CHAPTER-I

ACUTE TOXICITY
INTRODUCTION

Pollution of fresh water ecosystem is caused due to a variety of pollutants. In an agricultural country like India insecticides, pesticides, weedycides constitute the major components of aquatic pollutants. A laboratory investigation in fish has documented variety of physiological, biochemical, haematological and behavioral responses of fish under toxic stress of insecticides and pesticides.

Recent studies indicate that fish an extremely valuable resource, are quickly becoming scarce. One consequence of the scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are safe for fish and other aquatic life. Pesticides are the most hazardous chemicals to men with ambient. Insecticides are extensively used to protect agricultural crops against the damages caused by pests.

Due to advent of agricultural and industrial revolution, most of the water sources are becoming contaminated (Khare and Singh, 2002). Industrial discharges containing toxic and hazardous substances, including heavy metals (Gbem et al., 2001 and Woodling et al., 2001), contribute tremendously the pollution of aquatic ecosystem.

Because of industrialization the water of the streams, lakes and rivers are receiving an increasing load of industrial wastes. Besides polluting waters, in many cases this water kills the fish and other aquatic organisms. Fresh water are highly vulnerable to pollution since act as immediate sinks for the consequences of human activities always associated with the danger of accidental discharges or criminal negligence.

Fish culture adjacent to agriculture fields is a common phenomenon. This has resulted in the indiscriminate discharge of various
highly persistent pesticides into the aquatic environment adversely affecting the non-target organisms, particularly fish (Rao and Shameem et al., 2004).

Aquatic contamination of pesticides causes acute and chronic poisoning of fish and other organisms. The pesticides damage vital organs correlates (Omitoyin et al., 2006; Johal et al., 2007; Joshi et al., 2007 and Velmurugan et al., 2007).

The injuries due to insecticides to aquatic environments are incontestable. The significant increase of chemical emissions in the water resources has lead to deleterious effect for aquatic organisms. (Livingstone, 2001 and Matsumoto et al., 2006).

Direct or indirect discharge of the pesticides in river, ponds, reserviors and other freshwater bodies create a serious problem, due to toxic properties of these pesticides and their adverse effect on aquatic life, particularly fishes. In this context, it is necessary to study in detail the acute or short term effect of pesticides on fishes.

Primary effects such as the death of fish can be detected but secondary effects remain unnoticed. The ability to detect, identify and respond properly to natural chemical stimuli is an important component of the environmental physiology of fishes. The classic ecotoxicological approach to test aquatic toxicity is to measure the direct effect in simple experiments using death, more than that as not the endpoint.

The use of pesticides in agriculture, public health and forestry ultimately leads to the contamination of aquatic biotopes posing a great threat to the environment. These pesticides through surface runoff reaches unrestricted areas like ponds and rivers which alter the physicochemical properties of water and is toxic to aquatic organisms, (Kamble and Muley 2000; Bhalchanra et al., 2001; and Madhab Prasad et al., 2002).
Heavy metals from industries disturb the aquatic environment and leads to environmental health hazards (Shukla et al., 2007; Gupta and Srivastava, 2006; Agtas et al., 2007 and Yoon et al., 2008).

The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman et al., 2002). However, these chemicals may reach other ecological compartments as lake and river through rain and wind, affecting many other organisms away from the primary target.

Toxicity is the relative property of any chemical, which refers to its potential to cause harmful effect on a living being. It is the function of the concentration of the chemical and duration of exposure. Toxicity data are commonly used for comparing different kinds of chemicals. The nature and extent of toxicity varies due to the origin of toxicants. They may be natural, artificial or synthetic ones. Chemical pesticides are synthetic toxicants used as agrochemicals for controlling various kinds of pests including insects, rodents and other crop enemies. They enter the ecosystem from non-point sources such as agricultural runoff from land lead to pesticide residues in almost everything through soil and water.

Toxicity testing through fish bioassay is a simple basic laboratory tool for the detection, evaluation and abatement of water pollution. Assessment of toxicity in aquatic ecosystem is conventionally done through bioassay are testing procedures using fish as test organisms. Fish have been a popular and useful test organisms in aquatic toxicological studies with the logic, that if fish life is protected as well. The commercial importance of fisheries is also a factor that goes in favour of this choice (Mahananda, et al., 2008).
The population of aquatic environment with array of xenobiotic compounds has become a menace to the aquatic flora and fauna which is a problem of immediate concern. These contaminants are let out into the water bodies from industrial and agricultural areas and as most of them are highly persistent; their levels fast reach to life threatening in term of both space and time (Brack et al., 2002 and Diez et al., 2002). The recent development of biomarkers based on the study of the response of organisms to pollutants has provided vigilant contamination monitoring, (Korami et al., 2000).

The global pollution has become a serious issue since 1980’s of special concern are atmospheric pollution problems, the green house effect and the reduction of protective ozone layer (Bickham et al., 2000).

The global pesticidal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of water sources becoming contaminated. (Khare and Singh, 2002).

The blind and selfish human activities have resulted in inexplicable grave global concern for present human civilization in the form of environmental pollutants. These pollutants come from many different sources and enter the air, water and land in a variety of ways. Some of the common pollutants include nitrates from fertilizers in surface runoff (agriculture); insecticides and herbicides (agriculture); bacteria from sewage (population); heavy metals (mining); plant debris (logging); silt (land debris, logging); chemical waste (industry) and acids (industry). DDT, PCBs, and similar toxic and persistent organic pollutants tend to accumulate in living organisms and can reach harmful levels particularly in species at the top of food chains.

