LETHAL AND SUBLETHAL TOXICITY OF MONOCROTOPHOS AN ORGANOPHOSPHATE ON THE JUVENILES OF ROHU – *LABEO ROHITA* (Ham.) UNDER TROPICAL CONDITIONS

By

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THESIS

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MASTER OF FISHERIES SCIENCE

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DEDICATED TO MY PARENTS,
TEACHERS AND GOD

DECLARATION

I hereby declare that this thesis entitled **"Lethal and sublethal toxicity of monocrotophos - an organophosphate - on the juveniles of Rohu – *Labeo rohita* (Ham.) under tropical conditions"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

Place : Panangad
Date : 3/10/2000

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CERTIFICATE

Certified that this thesis, entitled **"Lethal and sublethal toxicity of monocrotophos - an organophosphate - on the juveniles of Rohu – *Labeo rohita* (Ham.) under tropical conditions"** is a record of research work done independently by Sri. **Ramani M. B.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associate-ship to him.

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1. INTRODUCTION

Population increase, rapid industrialization, intensive terrestrial farming system and wide spread health protection programmes have accelerated large-scale production and utilization of varied types of synthetic organic biocides in our country. An array of wide spectrum chemicals and their combinations are now being used as insecticides, fungicides, herbicides, nematicides, rodenticides and molluscicides. Many of the pesticides used are highly toxic and remain in the environment for a long time, causing pollution. Moreover, due to repeated application of pesticides their toxic residues in environment and biota have reached alarming concentration. Unfortunately, many of these toxic chemicals are also mutagenic, carcinogenic or teratogenic to human beings and hydrobionts of the biosphere. Constant use of pesticide helps certain pests to develop resistance against these chemicals after few applications.

Insects and other pests cause damage to the crop, results in loss of food grain production. The annual loss of food grains due to pests and diseases is about Rs. 6,000 crore and out of it insects and rodents alone cause 26 per cent of the loss. Due to this and as there is no other alternative including the biological control method for controlling pests, the use of pesticides has become an integral part of modern agriculture system. Many nations, inspite of knowing about the harmful effects of these chemicals, are still using them in large quantities.

Agricultural chemicals were introduced to the Indian market soon after the Second World War. Application of organochlorines such as DDT and HCH (Hexachlorocyclohexane) started during late 1940's and early 1950's followed rapidly by organophosphates and carbamates in 1960's and 1970's respectively. In 1977, about 38,000 tonnes of insecticides were used in India (David, 1978). Demands for pesticides in the year 1983 and 1987 were 72,000 and 1,00,000 tonnes respectively and in the end of 2,000 A.D., it will reach to 2,00,000 tonnes, which will be almost double the amount used during 1987 (Sharma, 1987). The average consumption of pesticides in our country increased from 3.2 g/ha in 1954-55 to 336 g/ha in 1980 (Chottoraj, 1987). In USA and European countries, its consumption rate is still higher (1490-1870 g/ha). Multiple cropping pattern, high yielding varieties of hybrid plants, improved horticulture and increase in cropped area in each year under integrated plant protection programme in our country, will intensify the consumption rate of pesticide near to the level of western countries or it may even go higher in future than the permissible amount. It is a great concern that in the coming years, the environment will be in greater risk than at present.

When we look into the types of pesticides and their groups, it is observed that insecticides alone account for 80 per cent. Again, among insecticides, Organochlorines (DDT, DDD, Aldrin, Dieldrin, Hexachlorane, Polychloropinene, Toxophane, etc.) share about 40 per cent. Next to it are organophosphates (Malathion, Parathion, Methylparathion, Fenthion, Thimet,

Monocrotophos, Dimethoate, Pythion etc.) and carbamates (Sevin, Sevimox, Carbofuran, Carbaryl. Aminocarb, Propoxur, Fenuron, Monuron etc) (Edwards, 1976). At present, about 1200 formulations based on 100 pesticides costing around Rs. 700 crores are marketed (Venkatraman and Krishnakumari, 1992). Their use is steadily increasing.

Pesticides reach water either through direct application or indirectly from agricultural fields, spray drift, rainwater, sewage and effluents from industries manufacturing pesticides or using them in their processes. It seems that significant proportion of pesticides reach the adjacent water bodies through agricultural run-off. Relatively, water-soluble pesticides are transported in a dissolved state, while the insoluble ones are transported after being bounded to the particulate matter. Many factors like the amount of suspended particulate matter, the absorption characteristics of solids, distance between the area of origin of pesticide and the receiving water body, angle of slope, vegetation covers and the time lapse between treatment and rainfall, influence the flow of toxicants to water bodies. A heavy rainfall, soon after pesticide application, washes away fairly large proportion of the pesticide with the run-off water. Aerial spraying is done for large area of forests and agricultural fields. It is estimated that generally less than 35 per cent of the pesticides used in aerial spraying reaches the target, the remaining being carried away into the atmosphere (Hindin *et al.,* 1966). Pesticides lost into atmosphere in the vapour phase generally come back to terrestrial system with the rainwater. Tarrant and Tatton (1968) detected

residues of BHC, Dieldrin and DDT in the rainwater collected in Shetland Islands. The agricultural chemicals have been found in the aquatic environment as a result of direct contamination, most often by movement into water due to careless handling. Pesticides are also applied directly to water to kill the undesirable organisms in order to restock more desirable fish species or plant crops such as water nut and lotus. This had been used in the Nalcha tank in the Dhar district (Madhya Pradesh) to some ponds having water nuts (ICAR, 1967). Dichlorvos, malathion and quinalphos are widely used for control of carp lice (Kalyanaraman and Manickavasgam, 1992). In sublethal concentration, living organisms survive for more hours with some metabolic alteration, but in acute toxicity organisms cannot survive for longer periods. Insecticides thus cause an imbalance of self-sustaining ecosystem and exert a great deal of stress on body growth, fertility and population size of fish and other organisms living therein.

Jhingran (1975) stated that there is occurrence of large number of fish mortality in the ponds and tanks due to pesticides in all parts of India. The fish fauna of low land paddy fields, where fingerlings of Indian Major Carps as well as airbreathing fishes are raised along with paddy crops are even not spared from insecticide effects. A great deal of attention has been paid for understanding the status of pollution in the river systems of India. The scientists of the Central Inland Capture Fisheries Research Institute have undertaken studies to find the impact of pesticides entering the different river systems of India on the aquatic biomass. They have conducted field

investigations in the areas of effluent outfall and also monitored areas in the down stream stretches in order to understand the fate of the pesticides so discharged. Further, efforts have also been made to gain an understanding into the effects of these factory effluents on the natural populations of the aquatic fauna. Several varieties of fishes including the economically important major carps were subjected to bioassay tests under the influence of pesticides.

The number of chemicals used as pesticides and insecticides are increasing year after year thereby causing fatal effects on fish fry and fingerlings, spawning as well as feeding grounds, restricting migration of fish, reducing disease resistance and deteriorating the quality of fish produce. Thus, insecticides are becoming potential dangers for aquaculture industry. Although careful studies are required on the effect of insecticides and pesticides on our cultivable fishes, very little well-defined studies towards this end have been conducted so far.

The seasonal utilization of paddy fields for fish culture is quite common in Kerala and West Bengal. In recent years, with the advent of high yielding varieties of paddy, the use of pesticides has become widely prevalent. Organophosphate pesticides are now being widely used for its less persistence in the environment. Malathion, phosphamidon and monocrotophos are the commonly used organophosphates in the paddy fields of Kerala. The indiscriminate use of these pesticides in the paddy fields ultimately pollutes the aquatic environment, affecting aquatic fauna.

Studies on the effect of malathion and phosphamidon on the cultivable species of carps in the paddy fields have been done by scientists such as Arora *et al.,* (1971), Toor and Kaur (1974) and Ballal *et al.,* (1990). But studies on the effect of monocrotophos on these fishes are meagre.

Keeping this in view, the present work entitled "Lethal and sublethal toxicity of Monocrotophos on the juveniles of Rohu – *Labeo rohita* (Ham.), under tropical conditions" has been undertaken as a preliminary study under laboratory condition. In this study monocrotophos (36 per cent SL) is chosen as test chemical for its wide use in paddy fields and an attempt has been made to study the lethal toxicity on the juveniles of *L. rohita* (Ham.) as this fish is suitable for toxicity monitoring (Ashraf *et al.,*1992; Nair and Sherief, 1998). Besides it is a potential species for paddy cum fish culture.

The present study intends to deal with aspects such as (1) the maximum acceptable toxicant concentration (MATC) or safe level for tropical fresh water fish. (2) the threshold levels for different biological and physiological factors like growth, food conversion ratio (FCR) and apparent digestibility co-efficient of dry matter and (3) the changes in biochemical composition of body due to chronic exposure to monocrotophos.

2. REVIEW OF LITERATURE

The studies on bioassay took a turn by the publication of the paper on "The Gold-fish as a test animal for toxicity" by Powers in 1917. He had drawn the attention of others by conducting the study on the relative survival time of fish and concentration of pollutants. Later, Belding (1927) pointed out that a number of factors including the species of fish used, its sex, age, size, general physical condition, the size of the container, the volume of the solution, oxygen content of the test solution and temperature might influence the result of the bio-assay test.

In the early half of 20th century, standard methods for bioassay test were suggested by Doudroff *et al.,* (1951). From mid 70's bioassay was used as a tool by physiologists. Later on, sequential testing system (EPA, 1978; EC, 1979 & OECD, 1979) starting with short term test followed by long term test, along with factors to determine safe concentration (Canton and Slooff, 1979) were established. In later days the effects of toxicants were studied on several aspects

2.1. Acute toxicity test

Acute toxicity test is carried out to determine the concentration of a particular chemical that would elicit a specific response to a test organism or population in relatively short period of time. The end point measured, in this test, is death or immobilization of test organism. This criterion was accepted

because these were easily determined and had obvious biological and ecological significance (Leeuwen, 1986). A large number of papers had been published on the acute toxicity of different toxicants and pesticides by Bhatia (1971), Toor and Kaur (1974), Hansen *et al.* (1975), Lloyd *et al.* (1976), Pillai *et al.* (1977), Julin and Sander (1977), Robert and Rosemarie (1978), Nagaratnamma and Ramamurthi (1981), Jayasankar and Mathu (1983), Akhtar and Shafi (1985), Kaviraj and Ghosh (1985), Ramprabhu *et al.* (1986), Mani and Konar (1986), Perschbacher and Sankar (1989), Saksena and Pandey (1991), Silva *et al.* (1993), Davies *et al.* (1994),Barry *et al.* (1995), Nair and Sherief (1998) , Sulekha *et al.* (2000) and Anna Mercy *et al.*(2000a,b &c).

2.2. Test fish

In short term bioassay test selection of test organism is of prime importance. For the selection of a standard fish, Adelman and Smith (1976) listed the following criteria: (1) must have a constant response to a broad range of toxicants, tested under similar conditions, (2) be available in large quantities, (3) be easy to handle,(4) be easy to transport, (5) must be available all through the year and the desired age must be readily available, and (6) such a species should complete its life cycle within one year or less.

Considering the relative merits of each fish species a fair varieties of fishes have been used in the laboratory for toxicity test of pesticides. Toxicity of various pesticides was studied by Wallen and Geer (1957) on *Gambusia*

affinis; *Lloyd* (1960), on *Salmo gairdneri;* Katz (1961) on *Gasterosteus aculeatus;* Hansen *et al.(1975)* on *Cyprinidon variegatus;* Robert and Rosemarie (1978) on *Salmo clarkii;* Vittozzi and Angelis (1991) on *Lepomis macrochirus.* In India many workers had selected a number of test fishes such as *Cirrhinus mirigala, Puntius ticto* and *Colisa faciata* (Bhatia, 1971a&b); *Cyprinus carpio* (Toor and Kaur, 1974; Nagaratnamma and Ramamurthi, 1981; Ramprabhu *et al.* , 1986); *G. affinis* (Pillai *et al., 1977); Ophiocephalus punctatus* (Rao and Rao, 1981); *Channa punctatus* (Siddique, 1984; Akhtar and Shafi, 1985; Inbaraj and Haider, 1986); *Saccobranchus fossilis* (Akhtar and Shafi, *1985); Tilapia mossambica* (Kaviraj and Ghosh, 1985; Mani and Konar, 1986); *L. rohita* (Ramprabhu *et al.* , 1986; Nair and Sherief, 1998; Sulekha *et al.,* 2000); *Catla catla, C. mrigala, Glossogobius spp., Ambasis spp., Notopterus spp.* (Ramprabhu *et al.* , 1986); *Oreochromis mossambicus* (Sarkar and Konar, 1990); *Clarias batrachus* (Thakur *et al.,* 1990); *Puntius conchonius* (Gill *et al.* , 1991) and *Anabas testudineus, Channa marulius, Etroplus maculatus* (Anna Mercy *et al.,* 2000a & b). In the present study, the Indian major carp *Labeo rohita* (Ham.) is chosen as test animal because this fish is suitable for toxicity monitoring (Ashraf, *et al.,* 1992; Nair & Sherief, 1998). Besides it is a potential species for paddy cum fish culture.

2.3. Pesticide

The Pesticide used in the present study is monocrotophos, which is an Organophosphate. Many scientists conducted experimental works on toxicity

of organophosphate to various fish species earlier. Toor and Kaur, (1974) on *C. carpio;* Julin and Sandar, (1977) on *S. gairdneri;* Nagartnamma and Ramamurthi, (1981) on *C. carpio*; Akhtar and Shafi, (1985) on *C. punctatus* & *Saccobranchus fossilis;* Inbaraj and Haider, (1986) on *C. punctatus* and Flores-nava and Vizcarra-quiroz, (1988) on *Cichlosoma urophthalmus.*

Toxicities of diazinon, fenitrothion, carbaryl, malathion and phosphamidon to embryonic stages of *C. carpio* were conducted by Kaur & Toor (1977). Ballal *et al.,* (1990) studied the lethal effect of ekalux on various growth stages of three Indian major carps and Vittozzi & Angelis (1991) collected comparative data on acute toxicity of two hundred organic chemicals to different fish species recommended by OECD for toxicity testing. But literature on the effect of monocrotophos on fishes are few except for De Silva and Ranasinghe, (1989); Abdel and Nasser, (1991); Anna Mercy *et al.,* (2000a,b & c) and Sulekha *et al.,* (2000).

2.4. Environmental parameters

Various environmental parameters influence the toxic effect of insecticides and other toxicants on aquatic organisms. Some environmental factors affect physiological functions of the organism directly and facilitate rapid penetration of toxicants while some other factors act indirectly by affecting toxicant in changing their concentrations.

2.4.1. Temperature.

As fish is a poikilothermic animal, metabolic activities depend upon water temperature. Doudroff (1957) had noted that the time of survival of fish in toxic solutions increased 50 per cent with a decrease in water temperature by 10°C. Brown *et al.,* (1969) observed a decrease in specific duration toxicity of phenol with increase in temperature. Metelev *et al.,* (1971) remarked that low water temperature acted as a mark for the aquatic organism in the presence of toxic substance and poisoning of fish became evident with an increase in water temperature. Vankhede *et al.,*(1985) found that median lethal level of fytolan toxicity decreased with increase in temperature in case of *Rasbora daniconius.* The rise in temperature from 15°C to 35°C reduced the 96 hrs LC_{50} value from 0.719 to 0.111 mg/l. Sankar (1990) found that toxic substance entered through gills or body surface at high temperature, which ultimately decrease physiological and enzymatic activities of the exposed organism. Cheng-Fang *et al.,* (1993) reported that the masoten concentration causing total mortality of the fish at 22°C was approximately five and three times higher than that of 30°C after an exposure period of 24 hr and 48 hr respectively. Samuelsen (1987) reported that the decrease of the half-life was more significant at 13.5°C than at 4.5° C. The studies of temperature-toxicity relationships have shown a distinctly positive temperature coefficient for some pyrethroids bioassay. (Sogorb *et al.,* 1988). Ferrando and Andreu (1989) reported that the effect of temperature on the toxicity of endosulfan increased when the water

temperature increased (positive temperature co-efficient) in case of eels. Toxicity generally increased with temperature increase, but decreased in the case of a few chemicals like D.D.T., Dimethin and Methoxychlor (Mayer and Ellersieck, 1988).

