DISCUSSION
Evaluation of pesticide toxicity (Determination of LC$_{50}$ values)

The gradual degradation of the aquatic ecosystem by dint of the excessive and indiscriminate use, has ultimately resulted in the judicious and careful application and use of pesticides to protect and preserve the aquatic fauna, especially the most massive population, the fishes. In this regard, it has became a necessity and significance to perform studies on the sub-lethal effects of these pesticides involving time course experiments which indicate the sequence of events in physiological and biochemical systems, providing information on the nature and completion of compensatory mechanisms during toxicant exposure. In fact, such sublethal studies are conspicuous by their absence especially in commercially important food fishes like the Indian major carps which are not only form an important link in aquatic food chains, but do have a very great economic value. Hence in this investigation, a sub-lethal effects in the form of physiological and biochemical responses of the Indian major carp, *Labeo rohita* was subjected to widely used fungicide, ziram.

The bioassesment of the toxicity of the pesticide with reference to aquatic biota is playing a crucial role in establishing the toxicity guidelines of chemicals to non-target species. The most generally accepted technique of evaluating the toxicity of pesticides is the determination of LC$_{50}$ values where the toxicity of the pesticide to aquatic organism is assessed by the concentration of the toxic compound in water that will kill half of the animals exposed for a specified period of time.
In the present study, the percent mortality in different concentrations of ziram showed straight line, when the log concentration of ziram was plotted against probit mortality in the fish (Fig. 2), thus it agrees probit analysis (Finney, 1964). Thus mean LC$_{50}$ values obtained through these sources were taken as the LC$_{50}$ of this carp. Thus the LC$_{50}$ values obtained through % kill and probit kill were given in (Fig 1 and 2). Thus the toxicity of insecticides to a particular aquatic organism is usually expressed in terms of LC$_{50}$ which represents the amount of poison required (mg/litre) to kill 50% of the test population, namely the fishes in the present study. It was reported that fungicides are toxic to fish (Banurekha and Shaik Dawood, 1999), to rats (Deichman, 1981), to humans (Burns, 1975; Cam and Nogogosyan, 1963; Cripps et al., 1984).

During the toxic evaluation of fungicide ziram, the symptoms of poisoning in the form of irritability, hypertoxibility and tremors of the whole body of the fish have been noticed at LC$_{50}$ level of exposure. Similar symptoms of poison are noted when pesticides are exposed to fishes earlier as reported by (Cremlyn, 1974; Jayantha Rao, 1983; Obilesu, 1987; Indira, 1985; Onnurappa, 1986; Giridhar, 1997). Pandey et al., (1987) documented damage of gill tissue on exposure to polluted water in the fish, Punctius ticto, formation of coagulation film (appearance of the mucus covering over the gill) and the colour of the gill lamellae changed from red to brown, indicating non-circulation of blood. Thus the data on LC$_{50}$ values of dithiocarbamate fungicide, ziram will be highly useful in the final evaluation of extent of pollution of aquatic environment by agricultural chemicals.
**Time course of fungicide exposure**

With the increase in industrialization and improvement in agriculture, the scientific world has inadvertently introduced pollution into the aquatic environment. Pesticides used in pest control have been found to produce many physiological and biochemical changes in freshwater organisms by influencing the activities of some enzymes and metabolite levels in different tissues. All the animals in the universe will consume oxygen for their life activities. The intake of oxygen is utilized in the metabolic reaction and release of energy, which is utilized for the life activities. Oxygen consumption indicates metabolic rate of an organism. The metabolic rate of the animal reflects the basic physiological status in turn indicates the health of an organism. In the present investigation, the rate of oxygen consumption in the major carp, *Labeo rohita* was noticed. During sublethal exposure period of Ziram initially increased at 24 hours period from its level in the control medium (freshwater without ziram). The inhibition of oxygen consumption of the fish in the further sub-lethal exposure was seen through the 7th day period and reached maximum suppression in the 15th day of the total 30-day exposure period. The initial elevation of oxygen consumption at 24 hours exposure period is due to the increased locomotor activity arising out of the animal tendency to tide over the toxic medium. The initial increase of oxygen consumption was supported by the observation of (Parvathi, 1992; Prasad and Bashamohideen, 1985; Ramalingam, 1988; Giridhar, 1997).