Rivers receive waste from industries like tanneries, fell mongers and leather dresses, food processing, gas works, oil and grease processing
and refining, scouring of cotton and wool, dry dying of cotton, wool jute and rayon, piggeries, slaughter houses, calico printing and from the manufacture of batteries, paints, light alloys, concrete, rubber, plastic, dyes, chemicals, glue, paper pulp and paper etc. beside these substances from industries, forestry and agriculture also add many toxic pollutants to freshwater. Water pollution in streams, rivers and lakes kill fish and other aquatic animals.

However, pesticide exposure causes severe alternations in the tissue biochemistry of fishes (Kumar and Singh, 2000; Tilak et al., 2003 and Mathivanan, 2004). In general the toxic effects will be more when two or more toxicants act together in a synergistic manner, (Sujatha, 2006).

Organophosphates (OP) are one of the most preferred pesticides due to their high effectiveness and low persistent in the environment. OP pesticides directly inhibit acetylcholineesterase enzyme activity in fishes and invertebrates (Fulton and Key, 2001; Rao et al., 2005 and Agrahari et al., 2006). Dimethoate having trade name rogor an organophosphate insecticide was first described by Hoegberg and Cassaday in 1951 and introduced to the market in 1956 (Pandey,2009). Rogor is an organophosphate with acetylcholinesterase inhibiting ability and it bears acute and chronic ability (Verma, 2007).

In India, the pollution problem becomes noticeable after growth of industrialization and increases in population with diversification of human activities. Our life depends upon water and man needs water for drinking, other domestic and industrial purposes. Undoubtedly, water pollution is an age-old problem, but in this modern age, the problem like population increase, sewage disposal, industrial effluents, radioactive materials, pesticides, heavy metals and number of other factors have
polluted our water resources as much as that about 70% rivers and streams not only of India but also of the world contain polluted water.

Aquatic toxicology is the branch of the science of ecotoxicology that is “multidisciplinary in scope and interdisciplinary in practice” (Rand et al., 1995).

Ecotoxicology was derived from the words ecology and toxicology. This term was introduced by Truhant in 1969, who defined it as the “study of the harmful effects of chemical upon ecosystem.” Ecotoxicology deals with movement of pollutants in air, water, soil sediment and through food chains, with chemical transformation. However pure toxicology regards the uptake, distribution, metabolism and excretion of xenobiotics in living organisms (Walker et al., 1996).

The term toxicity test and bioassay are frequently used interchangeably. Bioassays were normally associated with the measurement of drugs where the prime interest was to determine the degree of response of the test organisms and the strength of stimulus. (Sprague, 1973) defined bioassay as a test in which the strength of material is determined by the reaction of the living organisms to it. Some terms which are commonly used in toxicity studies are acute, sub-acute, lethal, sub lethal, short term and long term toxicity test. (Alderiece, 1967) suggested that there are only two categories acute toxicity which is usually lethal and chronic toxicity which may be lethal or sub lethal. The results of toxicity bioassay are generally reported in terms of median tolerance limit (TLM) or median lethal concentration (LC$_{50}$ or TLC). LC$_{50}$ is a concentration at which 50% of the treated animals or fishes survive or it is an interpolated value based on the percentage of fish surviving at two or more concentrations at which less than half of the test fishes survive. The period of the exposure for the study of toxicity test is
usually 24, 48, 72 and 96 hours. Standard methods, American Public Health Association stated that, “in a bioassay, experimental organisms are subjected to a series of a known or suspected toxicant under adequately controlled conditions. Acute toxicity involves the damage to the organisms by the fast acting mechanism, fatal unless the organism escapes the toxic environment at an early stage.”

The development of ecotoxicology has shifted from the measurement of acute, lethal effects of chemicals to the assessment of sublethal and chronic effects (Anonymous, 2001).

As a result of acute toxicity may result from contact severe damage. It is an imperative to recognize the nature of these aftermath to evaluate the hazard associated with the use of specific pesticides in the environment.

Acute toxicity tests have copious unlimited uses. The bioassay may be used in the determination in the toxicity of some material, the degree of toxicity, variability and the treatment methods. The other important use of acute toxicity test is the determination of relative sensitivity of various species to a pollutant and effects on their life stage.

Acute toxicity studies are helpful in recognition of the vulnerable species of an ecosystem that can be used as an indicator species for particular type of pollution. The acute toxicity study is a preliminary step to the concentration to be tested in short term (lethal) or long term (sublethal) exposure. The acute toxicity tests have been far more frequently employed than chronic exposure test, but they are ordinarily only the first step towards acquiring meaningful information that can be obtained through long term or chronic studies. The toxicity of pesticide component rely on number of factors such as size, sex, weight, age, and strains. It is
also influenced by environmental factors such as change in pH, DO, temperature, hardness and relative humidity.

In India, (Mathur, 1962) for the first time recorded the toxicological action of pesticides, DDT to fishes, *Ophiocephalus punctatus*, *Heteropneustes fossilis*, and *Trochojasater fascitus*.