2.4.2. Oxygen.

Oxygen deficient water influences the intensity of metabolism and reduces resistance of fish exposed to a large number of toxicants of organic and inorganic nature. Davies (1975) formulated minimum dissolved oxygen requirement of different fishes. His approach was to examine the threshold level of dissolved oxygen that caused definite change in some physiological parameters such as reduced swimming stamina, increase or decrease in metabolic rate and reduced blood oxygen saturation in fish.

Certain fishes are extremely resistant to very low levels of oxygen or even anoxia. Blazka (1958) reported that *C. carpio* could survive in the absence of oxygen during winter. Mathu (1967) reported that *R. daniconius* survived about 3 months at 33°C under anoxia. Rao and Rao (1981) found that the rate of oxygen consumption and ammonia excretion decreased in *O. punctatus* exposed to elsan toxicity at different time intervals. Thillart *et al.*, (1983) had observed that *Carassius auratus* survived up to 22 hrs at 29°C under total anoxia. The effect of sublethal concentration of malathion (2.0 mg/l) on respiratory surface area of *C. mrigala* (body weight 21.5 to 27.5 g) was studied by Roy and Dutta (1986). They found that the average length of gill filament of treated one increased to 1.182 times than the normal one and

15.21 per cent decrease in secondary lamella per unit length of filament. Sastry *et al.,* (1991) found that the rate of oxygen uptake by gill, liver and muscle of *C. punctatus* increased significantly when the fish was exposed to aldrin indicating that there was greater demand for oxygen, and the rate of oxygen consumption by the whole fish increased to 38.1 per cent. Effect of sublethal concentration of tannic acid on the oxygen consumption of *C. carpio* revealed that the oxygen consumption decreased with increasing tannic acid concentration and the recovery rate was good at 144 hrs in tannic acid free medium (Chockalingam *et al.,* 1990). Sastry and Siddique (1983) reported high levels of lactic acid and haemoglobin in the blood of *C. punctatus* chronically exposed to endosulfan. According to them, stress increased the lactic acid, which decreased the pH, probably causing high haemoglobin. High haemoglobin content enhanced the oxygen carrying capacity of the blood. The fish, therefore, exhibited increased respiratory movements. The oxygen consumption at higher concentration was lower than those in lower concentration in *Anabas testudineus* chronically exposed to lindane (Bhakthavathsalam and Reddy, 1983) and dichlorvos (Mohapatra and Noble, 1993). A reduction in dissolved oxygen from 100 to 50 per cent of air saturation value reduced the threshold LC_{50} of phenol by about 20 per cent (Lloyd, 1961). Brett (1979) concluded that an oxygen concentration close to 5 mg/l is critical for growth, below which a drop of 1 mg/l dissolved oxygen causes a 30 per cent reduction in growth rate. Similar observation was made by Nair and Sherief (1998) who reported that dissolved oxygen levels of 4.2 and 3.8 mg/l reduced the specific growth rates of *L. rohita* by 55

and 64 per cent of the control, suggesting a complimentary role for dissolved oxygen as a toxicant in phenol toxicity. The tissue relationship of *Tilapia mossambica* exposed to dichlorvos exhibited a relation with the age of fish (Rath and Misra, 1980). Further, the authors found higher oxygen consumption for the gill tissues of all the age groups compared to the brain and muscle tissue and an inverse relationship between oxygen consumption and body weight.

2.4.3. pH.

The effect of higher or lower concentration of hydrogen ions on sensitivity of fish to toxicants is reflected in changes in the intensity of general metabolism and gas exchange. A decrease in pH of water leads to a reduction in metabolism and vice-versa. Carps were found to be very sensitive to a pH below 4.8 and characterized by acidosis (Metelev *et al.*, 1971). Hydrolysis of pesticides at high pH caused greater toxicity in organisms (Bender, 1969). Bhatia (1971a & b) found that when *C. mrigala*, *P. ticto and C. faciata* were exposed to copper toxicity at three different pH levels, the three species differed in their tolerance to the copper concentrations. Johnson and Julin (1980) concluded from their static bioassay tests to fathead minnows *Pimephales promelas*, channel cat fish *Ictalurus punctatus* and blue gills *Lepomis macrochirus* that the water hardness and the pH did not bring about significant variations in the toxicity of toxaphene. Doe *et al.*, (1988) reported that the toxicity of aminocarb, a base increased significantly with increasing pH. Conversely, the toxicity of

the acidic pesticide 2, 4 – D increased with decreasing pH, but the toxicity of the neutral pesticide fenitrothion did not change significantly with changing pH. Oyen *et al.,* (1991) observed that mortality of common carp eggs was highest between the pH range of 4.75 and 5.2 and there was a delay in the rate of embryonic development. Also, they had observed a strong increase of spinal cord deformation of sac-fry occurring at a pH range of 5.5 to 4.75.

2.5. Proximate body composition

Many of the synthetic pesticides are metabolized within the system of the organism. Variations have been noted in the process of metabolism within different tissues of the organism, species, length of exposure and probably the type of habitat. Pesticides in the aquatic environment cause several effects on the physiological and biochemical aspects of fishes (Webb and Brett, 1973; Arunachalam and Palanichamy, 1982; Palanichamy *et al.,* 1986,1987; Baskaran *et al.,* 1987; Seshagiri Rao *et al.,* 1987; Vasanthi and Ramasamy, 1987 and Vasanthi *et al.,* 1990).

2.5.1. Protein.

Important works on the protein content are that of Ahmed, (1979) and Rath & Misra, (1980) on Tilapia *mossambica* using malathion and dichlorvos and Rao *et al.,* (1987) on *Sarotherodon mossambicus* using benthiocarb. Similar results are also reported in different fish by various authors, for example, Webb and Brett (1973) in *Oncorhynchus nerka;* Jayachandran and Chockalingam (1986) in *Channa punctatus;* Palanichamy *et al.,* (1987) in

Lepidocephalichthys thermalis; Malla Reddy and Bashamohideen, (1988) in *Cyprinus carpio;* Palanichamy *et al.,*(1990) and Arunachalam *et al.,* (1990) in *M. vittatus;* Vasanthi *et al.,* (1990) in *C. striatus, M. vittatus* and *Oreochromis mossambicus* and Nair & Sherief, (1998) in *L. rohita.*

2.5.2. Lipid.

Several investigators have arrived at a close correlation between the lipid content and the pesticide residues. The liver, a vital organ of lipid metabolism, is seriously affected by toxicants. Fish liver is a primary organ for detoxification (Hutterer *et al.,* 1969) and all toxicants pass through it for detoxification and disposal. Lipid contents of muscle, liver, gill and intestine of *M. vittatus* reduced with increasing concentrations of effluent. (Palanichamy *et al.,* 1990). Reduction of lipid content may have been due to the utilization of lipids for energy demand under stress condition. Working with *Channa punctatus,* Saroj Gupta, (1987) has reported that the lipid content of liver decreased with increasing concentrations of vegetable oil factory effluent. Similar results are also reported in fish by various authors, such as Rao and Rao (1979) and Rath & Misra, (1980) in *T. mossambica;* Palanichamy *et al.,* (1986) in *O. mossambicus;* Vasanthi & Ramaswamy, (1987) and Seshagiri Rao *et al.,* (1987) in *Sarotherodon mossambicus; Arunachalam et al.,*(1990) & Palanichamy *et al.,*(1990) in *M. vittatus;* Vasanthi *et al.,*(1990) in *C. striatus, M. vittatus and O. mossambica* and Nair & sherief , (1998) in *L. rohita.*

2.5.3. Carbohydrate.

Carbohydrate metabolism was adversely affected in the hepatic tissue by the organophosphate insecticide dimethoate in *C. batrachus* (Begum and Vijayaraghavan, 1995). Srivastava and Singh (1981) carried out investigations on the carbohydrate metabolism of *H. fossils* exposed to elevated sublethal concentrations of methyl parathion. The reduction of glycogen content might have been due to turnover of synthesized glycogen or a decreased rate of glycogenesis in order to meet the excess energy demand brought about by toxicant induced stress (Ghosh, 1987; Shobha *et al.,* 1989; Koundinya and Ramamurthy, 1979; Shiva Prasada Rao, 1980). Babu *et al.,* (1988) reported that the decreased levels of tissue carbohydrate and pyruvate could be due to their decreased synthesis as a consequence of toxic stress in *S. mossambicus* during Benthiocarbe exposure.

3. MATERIALS AND METHODS

The experiments on lethal and sublethal toxicity of monocrotophos on the juveniles of Rohu - *Labeo rohita* (Ham.) were conducted for 48 hours and 32 days respectively during November - December, 1999 following the method of Nair and Sherief, (1998).

3.1. Experimental laboratory

The experiment was conducted in the wet lab of the Department of Aquaculture of the College of Fisheries, Panangad, which has concrete floor with gentle slope, providing proper drainage to remove pesticide contaminated water to minimize the risk of hazards. There were provisions for water supply, lighting and adequate ventilation in the shed.

3.2. Experimental tanks

The experiments were designed to be conducted in two phases. The first experiment was acute bioassay in 5 litre capacity glass troughs for 48 hrs. Based on the results from the first experiment, the second experiment (sublethal bioassay) was done in circular flat bottom fibreglass reinforced plastic (FRP) tanks with the following specifications.

Diameter	:	55 cm
Height	:	35 cm
Capacity	:	83 lit.
Rim width	:	3 cm
Thickness	:	4 mm
Colour	:	Aquamarine

Clear filtered fresh water drawn from an open well was used for the experiment and the tanks were filled up to 21.0 cm height (i.e. 50 litres) after subjecting to a fine filtration using nylon bolting cloth.

3.3. Experimental animals

Eight-week-old juveniles of Rohu – *L. rohita* (Ham.) were obtained from the carp hatchery of the College of Fisheries, Panangad. The average size was 46.84 ± 0.52 mm and 1012.29 ± 43.12 mg. They were acclimated in well water in the laboratory conditions for 10 days prior to the start of the bioassay. During this period they were fed, *ad libitum*, once a day, on a pelleted carp feed.

3.4. Experimental diet

The diet was prepared by using 40 % clam meat as the chief protein source (Sherief, 1989), 25% wheat bran, 25% groundnut oil cake and 10 % tapioca flour as a binder. All the ingredients were passed through a 1 mm mesh sieve.

The diet was prepared by thoroughly mixing the dry ingredients and then adding water @ 1.3 litres per kg of ingredients or until a stiff dough resulted. This was then steamed in a pressure cooker at atmospheric pressure for 30 minutes. The dough was then cooled and extruded in a noodle making machine through a 3 mm diameter and the resulting 'sphagetti-like' strings were dried in a forced convention air dryer at 60°C for 6 hrs. After drying, the diet was broken up into convenient pellet sizes.

Sample of the diet was subjected to proximate analysis following AOAC (1984) procedure. The diet was stored at –20°C until fed.

The proximate composition of the formulated pelleted feed is given in Table:1.

Table: 1

Proximate composition of the formulated pelleted feed

Parameter	Per cent dry weight*
Moisture	9.00
Protein	41.93
Fat	6.95
Ash	4.29
Carbohydrate	37.83

*Dry weight basis

3.5. Pesticide used for the experiment

Monocrotophos is a water-soluble organophosphate concentrate containing 360 gm/kg monocrotophos {O, O – Dimethyl – O – (2 – methyl – carbonyl – 1 methyl vinil) phosphate} as active ingredient in a kg of product (m/m). This is equivalent to 400 gm monocrotophos in a litre of product (w/v). It is a broad-spectrum systemic and contact insecticide – cum-acaricide with long-term residual action. It is a product of Hindustan – Ciba – Geigy Limited, Bombay.

3.5.1. Preparation of test solution.

The dilution of this concentration is based on the formula,

$$\text{Vol. Of commercial formulation} = \frac{\text{Required vol. of stock solu}^{n} \times \text{Desired strength of stock solu}^{n}}{\text{Strength of commercial formulation}}$$

The test solution was freshly made up every day.

3.6. Experimental procedure

3.6.1. Lethal toxicity.

Preliminary 48 hours exploratory tests were conducted before fixing the desired concentrations for finding out the LC_{50} values of the pesticide on the above fishes. Based on the results of the exploratory tests seven concentrations of pesticide ranging from 70 mg/l (no morality) to 130 mg/l (100 % mortality) were selected for the final 48 hr LC_{50} tests. Static bioassay with toxicant replenishment every 4 hr interval was carried out in glass containers in triplicates with five litres of water accommodating 10 fishes each. A control without any contamination of pesticide was also kept. From the acclimated stock, ten healthy fishes were selected randomly and they were allowed to starve for 24 hr prior to the experiment. Mortality during 48 hr exposure period was recorded from each treatment. The 48 hr LC_{50} value of the pesticide was calculated using C - MSTAT probit analysis on computer.

3.6.2. Sublethal toxicity.

Based on the LC_{50} values so obtained seven nominal concentrations of the pesticide were selected for sublethal toxicity studies. One third of the 48 hr LC_{50} was taken as the maximum sublethal concentration (Konar, 1969) and the lower concentrations were chosen according to Sprague (1973). The experimental fishes were exposed to such sublethal concentrations for a period of 32 days. The concentrations of the pesticide used for sublethal exposure are given below:

Treatment		Concentration (mg/l)
T_1	:	0.0
T_2	:	2.0
T_3	:	5.0
T_4	:	10.0
T_5	:	15.0
T_6	:	20.0
T_7	:	30.0
T_8	:	40.0

Sublethal exposure was done in a static system where water and pesticide medium were renewed every 24 hrs to obtain the desired pesticide concentration. A control, free of pesticide was also maintained. The treatments were maintained in triplicates. 20 healthy fishes chosen at random from the acclimated stock were reared in 50 litres of water in

fibreglass tanks. The tanks were covered with plastic-mesh nets to prevent escape of the fishes by jumping. The ratio of the animal wet weight to water volume ranged from 0.389 to 0.451 gm/litre. The experiment was conducted at ambient temperature (28 ± 2° C). The dissolved oxygen, pH and temperature in the different treatments were measured immediately before and after the pesticide inoculation. While running the static bioassay, the animals were closely observed for the growth, general health, as well as to assess their number (survival).

3.6.3. Inoculation of test solution.

The test solution (i.e. stock solution) was inoculated into the tanks containing water by using 1ml and 5 ml graduated glass pipettes with a rubber device for pipetting solution in order to avoid hazard by sucking through mouth. After inoculation, the pesticide was thoroughly mixed by gentle stirring of the tank water.

3.6.4. Layout of the experiment.

Completely randomized design (CRD) was used for conducting the experiment. The test solution of monocrotophos was used at seven different dosages; thus there were eight treatments including control. Each treatment was replicated thrice, thereby involving 24 tanks. Each tank was marked as T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8 to represent the treatments and replication number as subscripts, after distributing the treatments randomly.

3.7. Sublethal bioassay tank management

3.7.1. Source of water for experiment.

Well water was used for acclimatization of fishes and for running the tests. Freshwater was filtered using nylon bolting cloth and aerated to saturation prior to use. The dissolved oxygen, pH and temperature were in the range of 7.5 – 8.2 mg/l, 7.5 – 8.0 and 28 ± 2° C respectively in the aerated well water.

3.7.2. Feeding.

The fish was fed once a day on a pelleted carp feed at 6 % of the body weight (dry food per whole wet body weight) adjusted against weekly weight measurements. The feed was given in a small petri dish kept at the bottom of the tank. Petri dishes were cleaned thoroughly before the next feeding.