After 24 hours there was a progressive decrease in the oxygen consumption through 7-day of exposure period and maximum percent inhibition was observed at
15-day of sublethal exposure period. This suppression in oxygen consumption may be due to continuous exposure of *Labeo rohita* to ziram, where in the activation of detoxifying enzymes might not have set in the organ of this fish. This suppression of oxygen is also may be due to the rupture in gill epithelium which was coincided with earlier reports of Jayantha Rao (1982), Natarajan (1984), Ramalingam (1980, 1985), Giridhar (1997).

From 15th day onwards the percent of oxygen consumption by fish, *Labeo rohita* recovered from its earlier suppression and reached almost to the level of control at 30-day exposure indicating that this fish, *Labeo rohita* has the capacity to compensate pollutional toxicity occurred at sub-lethal exposures of ziram most probably by enhancing the activation of detoxifying enzymes which bring about the biodegradation as reported by (Mukhopadhyay and Dehadri (1978). The sub-lethal concentration of ziram could cause a physiological system to oscillate outside its normal range of variation mostly suppressive yet with time, without suffering lasting effects the system could show return to the nearing normal state as seen in the present investigation. The *Labeo rohita* has got the capacity to compensate and adapt to the imposed fungicide toxicity.

**Fungicide exposure - oxygen consumption of the whole fish**

Oxygen consumption has been used as sensitive and good indicator of toxicity in aquatic animals exposed to pollutants in general (Sellers *et al.*, 1975; Bayne, *et al.*, 1980; Mahajan and Dheer, 1980, Bashamohideen and Subba Rao, 1982). It is also evident that pollutants gain entry largely through the gills of fishes (Premdas and
Anderson, 1963; Ferguson and Goodyear, 1967; Bashamohideen and Subba Rao, 1982; Indira, 1985). In the present study, where the rate of oxygen consumption is initially elevated at 24 hours exposure period and then suppressed during the other sub-lethal exposure periods like 7, 15, 30 days was reported by (Rajamannar and Manohor, 1992, 1998). However, the percent suppression in oxygen consumption is not consistent with increasing time of sub-lethal exposure of ziram. Hence, maximum suppression in fish was observed at the 15th day of the 30-day exposure period. But there was a rise at the 30th day exposure period from the earlier suppression. This may indicate that the ziram toxicity is reduced. And after 24 hours the decreased oxygen consumption observed may be due to tissue damage. The secretion of mucus layer over the gill lamella has observed during the toxicity and the coagulation of mucus in gills caused demolition of various important processes such as gas exchange, nitrogen excretion, salt balance and circulation of blood (Giridhar, 1997).

Increase of lactic acid in tissues is attributed to the inadequacy of oxygen supply in cells to cope up with complete breakdown of carbohydrates to carbon dioxide and water (Seskin and Levine 1947). Hence the persistence of tissue lactate in fishes exposed to DDT and malathion implies hypoxia. Significant decrease in the oxygen consumption of the whole animal and corresponding decrease in succinic dehydrogenase (SDH) enzyme activity in the liver and muscle of DDT and malathion treated fishes also support tissue hypoxia (Ramalingam, 1980,1985).

Further, in the fish, Labeo rohita when exposed to DDT, BHC, dichlorvos and monocrotophos showed significant initial increase in oxygen consumption
(Rajamannar and Manohar, 1992, 1998). The initial elevation in the oxygen consumption at 24 hours exposure period is due to increased locomotor activity arising out of the animal tendency to escape from the new medium which is a toxic medium and this situation is termed as 'Escape reaction' of the animal as suggested by (Potts, 1954; Gron, 1957; Bashamohideen and Parvatheswara rao, 1972, Saibala, 1988; Giridhar, 1997). After 24 hours there was a progressive decrease in the oxygen consumption through 7th day exposure period and maximum percent suppression was observed at 15th day of sub-lethal exposure period. The same was found in the findings of Indira (1985) on oxygen uptake efficiency of Catla catla on exposure to malathion. These findings in oxygen consumption indicate that the Labeo rohita undergoes compensating mechanism leading to homeostasis by varying the metabolic rate during ziram exposure and ultimately these variations in oxygen consumption could be attributed and befitting into the compensating mechanisms. These compensating mechanisms in the above studies including the present study could be possible, probably due to activation and enhancement of detoxifying enzymes which bring about biodegradation of the pesticide to reduce its toxicity by the way of recovery from 15th day suppression and such detoxication mechanism was reported by Mukhopadhyay and Dehadri (1978).

