"Studies on Influences of Organophosphate Pesticides on Reproductive Activities of Female Common Carp , *Cyprinus carpio communis*"

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By

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UNDER THE SUPERVISION OF

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Certificate

Certified that the dissertation entitled "Studies On Influences Of Organophosphate Pesticides On Reproductive Activities Of Female Common Carp, Cyprinus carpio communis" submitted by Mr. Farooq Ahmad Mir for the award of **M. Phil.** Degree in Zoology, is based on original research work carried out by him under our supervision. This dissertation has not been submitted in part or in full, to any University/Institution for any degree or diploma. The candidate has fulfilled all the statutory requirements for the submission of the dissertation.

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"In the name of ALLAH, most gracious, most merciful. Praise be to ALLAH, the cherisher and sustainer of the worlds; most gracious most merciful; master of the Day of Judgment. You do we worship, and your aid do we seek. Show us the straightway. The way of those on whom you have bestowed your grace, those who (portion) is no wrath, and who do not go astray" (Ameen)

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Chapter - 1

INTRODUCTION

Fishes are the earliest known vertebrates and flourished during the Devonian period, about 400 million years ago they form a highly successful group of animals comprising more than 40,000 species inhabiting all seas, rivers, lakes, canals, dams, muddy water, brackish water, estuaries and all places where there is water. A very wide distribution of fishes into a variety of habitats has resulted in numerous adaptations in their morphology, physiology and behavior.

Economically, fishes constitute a very important group of animals and provide a rich source of food, liver oil and a number of other by products, like fish meal, fish manure, Isinglass, etc.

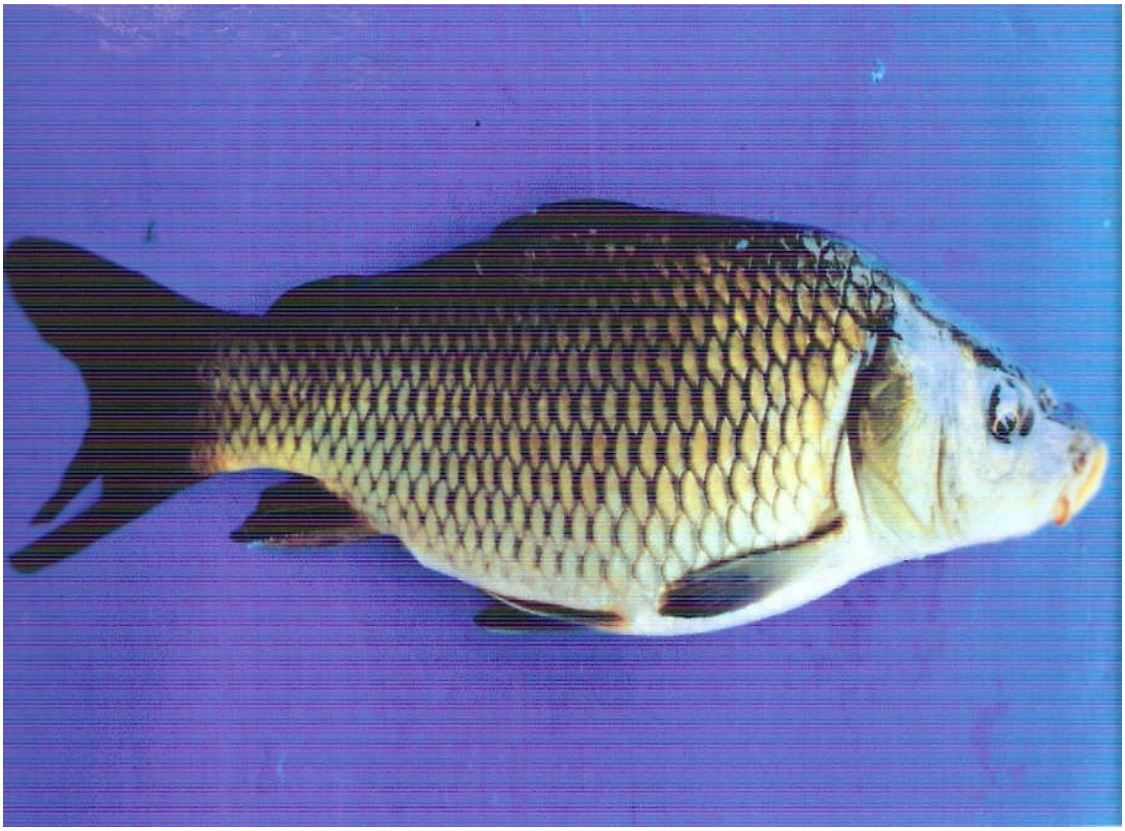
India is a land of diverse topography, climate and natural resources. There are 12 major rivers and 48 lesser rivers with a total catchment area of 277.6 million hectares. The immense freshwater habits harbor 587 species of freshwater finfish out of the total 218 species distributed in India of the estimated 2,1723 living species of fishes in the world (Nelson, 1984).

The valley of Kashmir is situated in the western Himalayas, between the latitudes of $32^0$17′ and $36^0$58′N and longitudes of $73^0$26′ and $86^0$50′ is 131 km in length and 40 km in width with an average elevation of 1600 meters above

sea level. The valley is surrounded on all sides by high mountains and abounds in a great array of freshwater habitats of lotic as well as lentic nature, which are inhabited by a number of fish species, including indigenous as well as alien.

The common carp, *Cyprinus carpio communis* is a highly palatable and preferred for culture due to its high growth rate and prolific breeding in confined water. The common carp, *C. carpio* is a native of the temperate regions of Asia especially of China (Gunther, 1886). Jenkins (1961) lists the black sea, the Caspian and eastwards to Turkestan as its original home. Schaperchaiis (1933) stated that it was a native of the mouth of the rivers which shed their water into the Caspian and the black sea. The earliest reference to the culture of common carp is from China as early as in 475 B.C. It is thus autochthonous to China and Russia, though according to Okada (1960), it originated from central Asia and was introduced into China and Japan in the Oriental region and into Greece and Europe through Rome, in ancient times. The original natural distribution of common carp was thus confined to narrow belt in central Asia within latitudes 35^o-50^oN and longitudes 30º-135^oE. The altitude generally is over 304m above sea level. At present, the common carp enjoys global distribution occurring in tropical as well as temperate regions acclimatized to a variety of habitats and extremes of environment (Alikunhi, 1996).

Two exotic varieties of common carp (Scale carp and mirror carp) were introduced in mid fifties by fishery department. The fish have got well established in the waters of the valley, especially, the lentic habitats and over shadow at present almost all the indigenous species. It has been reported by a number of workers (Das, 1963; Malhotra, 1970 and Sunder *et al*., 1977) that the introduction of carp species in Kashmir has proved detrimental to the endemic species of fish.



Female common carp *(Cyprinus carpio communis)*

Plate 1

Systematic position of Common carp, *Cyprinus carpio communis*

Phylum	-	Chordata
Group	-	Vertebrata
Series	-	Pisces
Class	-	Teleostomi
Sub-class	-	Actinoptergii
Order	-	Cypriniformes
Division	-	Cyprini
Family	-	Cyprinidae
Sub-family	-	Cyprininae
Genus	-	*Cyprinus*
Species	-	*carpio*
Variety	-	*communis*

Every species has an immense power of biotic potential, and if the conditions are favorable the population may increase to the explosive level. But, it seldom happens, because from the beginning of gametogenesis the attainment of maturity, there are a number of factors responsible for untimely death of embryos, juveniles and adults even before they start reproducing. Many commercial and productive fish species are adversely affected in modern times, due to severe, fast and undesirable changes detrimental to their surroundings, survival and viability.