3.7.3. Feed and faeces collection.

Feed remnants and faecal matter were collected by siphoning, every 24 hrs. The faeces naturally released by the fish could be easily detectable (Mukhopadhyay & Ray, 1997). Feed and faeces collected from each replicate treatment were pooled, dried at 100°C to a constant weight in an oven and stored for subsequent analysis. Pooled faecal samples for each replicate treatment were analysed separately.

3.7.4. Determination of mortality.

Fish mortality was determined once every 12 hrs by the response of the fish to a gentle touch in the opercle with a glass rod. Those fishes that failed to show any movement were declared dead and were removed.

3.8. Physicochemical parameters

Water quality parameters in the experimental tanks were measured by the following methods:

3.8.1. Dissolved oxygen.

Modified Standard Winkler's Method (Strickland and Parsons, 1972).

3.8.2. pH.

Universal pH Indicator Solution Method.

3.8.3. Temperature.

Using thermometer with an accuracy of 0.1°C.

The dissolved oxygen, pH and temperature were measured immediately before and after the pesticide inoculation.

3.9. Growth parameters

Growth measurements were made on 8^{th}, 16^{th}, 24^{th} and 32^{nd} day of experimental period.

Weight measurement.

All the fishes were weighed collectively and the mean weight was calculated to the nearest milligram on a monopan electronic balance

(Shimadzu–Labror AEU 130 V with an accuracy of 0.1 mg) after blotting off the excess moisture using filter paper. Fishes were handled carefully to reduce stress. Care was taken to see that the last feed was 24 hours prior to the growth measurements.

Determination of growth parameters.

3.9.1. Initial and final weight determination.

The initial and final wet weights were determined using the above method at the beginning and at the end of the experiment.

3.9.2. Mean wet weight gain.

It gives the increase in the weight of the fish during the experimental period. It was calculated by using the formula:

Mean wet weight gain = Final weight – Initial weight

3.9.3. Specific growth rate (SGR).

Growth performance can be measured in terms of specific growth rate since it is a more refined and improved growth index than absolute weight gain or percentage growth rate (Hepher, 1988). The specific growth rate (SGR, %/d) during the exposure period was calculated using the equation:

$$SGR = \frac{\ln W_f - \ln W_i}{\Delta t} \times 100$$

Where, ln = Natural Logarithm (base 'e')
W_i = Mean initial body weight
W_f = Mean final body weight
Δt = No. of days

3.9.4. Percentage wet weight gain (Somatic growth).

The percentage somatic growth is used to compare the somatic growth under different treatments which is calculated by using the formula:

$$\% \text{ Wet weight gain} = \frac{\text{Mean final body weight} - \text{Mean initial body weight}}{\text{Mean initial body weight}} \times 100$$

3.9.5. Food conversion ratio (FCR).

The food conversion ratio was calculated using the formula:

$$FCR = = \frac{\text{Amount of food consumed per fish (dry wt.)}}{\text{Weight increment per fish (wet wt.)}}$$

3.9.6. Food conversion efficiency (%).

Food conversion efficiency is the functional ratio of percentage to food conversion ratio (FCR). The FCE was calculated by using the formula:

$$FCE = \frac{100}{FCR}$$

3.9.7. Average daily food consumption (ADFC).

ADFC was calculated using the formula

$$ADFC = \frac{\text{Amount of food consumed}}{\text{Time (days)}}$$

3.9.8. Apparent dry matter digestibility (%).

Digestibility coefficient for dry matter was determined by direct quantitative feces collection method. Apparent dry matter digestibility (ADMD) was computed by following the procedure of Maynard & Loosli, (1969) and Nair & Sherief (1998).

$$ADMD = \frac{\text{Amount of food consumed - Feacal output}}{\text{Amount of food consumed}} \times 100$$

3.9.9. Survival rate (%).

Mortality observed during the exposure period was used to calculate the percentage survival at the end of the experiment by using the formula:

$$SR = \frac{\text{Initial No. – No. of dead animal}}{\text{Initial No.}} \times 100$$

3.9.10. Determination of maximum acceptable toxicant concentration level.

Determination of maximum acceptable toxicant concentration (MATC) level of the toxicant was determined using the formula:

$$MATC = (NOEC \times LOEC)^{1/2}$$

Where, NOEC = No Observable Effect Concentration
LOEC = Least Observable Effect Concentration

3.9.11. Determination of the application factor (AF).

The application factor was calculated by using the formula (Mount and Stephan,1967):

$$AF = \frac{MATC}{48 \text{ - hrs } LC_{50}}$$

3.10. Proximate analysis

Proximate analyses of the experimental diet and the initial fish body proximate composition were performed prior to the start of experiment. After the 32–d exposure the body proximate composition was analysed. Moisture

(loss of drying at 105°C for 12-h). Crude Protein (Kjeldahl nitrogen x 6.25) and ash contents (residue left after heating the sample at 550°C in a muffle furnace for 6-h) were determined by AOAC (1984) methods. Lipids were extracted using Soxhlet extraction method (AOAC, 1984).

Carbohydrate concentration was indirectly determined by using Knauer's procedure (Knauer *et al.,* 1994) as given below:

Percentage carbohydrate = 100 – (% Protein + % Lipid + %Ash)

3.11. Statistical analysis of the results

The treatment means of all indices, such as growth, specific growth rate, percentage growth increment, survival rate, food conservation ratio, average daily food consumption, apparent dry matter digestibility and food conversion efficiency were subjected to statistical analysis using one way analysis of variance on IBM-PC micro-computer based on CRD programmes.

The students' 't' test at 5% level of significance was used for finding the critical difference between the pair wise comparison of treatment values.

4. RESULTS

The results of the experiments are categorised conveniently under the following heads.

4.1. Lethal toxicity

4.2. Physicochemical parameters

- 4.2.1. Water temperature
- 4.2.2. pH
- 4.2.3. Dissolved oxygen

4.3. Sublethal toxicity

- 4.3.1. Growth increment
- 4.3.2. Percentage growth increment
- 4.3.3. Specific growth rate
- 4.3.4. Food conversion ratio
- 4.3.5. Food conversion efficiency
- 4.3.6. Average daily food consumption
- 4.3.7. Apparent dry matter digestibility
- 4.3.8. Survival rate
- 4.3.9. MATC and application factor

4.4. Carcass proximate composition

- 4.4.1. Initial carcass proximate composition of test animals
- 4.4.2. Final carcass proximate composition of test animals

4.1. Lethal toxicity

The mortality of Rohu juveniles during 48 hr exposure to various concentrations of monocrotophos is given in Table: 2.

The result of the probit analysis for the Rohu juveniles during 48 hr exposure to various concentrations of monocrotophos is given in Table:3. This is represented graphically on Fig.1.

The calculated 48 h LC_{50} value of monocrotophos of Rohu juveniles is 104.02 mg/l in static bioassay without aeration. The 95% fiducial limits ranged from 83.25 to 124.79 mg/l. The values of slope function (b) and intercept (a) are 11.12 and –17.43 respectively.

The calculated 48 h LC_{50} value of monocrotophos of Rohu juveniles is 104.02 mg/l in static bioassay without aeration. The 95% fiducial limits ranged from 83.25 to 124.79 mg/l. The values of slope function (b) and intercept (a) are 11.12 and –17.43 respectively.

4.2. Physicochemical parameters

4.2.1. Water temperature.

The range of temperature in the experimental tanks during the study period is given in Table: 4.

Minimum temperature recorded was 26.0°C and maximum temperature was 30.0°C. Weekly mean temperature values ranged from 27.59 to 28.62° C.

Table: 2

Mortality parameter for the Rohu (*L. rohita)* juveniles during 48-h exposure to various concentrations of monocrotophos

Particular	Toxicant concentration (mg/l)				
	Control	70	90	110	130
Per cent mean mortality ± SD	0.0 ± 0.0	3.33 ± 4.71	23.33 ± 4.71	60.0 ± 8.16	86.67 ± 4.71

Table: 3

Result of probit analysis for the Rohu (*L. rohita)* juveniles during 48 -h exposure to various concentrations of monocrotophos

Exposure period (hrs)	LC_{50} (mg/l)	95% Fiducial Limits*(mg/l)		Slope function (b)	Intercept (a)
		Lower	Upper		
48	104.02	83.25	124.79	11.12	-17.43
Regression equation: Probit y= -17.43 + 11.12 x Log_X					

*Significant at 5 % level.

Table: 4

Water temperature in the experimental tanks during the study period

Temperature (°C)	Weeks			
	1	2	3	4
Mean ± SD	28.62 ± 0.04	27.59 ± 0.05	28.17 ± 0.05	27.71 ± 0.06
Range	28.30 – 29.0	26.00 –29.00	26.4 – 30.00	26.0 – 29.30

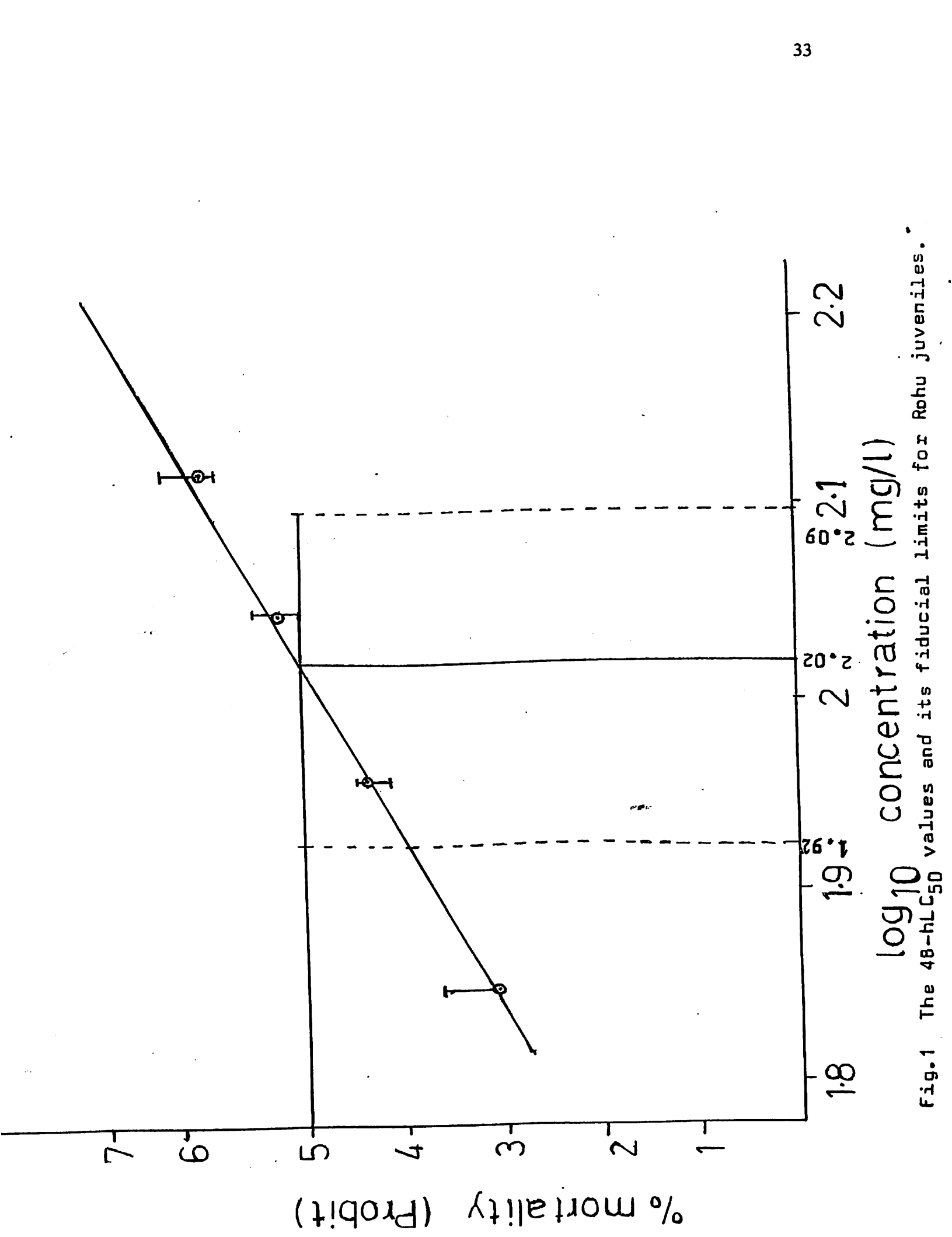

Fig.1 The 48-hLC$_{50}$ values and its fiducial limits for Rohu juveniles.

4.2.2. pH.

Range of pH in the experimental tanks is given in the Table: 5.

Minimum and maximum pH values observed during the study period were 6.5 and 8.0 respectively. Weekly mean pH values ranged from 7.36 to 7.39.

4.2.2. Dissolved oxygen.

Range of dissolved oxygen in the experimental tanks is given in Table: 6.

A minimum of 4.8 mg/l and a maximum dissolved oxygen content of 8.2 mg/l were obtained during the study period. Weekly mean values ranged from 6.87 to 6.91 mg/l .

4.3. Sublethal toxicity

The summarized results of growth parameters of Rohu juveniles due to 32 – days exposure to various sublethal concentrations of monocrotophos are presented in Table: 7.

4.3.1. Growth increment.

The data on mean wet weight gain of Rohu juveniles for the different sublethal exposures of monocrotophos are presented in Table: 8.

Compared to the control, juveniles exposed to the different concentrations of monocrotophos showed a lower wet weight gain. The

Table: 5

pH of Water in the experimental tanks during the study period

pH	Weeks			
	1	2	3	4
Mean ± SD	7.36 ± 0.12	7.39 ± 0.12	7.39 ± 0.12	7.39 ± 0.12
Range	6.5 – 8.0	6.5 – 8.0	6.5 – 8.0	6.5 – 8.0

Table: 6

Dissolved oxygen in the experimental tanks during the study period

Dissolved oxygen (ppm)	Weeks			
	1	2	3	4
Mean ± SD	6.91 ± 0.25	6.9 ± 0.31	6.87 ± 0.30	6.89 ± 0.32
Range	5.0 – 8.2	4.8 – 8.2	4.8 – 8.2	4.8 – 8.2

Table: 7

Growth parameters (Mean ± SD) for Rohu (*L.rohita)* juveniles during 32 days exposure to various sublethal concentrations of monocrotophos