Investigations on the effects of pesticides on fishes have been made by number of workers in foreign countries as well as in India. Scientist has been trying to correlate the effects of these pesticides/ pollutants/toxicants with the fish life. The acute toxicity test has been measured for many species in variety of ecological systems.

Numbers of workers like (Alabaaster and Abram, 1965; Mount and Warner, 1965 and Sprague, 1969, 1971, 1972 and 1973) have dealt with the pollution and its effect on fish. The efforts of these workers have gone a long way in standardizing the toxicity bioassay techniques used in the water pollution studies.

In recent years, extensive studies have been carried out on the acute toxicity of pesticides, (Santhakumar *et al.*, 2000) studied acute toxicity of monocrotophos on ethological responses of *Anabus testudineus* and behavioral changes. (Khillare and davane,1987-92) studied acute toxicity of endosulfan, malathion and sevin pesticides to *Puntius stigma* and reported environmental factors which effect the toxicity of pesticides. (Rahaman *et al.*, 2002) studied the effect of daizion 60 EC on *Anabus testudineus, Channa punctatus* and *Barbodes gonionotus*.

Various workers have reported acute toxicity of various pollutants to aquatic animals; (Rao and Ramaneswari, 2000; Wan *et al.*, 2000; Dede and Kaglo, 2001; Singh and Sandhu, 2001; Mazon *et al.*, 2002; Singh *et al.*, 2004; Mahalakshmi and Muniyan, 2005; Naik *et al.*, 2004; Monkidje,
Several workers have investigated the toxicity of pesticides in fish. (Singh et al., 2010) studied the acute toxicity and behavioral responses of common carp *Cyprinus carpio* to dimethoate. (Pandey et al., 2009) studied the acute toxicity bioassay of dimethoate on *Heteropneustes fossilis*.

Charjan et al., (2008) studied the organophosphorous pesticide, rogor to *Channa orientalis*. (Srivastava et al., 2010) find out acute toxicity and behavioral responses of *Heteropneustes fossilis* to dimethoate. (Pandey et al., 2008) studied the effect of temperature on mortality and behavioral responses in *Heteropneustes fossilis* exposed to dimethoate. (Bhandare et al., 2011) studied the short term toxicity of *Channa striatus* exposed to rogor. (Bhandare et al., 2011) studied the toxicity and behavioral changes in *Puntius stigma* exposed to rogor. (Kalavathy et al., 2001) studied toxic effect of the pesticide dimethoate on *Sarotherodon mossambicus*. (Marigoudar et al., 2009) studied cypermethrin induced respiratory and behavioral responses of *Labeo rohita*. (Rao and Shameem, 2004) investigate the acute toxic effect of carbaryl on the common carp, *Cyprinus carpio*. (Deshmukh and Sonawane, 2010) studied acute lethal toxicity of endosulfan on *Channa gachua*, and (Tilak and Rao, 2003) studied the *chloropyrifos* toxicity to fresh water fish.

The main purpose of toxicity test was to produce data concerning the adverse effect of rogor on freshwater fishes *Puntius stigma* and *Channa striatus*. The most common types of toxicity test with aquatic animals are the acute mortality test, which was usually conducted to obtain information about lethal concentration and to understand the
physiological process, tolerance limit of the fishes under stress conditions. Very little work has been carried on acute toxicity of rogor on freshwater fishes, *Puntius stigma* and *Channa striatus*. Therefore, the present investigation was undertaken to determine the relative toxicity of rogor to freshwater fishes, *Puntius stigma* and *Channa striatus*.
MATERIALS AND METHODS

Freshwater fishes *Puntius stigma* and *Channa striatus* were selected for bioassay studies to evaluate the toxicity of rogor.

The *Puntius stigma* (family Cyprinidae) and *Channa striatus* (Channidae), fishes were collected from Sukhana River flowing near Nipani, 25 km away from Aurangabad (M.S). These two fish species were easily available during present study.

The fishes were allowed to acclimatize to the laboratory conditions for four weeks before used for the tests. During the period of acclimatization, the fishes were fed on small pieces of live earthworms. Feeding was provided after every 24 hours before toxicity tests. During the acclimatization and acute toxicity tests filtered and aged water used for maintain the fish as it helps to stabilize its composition and to eliminate residual chlorine which considered highly toxic to fishes.

The fishes were maintained in sufficiently large aquaria so to prevent overcrowding, the acclimatized fishes were given artificial air by aerator. All the necessary precautions were taken in consideration to keep the aquaria clean and away from any mechanical disturbances. Glass of size (3*1*1* feet) were used as test container. Artificial aeration during the toxicity test was avoided.

The physico-chemical characteristics of test water have analyzed regularly during the test period following the standards described in (APHA, 2000).

The fishes, *Puntius stigma* and *Channa striatus* were selected for the tests. The *Puntius stigma* ranged from 7.5 to 8.5 cm in length and 4.5 to 5.5 gm in weight. The fishes, *Channa striatus* ranged from 14 to 16 cm in length and 30 to 32 gm in weight.
For the selection of test concentration, some pilot tests were carried out as it is essential to determine the range of toxicity of a particular pesticide. The range of concentration selected was resulted in zero to hundred percent mortality. In order to maintain the level of the toxicant in the water, it was changed at every 24 hours during the short term or 96 hours exposure.