Local fish populations, however, often are subject to sudden and large scale mortalities. This happens due to unusual natural causes, and such mortalities are beyond remedy. Then there are equally significant mortalities of fish population which stem from causes for which mankind is responsible. Near large centers of urban agglomerations and in proximity of mines, mills, industries and the like changes are brought in the chemical, physical and biological qualities of water so much as to cause mass killing of fish life.

Due to enormous growth in the human population in the last quarter of the 21st century when human population more than doubled in first 35 years demanded more space to live and increase in the production of agriculture. To increase the agriculture produce and to protect the crops from organisms which damage the agricultural crops, live stocks, plants and plant products etc. was the agenda of FAO and a number of methods have been evolved to get rid of these injurious pests. These included the pesticides of plant origin, inorganic chemical insecticides, organic chemical pesticides such as chlorinated hydrocarbon compounds e.g., DDT, BHC, Heptachlor DDT, BHC, Aldrin, Dieeldrin, Endrin, Diendrin, Chlordane, Lindane, Gammaxene, Toxaphene, Strobane etc., Carbamate pesticides e.g., SEVIN and organophosphate pesticides e.g. malathion , diazinon, Dimethoate, dichlorvos Malathion, Diazinon (Tik20), chlorothion, Fenthion, Fenitrothin, DFP (Di isopropyl florophosphate), TEPP (Tetraethylpyrophosphate), thio TEPP, OMPA (Octamethylpyrophosphoamide), Dichlorofenthion, trichlorfos, fenamiphos, ethyl parathion, eroxathion etc.

All these chemicals have acquired the attention of people due to their low cost, easy application, availability and quick action. These chemicals when applied in the agricultural fields make their in to the low lying water bodies including rivers, ponds, lakes etc.through surface run off after the rains.

The impact of hazardous chemicals, (including organophosphates), human interferences through modernization, urbanization and excessive use of agrochemicals, discharges or industrial effluents have certainly created some sort of stress into the aquatic society. Pesticides employed in pest control in India like

agricultural country have contaminated fresh water habitats remarkably. The problem of pesticide pollution and environmental hazards is quiet serious problem in India, due to acute biocidal effects of persistent chlorinated hydrocarbon. Chemicals organophosphates because of quick action, short half life and little bioconcentration (Stickle, 1974) are indiscriminately used in the field as a result of which wild life is often exposed to these pesticides. There are several reports to show that certain of organophosphates (Dimethoate, Dichlorvos, Diazion etc) in higher concentration are highly toxic to wild life even causing mortality in exposed animal population, with references to organophosphate pesticides. Acute toxicity studies in fish model have provided basic data such as nature of toxicant, susceptibility of individual species, lethal concentration to 50% of the exposed fish species (Lc50) determination of sub lethal concentration of the toxicant for further investigations.

The responsiveness of animals including fishes to organophosphate compounds ranges from altered metabolic activities to death (Anam and Mitra, 1995). On several occasions depending upon the level of exposure impaired reproductive activities following ingestion of organophosphate compounds have been reported in both female and male fishes.

The acquisition of pesticides by aquatic biota occurs by three ways (Kerr and Vass, 1973)

1. Direct uptake of contaminated food.
2. Direct absorption from water through gills.
3. Absorption through integument.

Each of the above processes varies with the environmental condition and type of animal. But absorption through gills and are the main routes in fish. In higher aquatic animals, the rate of exposure to a pesticide in the surrounding medium is taken as the measure of the rate of contact with both polluted water and contaminated food (Edwards, 1973).

In fish the uptake of pesticide taken place through absorption by gills (Holden, 1962; Murphy, 1971) and by ingestion of contaminated food (Grazenda *et al.,* 1970;

Macek, 1970). Residue uptake through gills is related to the metabolic rate and body size (Murphy, 1971).

The fishes are directly influenced due to these toxic chemicals which cause mortality and several disease as a result the growth is ceased. The breeding capacity of fishes is also hampered due to these toxicants. Behavioral activities of an organism represents the final integrated result of a diversity of biochemical and physiological processes of behavioral patterns are knowing to be highly sensitive to changes in the steady state of an organism (Warner *et al.,* 1996). Lots of scientist expressed the pattern of behavioral changes varies according to fish species, concentration of chemical (Pesticide), Physico-chemical conditions and on rate of excretion of fish species. Poisons with a restorative action, the symptoms are manifested primarily through their action on the nervous system; cause a rapid loss of equilibrium in fish (Metler *et al.,* 1971). Symptoms of furadon and Malathion toxicity were reported by Konar and Ghosh (1982). Within 24 hours of exposure to 0.5 ppm furadon, fish had slow opercular movement, lethargy and occasional jumping. Reports related to effects on fish reproduction are scarce and do not encompass the diverse range of events involved in reproduction such as the onset of puberty, gametogenesis, oocyte maturation, ovulation, spermiation, spawning, fecundity, fertilization, endocrinology of reproduction, and developmental events such as embryogenesis, hatching , and post hatching metamorphosis. Information on all these reproductive aspects is available in temperate zone fishes exposed to pesticides.

Since the lipid content of fish gonads increase tremendously during the reproductively active phase some times by more than 200 times (Lal and Singh, 1987a), lipophilic pesticide residues accumulate and increase in recrudescing fishes. These pesticides are therefore likely to interfere with gonadal activities.

Pesticide-Induced reproductive failure or dysfunction is evident from the available reports on Indian fishes (Singh and Singh, 1982b; Singh *et al.,* 1997). Pesticides have been reported to cause damage to gonads such as cytolysis of germ Cells, arrest of gametogenesis, Inhibition of steroidogensis, gamete maturation, release of gamete, spawning and hatching. Reproductive toxicity indicates changes on

the pattern of breeding response, on fecundity, on fertilization rate, hatchability of larvae and above all survivability of larvae, fry etc. though, it is a prime subject of research because it directly relates to productivity of fishes.

Hence, the present study was undertaken to investigate influence of the organophosphates pesticides on the reproductive activities of female common carp, *Cyprinus carpio communis*. Also, it may not be ruled out that organophosphate pesticides are potent antiacetylecholinesterase agents which phosphorylate the enzyme acetyl cholinesterase resulting in accumulation of endogenous acetylcholine and consequent disruption of neuro-function. Thus measurement of acetylcholine activities in different tissues are considered as marker of organophosphates neuro-toxicity associated with brain and reproductive organs of target fish. Nevertheless those who have worked on this aspect made appreciable advances in this subject. This study also covered the estimation of the toxicity of different sub lethal concentrations of Dimethoate (Rogor) and dichlorvos (Neon) to common carp which is a recommended fish species for bioassay experiments and abundantly used as a food source in Kashmir (India). On the basis of this study we can compare toxicity of these selected pesticides to other pesticides and can also use common carp as a model for other fish species. The reported results would be useful contribution in ecotoxicity risk assessment studies of these organophosphate pesticides as fish species.

Chapter - 2

REVIEW OF LITERATURE

Numerous studies are available reporting the effects of pesticides on reproductive activities in Indian fishes. The majority of these reports deal with histopathological changes in gonads and endocrine glands involved in the regulation of reproduction following treatment with different pesticides. Pesticides are reported to cause degenerative changes in gonads and arrest gametogenic processes either by acting directly on the gonads or by interfering with the secretary activity of the hypothalamo—hypophysal-gonadal/thyroid axis that regulates various reproductive events. Secretion of hormones such as gonadotropin releasing hormone, gonotropin, growth hormone, adrenocorticotropic hormone, testosterone etc are in general lowered leading to cessation of gametogenesis, vitellogenesis, oocytes maturation, ovulation spermiation etc. Adverse effects of pesticides have also been demonstrated on fecundity, fertilization, hatching and postembryonic development. The effects are highly variable and depend on the nature, dose and mode of application of the pesticides.