Parameters	Nominal monocrotophos concentrations (mg/l)						
	0.0 (T_1)	2.0 (T_2)	5.0 (T_3)	10.0 (T_4)	15.0 (T_5)	20.0 (T_6)	30.0 (T_7)
Initial weight (g)	1.02±0.02 ^{a}	1.06±0.04 ^{a}	1.03±0.05 ^{a}	1.03±0.03 ^{a}	1.01±0.04 ^{a}	1.06±0.04 ^{a}	1.04±0.06 ^{a}
Final weight (g)	1.70±0.06 ^{a}	1.68±0.09 ^{ab*}	1.55±0.05 ^{b**}	1.38±0.01 ^{c}	1.27±0.02 ^{d}	1.17±0.03 ^{de}	0.88±0.05 ^{e}
Mean wet wt. gain (g)	0.68±0.04 ^{a} (100.0)	0.62±0.07 ^{ab*} (91.18)	0.52±0.02 ^{b**} (76.47)	0.35±0.02 ^{c} (51.47)	0.25±0.02 ^{d} (36.76)	0.11±0.01 ^{e} (16.18)	−0.16±0.12 ^{f}
Specific growth rate (%/d)	1.58±0.05 ^{a} (100.0)	1.44±0.11 ^{ab*} (91.14)	1.28±0.08 ^{b**} (81.01)	0.93±0.07 ^{c} (58.86)	0.70±0.07 ^{d} (40.30)	0.31±0.03 ^{e} (19.62)	-
Percentage growth increment (%)	66.13±2.58 ^{a*} (100.0)	58.48±5.82 ^{b**} (88.42)	50.88±3.72 ^{c} (76.94)	34.38±3.08 ^{d} (51.99)	25.12±2.75 ^{e} (37.99)	10.29±1.09 ^{f} (15.56)	-
Food conversion ratio (FCR)	2.24±0.12 ^{a} (100.0)	2.36±0.20 ^{a} (105.36)	2.68±0.12 ^{a*} (119.64)	3.44±0.20 ^{b**} (153.57)	4.67±0.37 ^{c} (208.48)	10.16±1.06 ^{d} (453.57)	-
Food conversion efficiency(%)	44.77±2.32 ^{a} (100.0)	42.60±3.44 ^{ab*} (95.15)	37.39±1.74 ^{b**} (83.52)	29.12±0.81 ^{c} (65.04)	21.53±1.63 ^{d} (48.09)	9.95 ±0.97 ^{e} (22.22)	-
Average daily food consumption (mg/d)	1005.82±3.88 ^{a*} (100.0)	961.11±28.64 ^{b**} (95.56)	931.56±10.72 ^{bc} (92.62)	806.89±27.13 ^{c} (80.22)	791.89±3.72 ^{cd} (78.73)	712.89±5.45 ^{d} (70.88)	529.33±24.25 ^{e} (52.63)
Apparent dry matter digestibility(%)	68.16±2.73 ^{a} (100.0)	64.51±2.14 ^{a} (94.64)	62.40±3.58 ^{a} (91.55)	57.22±3.82 ^{a*} (83.95)	50.18±6.16 ^{b**} (73.62)	48.96±2.87 ^{b} (71.83)	48.33±11.71 ^{b} (70.91)
Survival rate	98.33±2.36 ^{a}	93.33±6.24 ^{a}	96.67±2.36 ^{a}	96.67±2.36 ^{a}	96.67±2.36 ^{a}	93.33±2.36 ^{a*}	70.0±10.80 ^{b**}

*NOEC

** LOEC

Numbers in parenthesis are percentage of control values

Values with different superscripts in the same row differ significantly (P< 0.05).

mean wet weight gain of the fish exposed to different concentrations of monocrotophos such as 2.0, 5.0, 10.0, 15.0 and 20.0 mg/l were found to be 0.62 , 0.52, 0.35, 0.25 and 0.11 g respectively. The highest mean weight gain was obtained in control i.e. 0.68 g. There was weight loss in 30 mg/l monocrotophos concentration.

The result of analysis of variance (ANOVA) of the data on the growth increment of Rohu juveniles is given in Table:9

Comparison of treatment means based on critical difference

Critical difference = 0.075

Treatment	: T_1	T_2	T_3	T_4	T_5	T_6
Mean	: 0.68	0.62	0.52	0.35	0.25	0.11

Underscored means are not significantly different.

Analysis of variance (Table: 9) and subsequent pair wise comparisons showed that the mean wet weight gain was significantly different in fish exposed to 5, 10, 15,20 and 30 mg/l monocrotophos when compared to control, but not significant in the control and the lower concentration of 2 mg/l.

Weekly progression of growth in wet wt. is represented in Fig.2 (scattered plot of the values in and around the 8^{th},16^{th} ,24^{th}, and 32^{nd} days is to avoid clustering and to clearly depict the standard deviation).

Table: 8

Growth increment in Rohu juveniles due to a 32 – days exposure to various Sublethal concentrations of monocrotophos

Treatment (mg/l)	Replication	Avg. initial wt. (g)	Avg. final wt. (g)	Net wet wt.gain (g)	Mean wet wt. gain ± SD
T_1 (0.0)	1	0.995	1.618	0.623	0.68 ± 0.04
	2	1.05	1.754	0.704	
	3	1.02	1.721	0.701	
T_2 (2.0)	1	1.114	1.760	0.646	0.62 ± 0.07
	2	1.029	1.560	0.531	
	3	1.042	1.728	0.686	
T_3 (5.0)	1	0.988	1.490	0.502	0.52 ± 0.02
	2	0.997	1.550	0.553	
	3	1.102	1.613	0.511	
T_4 (10.0)	1	1.003	1.380	0.377	0.35 ± 0.02
	2	1.075	1.40	0.325	
	3	1.014	1.372	0.358	
T_5 (15.0)	1	0.972	1.243	0.271	0.25 ± 0.02
	2	1.007	1.270	0.263	
	3	1.062	1.289	0.227	
T_6 (20.0)	1	1.010	1.128	0.118	0.11 ± 0.01
	2	1.092	1.203	0.111	
	3	1.085	1.183	0.098	
T_7 (30.0)	1	1.004	0.92	–0.084	–0.16 ± 0.12
	2	1.128	0.80	–0.328	
	3	0.984	0.91	–0.074	

Table: 9

Result of analysis of variance (ANOVA) of the data on the growth increment in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	137.3806	5	27.4761	82.3486*
Error	4.0039	12	0.3367	
Total	141.3845	17		

* Significance at 5% level

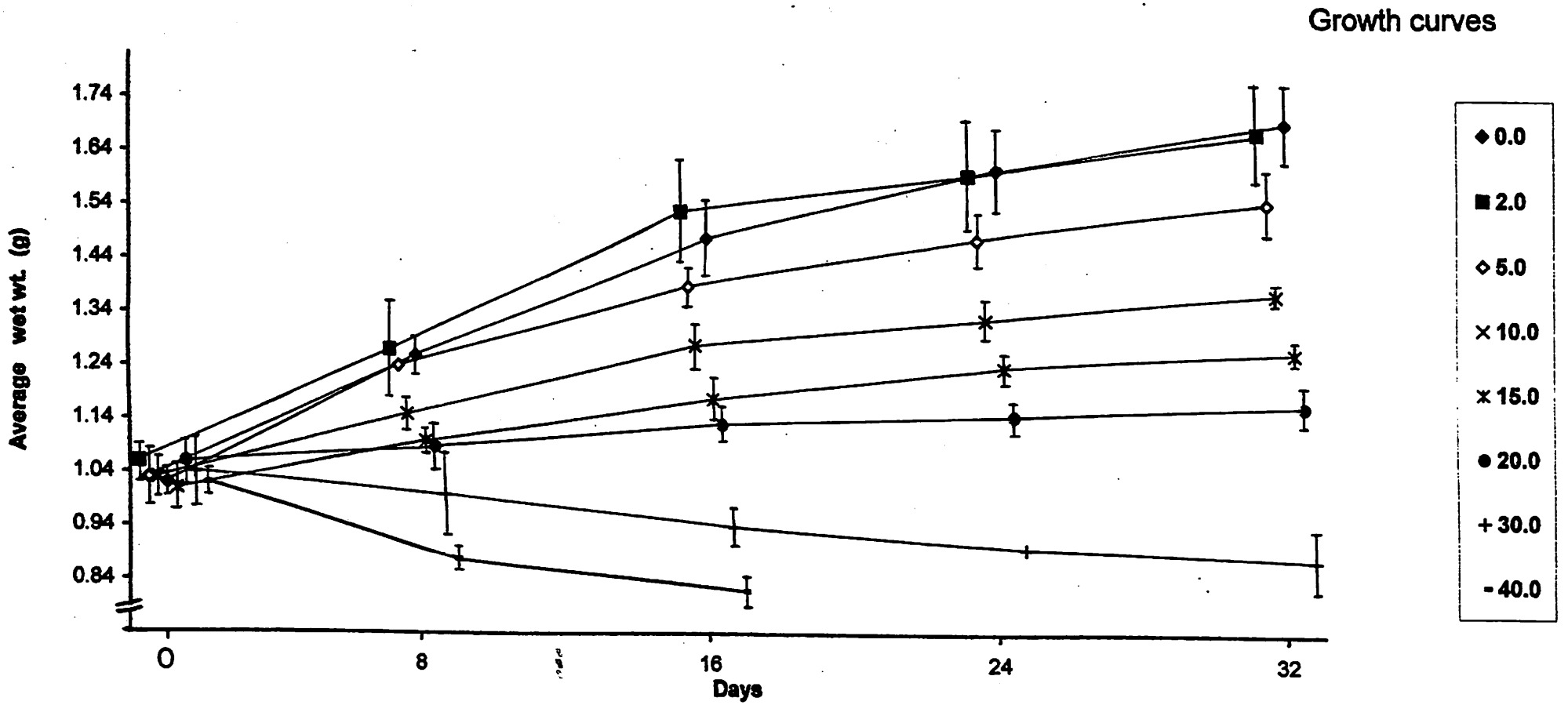

Fig-2 Weekly progression of Growth in wet weight of the rohu juveniles exposed to different concentrations of monocrotophos for the 32 day exposure period.

4.3.2. Percentage growth increment.

The data on the percentage of growth increment of Rohu juveniles for the different treatments containing different concentrations of monocrotophos are presented in Table: 10.

The highest percentage of growth increment was obtained in control, i.e. 66.13%. Fish exposed to the different concentrations of monocrotophos showed lower percentages of growth increment. The percentage of growth increment of the fish exposed to different concentrations of monocrotophos such as 2, 5,10,15 and 20 mg/l were found to be 58.47, 50.88, 34.38, 25.12, and 10.29 g respectively.

The result of analysis of variance of the data on the percentage of growth increment of Rohu juveniles is given in Table: 11.

Comparison of treatment means based on critical difference

Critical difference = 7.5763

Treatment	:	T_1	T_2	T_3	T_4	T_5	T_6
Mean	:	66.13	58.47	50.88	34.38	25.12	10.29

Analysis of variance (Table: 11) and subsequent pair wise comparisons showed that all the five treatments were found to be significantly different than the control and individual treatments were also found to be significantly different.

Table:10

The percentage of growth increment in Rohu (*L. rohita*) juveniles due to a 32 – days exposure to various Sublethal concentrations of monocrotophos

Treatment	Replication	Avg. initial wt.(g)	Avg. final wt.(g)	Percentage growth increment	Mean ± SD
T_1 (0.0)	1	0.995	1.618	62.61	66.13 ± 2.58
	2	1.05	1.754	67.05	
	3	1.02	1.721	68.73	
T_2 (2.0)	1	1.114	1.760	57.99	58.48 ± 5.82
	2	1.029	1.560	51.60	
	3	1.042	1.728	65.83	
T_3 (5.0)	1	0.988	1.490	50.81	50.88 ± 3.72
	2	0.997	1.550	55.47	
	3	1.102	1.613	46.37	
T_4 (10.0)	1	1.003	1.380	37.59	34.38 ± 3.08
	2	1.075	1.40	30.23	
	3	1.014	1.372	35.31	
T_5 (15.0)	1	0.972	1.243	27.88	25.12 ± 2.75
	2	1.007	1.270	26.12	
	3	1.062	1.289	21.37	
T_6 (20.0)	1	1.010	1.128	11.68	10.29 ± 1.09
	2	1.092	1.203	10.17	
	3	1.085	1.183	9.03	

Table: 11

Result of analysis of variance (ANOVA) of the data on the percentage growth increment in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	6819.803	5	1363.9610	75.2165*
Error	217.606	12	18.1338	
Total	7037.409	17		

* Significance at 5% level

Average weekly percentage of growth increment of the Rohu juveniles exposed to different concentrations of monocrotophos for 32 days is represented in Fig.3.

4.3.3. Specific growth rate (SGR).

The data on specific growth rate (SGR) of *L. rohita* juveniles for the different concentrations of monocrotophos are presented in Table: 12.

Compared to the control, fishes exposed to the different concentrations of monocrotophos showed a lower specific growth rate. The specific growth rate of the fish exposed to different concentrations of monocrotophos such as 2, 5,10,15 and 20 mg/l was found to be 1.44, 1.28, .0.93, 0.70 and 0.31% respectively. The highest percentage of specific growth rate was obtained to be 1.58 in control.

The result of analysis of variance of the data on the percentage specific growth rate of *L. rohita* juveniles is given in Table: 13

Comparison of treatment means based on critical difference

Critical difference = 0.1593

Treatment	: T_1	T_2	T_3	T_4	T_5	T_6
Mean	: 1.58	1.44	1.28	0.93	0.70	0.31

Underscored means are not significantly different.

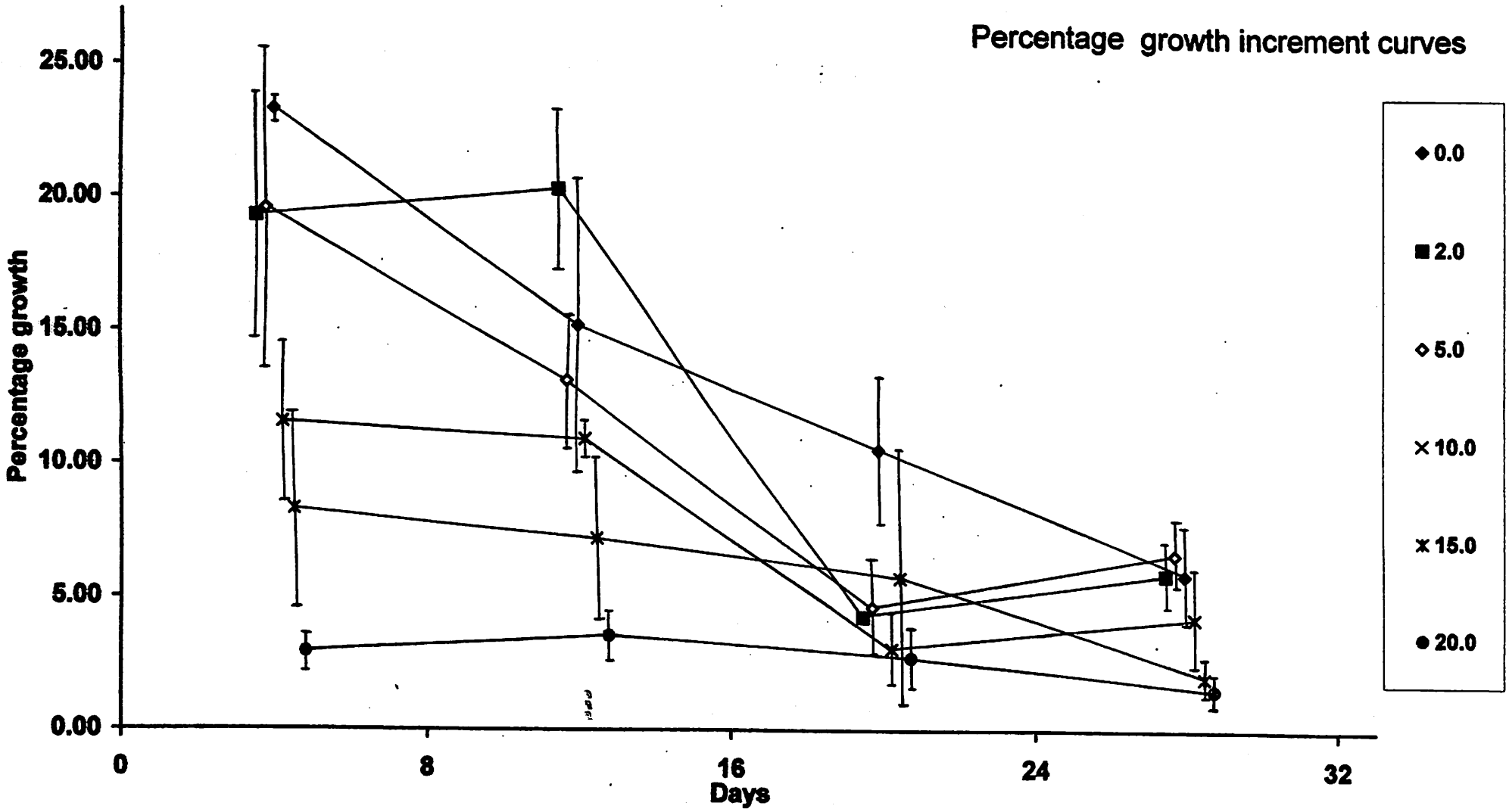

Fig-3 Average weekly Percentage growth increment of the rohu juveniles exposed to different concentrations of monocrotophos for the 32 day exposure period.