Toxicity was determined by static bioassay. To evaluate the toxicity, series of different concentration were prepared on the basis of the result based on the pilot tests. 10 healthy fishes of uniform size and weigh were exposed to various concentrations for 96 hours. Simultaneously the control groups were maintained with zero toxicant concentration under analogous conditions.

The bioassay tests were continued for 96 hours. The tests were started in the morning and observations on the behavior were noted. The observations on mortality and survival were noted after 24, 48, 72 and 96 hours. The fish was considered dead when there was no movement showed and lack of responses to mechanical stimuli. The dead fishes were removed from test container.

Each experiment was repeated twice to secure constant results. The data collected were the extrapolated statistically by means of the method and transforming the toxicity curve (% mortality / concentration) in to regression lines (mortality in probit / concentration i.e. probit kill / concentration) according to the methods of (Finney, 1971), which allows the calculation of average lethal concentration (LC$_{50}$) for 24, 48, 72 and 96 hours. Hence, present study, organophosphorus pesticide i.e. rogor was selected to evaluate its toxic effect on $Puntius stigma$ and $Channa striatus$. 
Organophosphate:

The organophosphate insecticides have several commonly used names such as organic phosphate, phosphorus insecticides, phosphate insecticides and phosphorus esters of phosphoric acid. They are all derived from phosphoric acid and are generally the most toxic of all pesticides to vertebrate animals.

![Phosphoric acid structure](image)

Indeed, they are related to the “nerve gases” by chemical structure and mode of action. The insecticidal action of these compounds was discovered (1942) in Germany during World War II in the study of materials closely related to the nerve gases, sarin, soman and tabun. Initially, the discovery was made in search of substitutes for nicotine, which was critically short supply in Germany. The organophosphate insecticides were derived from phosphoric acid and were related to the nerve gases. The organophosphates have two distinct features. Firstly they were generally much more toxic to vertebrates than the organochlorine insecticides and secondly they were non-persistent.

OP is the short form for organophosphate, which exerts action by tying up or inhibiting certain important enzymes of the nervous system, cholinesterase (ChE). Throughout the nervous system in vertebrates as well as insects, are, electrical switching centers, are synapses, where the electrical signal is carried across a gape to a muscle or another never fiber (neuron) by a chemical, in many instances, acetylcholine (ACh). After the electrical signal (nerve impulse) has been conducted across the gape by ACh, the ChE enzyme moves in quickly and removes the ACh so the circuit won’t be blocked. These chemical reactions happened extreme rapidly and goes on constantly under normal conditions. When OP enters the scene, they attach to
the ChE in a way that prevent them from removing the ACh and the circuit jams because of accumulation of ACh. What really this means in that the accumulation of ACh interferes with the neuromuscular junction, giving rise to rapid switching of voluntary muscle and finally to paralysis. This has of particular importance in proper functioning of the respiratory system.

**Rogor:**

[Roger, dimethoate, phosphamide, 0, 0-Dimethyl-5-(CN-methyl-crbamoylmethyl) dithiophosphate]

The rogor pesticide is a commercial grade pesticide, can be obtained from the market. The detailed structure and active gradient of the pesticide illustrated in Table No: 2.

Rogor was a systemic insecticide produced by reacting salts of dimethyldithio-phosphoric acid with N-methylchloroacetamide, in aqueous medium in the presence of some organic solvents. It is also produced by reacting 0-0-dimethyl-5-(carboxymethyl) or 5-(carboryloxy-methyl) dithiophosphate at low temperature with aqueous methylamine. Both phenyl and ethyl esters are used. However the rogor obtained by this method is less stable due to presence of impurities like methylamine traces which can decompose rogor easily. Pure rogor is a white crystalline substance with camphor like color. The technical material is yellowish brown (amber) colored, liquid with sulphurous acid smell. Pure rogor melts at $51^\circ\text{C}$ to $52^\circ\text{C}$. It is highly soluble in water (30 g/lit), and in most organic solvent but slightly soluble in paraffinic hydrocarbons.

When rogor is oxidized by various oxidizing agents or by the oxygen in the air (Oxidation by the oxygen in the air proceeds especially well in green leaves of the plant), it is converted to 0-0 dimethyl-5 (N- methyl-crbamoylmethyl) thiosphosphate. The hydrolysis of dimethoate takes place most quickly in alkaline medium. In acidic medium, it is more stable (50%
is hydrolyzed in 8 hours at 9.0 pH, and at 70°C but at 5.0 pH requires 21 hours). Depending on the dosage of rogor applied, it usually broken down in plants within 15 to 20 days, while applying rogor care should be taken that there was no water stress.

**TABLE NO 1: DETAILED STRUCTURE OF PESTICIDE UNDER TOXICITY TEST**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Trade name</th>
<th>Chemical name</th>
<th>Structural Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rogor</td>
<td>Dimethoate</td>
<td><img src="image" alt="Structural Formula" /></td>
</tr>
</tbody>
</table>
RESULTS

In the present study LC$_{50}$ values were decreased at 96 hrs and found to be increased during the exposure period of 24 hrs, so the values were recorded highest at 24 hrs and the lowest at the 96 hrs of exposure.