Some literature is available on the fish ovarian damage due to pesticide. In India, most of the ovarian research has been concentrated on air breathing fishes cat-fishes, carps with relation to effect of a number of organochlorine, carbamate and organophosphate pesticides. Kurlshrestha *et al.* (1984) worked on the exposure of endosulfon and carbaryl an the ovaries of *Channa striatus* (Bloch) and observed reduction in the number of oocytes, increased number of damaged oocytes, development of inter follicular species, reduction in gonado somatic Index, Fecundity, fertilization etc.

Weiss (1958) worked on the determination of cholinesterase in the brain tissue of three species of fresh water fish and its inactivation in vivo and reported that the exposure of fish to anti-acetycholinesterase substances depresses the level of brain

acetyl cholinesterase activity. When death of the fish takes place the level of ChE activity has fallen to about 30-60 percent of normal activity. If exposed fish are placed in fresh water the ChE activity of the brain returns over a period of time to normal level.

Boyd (1964) reported that, at concentration above the threshold toxicity, many organophosphate compounds caused large pregnant female of *Gambusia affinis* to abort.

Cope *et al*. (1970) reported that in experimental ponds, 2, 4-D butyl ester at the highest concentration caused a delay in the spawning of blue gills.

Carlson (1972) reported that the carbaryl (Sevin) pesticides cause prevention of reproduction in fathead minnow, *Pimephales promelas*.

Holden (1972) reported that brown trout eggs containing aldrin failed to hatch. Hatching of blue gill eggs exposed to different doses of a formulation of fenofrop (Kuron) was not affected, but all the fry hatching from eggs exposed to the highest concentration died.

Wilbur *et al*. (1973) reported that hatching of blue gill eggs exposed to different doses of a formulation of (kuron) was not affected, but all the fry hatching from eggs exposed to the highest concentration died.

Smith *et al*. (1974) described that eggs of DDT exposed adults of C*oho salmon* showed abnormal gastrulations, and 39% of them had vertebral deformities upon hatching and percent of deformed larvae was dose dependent.

Halter (1974) reported that hatchability of *Coho salmon* eggs, exposed to PCB's, was much reduced.

Rao (1974) compared the toxicities of organo-phosphorus and carbamate pesticides using *Panchax panchax* as the test fish. The 96-hour LC50 value by phosphamidon (Dimecron) DDVP, and Niwan was found to be 23.88, 23.04, 3.87 and 3.42 mg/l, respectively while that for the carbonate Cuman was only 1.94mg/l indicating that phosphamidon is the least and Cuman the most toxic.

Toor and Kour (1974) reported various adverse effects of several pesticides on hatchability and survival developing eggs and hatchlings in the common carp.

Freeman and Idler (1975) demonstrated reduced hatching of embryos by PCB in brook charr, *Salvenlinus fontinalis*.

Ghosh and Konar (1976) reported that the pesticides reduce breeding and fecundity in fishes.

Weis *et al*. (1976) worked on embryos of Atlantic silverside treated singly with P.P-DDT, Malathion or Carbaryl and observed that they reduced the survival time of embryos, even these effects were observed at concentrations that occur temporarily in the environment.

Saxena and Garg (1978) reported retardation of ovarian development by fenitrothious and carbaryl chemicals in *Salvenlinus fontinalis*.

Curtis (1978) reported that the ovipositor of *oryzias latipes* was suppressed heavily by 96 hour exposure of chlordecone.

Venugopalan *et al*. (1979) reported that the embryos, sac-fry, and larvae of caranx exposed to lindane had advanced hatching and larval characters.

Pandey and Shukla (1980) observed necrosis of seminiferous tubules, atrophy of interstitial cells, and thickening of sperm duct in 1, 2, 3, 4, 5, 6-hexachlorocyclohexane (γ-BHC) (2mg-1)-treated *Oreochromis mossambicus*

Siva Prasada (1980) demonstrated that the effect of organophosphorus like methyl parathion on the fish *Tilapia mossambica* and reported a decrease in the body weight.

Basha (1980) reported that the malathion, sevin and lindane caused decrease in the body weight of the fish *Tilapia mossambica*.

Yasuno *et al*. (1980) described that Fenitrolthion, at sublethal concentrations affected egg production whereas temephos at sublethal concentrations affected normal birth in *Oryzias latipes.*

Buckler *et al*. (1981) reported that the hatching success of fish eggs exposed to different concentrations of mirex increased and hatching of fat-head minnows eggs exposed to different concentrations of chlordecone was significantly lower than that of the controls.

Kabeer *et al*. (1981) investigated that exposure of fish *Tilapia mossambica* to a sublethal (2mg/litre/48) concentrations of marathon showed no significant changes in any of the physical parameters investigated like, body weight and body water content. While the oxygen consumption of the fish showed a consistent increase up to 24h and later declined to 8% suggesting the reduction of oxidative metabolism at the end of 48h.

Singh (1982a) studied the effects of pesticides such as malathion, paramar M-50, hexadrin and aldrin on ovarian P32 up take and found these pesticides effectively reduce gonadotropin recreation with consequent decrease in ovaprin P32 uptake in sham hypophy-sectomised fish.

Goodman *et al*. (1982) worked out that fecundity and fertility of female sheeps head minnows exposed to different doses of chlordecone shown marked reduction.

Pawar *et al*. (1983) observed ovarian damage in freshwater fish *Garra mullya* (sykes) when exposed to sumithion (pesticide).

Pandian *et al*. (1983) worked on the food utilization in the fish *Channa striatus* exposed to sublethal concentrations of DDT and methyl parathion and reported that sublethal concentrations of DDT and methyl parathion (MP) in the medium significantly affected the rates of feeding, absorption and conversion in *Channa striatus*. Fish exposed to chemical consumed 23 or 50% less food than those exposed to pesticide-free water; absorption rate also decreased from 120cal/g live fish/day in the control to 88 and 59 cal/g/day in pesticide exposed fish. Efficiency to convert the absorbed food into body substance dropped from 30% in the control to 6 and 12% in the exposed fish.

Kulshrestha, *et al*. (1984) worked on the exposure of endosulfon and carbaryl on the ovaries of *Channa straitus* (bloch) and observed reduction in no. of oocytes,

increased no. of damaged oocytes, development of interfollicular spaces, reduction in gonado somate index (GSI) etc.

Shukla *et al*. (1984) have noted decreased ovarian activity and atretic oocytes in *Saratherodon mossambica* exposed to melathion. Later in (1985) they reported that the DDT and endosulfan produced fibrogenesis and thickening of the ovarian tunica in *Tilapia*

Nagler (1984) investigated sublethal effect of pentachlorophenol (PCP) on ovarian development in rainbow trout, *Salmo gairdneri* and reported increase in intrafollicular spaces, thickening of ovarian wall and follicular atresia.

Kulshrestha *et al.* (1984) reported necrosis and fibrosis of ovarian connective tissue, dilatation of blood vessels, decrease in oocytes diameter and increase in intrafollicular space in *Channa striatus*.

Kumar *et al.* (1984) reported that the organophosphate and other pesticides enter water bodies as a consequence of rain and leaching from the soil or because it is carelessly discharged directly into aquatic ecosystems.

Ghosh *et al*. (1985) has described damage in ovary, viz. degeneration of follicular wall, ooplasm and connective tissue due to melathion toxicity on *Heteropneustes fossils*.

