (1988) had studied alterations in the gills of T. mossambica after the treatment of plant toxin. Other than this work there is no single investigation to show histological alterations caused due to plant toxin in the gills of fishes. In the present investigation we report the effects of fruit toxin of A. concinna on histopathology of undesirable freshwater fish T. mossambica.

In the treatment with lower concentrations of extract E_5 the histological changes include increase in the interlamellar space, reduction in the primary gill lamellae, displacement of the epithelium from the basement membrane, increase in the number of mucous secreting cells and acidophill cells, curling of secondary gill lamellae, enlarged pillar cells whereas in treatment with higher concentration of the extract E_5 these histological changes included the reduction in supporting bony elements, enlargement of distal gill lamellar blood spaces, increased subepithelial spaces, ruptured lamellar capillaries and detachment of gill epithelium. Such results were more or less similar to the changes caused by chemical substances in the earlier investigations.

b) Discussion on mucosubstance alterations :

Variety of mucosubstances are elaborated in the gills of T. mossambica such as :

i) Natural mucosubstances are elaborated by most of the epithelial while neutral and acidic (mixed) mucosubstances are present in a few epithelial cells indicating interconversion of both these types in these cells.

ii) Either neutral mucin, mixed mucins (acidic + neutral) sulfated mucins or carboxyl containing acidic mucins are present in the mucous cells.

iii) Only mixed mucosubstances are present in the basement lamina, in which both neutral and acidic mucins are present.

iv) Only strong sulfated acidic mucosubstances are elaborated by the gill rachis.

Interesting changes were observed in the mucosubstance elaboration by the gill elements during the treatment of sublethal concentrations of the phytotoxin initially epithelial cells, basement lamina and gill rachis contained very low concentration of mucosubstances in them, which increased moderately in the lower doses of plant toxin. Whereas in higher doses it became maximum in them. But this pattern was not observed in the pillar cells of the secondary filaments of the gills. These cells contained moderate concentrations in the normal conditions which immediately even after low dose treatment of plant toxin increased to maximum and in the higher doses their number and concentrations fall down to a minimum. This might be due to loss of pillar cells from the filaments (Skidmore and Tovel, 1972; Harold,

1987 and Bhosale, 1988) or due to disappearance of cytoplasmic organelles as in copper sulfate and zinc chloride treatment (Rajik et al., 1983).

Observation of particular type of mucosubstance within a specific cell type of the Gill in T. mossambica seen to be related with their certain functions. Rosen and Cornford (1971) found correlation between the mucus and its friction reducing properties. Gill mucins also function as a protective layer checking bacterial and fungal infections (Vanoosteen, 1957; Jansen,1976) such a protective function to the mucins has been attributed to invertebrate mucosubstances (Gottschalk, 1960). It is known that the mucosubstances of the lungs surface help in exchange of gases by keeping surface epithelium moist (Hoar, 1965). Similarly at the surface epithelial cells of gill, these mucosubstances might be involved in gaseous exchange. This view has been supported by observations that the acidic mucins have water binding capacity so that the film of water spreads over the gill surface, which in turn facilitates the exchange of gases (Munshi, 1979; Hughes, 1979; Hughes and Munshi, 1979). The acidic mucins in the gill epithelium might be useful in the transfer of cations across the epithelium (Kirschner, 1978) and hence might be helping in osmoregulation and electrolyte excretion (Ingale, 1981).

It is observed in the present investigation that the mucosubstances during the treatment increased and when the fish die, mucus with blood clots entangled in the gills. Elaboration of such bulk quantities of mucosubstances might be having some protective function upto certain limit of intoxication against chemical injury to the gill filaments and if that limit exceed the protective function against that injury must have lost and haemolysis takes place.

iii) Liver

a) Discussion on histopathological alterations :

Detoxification is one of the important functions of the liver. Many exogenous and endogenous toxic compounds are broken by the liver (Lagler et al., 1977) under normal conditions hepatocytic functions are not over-burdened but during intoxification in various toxic treatments hepatocytic functions are under a tress. Therefore, several histopathologic changes occurred in the liver due to the pollutants, inorganic ions and toxins, at gross tissue level as well as at cell level also (Eller, 1971; Bhaktavathsalam et al., 1982; Dubale and Shah, 1979). Liver histology was greatly altered in carps subjected to DDT, lindane and -HCH (Hexochlorocyclohexane) Studnicka et al., 1983). In copper sulphate and chloride intoxification, organelles of the cells disappeared (Rajik et al., 1983) whereas aroclor, 1254, poisoning showed vacuolation in hepatocytes of Salmo gairdneri (Shivarajah et al.,1978) EM study, upon aroclor, 1254, treatment showed enlargement of rough endoplasonic reticulum of hepatocytes (Shivarajah et al., 1978). Proliferation of fibroblasts (fibrosis) in hepatocytes were evident in cadmium treatment to Garra mullya (Wani and Latey, 1983), with mercury toxicity to Sarotherodon mossambicus (Naidu et al., 1983) and with plant toxin of L. latifolium treatment to bull calves (Kiptoon et al., 1982). The earlier investigators except Kiptoon et al. (1982) have studied changes with reference to chemical toxicity but not a single reference was found to show the histological changes due to plant toxin.