The results revealed that the water used for experiment did not contain any toxic substance. Initially no mortality was observed in control group. The LC$_{50}$ values of rogor under laboratory conditions to freshwater fishes, *Puntius stigma* and *Channa striatus* were exposed to rogor for 24hrs, 48hrs, 72hrs and 96 hrs have been recorded at 9 ppm, 8.31 ppm, 7.8 ppm and 7.1ppm whereas 16.8 ppm, 16.2 ppm, 15.6 ppm and 15 ppm respectively. Table nos. (7 to 16)

Fishes *Puntius stigma* and *Channa striatus* were exposed to lethal concentration of rogor for a short term exposure and were studied in terms of general behavior, rate of survival and mortality. The physicochemical characteristics of water were Temperature, 26±20C; Conductivity, 0.72µmhos/cm; Acidity, 4.2ppm; Alkalinity, 27ppm; pH, 7.1; D.O, 6.5; Total Hardness, 84ppm recorded (Table number 5) and Temperature, 26±20C; Conductivity, 0.70µmhos/cm; Acidity, 4.1ppm; Alkalinity, 30ppm; pH, 7; D.O, 6.7; Total Hardness, 85 ppm recorded (Table number 6). The LC$_{50}$ values for *Puntius stigma* and *Channa striatus* recorded in fig nos (1-8) along with regression results, calculated LC$_{50}$ varience, chi-square, and 95% fiducial limits were recorded in table nos (11and16).

Behavioural changes were as a tool for hazardous assessment of water pollution. In control group of fishes, normal behavior, such as constant opercular movement, steady balance, normal surfacing and non aggressive movement have been observed.
It was observed in the experiment that the exposed fishes exhibited altered behavior as compared with the control fishes. After addition of rogor in experimental set of aquaria, the fishes became restless, started moving to and fro in the water. They started coming to the surface of the water to inhale air and it looked as they were suffocating.

During acute toxicity test, fishes *Puntius stigma* and *Channa striatus* exhibited peculiar reaction after 24 hrs of exposure. At first fishes showed alter behavioural responses, restlessness and hyperactivity, erratic swimming, convulsions, jerky movement, disruption of schooling behaviour, rapid opercular movement and thick mucous covering over the whole body surface and loss of equilibrium.

The activity of the fish, *Puntius stigma* exposed to lethal concentrations of rogor showed high excitation of the fish as soon as they came in contact with pesticides. The fish showed increase in opercular activity, erratic swimming and jerky movement. The fish struggled hard for breathing sometimes engulfing atmospheric air and avoided toxic medium. The fishes have tried to leap out of toxic medium. At the end of short term exposure, it was found that the colour of the fishes became dark. Besides this, there was a thick mucous covering over the whole body surface and gills.

In case of fish, *Channa striatus*, exposed to lethal concentration of rogor showed high excitation, but the movement of the fishes were not rapid. The fish showed increased opercular movement, loss of balance, surfing activity were observed. At the end of short term exposure, it was found that, there was thick mucus covering all over the body surface and gill.
Fish secretes copious amount of mucus, as a defense mechanism to neutralize the effect of rogor, which gradually covers all over the body, gills and the buccal cavity.

The LC$_{50}$ values and exposure period showed a direct relationship, therefore it was concluded from the present study that the *Puntius stigma* and *Channa striatus* were very sensitive to the rogor as evident regarding the behavioural study.
DISCUSSION

The results revealed that the water used for experiment did not contain any toxic substance. Initially no mortality was observed in control group. Further, increased concentration lead increase in mortality. Fishes were exposed to lethal concentrations of rogor for acute test exposures were studied in terms of behavior, rate of survival and mortality. Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicants, its concentration and duration of exposure.

The LC$_{50}$ values of fresh water fishes, *Puntius stigma* and *Channa striatus* exposed to rogor for 24hrs, 48hrs, 72hrs and 96 hrs have been recorded at 9ppm, 8.31ppm, 7.8ppm and 7.1 ppm whereas 16.8ppm, 16.2ppm, 15.6ppm and 15 ppm respectively. The LC$_{50}$ values, regression results have calculated to support present observations presented (Table nos 7 - 16).

The LC$_{50}$ values were decreased with increased exposure period. In the present investigation LC$_{50}$ values were decreased at 96 hrs and found to be increased during the exposure period of 24 hrs.

LC$_{50}$ values have been determined for different fishes in relation to different pesticides by previous workers. These values differ greatly from one animal to another. Such difference in the toxic values could be attributed to various factors such as variability in bioassay technique, difference in size and weight of test fishes (Reddy and Shembekar; 2009).

The LC$_{50}$ values of Dimethoate for certain air-breathing fishes were reported to be very high, as in *Clarias batrachus*, [Begum, 1995]. It is 65 mg/l for 96 hrs,

The 96 hrs LC$_{50}$ value of dimethoate for *Lebistes reticulatus* has been reported as 19 mg/l by (Gupta, 1984).
De Mel and Pathiratne, (2005) studied toxicity assessment of insecticides commonly used in rice pest management to the fry of common carp, *Cyprinus carpio* and observed 96 hrs LC$_{50}$ value for dimethoate to fish was 26.11 mg/l.

Swaileh, (2006) reported the LC$_{50}$ of dimethoate for 96 hrs exposure to the fish *Nile tilapia* (*Oreochromis niloticus*) is 40 mg/l.