Haider *et al*. (1985) reported that the exposure of *Channa punctatus* to cythion (2mg/l) and *Mystus vittatus* to chlorfenvinphos (0.003mg/l) tetrachlorvinphos (0.15mg/l), mevinphos (0.00012mg/l) and malathion (2.5mg/l) arrested ovarian development and treated ovary contained only stage I oocytes while controls showed large number of stages II and III oocytes. Later in (1986), they also demonstrated that organophosphates such as malathion, mevinphos, chlorfeninphos and tetrachlorvinphos significantly reduced the rate of GVBD in oocytes of the cat fish, *Mystus vittatus* in vitro.

Kling (1986) worked vividly on the toxic effects of sublethal doses of Labaycid upon the ovaries in *Sarotheroden leucostictus* and observed that exposure caused

total artesian, inhibition of vitellogenesis in the gonads and decreased gonado-somatic index (GSI).

Macek (1986) described that following the feeding of DDT to brook trout at different doses for 22 weeks, the fishes fed with lower doses showed significantly higher no. of ova than those of higher doses.

Raj *et al*. (1987) studied the effects of long term exposure to cythion on the reproduction of the teleost fish, *Channa punctatus* (Bloch) and reported that fish exposed to 2.0mg/l of commercial cythion continuously for 6 months, exhibited a significant inhibitions of gonadal development and gondosomatic index. The immature oocytes of the experimental fish exhibited cytoplasmi proteinaceous inclusion bodies, which ultimately led to their degeneration.

Khan (1987) worked on toxic effects of mercuric chloride on sperm and egg viability of two populations of mummichog, *Fundulus heterocltus* and reported that the 0.01ppm mercuric chloride (Hg) for 2 min caused a significant reduction in fertilization success and sperm mortality.

Ansari *et al*. (1987) worked on melathion toxicity on the ovary of zebra fish, *Brachydanio rerio* and observed histomorphological damage, besides increased number of atretic follicles.

Lal *et al*. (1987 b,c,d) reported that the pesticide induced interference in the production of villogenin and lipid derived energy generation in the cat fish *Clarias batrachus.*

Ram *et al*. (1988) reported that the exposure of *C. batrachus* to emisan resulted in cellular pycnosis and nuclear necrosis in neurons of *Nunclens preopticus.*

Pandey (1988) worked on the impact of endosufan (Thiodon) EC 35 on dynamics of oocyte development in the teleostean fish, *Colisa* (Trichogaster) *fasciatus* and described that ovarian activity was retarded greatly, the diameter of oogonia and stage I oocytes was greatly reduced and ovarian wall became thick.

Kumar *et al*. (1988) reported increased oocyte artesia in aldrin treated (0.05mg/l) *Puntius conchonius*.

Dey *et al*. (1989) reported ovarian damage to C*hanna punctatus* after chronic exposure to low concentration of elsan, mercury and ammomia.

Singh (1989) reported malathion-induced disruption of vitellogensis in *Monopterus albus*.

Krishnan *et al*. (1989) studied the toxic and sublethal effects of endosulfan and carbaryl on growth and egg production of cladoceran, *Moina micrura* and reported that the commulative growth and egg production during life were inhibited to 31.8 and 20.7% in end sulfa and 28.2 and 13.7% in carbaryl-treated animals, respectively. The growth coefficient (K value) and the intrinsic rate of natural increase per day (r value) of control *M*. micrura were 0.144 and 0.377, respectively; these decreased to 0.121 and 0.364, in carbaryl and 0.073 and 0.356 in endosulfan treated animals respectively

Anjali *et al*. (1990) studied effect of sublethal does of three pesticides on the ovary of a carp minnow, *Rasbora daniconius* and reported that the exposure to endosifan, carbiofuran and methyl parathion produced several deleterious effects. The peritoneal lining was saveraly damaged on prolonged exposure to methyl parathion but endosulfan and carbofuran exposure did not have any effect.

Rastogi *et al*. (1990) reported sublethal doses of DDT and endoslfan pesticides caused necrosis and fibrosis of ovarian connective tissue, dilation of blood vessels, and decrease in oocyte diameter and increase in intrafollicular space in *Rasbora daniconius*.

Kirubagaran *et al*. (1990) demonstrated an increase in brain dopamine (DA) and norepinephrine (NE), and a decrease in serotonin (5-HT) and monoamine oxidase (MAO) in *C. batrachus* treated with emisan-6, a commercial fungicide.

Singh *et al*. (1991, 1992, 1993) studied extensively on the impact of gamma BHC to the steroid hormonal profiles of *Heteropneustes fossils* and correlated it to ovarian damage and showed that pesticides suppressed the levels of these hormones,

viz. testosterone (T) estradsol-17beta, 17alpha etc. and described that ovine LH releasing hormone and mystics gonadotropin has modulatory role in it.

Burdick *et al*. (1992) reported that the organophosphate pesticides cause reduction in reproductive efficiency in brown trout, *Salmo trutta* and breek charr, *Salvelinus fontinalis*.

Pavlov *et al*. (1992) studied the effect of DDVP, an organophosphorus insecticide on feeding behaviour and brain acetyl cholinesterase activity in bream, *Abramis brama* (L.) and reported that DDVP exposure resulted in decreased amount of food consumed and inhibited brain acetylcholinesterase activity.

Chirashree *et al*. (1992) described non lethal concentrations of pesticide impair ovarian function in the freshwater perch, *Anabas tastudineus*, and reported that decrease in GSI continued until the end of the exposure period, the decline in the GSI was highest on the 60^{th} day of exposure which coincided with the pre-spawning phase of the fish.

Sukumar *et al*. (1992) reported that the carbamates such as carbaryl and carbofuran induce degenerative change in fish *Colisa lalia* ovary.

Sharmistha *et al*. (1992) reported a drastic reduction in plasma vitellogenin following endosulfan treatment to *Clarias batrachus*.

Choudhury *et al*. (1993) observed a significant rise in estradrol-17B in the perch *Anabas testudineus* exposed to the organophosphate metacid-50 (0.00016mg/l) or carbaryl (1.7mg/l) for 15 days.

Kaur *et al*. (1993) found no effect on hatchability of eggs at very low concentration of carbaryl (0.05mg/l) carbofuran (0.01mg/l) and malathion (0.1mg/l). However higher concentrations (carbaryl 3-5mg/l, carbofuran 2-4mg/l, malathion 25-30mg/l and phosphandion 300-400mg/l) caused egg development to arrest prior to the closure of the blastopore and heavy mortality (>50%) occurred at this stage indicating the greater sensitivity of early embryonic stages to the pesticide.

Rodrigues (1994) reported that the organophosphorus contamination has been found in environments, elements of food chain and humans.

Srivastava *et al*. (1994) described increased oocyte atresia in chlordane exposed *H. fossilis*.

Read *et al*. (1995) reported that streams and rivers are generally the collecting environments for the pollutants such as agricultural pesticides run-off, Industrial discharges, and domestic waste water.

Chatterjee *et al*. (1997) reported that the stage I primary oocytes were predominantly higher in carbofuran-treated *H. fossilis* than the prevalent stage II and III oocytes in the control animals.

Pan (1998) studied the inhibition of brain acetylcholinesterase activity of juvenile largemouth bass *pterus salmoides* by sublethal concentrations of diazinon and reported that juvenile brain acctyhilinesterse activities were significantly inhibited by sublethal dozes of diazinon.