Table:12

Specific growth rate in Rohu (*L. rohita*) juveniles due to a 32 – days exposure to various Sublethal concentrations of monocrotophos

Treatment	Replication	Avg. initial wt. (g)	Avg. final wt. (g)	SRG (%/d)	Mean ± SD
T_1 (0.0)	1	0.995	1.618	1.52	1.58 ± 0.05
	2	1.05	1.754	1.60	
	3	1.02	1.721	1.63	
T_2 (2.0)	1	1.114	1.760	1.43	1.44 ± 0.11
	2	1.029	1.560	1.30	
	3	1.042	1.728	1.58	
T_3 (5.0)	1	0.988	1.490	1.28	1.28 ± 0.08
	2	0.997	1.550	1.38	
	3	1.102	1.613	1.19	
T_4 (10.0)	1	1.003	1.380	1.0	0.93 ± 0.07
	2	1.075	1.40	0.83	
	3	1.014	1.372	0.95	
T_5 (15.0)	1	0.972	1.243	0.77	0.70 ± 0.07
	2	1.007	1.270	0.73	
	3	1.062	1.289	0.61	
T_6 (20.0)	1	1.010	1.128	0.38	0.31 ± 0.03
	2	1.092	1.203	0.30	
	3	1.085	1.183	0.27	

Table:13

Result of analysis of variance (ANOVA) of the data on the specific growth rate in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	3.5272	5	0.7054	87.9956*
Error	0.0962	12	0.0080	
Total	3.6234	17		

* Significance at 5% level

Analysis of variance (Table 13) and subsequent pair wise comparisons showed that the specific growth rate was significantly different in fish exposed to 5, 10,15, and 20 mg/l monocrotophos, but not significant in lower concentration of 2 mg/l.

Average weekly specific growth rate values of the Rohu juveniles exposed to different concentrations of monocrotophos for 32 days is represented in Fig. 4.

4.3.4. Food conversion ratio (FCR).

The data on the food conversion ratio in *L. rohita* juveniles due to 32 – days exposure to various sublethal concentrations of monocrotophos is presented in Table:14.

Compared to the control, those exposed to the sublethal concentrations of monocrotophos showed a higher food conversion ratio. The food conversion ratio of the fish exposed to different concentrations of monocrotophos such as 2, 5, 10, 15, and 20 mg/l was found to be 2.36, 2.68, 3.44, 4.67 and 10.16 respectively. The lowest FCR was obtained to be 2.24 in control.

The result of analysis of variance of the data on the food conversion ratio of *L. rohita* juveniles is given in Table: 15

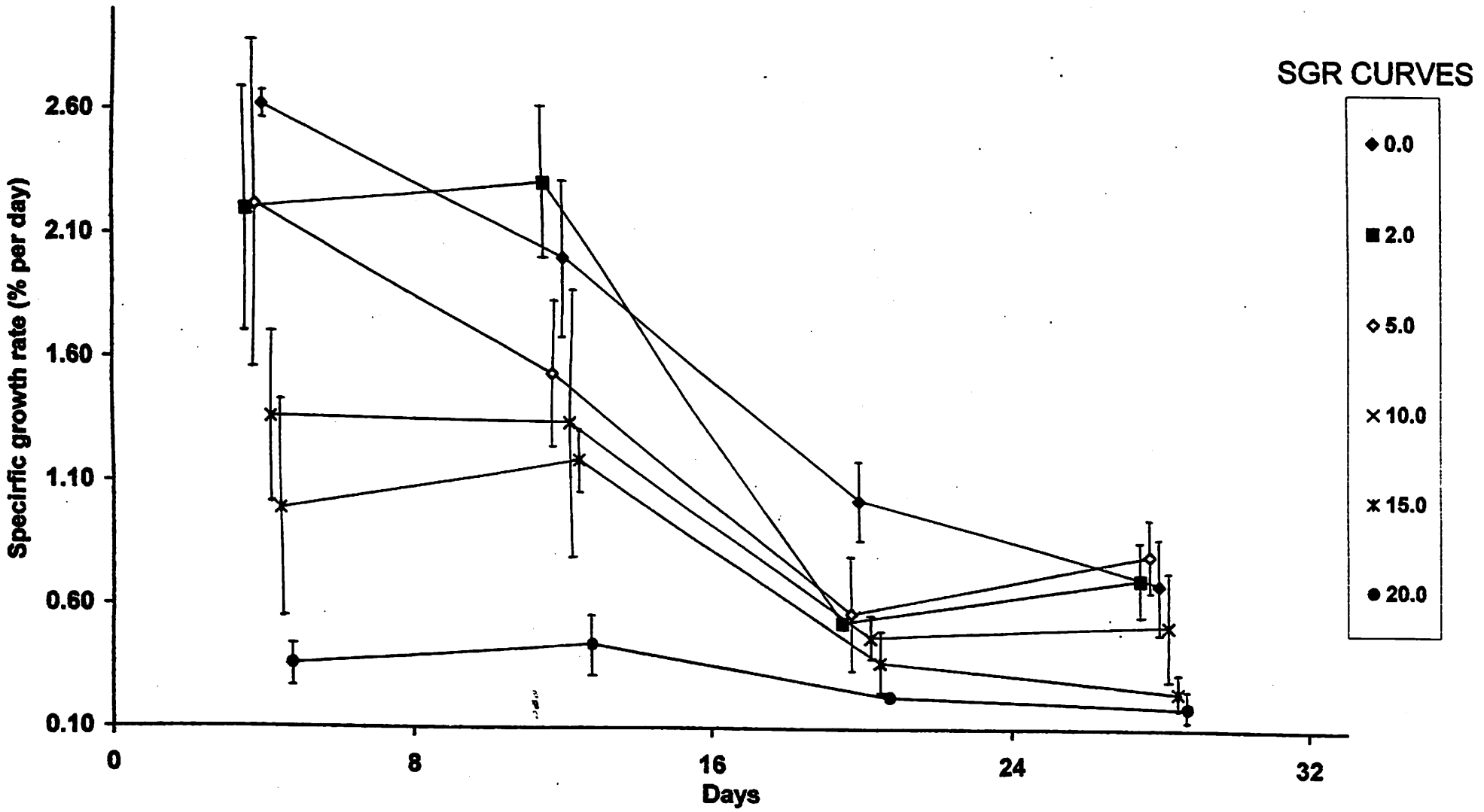

Fig-4 Average weekly SGR values of the rohu juveniles exposed to different concentrations of monocrotophos for the 32 day exposure period.

Comparison of treatment means based on critical difference

Critical difference = 1.0277

Treatment	: T_1	T_2	T_3	T_4	T_5	T_6
Mean	: 2.24	2.36	2.68	3.44	4.67	10.16

Underscored means are not significantly different.

Analysis of variance (Table: 15) and subsequent pair wise comparisons showed that the food conversion ratio was significantly higher in fish exposed to 10, 15, and 20 mg/l monocrotophos, but not significant in the control and the lower concentration of 2 and 5 mg/l.

Average weekly food conversion ratio of the Rohu juveniles exposed to different concentrations of monocrotophos for 32 days is represented in Fig.5.

4.3.5. Food conversion efficiency (FCE).

The data on the food conversion efficiency in *L. rohita* juveniles due to 32–days exposure to various sublethal concentrations of monocrotophos are presented in Table:16.

Mean values for food conversion efficiency for the fish exposed to various sublethal concentrations of monocrotophos was lower than the control. The food conversion efficiency of the fish exposed to different concentrations of monocrotophos such as 2, 5, 10, 15, and 20 mg/l was found to be 42.6,

Table:14

Food Conversion Rate (FCR) in Rohu juveniles due to a 32 – days exposure to various Sublethal concentrations of monocrotophos

Treatment	Replication	Avg. wet wt. gain per fish (g)	Amount of food consumed (dry wt.) per fish (g)	FCR	Mean ± SD
T_1 (0.0)	1	0.623	1.501	2.41	2.24 ± 0.12
	2	0.704	1.521	2.16	
	3	0.701	1.507	2.15	
T_2 (2.0)	1	0.646	1.490	2.30	2.36 ± 0.20
	2	0.531	1.400	2.63	
	3	0.686	1.479	2.16	
T_3 (5.0)	1	0.502	1.380	2.75	2.68 ± 0.12
	2	0.553	1.390	2.51	
	3	0.511	1.420	2.78	
T_4 (10.0)	1	0.377	1.260	3.34	3.44 ± 0.20
	2	0.325	1.160	3.57	
	3	0.358	1.126	3.40	
T_5 (15.0)	1	0.271	1.190	4.39	4.67 ± 0.37
	2	0.263	1.165	4.43	
	3	0.227	1.181	5.20	
T_6 (20.0)	1	0.118	1.130	9.58	10.16 ± 1.06
	2	0.111	1.027	9.25	
	3	0.098	1.141	11.64	

Table:15

Result of analysis of variance (ANOVA) of the data on Food Conversion Ratio in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	137.3806	5	27.4761	82.3486*
Error	4.0039	12	0.33670	
Total	141.3845	17		

* Significance at 5% level

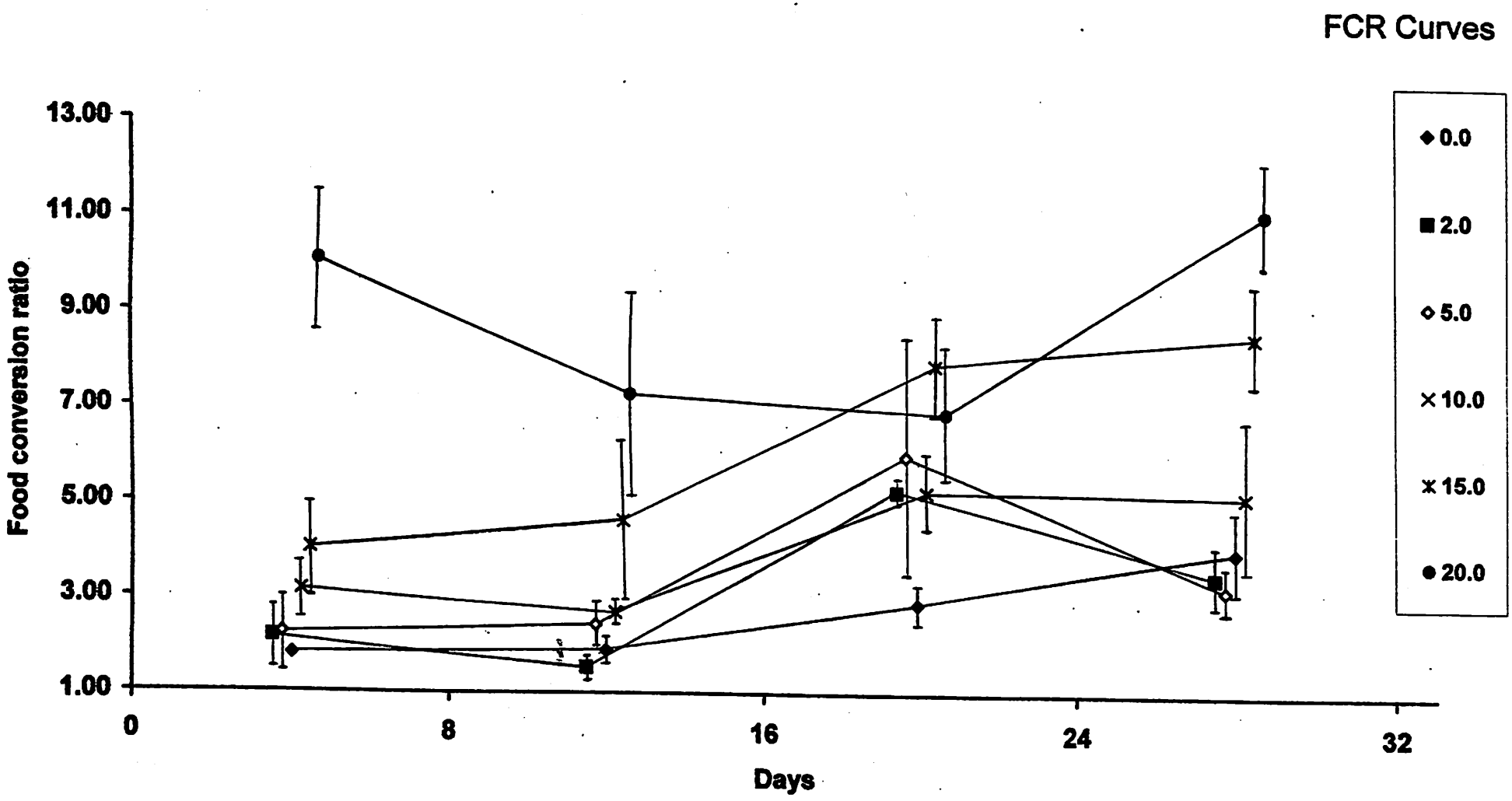

Fig-5 Average weekly FCR values of the rohu juveniles exposed to different concentrations of monocrotophos for the 32 day exposure period.

37.39, 29.12, 21.53 and 9.95 % respectively. The highest food conversion efficiency was obtained to be 44.77% in control.

The result of analysis of variance of the data on the food conversion efficiency of *L. rohita* juveniles is given in Table: 17.

Comparison of treatment means based on critical difference

Critical difference = 4.3946

Treatment	: T_1	T_2	T_3	T_4	T_5	T_6
Mean	: 44.77	42.60	37.39	29.12	21.53	9.95

Underscored means are not significantly different.

Analysis of variance (Table: 17) and subsequent pair wise comparisons showed that the food conversion efficiency was significantly different in fish exposed to 5, 10, 15, and 20 mg/l monocrotophos. But not significant in the control and the lower concentration of 2 mg/l .

Average weekly food conversion efficiency of the Rohu juveniles exposed to different concentrations of monocrotophos for 32 days is represented in Fig.6.

4.3.6. Average daily food consumption(ADFC).

The data on the average daily food consumption in *L. rohita* juveniles due to 32 – days exposure to various sublethal concentrations monocrotophos are presented in Table: 18.