Reddy and Shembekar, (2009) acute toxicity test of dimethoate on freshwater fish Channa gachua was studied. The LC$_{50}$ values were determined at 24hrs, 48hrs, 72hrs and 96 hrs. were found to be 31.29ppm, 25.65ppm, 23.82ppm and 20.25 ppm respectively. whereas in *Heteropneustes fossilis* [Pandey et al., 2009], Very low LC$_{50}$ values for 24hrs, 48hrs, 72hrs and 96 hrs dimethoate exposure was recorded as 3.38, 3.23, 3.08 and 2.98 mg/l.

Sing *et al.*, (2010) observed acute toxicity and behavioral responses of common carp *Cyprinus carpio* (Linn.) to an organophosphate (dimethoate). The fingerlings were exposed to dimethoate to determine LC$_{50}$ values for 24hrs, 48hrs, 72hrs and 96 hrs was found to be 1.84, 1.78, 1.68 and 1.61 mg/l respectively.

Vittozzi and Angelis, (1991) reported 0.78 mg/l and 0.79 mg/l as 96 hrs LC$_{50}$ values of dimethoate for Bluegill and Trouts respectively.

Very high LC$_{50}$ values of different toxicants were observed by, (Vasait and Patil, 2005). The toxicity of Monocrotophos on the *Nemacheilus botia* has been studied. The LC$_{50}$ values were found 49.6ppm and 42 ppm for 7 and 14 days exposure period. The result indicates decreased LC$_{50}$ value with concentration duration of exposure increased.

The acute toxicity studied of chromium on freshwater fish, *Mystus vitatus*, for a period of 24hrs, 48hrs, 72hrs and 96 hrs and reported LC$_{50}$
values as 82.79, 72.11, 64.42 and 61.67 mg/l respectively (Shivkumar et al., 2006).

Whereas, very low LC$_{50}$ values of pesticides were observed by, (Mohite et al., 2004) studied the LC$_{50}$ dose and lethal concentration for phosphamidon and endosulfan was found to be 9.82, 25.12 mg/l and 0.28, 1.24 mg/l respectively.

The freshwater fish *Channa punctatus* was exposed to organochlorine insecticide Kelthane for 24hrs, 48hrs, 72hrs and 96 hrs were 0.72ppm, 0.74ppm, 0.75ppm and 0.75 ppm respectively shown by (Rao et al., 2006).

The acute lethal toxicity of endosulfan on *Channa gachua* exposed to 24hrs, 48hrs, 72hrs and 96 hrs LC$_{50}$ were 0.0049ppm, 0.0042ppm, 0.0038ppm and 0.0035 ppm respectively were shown by (Deshmukh and Sonawane, 2010).

The LC$_{50}$ values of lindane under normal laboratory conditions to *Rasbora daniconius* for 24hrs, 48hrs, 72hrs and 96 hrs have been recorded 0.0080ppm, 0.0075ppm, 0.0070ppm and 0.0060 ppm, whereas for dimethoate the LC$_{50}$ values were 0.098ppm, 0.082ppm, 0.074ppm and 0.058 ppm respectively (Gaikwad, 2003).

The LC$_{50}$ values of diazion 60 EC to *A. testudineus, C. punctatus* and *B. gonionotus* were reported earlier 6.55ppm, 3.09ppm and 2.27 ppm for 96 hrs exposure (Rahman et al., 2002).

The *Channa striatus* in the present study, results were slightly lower than the LC$_{50}$ values shown by (Agrahari et al., 2006) biomarkers of monocrotophos in a freshwater fish *Channa punctatus* (Bloch) for acute exposure to 18.56ppm at 96 hrs.

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicants, its concentration and duration of exposure.
The toxicity of chemicals to aquatic organisms has been shown affected by age, size and health of the species (Abdul-Farah et al., 2004).

In *Puntius stigma* the results in the present study were slightly higher than the LC$_{50}$ values shown by earlier worker (Ramesh and saravanan, 2008) the acute toxicity of an organophosphorous insecticide chlorpyrifos on economically important freshwater teleost fish *Cyprinus carpio*. The 24 hrs LC$_{50}$ was 5.28 ppm.

The difference in toxicity to the different species mentioned above due to differences in absorption of pesticide, their accumulation, biotransformation and excretion. Differences in metabolic pathways among species may result in different patterns of biotransformation leading to more or less toxic metabolites (Wasu et al., 2009).

The malathion toxicity on fingerlings of air breathing cat fish, *Heteropneustes fossilis*. Toxicity for a period of 24hrs, 48hrs, 72hrs and 96 hrs was found to be 19.0ppm, 18.0ppm, 17.5ppm and 17.0 ppm in developmental stage while later in adult fish it increased to 35.4ppm, 24.0ppm, 35.5ppm and 33.0ppm respectively (PankajKumar et al., 2004).

Similar observations were observed in, *Cirrhinus mrigala* (Verma et al., 1978) reported LC$_{50}$ of malathion as 0.88 ppm at 96 hrs, he further reported LC$_{50}$ for 24hrs, 48hrs, 72hrs and 96 hrs to different fishes *Clarias batrachus*, 20.41ppm, 18.00ppm,17.18ppm and 16.10 ppm. *Heteropneustus fossilis*, 18.49ppm,17.18ppm, 16.18 ppm and 15.00 ppm and *Puntius sophore* 8.15ppm, 7.902ppm, 7.8ppm and 7.45 ppm respectively.