Jones *et al*. (1998) observed the effects of carbaryl, permethrin, 4-nonylphenol, and copper as muscarinic cholinergic receptors in brain surrogate and listed fish species and reported that down regulation of MchR occurred in all warm water species (fat-head minnow, surrogate razorback sucker, *bonytail chub*, Colorado squawfish), except Colorado squawfish, and at carbaryl concentrations similar to those causing down regulation observed in rainbow trout-Permethrin exposure resulted in down regulation in fathead minnow and razorback sucker, but the concentrations required for observation of this phenomenon were much greater than observed in cold water species (rain bow trout surrogate, apache trout, lanhan trout). Copper exposure caused a decrease in brain MChR in rainbow trout and apache trout.

Gruber *et al*. (1998) studied effects of organophosphate and carbonate insecticides in agricultural water and cholinesterase inhibition in common carp, *Cyprinus carpio communis* and reported that the depressed AChE activity in brain tissue. Neither sex nor size appears to be a covariable in the analysis.

Hazarika (1998) worked on the toxicological impact of different sublethal doses of BHC on the ovary of air-breathing catfish, *Heteropneustes fossils* (bloch) and investigated detailed histopathological changes.

Peezely (1998) described effects of a fungicide (VITAVAX 200EF) on the reproductive-endocrine functions in Mallards and observed ovarian changes and changing pattern of steroids level.

Oern *et al*. (1998) did extensive research of the impact on reproduction of an orally administered mixture of selected PCBS in zebra fish, *Danio rerio* and observed that egg production was reduced in all three groups exposed and intermediate and higher groups contained a reduced number of nature oocytes.

Oern *et al*. (1998) reported no differences in hatching frequency or medium hatching time were recorded in zebra fish, *Danio rerio*, which were orally exposed to a mixture of 20 PCB's in three different dose levels.

Ghosh *et al*. (1999) demonstrated that metacid 50 arrested gonadotropin-releasing hormone (GnRH) induced final oocyte maturation in freshwater perch *Anabas testudineus*.

Zagatto (1999) reported that the toxicity tests with embryos and larvae are valuable for assessing potential impacts on growth, reproduction and survival of organisms in polluted environments and are important tools for good environmental monitoring.

Golovanova *et al*. (1999) studied in vitro effects of cadmium and DDVP (dichlorovos) on intestinal carbohydrase and protease activity in fresh water teleosts and observed that the total amylolytic activity in borbot, crucian carp and common carp, Sucrase activity in blue bream and total proteolytic activity in burbot and pike were significantly decreased by cadmium at 50mg/l. DDVP (at 2mg/l) caused a significant decrease in total proteolytic activity in pike, but had no effect on either protease or carbohy- drase activities in other fish species.

Nathaniel *et al.* (2000) reported that the Diaznon disrupts anitipredator and homing behviours in Chinook salmon, *Oncorhynchus tshawytscha.*

Chuiko (2000) worked on comparative study of acetylcholinesterase and butyrylcholinesterase in brain and serum of several fresh water fish by DDVP, an organophosphorus pesticide-reported that brain acetylcholinesterase activity varied among fish species approximately 10-fold, ranging from 192.6 to 1353.2µ mol/g/h respectively in perch and white fish. All cyprinids had higher brain acetycholinesterase activity than these other fish families. Serum acetycholinesterase activity was 100-fold lower than in brain. Serum butyrlylcholinesterse activity was found only in cyprinids with the exception of the common carp. It varied from 163.8 to 970.3µ mol/g, respectively in roach and bleak.

Bhuiyan *et al*. (2001) studied the effects of Sumithion on the histological changes of spotted murrel, *Channa punctatus* (Bloch) and reported that in sumithion treated fishes rupture of blood vessel, pyknosis, mild necrosis and vacuolation of the liver.

Phillips *et al*. (2002) studied the acute toxicity and cholinesterare inhibition in larval and early juvenile walleye exposed to chlorpyrifos and reported that pro-larvae (yolk, sac, endogenous feeding stage) were least sensitive to chloropyrifos (median lethal concentration [LC 50] = 225-316µl) and post larvae I (oil globule, exogenous feeding stage) were less sensitive (LC50=12-13µg/l) than post larvae II (oil globule absent; LC50=12-13µg/l), juvenile fish were less sensitive that post larval stages, but did not differ significantly among the juvenile ages tested.

Erwin *et al*. (2003) studied the effects of chronic exposure of parathion M. acetylcholine esterase inhibition and increased food consumption rate in the zebra fish, *Danio rerio* and reported that inhibition rate was significant above 0.9 µ g/l after 144 days and above 4.3 µg/l after 250 days of exposure, while as survival, growth and reproduction were not affected the same extent as acetylcholine esterase activity.

Chciko *et al*. (2003) observed acetylcholine esterase and butyrylcholine esterase activities in brain and plasma of fresh water teleosts and reported that brain acetycholinesterase activity varied among fish species approximately 15-fold ranging

from 138 to 2011 µmol/g/ hr. All cyprinids had higher brain AChE activity than other fish families.

Tripathi *et al*. (2003) studied toxic effects of dimethoate (organophosphate) on the Metabolism and Enzyme system of fresh water teleost fish *Channa punctatus* and reported effect of dimethoate on carbohydrate and nitrogenous metabolism in muscle liver and gonad tissue. Besides, enzymatic activities such as acetycholinesterase lactate and lactic dehydrogenase activity was also found inhibited.

Kuz *et al.* (2003) studied the effects of adrenaline and piracetam on fish feeding behaviour and reported intraperitoneal administration of adrenaline causes an increase of the latent feeding period in goldfish and carp regardless of the season. Piracetam in summer has no effect on the rate of feeding response in carp and decrease the latent feeding period in goldfish, white in autumn it slightly decreases the latent feeding period in the former species and restores the active feeding in the latter.

Behra *et al.* (2003) studied on the use of zebra fish mutants to identify secondary target effects of acetylcholiesterase inhibitors and reported that the inhibitors block zebra fish AchE effectively at µ-nanomolar ranges.

Dutta *et al*. (2003) studied effects of endosulfan on brain acetylcholicsterare activity in juvenile blue gill sunfish and reported that based on exposure durations of 24, 48, 72 and 96h and 1 week at 1 µg/l, stepwise decreases in acetycholinesterase activity were noted, corresponding to 3.57%, 12.65%, 14.23%, 16.31% and 23.11% inhibition respectively. These changes in AChE activities will certainly affect the normal behaviour of the juvenile blue gill which is detrimental to their very existence in the natural habitat.

Scott *et al*. (2004) studied the effects of environmental pollutants on complex fish behaviour and reported that many toxicants disrupt complex fish behaviours such as predator avoidance, reproductive behaviour and social behaviours. Toxicant exposure often completely eliminates the performance of behaviours that are

essential to fitness and survival in natural ecosystem, frequently after exposures of lesser magnitudes than this causing significant mortality.

Chindah *et al*. (2004) evaluated the acute and sublethal toxicity of an organophosphate pesticide (chloropyrifos) of the juvenile of *Tilapia guineansis* and reported that operculum beat frequency and tail beat frequency were significantly affected by the exposure and progressive reduction in the no. of leucocytes and erythrocytes indicating that the fish has become anemic.

Chandra *et al*. (2004) observed retardation in the onset of first ovarian maturity in carbofuran treated common carp, *cyprinus carpio*.

Singh *et al*. (2004) recorded a significant reduction in circulating gonadotropin levels in response to hexachlorocyclohexane treatment in *H. fossilis*.

Adhikari *et al*. (2005) studied the effect of cypermethrin on breeding performances of a freshwater fish, *Labeo rohita* (Hamilton) and reported that the significant reduction ($P<0.01$) of total no. of eggs, total amount of egg (litre) total amount of egg (Litre per kilogram of body weight), fertilization percentage and expected fertilized egg number at the concentration of 0.40 and 0.80µl/l of cypermethrin. Also, the reduction in hatching percentage, expected no. of hatching and expected number of hatched larvae were significantly different ($P<0.01$) between the treatment and the control at all cypermethrin concentrations. No significant differences for the 96h survivability of hatched larvae were reported at 0.16 and 0.40µl /l levels of cypermethrin, where as significant difference ($P<0.05$) were reported at 0.80µl/l.