Different changes in the present investigation are sighted as, displacement of hepatocytes, aggregation of cytoplasmic contents of hepatocytes, swollen hapatocytes with vacuolization with loss of cell boundaries, disruption of sinusoids and lastly leading to deformation of liver histology. These observations coincide with observations of Shivarajah et al. (1978) and Wani and Latey (1983).

During intoxication it was appeared that the plant toxin might be entering into the hepatocytes and R.B.Cs. because of which cells became enlarged. During higher doses, more toxin enter and cells get further enlarged, exceeding certain limit and cells rupture causing deformities in the liver histology. The vacuolization, and aggregation were the consequent effects due to the swelling and rupturing of the hepatocytes. Similar necrotic and cirrhosis formation effects had also been observed by other investigators (Sastri and Sharma, 1978; Gupta and Singh, 1982; Chatterjee et al., 1983). The enzymatic effect would throw some light on the action of plant toxin on hepatocytes. Such work have not been included in the present investigation, although some work on enzymes concerned with such toxicological functions is in progress, in this laboratory. In the preliminary investigation it had been observed that phytotoxin affects the carbohydrate digesting enzymes of the liver.

b) Discussion on mucosubstance alterations :

Presence of glycogen in hepatocytes was proved by PAS reactivity and malt diastase digestion test, whose concentration varied with different concentrations of the plant toxin. In the low doses mucosubstances changes

were moderate but during higher doses maximum glycogen was lost. This change might be due to the fact that as the fish accumulates the poison in the liver and become lithargic, probably due to impairment of cholinesterase enzyme system of the nervous system as indicated by Ramanujam and Ratha (1983), Chopra et al., (1958) and Virdi (1982) and food intake is greatly reduced. During this stress period, great amount of energy required might have come from this stored glycogen in liver and that is why in high dose treatment of plant toxin glycogen might be depleted.

During phytotoxin treatment to fish, there is no appreciable change in the acid mucosubstances in the liver. With present knowledge the function of acid mucosubstance can not be ascertained.

iv) Kidney.

a) Discussion on histopathological alterations :

Toxins after detoxification and nitrogenous waste products are brought into the circulation and through blood brought to the kidney for their excretion. Since this unusual work performed by the kidneys, certain structural changes ought to occur. Such changes are less in low concentrations while these are drastic in high concentrations of toxin treatment, leading to kidney failure and death of the fish (Mathur, 1969; Holden, 1965). Many chemical toxicities produce drastic changes in the kidney histology, copper sulphate and zinc chloride produce necrosis of the glomeruli (Kumar and Pant, 1981; Rajik et al., 1983). Organic pollutants produce enormous swelling of the glomeruli, thickening of the endothelial wall of the capillaries and increases in the haemopoitic mass in Widow tetra (Amininkutty and Rege, 1978).

Thiodon toxication and maltation produces necrosis in C. punctatus (Dubale and Shah, 1984) and development of lesions in the kidney (Chaturvedi and Saxena, 1978).

There is interesting observation due to Amaranthus retroflexus poisoning to swine and pigs. The histologic lesions in the kidneys of affected animals were characterized by hydropic degeneration and coagulative necrosis of both proximal and distal convoluted tubules. Glomeruli were shrunken and were apparently increased in cellularity. There was dialation of Bowman's capsules. Many tubular proteinaceous casts were observed in the distal and collecting tubules (Buck et al., 1976). Exactly similar results were obtained in the toxicological investigation on L. eriocephalus and S. laurifolius plant toxin during intoxication to fish T. mossambica. The histological alterations observed in the glomerular size, degeneration of basement membrane and development of intertubular space, histologic alteration in proximal and distal tubules and Malphigian bodies which showed swelling of distal tubular cells and severe necrosis, dialation of Bowman's capsules and shrunken glomeruli.

In the present investigation histopathological alterations in the various kidney elements were similar to those observed by Harold (1987), during intoxication due to toxin of leaves of L. eriocephalus and by Bhosale (1988) during intoxication due to toxin of fruits S. laurifolius.