The experiments were designed to investigate toxic effect of rogor pesticide to fish *Channa orientalis*. The LC$_{50}$ values for 24hrs, 48hrs, 72hrs and 96 hrs exposure periods were recorded 5.82, 5.50, 5.50 and 5.00 ml/l respectively (Charjan et al., 2008).
The LC$_{50}$ values of dimethoate to the freshwater catfish *Heteropneustes fossilis* at various exposure periods are 15.92 mg/l for 24 hrs; 13.42 mg/l for 48 hrs; 12.39 mg/l for 72 hrs and 11.34 mg/l for 96 hrs, respectively observations recorded by (Srivastava *et al.*, 2010).

The LC$_{50}$ value of dimethoate for *Colisa fasciatus* as 13.0 mg/l for 24 hrs, 11.4 mg/l for 48 hrs, 10.0 mg/l for 72 hrs and 9.3 mg/l for 96 hrs, reported by (Shukla, 1995).

Acute toxicity of organophosphate pesticide Metasystox on freshwater fish, *Nemacheilus botia* to evaluate median lethal concentration for 24hrs, 48hrs, 72hrs and 96 hrs, The LC$_{50}$ values were 10.3 ppm, 9.131 ppm, 7.884 ppm and 7.018 ppm after exposure to 24hrs, 48hrs, 72hrs and 96 hrs respectively (Nikam *et al.*, 2011).

The acute effect of carbaryl on the common carp *Cyprinus carpio*, were observed the 96 hrs LC$_{50}$ value was 14.64 ppm (Rao and Shameem, 2004).

The LC$_{50}$ values of dimethoate for air-breathing teleost *Channa punctatus* it was 17.9 mg/l for 96 hrs, (Srivastava and Singh, 2001). The LC$_{50}$ values observed in *Channa punctatus exposed to carbaryl* was 8.5 ppm [Sambasiva Rao, 1999].

The LC$_{50}$ value of Metasystox and sevin to freshwater teleost, *Mystus vittatus* LC$_{50}$ values were recorded 6.5 ppm and 11.5 ppm for 30 days exposure by (John, 2007). The 96 hrs LC$_{50}$ value for *Channa punctatus* were recorded as 6.61 ppm, for malathion toxicity (Pandey *et al.*, 2005).

The acute toxicity due to organophosphate Nuvan to the grass carp *Ctenopharyngodon idella* LC$_{50}$ values were recorded were 13.1 ppm, 10.9 ppm, 9.8 ppm and 6.5 ppm at 24hrs, 48hrs, 72hrs and 96 hrs respectively (Tilak and Kumari, 2009).
The toxicity of carbaryl and malathion to *Clarius batrachus* was studied for 48hrs and 96 hrs, the LC$_{50}$ values of carbaryl for 48rs and 96 hrs was 13.24ppm and 5.248 ppm and for malathion 48hrs and 96 hrs LC$_{50}$ was 0.31ppm and 0.25 ppm respectively (Wasu *et al.*, 2009).

The toxicity test of fenvalerate was conducted for freshwater fishes, *Labeo rohita (Ham)*, *Catla catla*, *Cirrhinus mrigala*, *Aplocheilus punchex* and *Ctenopharygodon idellus*. The static and continuous flow through test was employed to determine the LC$_{50}$ for 24hrs, 48hrs, 72hrs and 96hrs to test fish. It has been observed that *Cirrhinus mrigala* was more sensitive to this pesticide followed by other fishes, (Tilak *et al.*, 2001).

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicants, its concentration and duration of exposure. The evaluation of LC$_{50}$ concentration of pollutants was important step before carrying out further studies on physiological changes in the animal (Nikam, 2011). The percent survival rate of the fish decreased with increasing concentration and period of exposure. The LC$_{50}$ values were decreased gradually as the period goes on increasing. Therefore for a single toxicant, on the fish species different LC$_{50}$ values have been recorded.

In *Catla catla* (Kumar and Singh, 2000) the LC$_{50}$ values for 96 hrs was reported very low as 0.007 ppm exposed to dimethoate. The 48 hrs LC$_{50}$ of carbaryl to *Clarius batrachus* was 13.24 ppm and for 96 hrs LC$_{50}$ it was found 5.248ppm (Wasu *et al.*, 2009)Which were relatively less than *C. striatus* and higher than *P. stigma* in the present study. He is also reported the LC$_{50}$ values of malathion for 48 hrs and 96 hrs. The LC$_{50}$ values were 0.31 ppm and 0.25 ppm, which is relatively less than *C. striatus* and *P. stigma*. 
Many workers have been reported the toxicity of different pollutants to different fishes. (Pashine and Kurve, 2000; Jiraungkoorskul et al., 2003; Jagatheeswari, J 2004; Vutukuru et al., 2005; Emad et al., 2005; Boran et al., 2007; Glusczak et al., 2007; Yadav et al., 2007; Bringolf et al., 2007; Ayoola 2008; Akter et al., 2008; Bhakya and Baskaran, 2008; Langiano et al., 2009; and Malla et al., 2009; Halappa and David, 2009; Magar and Waghmare, 2010 and Nwani et al., 2010).