Auta *et al*. (2006) studied the sublethal effect of dimethoate on growth and food utilized of *Oreochromis niloticers* (Trewavas) and reported the growth rates were significantly reduced in fish exposed to sublethal concentration of pesticide, the food utilization showed that the control fish gave the best FCR, PER, APP, NPU, and PPV, all which were significantly higher ($p<0.05$) than the group of fish exposed to the toxicant. The study showed that the dimethoate caused various physiological disorders in the fish.

Vankateswara (2006) studied sublethal effects of profenofos on locomotive behaviour and gill architecture of the mosquito fish, *Gambusia affinis* and reported that the sublethal concentration of 13mg/L (1/5 of LC 50) altered locomotive behaviour such as distance traveled and swimming speed in expired fish due to inhibition in the activity of acetylcholine esterase and deformities in the primary and secondary lamella of gill.

Nevin *et al*. (2006) studied neurotoxicity evaluation of the organofluorine pesticide etoxazole in the brain of *Oreochromis niloticus* and reported that at the sublethal concentrations (.27, .54, .81, 108, 1.35mg/l) and exposure duration (1, 7, 15 days) tested, etoxazole has no inhibitory effect on the acetycholinesterase and sodium potassium activated adenosine triphosphatase activities.

Adhikari *et al*. (2006) worked out effect of cyper-methrin on breeding performances of a fresh water fish, *Labeo rohita* (Hamiltion) and reported that significant reduction ($P<0.01$) of the total no. of eggs, total amount of egg (litre), fertilization percentage and expected fertilized egg number at the concentration of 0.40 and 0.80µl/l of cypamethrin.

Campagma *et al*. (2006) studied the effect of dimethoate 40% on eggs and larvae of *Prochildus lineatus* and observed that the 48h LC50 for eggs is higher than 16.0µg/l, whereas for recently hatched larvae it was found to be significantly lower (11.81µg/l). Larval mobility was also found reduced by this insecticide.

Lal (2007) reported pesticide-induced reproductive disfunction in Indian fishes by lowering secretion of hormones such as gonado-tropin releasing hormone (GnRH), gonadotropin, growth hormone, adrenocorticotrophin hormone, testosterone, estrogen and thyroid hormones, leading to cessation of gametogenesis, vitellogenesis, oocyte maturation, ovulation, etc.

Rodrigo *et al*. (2007) studied effects of dicholorovos on the acetylcholinesterase from tambaqui, *Colossoma macropomum* brain and reported that the inhibitory effect of dichlorovos on AChE activities at concentrations 001 to

10ppm, the effect followed on exponential decay model (Y = 9.420+26.192e) (-X 15.380), r^2 = 0.989), presenting LC50 of 0.081ppm (0.368 µ mol/L).

Vivas *et al*. (2007) studied short-term effects of quirlan (R) (chlorfeminphos) on the behaviour and acetylcholinesterase activity of *Gambusia holbrooki*, and reported that the chemical showed high toxicity to *G. holbrooki* by significantly impairing all behavioral responses (location in the test vessel, activity excitability swimming and feeding), exhibiting a time dependent pattern. A strong inhibition of AChE was observed in fish exposed to chlorfeminphos (LC50 = 3.55mug L. Behavioural impairment was registered in fish with >40% AChE inhibition levels, while mortality was only observed in fish exhibiting AChE inhibition levels > 80%.

Salvo *et al*. (2008) studied the effects of endosulfan sublethal concentration on carp (*Cyprinus carpio*, Linnaeus, 1758): morphometries histologic, ultra structural analysis and cholinesterase activity valuation and reported that the hepatic somatic index (HSI) and the liver weight showed smaller values when compared with the control groups, besides the histopthological and ultra structural alterations also observed. No significant alteration in the cholinesterase activity of both brain and striated muscle has been observed.

Vineet *et al*. (2008) studied the behaviour and respiratory dysfunction as an index of malathion toxicity in the fresh water fish, *Labeo rohita* (Hamilton) and reported that the carp in toxic media exhibited irregular, erratic and darting, swimming movements, hyper excitability, and loss of equilibrium and sinking to the bottom which might be due to inactivation of AChE activity.

Khalid *et al*. (2008) studied the ethological response and haematological and biochemical profiles of carp, *cyprinus carpio* exposed to trichlorfos, and reported that the erythrocyte count haremoglobin concentration and haematocrit values increased whereas leukocyte count dropped down after trichlosfas exposure. A significant reduction in the acetylcholinesterase activity in the brain tissue of the fish exposed to tricholorfos was also registered.

Kristen *et al*. (2008) studied the effects of diazinon exposure in hybrid striped Bass on biochemical and behavioural aspects and reported that the sublethal exposure to diazinon, an organophosphate pesticide, may lead to feeding behaviour abnormalities in hybrid striped bass through inhibition of brain acetylcholine esterase activity.

Singh *et al*. (2009) studied acute toxicity and behavioural responses of common carp *Cyprinus carpio* (Linn.) to an organophosphate (dimethoate) and reported that test fish exhibited erratic swimming, increased surfacing, decreased rate of opercular movement, copious mucous secretion reduced agility and inability to maintain normal posture and balance with increasing exposure time.

Ismail *et al*. (2009) evaluated the acute toxicity of profenofos and its effects on the *cyprimus carpio*, and reported that the behavioral responses of fish exposed to profenofos included loss of balance, moving in spiral fashion with sudden jerky movements, lying on their sides and rapid flapping of the operculum with the mouth open.

Hanson *et al*. (2009) studied the uptake and toxicity of same pesticide on three fresh water fish *Oreochromus niloticus*, *Clarias gariepinus* and *Chrysicthys nigrodigitatus* and reported that the pesticides had adverse effects on the general growth and reproduction of fishes, gonadosomatic indices also showed that the pesticides affected the development of the body the gonads and their reproduction.

Weiss (2009) worked on the determination of cholinesterase in the brain tissue of three species of fresh water fish and its inactivation in vivo and reported that the pesticide treated fish showed abnormal behaviour including loss of balance staying motionless in group at bottom, lying laterally at bottom, swimming in spiral fashion with jerks revolving in water opened north and rapid opercular movements.

Chapter - 3

MATERIALS AND METHODS

3.1 TEST ORGANISMS

Females of common carp (*Cyprinus carpio conmunis*) were used as test organisms for several reasons.

- Ecotoxicological studies of common carps are of potentially great importance, as they have a wide distribution throughout India including Kashmir water bodies.
- They are designated as toxicity test fish by United States Environmental protections agency (U.S. EPA, 1979).
- *Cyprinus carpio communis* is a representative of an ecologically important group.
- It occupies a position within a food chain leading to man.
- It is widely available, amendable to laboratory tests, early maintained and genetically uniform, and
- There is an adequate background data on the organism (*Cyprinus carpio communis*) i.e. physiological, genetics, Taxonomy, Embryology etc.

The fish is voraciously omnivorous; efficiently converting the food ingested, into flesh, grows very fast and is prone to artificial feeds. It naturally breeds in confined waters, spawning occurs in shallow marginal weed infected areas. Breeding season is mid January to March and again July to August.

Fish for the present work were procured from the local market throughout the year 2009, for the experimentation of different parameters related to reproductive activities of the fish. Such as effect on GSI, ova diameter, ovary weight acetychlolinesterase activity in ovary and brain and like.