At 24 hours the animal might have experienced the fungicide loading toxicity in due course the animal slowly accommodates its metabolic pathway towards encountering fungicide present in the aquatic media. At 24 hours exposure period the animal struggle to tide over the pollutant effect. At 7th and 15th day reflects both toxic
and adaptive phases indicating intermediary phenomenon. After 24 hours there was a progressive decrease in $O_2$ consumption in through 7th day exposure period and maximum percent suppression was observed at 15th day sublethal exposure period. Thus there is a fairly good amount of recovery in oxygen consumption of fish at the 30th day of exposure of the fungicide. Thus the ratio of oxygen consumption of Labeo rohita species seem to serve as a very good indicator of the fungicide pollution that would effect the physiological and biochemical processes of fish.

**Fungicide exposure- opercular activity**

The variations in the rate of opercular movements may due to physiological and biological changes. The rate of opercular activity is decreased under ziram exposure. This is a parlance with the decreased oxygen consumption. This correlation is because when opercular movements are decreased, the rate of water flow onto the gills surface area is increased with the unit time decreases and hence the possibility for potential oxygen consumption within the unit area and time is decreased. Similar observations were made by Koudinya (1978), Jayantha Rao (1982), Narasimha murthy (1983), Indira (1985), Saibala (1988), Rajamannar and Manohar (1998). Thus the fungicide ziram inhibits energy metabolism of fish. So the decline in the opercular activity under pollutant toxicity served as good indicator in fishes.

In the present study, the impact of ziram on above physiological activities at the period of 24 hours depicts that there was a sudden rise in all physiological parameters that is an increase in oxygen consumption and opercular rate. After 24 hours there was a progressive decrease in the rate of opercular activity and maximum
% suppression was observed on the 15th day fungicide sub-lethal exposure period. At the end of 30th day exposure period, the opercular activity reached nearer normal level. Similar changes in fishes exposed to sun-lethal concentrations of pesticides were observed by Shanwaz and Bashamohidden, 1985; Indira, 1985; Onnurappa, 1986, Saibala, 1988).

The variations in oxygen consumption and rate of opercular movements were supported by number of workers Rama murthy and Koundinya (1979, 1980) for sevin and phosphomidon on other fishes. The decrease in oxygen uptake by gills resulted in hypotoxic and anoxic in which gill tissue not only suffer from oxygen 'debt' (Natarajan, 1983) but losses effective mechanism for remaining CO₂ from blood. The inhibition on oxygen consumption in sub-lethal concentrations may be due to disintegration or rupture of respiratory epithelium and also due to coagulation film 'anoxia' in which mucus is lost from gill as a result of which absorption of oxygen from surrounding is adversely effected. Therefore in Labeo rohita, ziram may alter the opercular activity which in turn has profound influence on oxygen utilization dynamics of fish. Preliminary studies on the opercular activity is made in the case of common carp subjected to malathion exposure on 30 days of sublethal exposure indicating that the organs are recovered from pesticide toxicity (Indira, 1985). Thus, the changes in oxygen consumption and opercular activity could reflect the effect of pollutional toxicity of pesticides on the aquatic fauna especially in fishes.
Biochemical Responses of *Labeo rohita*

**Fungicide exposure - Carbohydrate metabolism**

Carbohydrates beside their structural role in cell also serve as a reservoir of chemical energy, to be increased or decreased according to the metabolic state of the cell. Carbohydrates get oxidized to provide energy for other metabolic processes. Carbohydrates are utilized by cell mainly in the form of glucose (Harper *et al.*, 1979). They are usually stored in the liver, as a polysaccharide compound, glycogen. The energy derived from the oxidation of carbohydrate is of prime importance for the survival of the animal. Impairment of carbohydrate metabolism in a variety of pathological disorder is well known (Harper *et al.*, 1979). The disturbances in the carbohydrate metabolism caused by action of toxic compound and the complimentary shift from anaerobic segment appears to be essential in the cell and tissue metabolism (Vasilos *et al.*, 1976).