In the present study it has been observed that percent mortality increases with increase in concentrations of pesticides as documented earlier by (Oti, 2002; Ayuba and Ofojekwu, 2005).

Behavioural changes were physiological responses shown by the animal, which were often used as the sensitive measure of stress syndrome in the organisms experiencing it, consequently the behavioral changes were observed in control and test fishes.

Control fishes: The control fishes behaved in a natural manner i.e., they were active with well coordinated movements. Control fishes maintained a fairly compact school, covering about one third of the bottom during the tank area. Fishes were observed to scrap the bottom surface. They were sensitive to any mechanical stimulation and moved to bottom of the tank. Except a less response to form a dense school towards the end of the study, no other extraordinary behavior was observed.

In the experimental fishes, *Puntius stigma* and *Channa sriatus* the disruption of schooling behavior of the fish, due to the lethal and sub lethal stress at the toxicant, results increased swimming activity and increased expenditure of energy (Venkatarathnamma et al., 2008). The study on behaviour and respiratory dysfunction as an index of malathion toxicity in *Labeo rohita* clearly reported that while the control fish were active with controlled and co-ordinated movements, the toxicant exposed
fish exhibited irregular, erratic and darting movements, loss of equilibrium due to inhibition of AchE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyperstimulation (Patil and David, 2008).

Recent studies on acute toxicity and responses of common carp, *Cyprinus carpio* to an organophosphate, Dimethoate reported erratic swimming of the test fish, increased surfacing, decreased rate of opercular movement, copious mucous secretion, reduced agility and inability to maintain normal posture and balance with increasing exposure time (Pandey *et al.*, 2009).

In the various behavioral anomalies in fish exposed to different toxicants in general include initial increase in opercular movements followed by steady decrease with increase duration of exposure (Shivkumar and David, 2004), gulping air at the surface, swimming at the water surface, disrupted behaviour and easy predation (Ural and Simsek, 2006). Gulping of air may help to avoid contact of toxic medium. Surfacing phenomenon might be a demand of higher oxygen level during the exposure period (Katja *et al.*, 2005). Finally, fish sunk to the bottom with the least opercular movements and die with their mouth opened.

Many researchers have worked on toxicity and behavioural study of fish due to toxicants (Santhakumar *et al.*, 2000; Kalavathy *et al.*, 2001; Parma de Croux *et al.*, 2002; Rao *et al.*, 2003; Almazan-Rueda *et al.*, 2004; David *et al.*, 2004; Shivkumar and David, 2004; Katja *et al.*, 2005; Mushigeri and David, 2005; Prashanth *et al.*, 2005; Dube and Hosetti, 2010;).

The exposure of fish to different concentrations of rogor shows altered behavioural responses. The restlessness and hyperactivity in fish may occur due to the inactivation of acetylcholinesterase, leading to...
acetylcholine at synaptic junctions (Fulton et al., 2001). Stimulation of peripheral nervous system which results into increased metabolic activities. Disruption of schooling behaviour of fish due to the lethal and sub lethal stress at the toxicant, results in increased swimming activity and entails increased expenditure of energy (Venkata Rathnamma et al., 2008). The abrupt erratic and jerky swimming may be due to inhibition of the AchE in the brain and neuromuscular junctions, (Rao et al., 2005 and Agrahari et al., 2006).

The opercular movement in the fishes has been reported to increase the exposure of toxicants (Pandey et al., 2005 and Yadav et al., 2007). Contrary to it, the opercular movement in fish showed marked decrease exposed to dimethoate (Pandey et al., 2009).

Fishes exposed to lethal concentrations of rogor for 24 hrs, at first fishes showed increased opercular movement. This could be due to clearance of the accumulated mucus debris in the gill region for proper breathing. At the inception the fishes suffocate and try to come at the surface for gasping the air. The fishes are avoiding toxic water with fast swimming and jumping faster. Presence of over secretion of mucous on the body was found and irregular swimming activity was noticed. Behavioural evidences in the present study are correlated to some extent with the work of earlier workers (Krishnamurthy and David 2010; Marigoudar et al., 2009; Susan et al., 2010; Rao et al., 2009; Marigoudar (b) et al., 2009; Shailender Singh et al., 2007; Pathan T.S, 2010; Singh et al.,2010; Singh et al.,2009; Pandey et al, 2009; and Paithane, 2010 ).

The behavioural changes are the obvious of the motivational biochemical, physiological and environmental influence state of the fishes. An erratic swimming of the treated fishes showed loss of equilibrium. It is proved that the region in the brain associated with the
The erratic swimming, rapid jerky movements and convulsions before death were evident and serve asphyxiation as indicated by gasping to death (Kalavathy, 2001). The present study has been concluded that fishes were highly sensitive to the rogor. Moreover, this study reveals that rogor is more toxic to *Puntius stigma* than *Channa striatus*.

Toxicity test are a fundamental appliance for ecological risk assessment of toxic compounds. In order to increase the reliability test results, both the control and experimental conditions can be improved and the variance of test media can be analyzed with statistics. An inference has been drawn from the present study, the mortality increases with increasing rogor concentration up to 24hrs. after that concentration decreases with an increase in exposure period indicating that the toxic effect of rogor is not only dose dependent, but it has been found time dependant also.