3.2 TOXICANTS (Test substances)

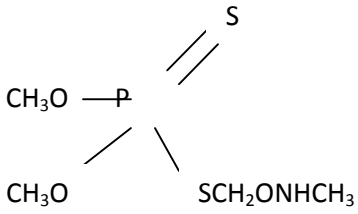
For the present study, Dimethoate (Rogor) and Dichlorvos (DDVP) were chosen as toxicants based partially on the probability of their having reproductive effects. These are employed routinely in the integrated farming practice to protect crops and animals from insects, weeds and diseases. The liberal use of these organophosphate pesticides at different stages of crop production, starting from seed processing to storage of agricultural produce is posing great danger to aquatic environment.

These organophosphate pesticides are more frequently used because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment.

IUPAC Name = dimethyl S- (Nmethyl. Carbanoyl methyl) phosphor-dithioate

Trade Name = Rogor, cygon, perfekthion etc.

Structural Formula =



Dimethoate

Molecular formula : $C_5H_{12}NO_3PS_2$

Molecular weight : 229.3 g/mol

Solubility in water : 90% (Mangle. G. 1987)

Vapour pressure : 98.0% (Tecter, D. 1988)

Dimethoate formulations are used to control a wide range of Acari, Aphididae, Aleyrodidae, Cleoptera, Diptera, Collembola, Lepidoptera and thysanoptera in cereals, citrus, coffee, cotton, fruit, grapes, pastures, potatoes, pulses and vegetables. They are also used for control of flies in animal houses. Dimethoate is a systemic

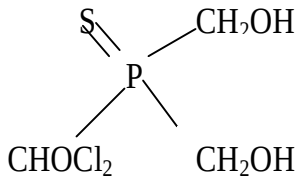
insecticide and a acaricide with contact and stomach action. It acts as a cholinesterase inhibitor (Tomlin 1997)

Dichlorvos: (DDVP)

IUPAC Name = DDVP (0,-o-dimethyl-0-2, 2-dichlorovinyl phosphate (USEPA, 2007)

Trade Name = Neuon

Structural Formula =



Molecular formula : $C_4H_7Cl_2O_4P$

Molecular weight : 220.98 g/mol

Solubility in water : 10 g/l at 20^0C

Vapour pressure : 20^0C 0.012 mmHg

Dichlorvos an organophosphate insecticide is used as an agricultural insecticide on crops, stored products, and animals. It is used as an insecticide for slow release on pest-strips for pest control in homes. It is also used as an antihelminthic (de-worming agent) for dogs, swine and horses as a botacide; agent that kills fly larvae (USEPA, 1994).

Detailed risk characterization of dichlorovos has been well documented in CEPA (1996), its toxicological profile in ATSDR (1997) and environmental assessment in APVMA (2008).

Dichlorvos specifically inhibits cholinesterase enzymes. It is poisonous if swallowed, inhaled or absorbed through the skin.

Atropine and pralidoxime are specific antidotes and artificial respiration may be needed (WHO, 1999, Gupta, 2006).

3.3 EXPERIMENTAL HABITAT

Fiber glass aquaria of the size 24"×12"×18" were set up in the ichthyology laboratory, Department of Zoology University of Kashmir. All the aquaria were of the capacity of 60 liters. Aquaria were provided with all the necessary equipments such as aerators, artificial light, and facial matter extraction tube water removing pipes to maintain the natural possible conditions for the test organism.

One aquarium was kept control and six aquaria contained fish exposed different sublethal concentration of the desired toxicant (test substances).

3.4 FISH TOXICITY TEST

The purpose of this test is to determine the sublethal toxicity of a substance to fish in fresh water. It is desirable to have, as far as possible information as water solubility, vapour pressure, chemical stability, dissociation constants and bio-degradability of the substance to help in the selection of the most appropriate test method (Static, semi-static and flow through) for ensuring satisfactorily constant concentrations of the test substance over the period of the test.

Additional information (for instance structural formula, degree of purity, nature and percentage of significant impurities, presence and amounts of additives and water pollution coefficient), should be taken into consideration in both the planning of the test and interpretation of the results.

3.5 DEFINITIONS AND UNITS

Acute toxicity is the discernible adverse effect induced in an organism within a short time (days) of exposure to a substance. In the present test, acute toxicity is expressed as the median lethal concentration (LC_{50}) that is the concentrations in water (test medium) which kills 50% of a test batch of fish within a continuous period of exposure. All concentrations of the test substance are given in weight by volume (milligram per liter).

3.6 PRINCIPLE OF THE TEST METHOD

The fish were exposed to the test substance added to water at a range of concentrations for a period of 96 hours. Mortalities were recorded at least at 24 hour intervals, and the concentration killing 50% of the fish (LC_{50}) at each observation time were calculated where possible.

Information on the test substance

It is necessary to know the water solubility of the substance under the conditions of the test. A reliable analytical method for the quantification of the substance in the test solution must also be available.

3.7 VALIDITY OF THE TEST

For a test to be valid the following conditions were fulfilled.

- The mortality in the control (s) was not allowed to exceed 10% (or one fish if less than ten are used) at the end of the test.
- Constant conditions were maintained as far as possible throughout the test.
- The dissolved oxygen concentration was at least 60 percent of the air saturation value through out the test.
- The concentration of the test substance was satisfactorily maintained and preferably it was at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentrations was greater than 20% results were mainly based on the measured concentration.

3.8 DESCRIPTION OF THE METHOD

Apparatus

Normal laboratory equipment and especially the following were employed:

a) Oxygen meter/equipment for determination of oxygen of H_2O
b) Equipment for determination of hardness of water.
c) Adequate apparatus for temperature control.
d) Tanks made of chemicals insert material and of a suitable capacity in relation to the recommended loading.

Holding of fish

All fish (test organism) were obtained and held in the laboratory for at least 20 days before they are used for testing. They were held in water of the quality used in the test for at least seven days immediately before testing and under the following condition.

Light : 12 to 16 hours photo period daily.

Temperature : Appropriate to the species.

For *Cyprinus carpio*, recommended test temperature range is 20-24^{o}C (OECD, Paris 1981)

Oxygen concentration : At least 80 percent of air saturation value

Feeding : Three times per week or daily until 24 hours before the test is started.

Following a 48-hour settling in period, mortalities were recorded and the following criteria applied.

- Mortalities of greater than 10% of population in seven days: regulation of entire batch.
- Mortalities between 5 and 10% of population: acclimatization continued for seven additional days.
- Mortalities of less than 5% of population: acceptance of batch.

3.9 WATER (Test medium)

- Good quality natural water or reconstituted water was preferred, although drinking water (dechlorinated if necessary) may also be used.
- Waters with total hardness of between 10 and 25mg $CaCO_3$ per liter, and with a pH 6.0 to 8.5 was preferable.

3.10 Test solution

- Test solutions of the chosen concentrations were prepared by dilutions of stock solution.

- Test solution of Dimethoate and Dicholorvos were prepared in water into the three different concentration of each as:

Dimethoate (Rogor)

LC_{50} = 1.70mg/l

50% of LC_{50} = 0.85 mg/l

70% of LC_{50} = 1.20mg/l

90% of LC_{50} = 1.53 mg/l

Dichlorvos (DDVP)

LC_{50} = 1.30mg/l

50% of LC_{50} = 0.65mg/l

70% of LC_{50} = 0.90mg/l

90% of LC_{50} = 1.17mg/l

- The test was carried out without adjustment of pH. If there was evidence of marked change in the pH of the tank water after dilution of the test substance, it is advisable that the test be repeated, adjusting the pH of the stock solution to that of the tank water before dilution of the test substance.
- This pH adjustment was made in such a way that the stock solution concentration was not changed to any significant extent and that no chemical reaction or precipitation of the test substance was caused HCl and NaOH are preferred.