Table:16

Food Conversion Efficiency (FCE) in Rohu juveniles due to a 32 – days exposure to various Sublethal concentrations of monocrotophos

Treatment	Replication	FCR	FCE (%)	Mean ± SD
T_1 (0.0)	1	2.41	41.49	44.77 ± 2.32
	2	2.16	46.30	
	3	2.15	46.51	
T_2 (2.0)	1	2.30	43.48	42.60 ± 3.44
	2	2.63	38.02	
	3	2.16	46.30	
T_3 (5.0)	1	2.75	36.36	37.39 ± 1.74
	2	2.51	39.84	
	3	2.78	35.97	
T_4 (10.0)	1	3.34	29.94	29.12 ± 0.81
	2	3.57	28.01	
	3	3.40	29.41	
T_5 (15.0)	1	4.39	22.78	21.53 ± 1.63
	2	4.43	22.57	
	3	5.20	19.27	
T_6 (20.0)	1	9.58	10.44	9.95 ± 0.97
	2	9.25	10.81	
	3	11.64	08.59	

Table:17

Result of analysis of variance (ANOVA) of the data on Food Conversion Efficiency in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	2703.338	5	540.6676	88.6184*
Error	73.213	12	6.1011	
Total	2776.551	17		

*Significance at 5% level

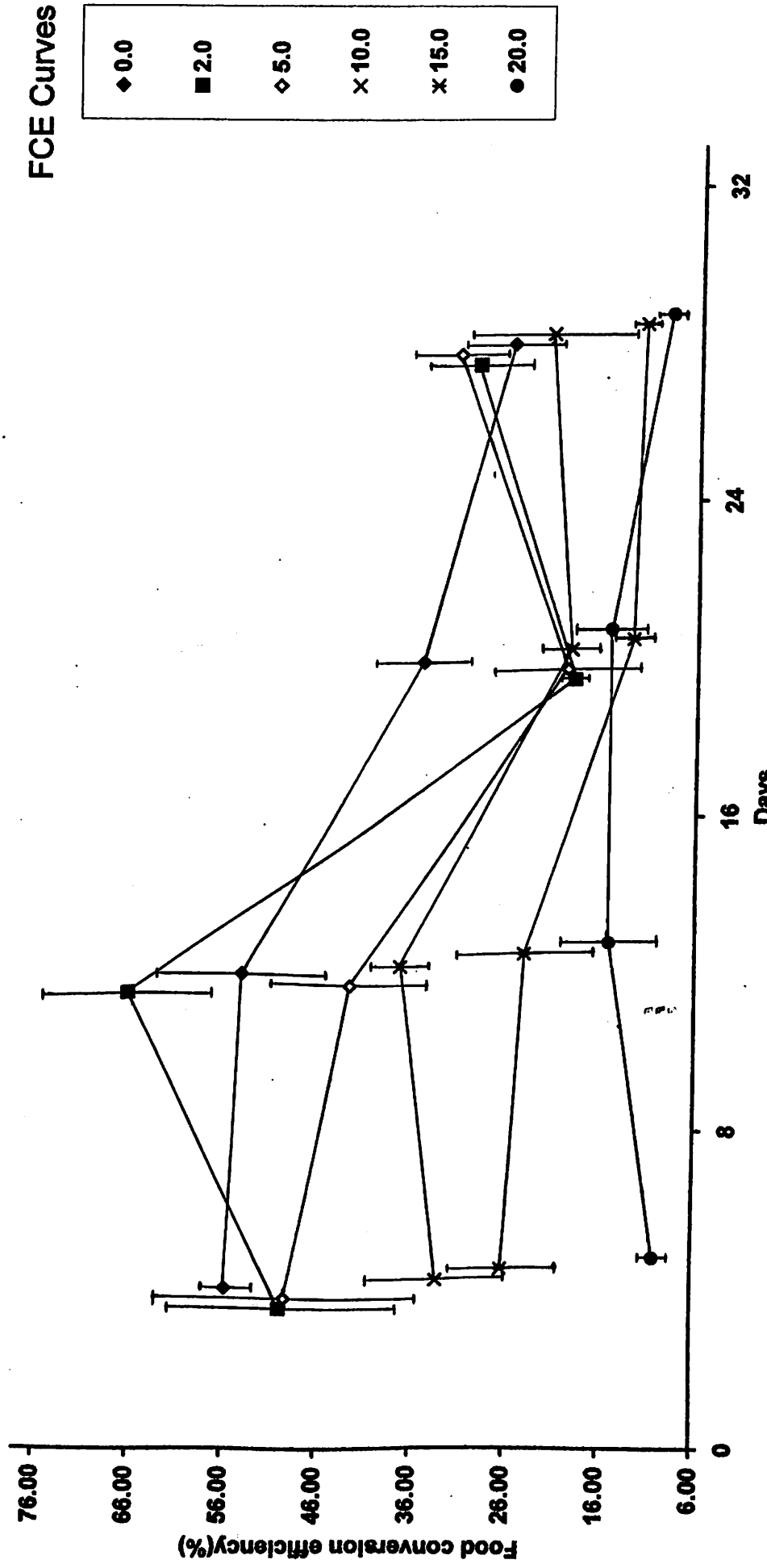

Fig-6 Average weekly FCE values of the rohu juveniles exposed to different concentrations of monocrotophos for the 32 day exposure period.

Mean values for average daily food consumption for the fish exposed to various sublethal concentrations of monocrotophos were lower than the control.

The result of analysis of variance of the data on the average daily food consumption of *L. rohita* juveniles is given in Table: 19.

Comparison of treatment means based on critical difference

Critical difference = 39.0333

Treatment :	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Mean :	1005.82	961.11	931.56	806.89	791.89	712.89	529.33

Underscored means are not significantly different.

Analysis of variance (Table: 19) and subsequent pair wise comparisons showed that the average daily food consumption was significantly lower in all the sublethal concentrations of monocrotophos than in the control.

Average weekly ADFC of the Rohu juveniles exposed to different concentrations of monocrotophos for 32 days is represented in Fig.7.

4.3.7. Apparent dry matter digestibility (ADMD).

The data on apparent dry matter digestibility (ADMD) of Rohu juveniles for the different treatments containing various sublethal concentrations of monocrotophos are presented in Table: 20.

Table: 18

Average daily food consumption in *L. rohita* juveniles due to a 32 – days exposure to various sublethal concentration of monocrotophos

Treatment	Replication	Amount of food consumed per day (mg)	Mean ± SD
T_1 (0.0)	1 2 3	1001.25 1010.73 1005.47	1005.8 ± 3.88
T_2 (2.0)	1 2 3	983.33 920.67 979.33	961 ± 28.64
T_3 (5.0)	1 2 3	923.0 925.0 946.67	931.56 ±10.72
T_4 (10.0)	1 2 3	837.67 771.67 811.33	806.89 ± 27.13
T_5 (15.0)	1 2 3	796.0 792.67 787.0	791.88 ± 3.72
T_6 (20.0)	1 2 3	706.33 719.67 712.67	712.89 ± 5.45
T_7 (30.0)	1 2 3	556.0 534.67 497.33	529.33 ± 24.25

Table:19

Result of analysis of variance (ANOVA) of the data on the average daily food consumption in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	1349.3710	6	224.8952	77.1168*
Error	40.828	14	2.9163	
Total	1390.199	20		

* Significance at 5% level

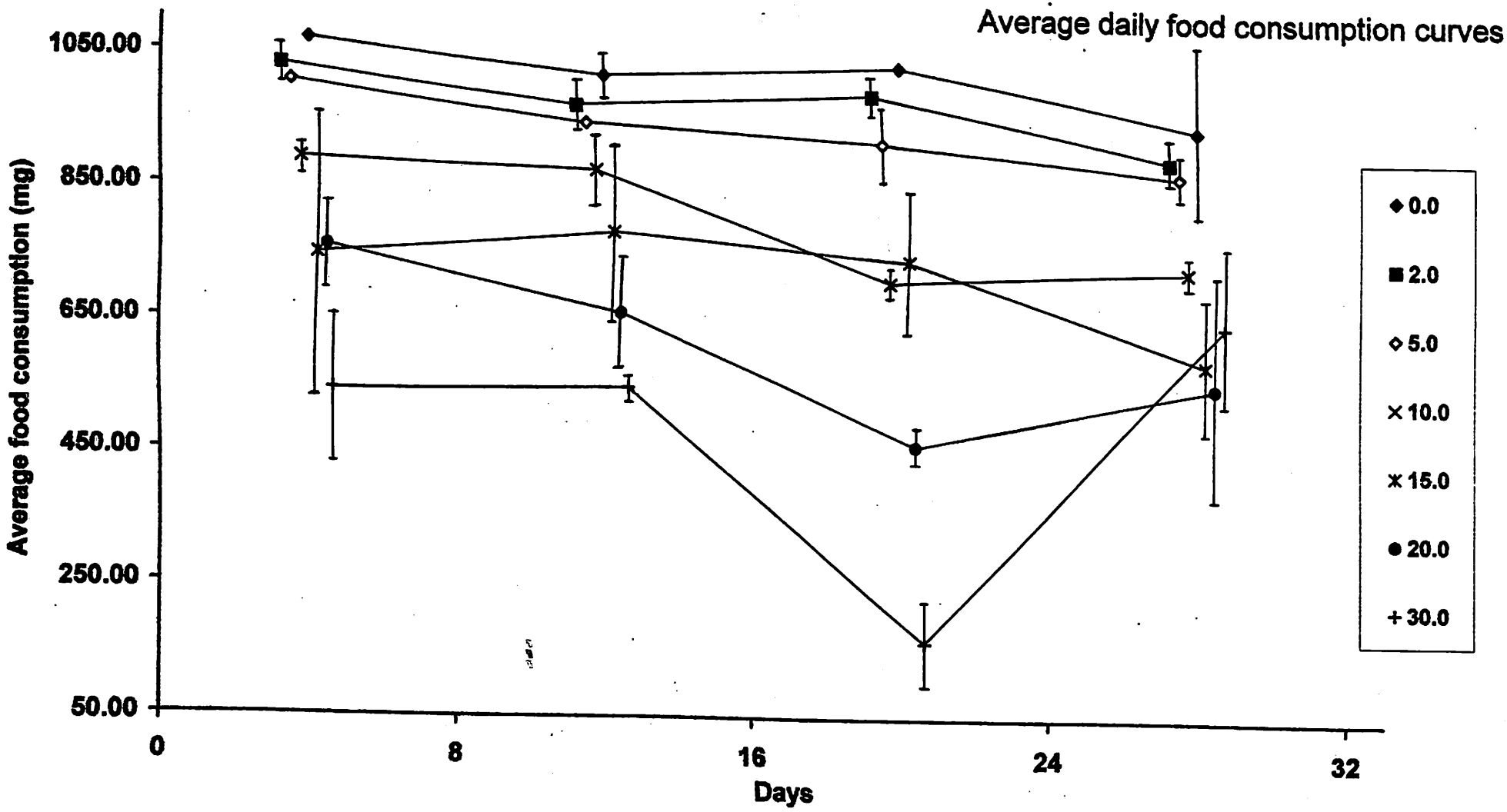

Fig-7 Average weekly ADFC values of the rohu juveniles exposed to different concentrations of monocrotophos for the 32 day exposure period.

Compared to the control fish, the apparent dry matter digestibility was reduced in the fish exposed to various sublethal concentrations of monocrotophos.

The result of analysis of variance (ANOVA) of the data on the apparent dry matter digestibility of Rohu juveniles is given in Table: 21.

Comparison of treatment means based on critical difference

Critical difference = 12.0992

Treatment :	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Mean :	68.12	64.51	62.40	57.22	50.18	48.96	48.33

Underscored means are not significantly different.

Analysis of variance (Table: 21) and subsequent pair wise comparisons showed that the apparent dry matter digestibility was significantly different in fish exposed to 15, 20 and 30 mg/l monocrotophos when compared to control. But not significant in the control and the lower concentrations of 2, 5 and 10 mg/l.

4.3.8. Survival rate (SR).

The data on the percentage survival of *L. rohita* juveniles due to 32 – days exposure to various sublethal concentrations of monocrotophos are presented in Table: 22.

Table:20

Apparent dry matter digestibility (ADMD) of Rohu juveniles for the different treatments containing different sublethal concentrations of monocrotophos

Treatment	Replication	Apparent dry matter digestibility (%)	Mean ± SD
T_1 (0.0)	1 2 3	70.62 64.35 69.50	68.15 ± 2.73
T_2 (2.0)	1 2 3	65.10 61.65 66.79	64.51 ± 2.14
T_3 (5.0)	1 2 3	57.72 63.05 66.42	62.40 ± 3.58
T_4 (10.0)	1 2 3	61.74 52.39 57.53	57.22 ± 3.82
T_5 (15.0)	1 2 3	43.12 58.12 49.30	50.18 ± 6.16
T_6 (20.0)	1 2 3	45.37 52.39 49.11	48.96 ± 2.87
T_7 (30.0)	1 2 3	45.46 63.89 35.64	48.33 ± 11.71

Table:21

Result of analysis of variance (ANOVA) of the data on the apparent dry matter digestibility in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	1189.164	6	198.194	4.1528*
Error	668.156	14	47.7255	
Total	1857.320	20		

* Significance at 5% level

After 19 days exposure, 100% mortality occurred at 40 mg/l monocrotophos test concentration. Percentage survival after 32 – days was 98.33, 93.33, 96.67, 96.67, 96.67,93.33 and 70.0% at 0.0, 2.0, 5.0, 10.0 15.0, 20.0 and 30.0 mg/l monocrotophos respectively.

The result of analyses of variance of the data on percentage survival of *L. rohita* juveniles is given in Table: 23.

Comparison of treatment means based on critical difference

Critical difference = 10.9774

Treatment :	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Mean :	98.33	93.33	96.67	96.67	96.67	93.33	70.0

Underscored means are not significantly different.

Analysis of variance (Table 23) and subsequent pair-wise comparisons showed that the percentage survival was significantly different at 30 mg/l monocrotophos concentration than in the control and the lower concentrations.

The weekly progression of percentage survival of the Rohu juveniles exposed to different concentrations of monocrotophos for 32 days is represented in Fig.8.

Table:22

Percentage Survival in *Labeo rohita* juveniles due to a 32 –d exposure to various Sublethal concentrations of monocrotophos

Treatment	Replication	Percentage Survival	Mean ± SD
T_1 (0.0)	1 2 3	100.0 95.0 100.0	98.33 ± 2.36
T_2 (2.0)	1 2 3	95.0 85.0 100.0	93.33 ± 6.24
T_3 (5.0)	1 2 3	100.0 95.0 95.0	96.67 ± 2.36
T_4 (10.0)	1 2 3	100.0 95.0 95.0	96.67 ± 2.36
T_5 (15.0)	1 2 3	100.0 95.0 95.0	96.67 ± 2.36
T_6 (20.0)	1 2 3	95.0 95.0 90.0	93.33 ± 2.36
T_7 (30.0)	1 2 3	85.0 60.0 65.0	70.0 ± 10.80

Table:23

Result of analysis of variance (ANOVA) of the data on percentage survival in Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	1778.578	6	296.4297	7.5455*
Error	550.0	14	39.2857	
Total	2328.578	20		

* Significance at 5% level

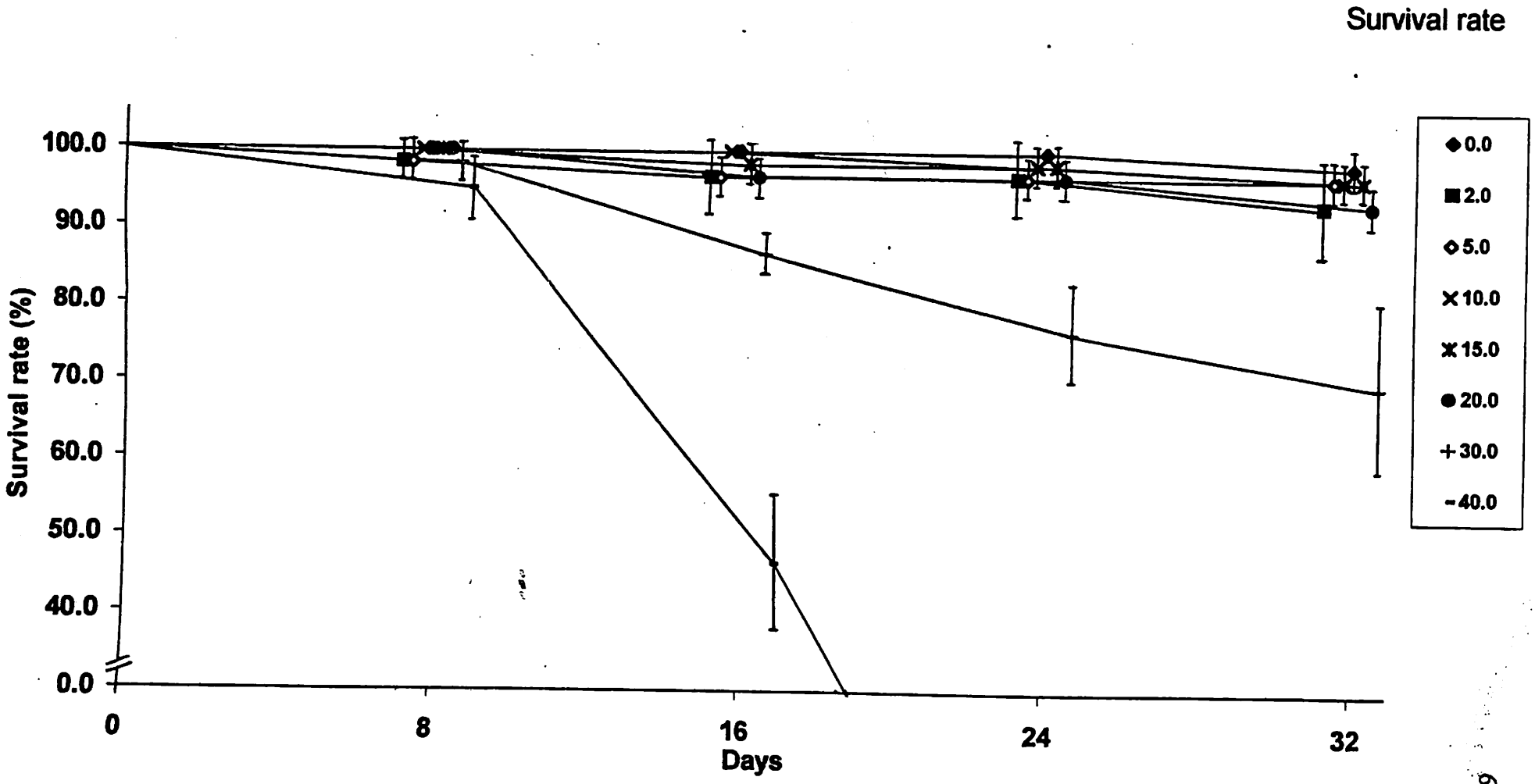

Fig.8 Weekly progression of percentage survival of the Rohu juveniles exposed to different concentrations of monocrotophos for the 32 days exposure period.