Such histologic alterations must have been occurring in the kidney as an initial shock by the active principle of the A. concinna circulating through the blood. After entering into the kidney, it accumulates and increases in concentration causing necrotic effects. Secondly this active

principle present in the fruits of this plant must be of larger size with high molecular weight which must have caused such histologic ill effects in the kidney.

b) Discussion on mucosubstance alterations :

Kidney is the target organ of the biochemists and histochemists working on toxicological problems. It is because after liver, degraded toxic compounds are brought to the kidney for their disposal to the outside of the body. The presence of mucosubstances in the kidney tubules of adult vertebrates is well known due to the work by Longley and Fisher (1954,1956) and Longley et al. (1963). Longley and his coworkers suggested the role of mucins as protective or hydrophilic colloids in preventing the precipitation of solutes from the urine or in maintaining its fluidity or mobility. But there is not a single investigation to show the role of mucosubstances in the kidney during the phytotoxin treatment to the fish.

Having this view in mind, the present investigation on mucosubstances in the kidney tissues of T. mossambica in the fruit toxin of A. concinna treatment was undertaken. Such study revealed that the glycogen and acidic mucosubstances are in the Bowman's capsular cells which immediately increased maximum even with low doses toxin treatment and at higher doses steadily diminished which might be due to shrinkage of glomeruli and dilation of the Bowman's capsule during the treatment.

Acidic mucosubstances are present in the proximal tubules. Davis (1954) stated that some protein which escape into urine is reabsorbed by

the proximal tubules. This reabsorption function might possibly be performed by these acidic mucosubstances found in the present investigation. Longley and Fisher (1963) and Butt and Hauser (1952) have suggested the functional role for mucosubstances as protective colloids in the prevention of stone formation. In the light of existing literature and with the help of theoretical background, an attempt has been made in this contribution to correlate the occurrence of acid mucosubstance with the osmoregulatory function of the kidney. But the role of mucosubstances which increased during the initial phases of toxin treatment and decreased after treatment of high doses so also the role of sulfomucins in the distal tubules can not interpreted with the observations of the present investigation. It requires further extensive histochemical and biochemical studies at a comparative level on the renal mucosubstances of different fishes subjected to different toxins of plant origin.

v) Intestine

1) Discussion on Histopathological alterations :

The histopathological changes are mainly concerned with the changes in the thickness of various tissues and cells, vacuolization and sloughing off tissues, rupturing of the villi, increase in number of mucous secreting cells etc. in the intestine due to the plant toxin A. concinna intoxication. These changes are dose and time dependent. In lower concentrations slight changes are seen whereas at higher concentrations the severity in such changes increases. Similar type of histopathological changes due to the pure phenol

at 100 ppm after 144 hrs exposure have been reported by Chaterjee et al., (1983) in H. fossils. They observed ruptured mucosal folds, aggregation of mucous cells near the villi surface and ruptured villi also. Exactly identical reports in S. gairdneri (Brown and Shurben, 1972) and in S. mossambicus (Devi, 1986) have been recorded.

To certain extent tissues develop resdistance by increasing number of mucous secreting cells upto the certain limit of concentration of the toxin, beyond that tissues rupture and lesions occurred.

In the present investigation a fruit toxin from A. concinna is used because no one has tried to see impact of this toxin on the intestine of T. mossambica. The histopathological changes due to fruit toxin A. concinna includes thickening of muscularis increase in number of goblet cells and broken of serosa at low conc. 200 ppm.; acute proliferation of mucosal cells, bifurcated villi, vacuolation and separation of submucosa and disorganised muscularis at conc. 250 ppm.; sloughing off mucosal layer and degenerated circular muscle fibres at 300 ppm. and pycnotic nuclei in mucosal cells, occurrence of dead cell debris in the lumen at conc. 350 and 400 ppm.

2) Discussion on mucosubstance alterations :

The intestine of fish T. mossambica is one of the most important mucinous tissues and thought to play an important role for the maintenance of the internal environment. Florey stated that mucosubstances secreted by intestinal goblet cells have proved to be sulfated in all species that have been examinery (Florey, 1962).

In some earlier studies, the influence of $HgCl_2$ was observed to cause hyperactivity of mucous secreting cells in intestine of C. punctatus (Sastry

and Gupta, 1978; Bhosale, 1988) and sialic acid changes in intestine of C. batrachus due to malthian (Mukhopadhyay and Dehadrasi, 1980).

In the present investigation the neutral mucins in connective tissue of submucosa and serosa and glycogen concentration in muscularis were increased when fish was exposed to the 200 ppm plant toxin A. concinna. The sulfomucins in the goblet cells were increased two or three times in conc. 300 ppm. Natural mucins in the epithelial cells were also observed to be increased at conc. 300 ppm. There is a depletion of glycogen during the period intoxication, this might have occurred due to the utilization of carbohydrate. Except the above report probably the present work forms a pioneering histochemical study on mucosubstances and their changes in the fresh water undesirable fish T. mossambica during plant toxin A. concinna intoxication. The high concentration of mucins and large number of goblet cells in the large intestine are known to play role in lubrication of gastro-intestinal tract, help in formation of faecal ropes and defecation and possibly also play role in protection against chemical injury which is produced due to bacterial action and toxic chemicals.

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