The variations in the carbohydrate metabolism to meet the changing energy demands may be expected in animals during the toxicity. Studies of this nature involved in aquatic animals exposed to environmental pollutants have been carried out by many workers (Parvathi, 1982; Baghya Lakshmi, and Rama murthy, 1984; Indira, 1985; Basha mohideen and Pavathi, 1988; Saibala, 1988). From the studies of Ramu and Drexter (1973) it has been established that insecticides could increase the blood glucose and decrease in liver and muscle glycogen. There was a general decline in the liver carbohydrate content of fish exposed to sublethal concentrations of endosulfan. Here the carbohydrate content dropped significantly below the control.
level with an increase in the pesticide concentration after 7, 15 and 30 days of exposure. This confirms the findings of Murthy and Devi (1982). Similar trend was also observed in the endosulfan treated *Barbus stigma* (Manoharan and Subbiah, 1982). Umminger (1970) found that carbohydrates represent the principal and immediate energy precursors for fishes exposed to stress conditions while proteins being the energy source to spare during chronic periods of stress. Generally more energy is needed to mitigate any stress condition. This energy may be obtained from carbohydrates, proteins or lipids (Ganesan et al., 1989).

As the activity of respiratory enzymes is known to imply the tissue oxygen levels, studies on enzymes systems in fishes exposed to toxicants were carried out by (Natraja, 1980; Sastry and Malik, 1982; Sastry and Singh, 1982; Ramalingam, 1985; Prabhakar, 1988; Samuel et al., 1989, Rajamannar and Manohar, 2000). In the present study, a maximal decrease of liver glycogen and muscle glycogen was observed at 7th day. A similar decrease in blue gills treated with 2,4 D (PGBB) (Cope et al., 1970). Similar findings were reported by Koudinya and Ramamurthy (1979, 1980; Sivaprasada Rao and Ramana Rao, 1979; Jayantha Rao et al., 1981; Verma et al., 1983; Indira, 1985; Begum and Vijayaraghavan, 1994, 1999). The possible reason for hyperglycemia during pesticide toxicity is hypoxia in islets of langerhans of pancreas could lead to decrease insulin resulting hyperglycemia and decreased liver and muscle glycogen. Further, increase in blood glucose level and fall in muscle glycogen levels is indicative of increase in the rate of glycogenolysis (Begum and Vijayaraghavan,
1994). In the present study, the hyperglycemic condition may be due to the stimulation of pancreatic hormonal secretion, glycogen by pesticides (Harper, 1978).

Glycogen is considered to be the major source of energy in animal tissues and maintenance of glycogen reserves is an essential feature of normal metabolism (Turner and Manchester, 1972). Reports on the impairment of carbohydrate metabolism in aquatic organisms subjected to different group of pesticides are sparingly available (Grant and Schoettger, 1972; Srivastva and Singh, 1981; Singh and Srivastva, 1982; Indira, 1985).

It has been noticed that muscle glycogen decreased in all the periods of sub-lethal exposures of ziram. The decrease in muscle glycogen may mean either decrease in the rate of its synthesis or increase in the rate of its metabolic utilization. But, in some sub-lethal concentration of the pesticides, the conversion of glycogen to lactate might be inhibited as indicated by the decreased LDH activity. This may possibly lead to the accumulation of pyruvate in the muscle. The liver glycogen content was decreased in fish, *Labeo rohita* because of an extensive utilization of energy stores. This marked glycogenolysis in liver after 30 days sub-lethal exposure of fungicide toxicity was probably caused by a toxicity induced increase circulating catecholamines. The amount of decrease of glycogen in liver suggests that higher energy demands of the fish was occurred. The increase in energy demand is followed by and increase in blood glucose. These changes may suggest that glycogenolysis might be a mechanism utilized by the fish to tide over the toxic effects of ziram. There was a general decline in the liver and muscle glycogen content of fish exposed
to sublethal concentration of endosulfan (Murthy and Devi 1982). This supports that there is decrease in liver and muscle glycogen and supports the present study.

**Fungicide exposure - Succinate dehydrogenase (SDH) activity**

The fungicide toxicity inhibit SDH activity in different tissues of fish, Labeo rohita indicates the depression of cellular metabolism in fish. Being a key enzyme in the TCA cycle it is logical to assume that with the inhibition of SDH activity the metabolic pathway might have turned anaerobic to meet the increases energy demands during the fungicide stress. Since, this enzyme also plays an important role in osmoregulation (Bashmohideen and Parvatheswara Rao, 1979) its depression would have disrupted the osmoregulatory machinery coinciding with the results. There are reports from different workers in different fishes exposed to various pesticides (Vijayalakshmi, 1980; Koudinya, 1984; Rama murthy, 1981; Sastri and Siddiqui, 1983; Natarajan, 1981; Ramalingam, 1985; Indira, 1985; Vasanthi and Ramaswamy, 1987; Rani et al, 1989; Tripathi, 1990).