4.3.9. MATC and application factor (AF).

MATC for each of the parameter is calculated separately using the formula:

$$MATC = (NOEC \times LOEC)^{1/2}$$

The results are given in Table: 24.

The MATC of monocrotophos to Rohu juveniles based on the growth parameters like SGR and FCE is 3.16 mg/l under a 32 – days static bioassay (with 24 – h replenishment) at 28 ± 2° C being the most sensitive of the biological parameters studied. Percentage survival as an end point gave an MATC of 24.5 mg/l. Percentage survival of Rohu juveniles is the least sensitive of the biological parameters studied whereas SGR & FCE are the most sensitive parameters. Based on the 48 – h LC_{50} values for Rohu juveniles, the application factor range from 0.03 (most sensitive) to 0.24 (least sensitive) and based on the 96 – h LC_{50} value the application factor range is 0.07 to 0.53 respectively.

Table: 24

Maximum Acceptable Toxicant Concentration (MATC) values (Safe level concentration) calculated based on the growth parameters for Rohu juveniles and their corresponding application factors

Parameters	Pesticide	NOEC mg/l	LOEC mg/l	MATC mg/l	48-h LC_{50} mg/l	AF= MATC / 48 h LC_{50}	96-h LC_{50} mg/l*	AF= MATC / 96 h LC_{50}
Growth increment	Monocrotophos	2.0	5.0	3.16 (2.0 - 5.0)	104.02	0.03 (0.02 – 0.05)	46.34	0.07 (0.04 – 0.11)
SGR	Monocrotophos	2.0	5.0	3.16 (2.0 - 5.0)	104.02	0.03 (0.02 – 0.05)	46.34	0.07 (0.04 – 0.11)
FCR	Monoc rotophos	5.0	10.0	7.07 (5.0 – 10.0)	104.02	0.07 (0.05 – 0.10)	46.34	0.15 (0.11 – 0.22)
FCE	Monocrotophos	2.0	5.0	3.16 (2.0 - 5.0)	104.02	0.03 (0.02 – 0.05)	46.34	0.07 (0.04 – 0.11)
ADMD	Monocrotophos	10.0	15.0	12.25 10.0 – 15.0)	104.02	0.118 (0.10 – 0.14)	46.34	0.26 (0.22 – 0.32)
Survival rate	Monocrotophos	20.0	30.0	24.5 (20.0 – 30.0)	104.02	0.236 0.20 – 0.30)	46.34	0.53 (0.43 – 0.65)

* Sulekha *et al.*, (2000)

4.4 Carcass proximate composition

4.4.1. Initial carcass proximate composition of test animals.

The carcass proximate composition of the test animals prior to start of the experiment is given in Table: 26.

The moisture level was 77.0% whereas crude protein content and crude fat content were 14.72 % and 4.72% respectively, while the percentage of ash was 2.29. The percentage of carbohydrate content was 1.27.

4.4.2. Final carcass proximate composition of test animals.

The carcass proximate composition of *L. rohita* juveniles at the end of the 32 – days exposure to various sublethal concentrations of monocrotophos are given in Table : 25.

Crude protein, crude fat and carbohydrate contents of the whole body of *L. rohita* decreased when fish were exposed to various sublethal concentrations of monocrotophos. The moisture and ash levels were found increased with increasing concentrations of monocrotophos

The results of the analysis of variance (ANOVA) of moisture crude protein, crude fat, ash and carbohydrate are presented in Table: 27, 28 , 29 , 30 and 31 respectively.

Comparison of treatment means for moisture based on critical difference

Critical difference = 0.9895

Treatment :	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Mean :	76.5	77.84	77.86	78.13	79.69	80.23	81.97

Underscored means are not significantly different.

Comparison of treatment means for crude protein based on critical difference

Critical difference = 0.5936

Treatment :	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Mean :	14.86	13.76	13.73	13.01	12.36	11.85	10.59

Underscored means are not significantly different.

Comparison of treatment means for crude fat based on critical difference

Critical difference = 0.3267

Treatment :	T_1	T_3	T_2	T_4	T_5	T_6	T_7
Mean :	4.7	4.21	4.18	3.83	3.04	2.57	2.17

Underscored means are not significantly different.

Comparison of treatment means for ash based on critical difference

Critical difference = 0.3944

Treatment:	T_1	T_3	T_2	T_4	T_5	T_6	T_7
Mean	: 2.35	2.88	2.97	3.65	3.67	4.17	4.48

Underscored means are not significantly different.

Comparison of treatment means for carbohydrate based on critical difference

Critical difference = 0.2271

Treatment :	T_1	T_4	T_3	T_2	T_5	T_6	T_7
Mean :	1.58	1.38	1.33	1.30	1.23	1.19	0.83

Underscored means are not significantly different.

The carcass proximate composition at the end of the 32 – days exposure period between control fish and those exposed to various sublethal concentrations was significantly different. The moisture and ash levels were significantly high; and the crude protein, crude fat and carbohydrate contents were significantly low at various sublethal concentrations of monocrotophos.

Table: 25

Carcass proximate composition (Mean ± SD)* for Rohu juveniles at the end of the 32 – days exposure to various sublethal concentrations of monocrotophos

Parameters	Nominal monocrotophos concentration (mg/l)						
	0.0 T_1	2.0 T_2	5.0 T_3	10.0 T_4	15.0 T_5	20.0 T_6	30.0 T_7
Moisture	76.5 ± 0.65 [a]	77.84 ± 0.58 [b]	77.86 ± 0.54 [b]	78.13 ± 0.17 [bc]	79.69 ± 0.56 [c]	80.23 ± 0.17 [cd]	81.97 ± 0.27 [d]
Crude protein	14.86 ± 0.48 [a]	13.76 ± 0.41 [b]	13.73 ± 0.31 [b]	13.01 ± 0.09 [c]	12.36 ± 0.25 [d]	11.85 ± 0.04 [d]	10.59 ± 0.14 [e]
Crude fat	4.7 ± 0.18 [a]	4.18 ± 0.28 [b]	4.21 ± 0.10 [b]	3.83 ± 0.11 [c]	3.04 ± 0.14 [d]	2.57 ± 0.07 [e]	2.17 ± 0.03 [f]
Ash	2.35 ± 0.12 [a]	2.97 ± 0.24 [b]	2.88 ± 0.10 [b]	3.65 ± 0.17 [c]	3.67 ± 0.31 [c]	4.17 ± 0.15 [d]	4.48 ± 0.08 [d]
Carbohydrate	1.58 ± 0.16 [a]	1.30 ± 0.14 [b]	1.33 ± 0.07 [b]	1.38 ± 0.12 [a]	1.23 ± 0.03 [b]	1.19 ± 0.03 [b]	0.83 ± 0.10 [c]

** Average of three values (expressed on wet weight basis).*

Table: 26

Initial carcass proximate composition of the test animals

Parameter	Per cent body weight*
Moisture	77.0
Protein	14.72
Fat	4.72
Ash	2.29
Carbohydrate	1.27

* Wet weight basis

Result of analysis of variance (ANOVA) of the data on carcass proximate composition for Rohu juveniles due to 32- days exposure to various sublethal concentrations of monocrotophos

Table:27

ANOVA of moisture content

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	60.3828	6	10.0638	31.5286*
Error	4.4688	14	0.3192	
Total	64.8516	20		

* Significance at 5% level

Table:28

ANOVA of crude protein content

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	35.7717	6	5.9620	51.8947*
Error	1.6084	14	0.1149	
Total	37.3801	20		

* Significance at 5% level

Table:29

ANOVA of crude fat content

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	16.0360	6	2.6727	76.8082*
Error	0.4872	14	0.0348	
Total	16.5232	20		

* Significance at 5% level

Table:30

ANOVA of ash content

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	10.2841	6	1.7140	33.7964*
Error	0.7100	14	0.0507	
Total	10.9941	20		

* Significance at 5% level

Table:31

ANOVA of carbohydrate content

Source of variance	Sum of squares	Degrees of freedom	Mean S. S.	F
Treatments	0.9609	6	0.1601	9.5246*
Error	0.2354	14	0.0168	
Total	1.1963	20		

* Significance at 5% level

5. DISCUSSION

5.1. Lethal toxicity

Earlier studies have shown that the lethal concentration of monocrotophos (48 h LC_{50}) is 16.5 mg/l in post-yolk fry and 45.0 mg/l in 3-week old fry of *O.niloticus* (De Silva and Ranasinghe, 1989), 145 mg/l in *T.nilotica* at pH 6.6 ± 0.2 (Abdel and Nasser, 1991), 9.64 mg/l in *H. fossils* (Anna Mercy, 1999) , 28.52 mg/l in *C. marulius* (Anna Mercy *et. al.,* 2000a) and 94.16 mg/l in *A. testudineus and* 3.33 mg/l in *Etroplus maculatus* (Anna Mercy *et. al.,* 2000b). Sulekha *et al.,* (2000) reported that the 96 h LC_{50} values for the juveniles of Rohu and Mrigal were 46.34 and 42.32 mg/l monocrotophos respectively.

In the present study the 48 h LC_{50} value of monocrotophos for *L. rohita* juveniles was 104.02 mg/l at 28 ± 2° C. This shows that Rohu juveniles are more resistant to monocrotophos when compared to juveniles of *O.niloticus, A. testudineus, Etroplus maculates, C. marulius* and *C. mrigala.* Arora *et al.,* (1971) treated four carp species with malathion and found that the 24 and 96 hr LC_{50} values for Rohu were 7.15 and 5.05 mg/l respectively. According to them Rohu was the most resistant among the four carp species. Ballal *et al.,* (1990) studied the effect of Ekalux in Catla, Rohu and Mrigal in different life stages from egg to juveniles. It showed that the 28 h LC_{50} values for the eggs of Catla, Rohu and Mrigal were 3.5, 4.0 and 3.25 mg/l respectively. The 60 h LC_{50} values for the hatchlings were 1.0 mg/l for Catla and Mrigal and 1.4 mg/l for

Rohu suggesting that Rohu is comparatively more resistant than both Catla and Mrigal. The 96 h LC_{50} recorded for Rohu fry was 1.01 mg/l , while it was 0.975 mg/l for Catla and Mrigal. Based on the present study, monocrotophos is found to be much less toxic than malathion and ekalux. According to the different rankings of toxicity, toxicants causing mortality of fish at concentrations > 100 mg/l are considered as 'mildy toxic' (Dongherti, 1951) or low toxic (Koesoemadinata and Djajadireja, 1976).

5.2. Physicochemical parameters

5.2.1. Temperature.

Temperature below 16°C or above 40°C are reported to be lethal for Indian Major Carps, optimum being 20 - 37°C (Srivastava, 1985). The rise in temperature from 15°C to 35°C reduced the 96 hr LC_{50} value from 0.719 to 0.111 mg/l in the case of *Rasbora daniconius* (Vankhede *et al.,* 1985). The weekly range of temperature observed during the present experimental period was 28 ± 2° C. The value recorded is within the optimal range suggested for the growth of *L. rohita* juveniles (Srivastava, 1985).

5.2.2. pH.

In the present study, pH of water was almost uniform in all the experimental tanks and varied between 6.5 to 8.0. Within the range of pH 6.5 – 8.5, there was little or no difference in toxicity of monohydric phenols to rainbow

trout (Herbert, 1962). From the static bioassay tests to fathead minnows *Pimephalus promelas*, channel cat fish *Ictalurus punctatus* and blue gills *Lepomis macrochirus* Johnson and Julin (1980) concluded that water hardness and pH did not bring about significant variations in the toxicity of toxaphene. According to Srivastava, (1985) neutral or slightly alkaline (7 – 8 pH) water is more productive than acid water.

5.2.3. Dissolved oxygen.

The weekly range of dissolved oxygen observed during the present experimental period was 4.8 to 8.2 mg/l. The oxygen level never fell below 60% of the saturated level in any of the test concentrations. From three well documented studies on large mouth bass, common carp and coho salmon, Brett (1979) concluded that an oxygen concentration close to 5 mg/l was critical for growth, below which a drop of 1 mg/l dissolved oxygen caused a 30 % reduction in growth rate. Rao and Rao (1981) found that the rate of oxygen consumption decreased in *Ophiocephalus punctatus* exposed to elsan toxicity at different time intervals. Sastry and Siddique (1983) found high levels of lactic acid and haemoglobin in the blood of *Channa punctatus* chronically exposed to endosulfan. According to them, stress increased the level of lactic acid which decreased the pH probably causing high hemoglobin production. High haemoglobin content enhanced the oxygen carrying capacity of the blood, but

Sastry *et al.,* (1991) reported that there was greater demand for oxygen and the rate of oxygen consumption was higher when the fish was exposed to aldrin.

5.3. Sublethal toxicity

5.3.1. Growth.

In the lower concentration of 2 mg/l monocrotophos , the growth rate and specific growth rate did not vary significantly from those of control fish. In the higher concentrations of 5, 10, 15 and 20 mg/l monocrotophos , growth rate and specific growth rate were significantly reduced to 23.5, 48.5, 63.2 & 83.8%, and 19.0, 41.1, 55.7 & 80.4 %/day respectively when compared to control. The percentage growth increment were reduced by 62 and 85% in Rohu exposed to 15 & 20 mg/l monocrotophos. Fishes in these higher concentrations showed a marked decrease in food consumption and food conversion efficiency leading to a significantly reduced growth rate, specific growth rate and percentage growth increment. Weight loss of Rohu juveniles was registered when the fish was exposed to 30 mg/l monocrotophos.