In the present study, the reason for initial increment of 24 hours period in SDH activity might be due to the enhancement in oxygen up take (Prosser, 1973). The % suppression on 7th day of fungicide toxicity, ziram might have been caused due to tissue damage as also suggested by Girija Moses (1984). Similar results are reported by Jayanyha Rao et al (1984) in *Tilapia mossambica* under heptachlor and Metaxystox exposure. Shifts in the enzymes like SDH and LDH in cellular components of different organs of fish, coupled with the changes in the oxygen
consumption at organismal level, can be correlated to the energetic aspects of the fish exposed to fungicide toxicity.

In the present study, maximal % suppression in the enzyme SDH activity was observed at the 7th day of fungicide exposed fish. The maximal % suppression due to fungicide toxicity was noticed in the gills of this carp, hence gill which is predominantly an oxidative tissue is thus effected maximally of all the tissues and it is possible because toxicants gain entry largely through the gills of fishes (Holden, 1962; Premdas and Anderson, 1963; Fergueson and Good Year, 1967).

At 24 hours there was an elevation in the SDH activity and at 7th day there was a maximum % decrease in SDH activity. Further there was a recovery during the course of 30 days and reaching the approximate normal level. Further, the recovery at 30 days in different tissues is also in the same above order. Thus, the overall assessment of the differences in the SDH activity in tissues of *Labeo rohita* in terms of suppression and recovery of SDH enzyme is found to be highly significant. The recovery in the SDH activity is attained below the control at 30 day period because of adaptation. Thus it is evident that fungicide toxicity is a physiological load on the carp where there is inhibition of the metabolism during initial exposure of ziram.

**Lactate dehydrogenase (LDH) activity - Fungicide exposure**

It has been reported that LDH activity elevate under anaerobic conditions especially in pathological state (Harper, 1977). This elevation of LDH activity is in compensation to the depressed SDH activity in the tissues of carp, *Labeo rohita* exposed to fungicide, ziram. The elevation of LDH clearly indicates that the pyruvate,
the end product of glycolysis is not routed to Krebs cycle, but routed to glycolysis. The unequal depression of SDH activity and elevation of LDH activity indicate favours in anaerobic metabolism in ziram to meet the energy demands. This elevation of LDH activity in the tissues, may suggest that the pathway turned anaerobic to meet the increased energy demands during fungicide toxicity. The LDH activity was significantly enhanced in all the tissues analyzed during the fungicide exposure periods. It is interest to note in the context that the damage to mitochondria causes the suppression in SDH activity and extensive proteolytic activity are related to the alternation in membrane permeability and the increase in permeability of cells may be characterized by the rise in LDH activity (Fluke, 1972). The two uptake of oxygen by various tissues also corroborated the decreased oxidation of important substrates such as succinate and pyruvate. This has been reported by number of workers similar enzymatic dysfunction and development and of anaerobic conditions during pesticide toxicity conditions were shown for the air breathing fish, *S. fossilis* (Delela *et al.*, 1980). Further, the recovery in LDH activity significantly noticed in all the tissues at 30 day period, it might be due to adaptation without physiological load and exemplified by the complete recovery in LDH activity in all tissues. Similar observations were reported (Fluke, 1972) in liver and muscle of *Clarias batrachus* exposed to three pesticides and there is substantial support to the present observation.

Lactate dehydrogenase forms the center of a delicately balanced equilibrium between catabolism and anabolism of carbohydrates. In the present study, stimulation of LDH and the rapid rate of glycolysis observed indicate that the end product of
glycolysis, pyruvate was not routed to Krebs cycle but through the lactic acid cycle under hypoxic conditions, leading to the accumulation of lactic acid. Finally the carbohydrate metabolism is highly involved in the organs of fish, *Labeo rohita* on exposure to sublethal concentration of ziram. Decrease in carbohydrate content, oxygen consumption, opercular movement, SDH and increase in LDH may help the animal to develop compensatory mechanism in meeting the sublethal toxicity on prolonged exposures. In conclusion the shifts in carbohydrate metabolism are to compensate with the situation. Metabolic compensation involves breakdown and synthesis of products necessary to cope up with altered situation and such a phenomenon is known as turnover. The present study shows that ziram reduces oxidative metabolism in the gill, liver and muscle tissue of *Labeo rohita*. Consequently this fish switch over to anaerobiosis as evidenced from the increased lactate content in the tissues.