While studying the toxicity of whole bleached Kraft mill effluent on under-yearling sockeye salmon, Webb & Brett (1972) suggested that the results of elevated maintenance energy demands on fish could be the reason for reduced growth rate. Webb and Brett (1973) have also suggested that both growth rate and conversion efficiency of under-yearling sockeye salmon were reduced in the sodium pentachlorophenate – exposure phase at concentrations greater than 2

ppb and, to a lesser extent, in the post exposure phase. Decreased growth rate has been reported in *Mystus vittatus* exposed to carbaryl by Arunachalam *et.al.,(*1980). Weight loss in common carp exposed to 12.5 mg/l phenol (for 2 – months) and 20% growth reduction in rainbow trout exposed to 1.5 mg/l phenol (for 18 weeks) were reported by Albaster & Lloyd (1982). Similar result has also been reported in *Barbus stigma* exposed to endosulphan (Manoharan & Subbiah, 1982). Growth of *Lepidocephalichthys thermalis* was significantly reduced to 1.0 and 0.4 mg/g fish per day in maximum sublethal concentration of ekalux (0.008 mg/l) and thiodon (0.0004 mg/l) respectively, than the control fish (Palanichamy *et al.,* 1986). Hansen & Cripe (1991), while studying the inter-laboratory variations in the results of the early life stage toxicity test using sheepshead minnows (*C. variegatus),* reported that chronic exposure to endosulfan significantly decreased weight, resulting in reduced growth rate. They also suggested that concentrations that affect weight in all acceptable tests were more sensitive in 6 to 9 µg/l for pentachlorobenzene. Studying the toxicity of phenol on Rohu juveniles, Nair & Sherief (1998) reported that the growth rate and SGR were reduced by 62 & 70% and 55 & 64 %/d respectively, when fish were exposed to 5 & 10 mg/l phenol concentrations.

5.3.2. Food conversion ratio and efficiency.

In the lower concentration of 2.0 and 5.0 mg/l monocrotophos, the values of FCR did not vary significantly from that of control fish. In the higher

concentrations of 10, 15 & 20 mg/l monocrotophos, FCR increased significantly to 3.44, 4.67 & 10.16 respectively, showing a clear decrease in food conversion efficiency. Significant differences in the FCR of Rohu exposed to 10,15 & 20 mg/l monocrotophos concentrations were the direct result of reduced food consumption and food conversion efficiency as was observed in pinfish (*Lagodon rhomboides)* exposed to sublethal concentrations of bleached Kraft mill effluent (Stoner & Livingston, 1978) and in Rohu juvenile exposed to sublethal concentrations of phenol (Nair and Sherief, 1998).

Palanichamy *et al.,* (1986) have also reported that the conversion efficiency significantly reduced to 47% and 67%, when *L. thermalis* was exposed to 0.008 mg/l ekalux and 0.0004 mg/l thiodon, respectively. Ramakrishnan *et al.,* (1991) reported that the conversion efficiency of *C. carpio var. communis* significantly decreased from 100% (control) to 43%, when the fish was exposed to 0.002 mg/l decis, a pesticide. Similar decrease in FCE with increasing concentration of different toxicants has been reported by Nagendran & Shakuntala (1979) expsoing *Punctatus. ticto* to sodium pentachlorophenate; Manoharan & Subbiah (1982) exposing *Barbus stigma* to endosulphan and Arunchalam *et al.,*(1990) exposing *Mystus vittatus* to malathion, thiodon & carbaryl.

5.3.3. Average daily food consumption (ADFC).

In the present study, even at lower concentration of 2.0 mg/l monocrotophos, the value of ADFC varied significantly from that of control fish.

In the higher concentrations of 10,15, 20 & 30 mg/l monocrotophos, ADFC were significantly reduced to 80.22, 78.73, 70.88 & 52.63 % of control respectively. Farmanfarmain *et al., (1980)* reported that intestinal absorption was inhibited by toxicants. Due to the inhibition of intestinal absorption, the efficiency of absorption of digested food also might have decreased and this might have resulted in poor food intake.

5.3.4. Apparent dry matter digestibility (ADMD).

In the lower concentrations of 2, 5 and 10 mg/l monocrotophos, the values of apparent dry matter digestibility (ADMD) did not vary significantly from that of control fish. In the higher concentrations of 15, 20 and 30 mg/l monocrotophos, digestibility of dry matter was significantly reduced to 73.62, 71.83 & 70.91 % respectively.

5.3.5. Survival rate (%).

In the lower concentrations of 2, 5, 10, 15 and 20 mg/l monocrotophos, the values of survival rate did not vary significantly from that of control fish. There was no significant difference in the survival rate between the lower concentrations also. But in 30 mg/l concentration the survival rate was 70 %. This is statistically different from control. Fishes exposed to 30 mg/l showed a reduction in ADFC (52.63%), ADMD (70.91%) and they showed reduction in weight at the end of the first week itself.

Working on ekalux stress, Ballal *et al.*,(1990) reported that Rohu is comparatively more resistant than Catla & Mrigal. They have also suggested that Rohu continued to maintain its position as the most resistant species even when it reaches the fry stage. Similar observations have also been made by Arora *et al.*, (1971) and Kumar (1983) using malathion and Sulekha *et al.,(2000)* using monocrotophos. Juveniles of *L. rohita* are good test animals as they yield relatively consistent results in toxicological assays and provide a steady control survival under tropical conditions as also shown by Ashraf *et al.*,(1992) and Nair & Sherief (1998).

5.4. Carcass proximate composition

Protein, lipid and carbohydrate, which constitute the major component of the body, play an important role in body composition and energy metabolism. This is affected by environmental factors like water pollution (Palanichamy, *et al.*, 1986). In the present study, even at lower concentrations of 2 & 5 mg/l monocrotophos, the values of protein, carbohydrate and lipid varied significantly ($p<0.05$) from those of control fish, though statistically there was no significant difference ($p<0.05$) between 2.0 and 5.0 mg/l concentrations. In the higher concentrations of 10, 15, 20 & 30 mg/l monocrotophos, crude protein content was significantly reduced to 13.01, 12.36, 11.85 & 10.59% respectively. Crude fat content was also significantly reduced to 3.83, 3.04, 2.57 & 2.17% respectively; and carbohydrate reduced to 1.38, 1.23, 1.19 & 0.83 respectively.

Decrease in the protein, fat and carbohydrate content of Rohu exposed to different sublethal concentrations in the present investigation indicated the utilization of all these energy components when fish is under stress. It does not mean that all the substances are simultaneously utilized. When the principal and immediate energy source gets depleted, the other sources exhibit a proportional depletion as the metabolism of these energy components are inter-linked through a common metabolic pathway i.e., the tricarboxylic acid (TCA) cycle (Arunchalan *et al.* 1990).

Significant differences in the carcass proximate composition of Rohu juveniles exposed to 10, 15, 20 & 30 mg/l monocrotophos may be the direct result of significantly reduced food consumption and food conversion efficiency as was observed in pinfish (*Lagodon rhomboides)* exposed to sublethal concentrations of bleached kraft mill effluent (Stoner & Livingston, 1978) and in Rohu juveniles exposed to sublethal concentration of phenol (Nair & Sherief, 1998). Significant depletion in body protein and fat is exhibited in the higher monocrotophos concentration (> 10 mg/l) to meet energy demand due to the toxicant stress, resulting in significantly reduced specific growth rate in the present study. Similar findings on depletion of body energy components of fresh water fishes during chronic exposure to different pesticides are those of Rao & Rao, (1979); Rath & Misra, (1980); Palancichamy *et al.,* (1986, 1987); Vasanthi

and Ramaswamy, (1987); Seshagiri Rao *et al.,* (1987); Babu *et al.,*(1988) Malla Reddy & Bashamohideen (1988) and Begum & Vijayaraghavan (1995).

Significantly increased level of moisture in fishes exposed to all concentrations of monocrotophos in the present study may be due to the decreased food intake and accumulation of water to varying degrees, as suggested by Soulter & Huggins, (1977) when they starved *Agonus cataphractus* for varying period. Increase in moisture content in Rohu juveniles due to chronic exposure to phenol has also been reported by Nair & Sherief (1998).

5.5. Maximum acceptable toxicant concentration and application factor

In the lowest concentration of 2 mg/l monocrotophos, the values of growth increment, SGR and FCE did not vary significantly from those of control fish. The MATC for monocrotophos to Rohu juveniles based on the major biological parameters of SGR and FCE is 3.16 mg/l at 28 ± 2° C. In the present study SGR and FCE gave the most sensitive MATC values among the different growth parameters studied. Percentage survival as an end point gave an MATC of 24.5 mg/l being the least sensitive of the biological parameters studied, showing a factor of 'x8' when compared to 'growth'. Kristensen (1994) in his review concluded that in 75% of the reported experiments on teleostean embryo-larval

stage 'survival' as and point was within a factor of 'x3' of the lowest LOEC recorded for the different end points studied including 'growth'.

The application factors derived by various investigators for fresh and salt water fishes exposed to different pesticides compare well with that derived for Rohu juveniles exposed to monocrotophos in the present study (Table: 32).

The MATC of monocrotophos to Rohu free embryo/apterolarva is found to be 0.346 mg/l (Anna Mercy *et al.,* 2000c). In the present study the MATC value is 3.16 mg/l much higher (x9) than that for embryo/apterolarva clearly indicating that the juvenile period is much less sensitive to monocrotophos. Greater sensitivity of young striped mullet than older juveniles to mirex and methoxychlor was reported by Lee *et al.,* (1975). Kristensen (1994) in his review states that teleost early life history stages (egg – embryo-larva) are almost always the most sensitive to the impact of toxicants, the juveniles being much less sensitive.

Table : 32

Comparison of application factor derived for Rohu juveniles with monocrotophos in the present study with those derived by other investigators for fresh and salt water fishes exposed to different pesticides.

Pesticide	Species	96-h LC_{50} (µg/l)	MATC (µg/l)	Application factor	Reference
Endrin	*Cyprinodon variegatus*	0.34	0.12 to 0.31	0.35 to 0.91	Hansen & Parrish, 1977
	Jordanella floridae	-	-	0.25	
Haptachlor	*Cyprinodon variegatus*	10.5	0.97 to 1.9	0.09 to 0.18	
	Pimephales promelas	-	-	0.07	Macek *et al.*, 1976
Methosychlor	*Cyprinodon variegatus*	49.0	12 to 23	0.24 to 0.47	Hansen & Parrish, 1977
Malathion	*Cyprinodon variegatus*	51.0	4 to 9	0.08 to 0.18	
	Pimephales promelas	-	-	0.05	Mount & Stephan, 1967
	Lepomis macrochirus	-	-	0.04	Eaton, 1970
	Jordanella floridae	-	-	0.02	Hansen & Parrish, 1977
Carbofuran	*Cyprinodon variegatus*	386.0	13 to 23	0.04 to 0.06	
Carbaryl	*Pimephales promelas*	9.0	0.21 to 0.68	0.023 to 0.075	Carlson, 1971
Monocrotophos	*L. rohita* (Free embryo)	-	0.346 mg/l	-	Anna Mercy *et al.*, (2000c)
	L. rohita (juvenile)	46.34 mg/l*	2 to 5 mg/l	0.04 to 0.11	Present study

*Sulekha *et al.*, (2000)

6. SUMMARY

The present study was aimed at evaluating the effect of different sublethal concentrations of monocrotophos on growth increment, specific growth rate (SGR), food conversion ratio (FCR), food conversion efficiency (FCE), average daily food consumption (ADFC), apparent dry matter digestibility (ADMD) and survival rate of Rohu - *L. rohita* juveniles. The methodology, important results and conclusions of the study are as follows:

1. Acclimated 8 – week old juveniles of Rohu – L. rohita (Ham.), average size of 46.84 ± 0.52 mm and 1012.29 ± 43,12 mg were obtained from the carp hatchery of the College of Fisheries, Panangad.

2. The calculated 48 h LC_{50} value of monocrotophos of Rohu juveniles is 104.02 mg/l in static bioassay without aeration. The 95% fiducial limits ranged from 83.25 to 124.79 mg/l.

3. The different sublethal test concentrations of monocrotophos used in the experiment were 0, 2, 5, 10, 15, 20 and 30 mg/l designated as T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively.

4. The duration of the experiment was 32 – days and the experiment was done in triplicate in order to find out the consistency of the result.

5. The animals were fed *ad libitum* once a day, on a pelleted carp feed. The different biological parameters monitored in order to find out the effect of monocrotophos on Rohu juveniles were growth increment, SGR, FCR, FCE, ADFC, ADMD and survival rate.

6. The various observations on the water quality parameters like temperature, pH and dissolved oxygen were found to be well within the tolerance limits for the optimum growth of Rohu juveniles.

7. There was no significant difference between the control and 2.0 mg/l monocrotophos concentration on SGR and FCE of the Rohu juveniles. All Concentration above 5.0 mg/l showed significant difference when compared to control values.

8. Based on these important biological factors the NOEC (No Observable Effect Concentration) and LOEC (Least Observable Effect Concentration) are 2.0 and 5.0 mg/l respectively.

9. The maximum acceptable toxicant concentration (MATC) of monocrotophos to Rohu juveniles based on the biological parameters like SGR and FCE is 3.16 mg/l (2.0 – 5.0 mg/l) under 32 – days static bioassay (with 24 h replenishment) at 28 ± 2°C, being the most sensitive of the biological parameters studied.

10. Percentage survival of the juveniles as an end point gave an MATC of 24.5 mg/l being the least sensitive of the biological parameters studied.

11. Based on the 48 h LC_{50} value for Rohu juveniles the application factor (AF) ranged from 0.03 (SGR, FCE) to 0.24 (survival) and based on the 96 h LC50 value the application factor range is 0.07 to 0.53 respectively.

12. Decrease in the protein, fat and carbohydrate content of Rohu juveniles exposed to different sublethal concentrations in the present investigation indicates the utilization of all these energy components when fish is under toxicant stress. Significant depletion in body protein and fat is exhibited in the higher monocrotophos concentration (> 10 mg/l) to meet energy demand due to the toxicant stress.

13. Significantly increased level of moisture in Rohu juveniles exposed to all concentrations of monocrotophos in the present study may be due to the decreased food intake and accumulation of water to varying degrees.

14. Based on the present investigation and related studies in the Department of Fishery Biology, the Rohu juvenile life-history period is found to be much less sensitive to monocrotophos toxicity when compared to the embryonic and larval periods.

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LETHAL AND SUBLETHAL TOXICITY OF MONOCROTOPHOS AN ORGANOPHOSPHATE ON THE JUVENILES OF ROHU – *LABEO ROHITA* (Ham.) UNDER TROPICAL CONDITIONS

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ABSTRACT OF A THESIS

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8. ABSTRACT

The seasonal utilization of paddy field for fish culture is quite common in Kerala and West Bengal. In recent years, with the advent of high yielding varieties of paddy, the use of pesticide has become widely prevalent. Monocrotophos is one of the commonly used organophosphate pesticides in the paddy fields of Kerala. The present study was designed to arrive at an experimentally determined application factor for fixing tentative water quality criteria for monocrotophos under tropical conditions. Juvenile Rohu *Labeo rohita* (46.82 ± 0.52 mm total length, 1012.29 ± 43.12 mg wet weight) were subjected to static lethal and sublethal bioassay. The 48 h LC_{50} value was found to be 104.02 mg/l at 28 ± 2°C. Juvenile Rohu were exposed to sublethal monocrotophos concentrations (2, 5, 10, 15, 20 and 30 mg/l) for 32 days at 28 ± 2°C without aeration. Treatment media were replaced every 24 hr. Fish were fed a pelleted diet at 6% wet body weight per day. Rohu exposed to 5, 10, 15, 20 and 30 mg/l monocrotophos showed significantly lower mean wet weight gain, specific growth rate (SGR) and food conversion efficiency (FCE). But fishes of lower monocrotophos concentration of 2.0 mg/l were not significantly different from the control. Juveniles exposed to 2, 5, 10, 15, 20 and 30 mg/l monocrotophos concentrations had higher moisture and ash content, and lower protein, lipid and carbohydrate contents, as body nutrients were depleted. The maximum acceptable toxicant concentration for juvenile Rohu was 3.16 mg/l for SGR and FCE as end points (the most sensitive) and 24.5 mg/l for percentage survival as an end point (the least sensitive) indicating application factors of 0.03 and 0.24 respectively.