3.11 PROCEDURE

Conditions of exposure

Duration	:	preferably 96 hours
Loading	:	Maximum loading of 1.0g fish/liter for static test was used.
Light	:	12 to 16 hours photo period daily.

Temperature	:	20-24^{0}C
Oxygen concentration	:	Not less 60% of the air saturation value. Aeration can be used provided that it does not lead to a significant loss of test substance.
Feeding	:	None
Distribution	:	Disturbances that would change the behavior of the fish were avoided

3.12 NUMBER OF FISH

At least 7 fishes were used at each concentration and in the controls.

3.13 TEST CONCENTRATION

At least 3 concentrations in geometric series with a factor preferably not exceeding 2.2 were used for the test substance (Dimethoate and dichlorvos). Dimethoate concentrations used in the present investigation were 50%, 70% and 90% of LC_{50} value (1.70mg/l) as 0.85mg/l, 1.20mg/l and 1.53mg/l respectively.

Dichlorovos concentration were prepared as 50%, 70% and 90% of the LC_{50} (1.30mg/l) as 0.65 mg/l, 0.90mg/l, 0.1.17mg/l respectively.

Observations

The fish were inspected at least after 24, 48, 72 and 96 hours. Fish were considered dead if there were no visible movement (e.g. gill movement) and if touching of the caudal peduncle produces no reaction.

- Dead fish were removed when observed and mortalities were recorded.
- Observations at three and six hours after the start of the test were done regularly.
- Records were kept of visible abnormalities (e.g. loss of equilibrium, swimming behaviour, respectively function, pigmentation etc).

- Measurement of the PH, dissolved oxygen temperature and hardness were carried out regularly.

Determination of some physico-chemical properties of water

Determination of some physico-chemical properties of test medium such as pH, temperature, light penetration, dissolved oxygen, total hardness as recommended by the OECD Guideline for testing of chemical (1992).

Physico-chemical properties test medium measured after APHA.

3.14 DISSOLVED OXYGEN

Dissolved oxygen by modified Winkler's method

Principle

Oxygen combines with manganous hydroxide to form higher hydroxide, which on acidification liberates iodine equivalent to that of oxygen fixed. This iodine is titrated by standard thiosulphate titrant using starch as indicator.

$M_nSO_4 + 2\ KOH \longrightarrow M_n(OH)_2 + K_2SO_4$

$M_n(OH)_2 + O \longrightarrow M_nO(OH)_2$

$M_nO(OH)_2 + 2H_2SO_4 + 2KI \longrightarrow MnSO_4 + K_2SO_4 + 3H_2O + I_2$

Requirement

Sodium thiosulphate titrant (0.025N), manganous sulphat solution, alkaline iodide, azide solution, starch indicator, concentrated sulphuric acid, narrow mouth 250 ml BOD bottles, measuring cylinder, burette stand.

Method

The following steps were followed:

1. Collected the sample (test medium) in a 250 ml narrow mouth glass bottle without bubbling.

2. Dispensed 2 ml each of manganous sulphate and alkaline lodide-azide solution, one after the other, right at the bottom of the bottle with separate pipettes.
3. Shaken the bottle upside down at lest 5 times and allowed the brown precipitate to settle.
4. Dissolved the precipitate by adding 2 ml of concentrated sulphuric acid and shaken the stoppered bottle.
5. Took the suitable aliquot (50ml) in flask and titrated with thiosulphate solution with swirling so that the colour changed to pale straw.
6. Added two drops of starch solution and titrated further till the blue colour disappeared.
7. Noted the total amount of titrant used in the process and calculate the dissolved oxygen content.

$$\text{DO in mg /l} = \frac{(8\times 10000\times N)V}{V}$$

Where V = Volume of sample

V = Volume of titrant used

N = Normality of titrant

Result = DO mg/l

3.15 TOTAL HARDNESS

Total hardness in waters is the sum of the concentrations of metallic cations present in it. In most fresh waters nearly all of the hardness is imparted by the calcium and magnesium ions.

According to the OECD, 1992 guidelines for fish toxicity tests, the test medium (water) with total hardness of between 10 mg and 250 mg $CaCO_3$ per liter is preferred. The total hardness of the test medium used (tap water) was determined by following method.

Requirement

- Standard EDTA titrant (0.01M)
- Erichrome black T indicator,
- Ammonia buffer, Erlenmeyer flask,
- Burette, stand, measuring cylinder.

Method

Take 50 ml sample in flask add 1ml of ammonia buffer and 5 drops of indicator solution

↓

The colour of the sample turns wine red

↓

Titrate with EDTA solution until clear blue colour appears.

↓

Note readings and calculate total hardness as mg/l of $CaCO_3$.

Calculation

Total hardness as mg/l $CaCO_3$ = ml of titrant used × 1000 ml of sample

Result: Total hardness as mg/l of $CaCO_3$

3.16 HYDROGEN ION CONCENTRATION (pH)

Chemical properties of the water not only alter the physical properties of the medium but also have significant bearing on the distribution and metabolic activities of the life forms, these in turn tend change them in due course of time.

pH is the measure of the relative acidity or alkalinity of water and represents the negative logarithm of hydrogen ion concentration in it.

$pH = \log 10 (H^+)$

$= \log 10 (H^+)$

$(H^+) = \text{Log } 10*1/H^+$

Electrometric Method

Activity of hydrogen ions in a solution is measured as the difference in e.m.f of glass electrodes with that of a calomel reference electrode over a scale calibrated directly in pH units.

According to OECD, (1992) guidelines for fish toxicity test, the test medium (water) with a pH of 6.0 to 8.5 are preferable.

Requirement

pH meter, combined electrode, buffer solutions of pH 4.0 and 9.2.

3.17 WATER TEMPERATURE

The temperature of water in each aquaria was measured by a good grade mercury thermometer ranging from 0^oC to 50^oC.

Temperature of the test medium ranged from 20-24^oC. In summer (June-August) temperature was maintained at the range (20-24^oC) by using electronic fans.

3.18 EXPERIMENTAL FISH

Healthy female *Cyprinus carpio communis* were obtained from Hazratbal Market, Kashmir and brought to the laboratory in plastic buckets with sufficient air. The plastic buckets were opened and the fish specimens were shifted to the glass aquaria for 20 days to be acclimatized and to eliminate transport-induced stress and allow for capture induced mortalities prior to pesticide exposure.

The specimens were about 09 ± 1.05 cm in length and 50±1.02 gm in weight. New supplies of fish were obtained monthly so that the fish material was seldom kept in the laboratory aquaria longer than one month.

3.19 ADMINISTRATIONS OF PESTICIDES

The common organophosphate pesticides used for this study were dimethoate (Rogor) and dichlorvos (DDVP) 'Neon' pesticides were administered to glass aquaria containing the experimental fish (*Cyprinus carpio communis*). The pesticides were mixed thoroughly with test medium by glass rod without disturbing the test animal. Thus, the pesticides were imbibed via the gastrointestinal tract and the surface of gills and skin of the experimental fish.

3.20 DETERMINATION OF LC_{50}

Toxicity was determined by renewal of static bioassay. All experiments were conducted in 5 rectangular glass aquaria ($24^{"} * 12^{"} * 18^{"}$) containing 36 liters of dechlorinated water to which volumes of dimethoate and dichlorovos was added (into two separate experimental designs) to achieve different concentrations of the toxicant. Thirty minutes after preparation of test solution, 10 experimental fishes were carefully placed into each replicate tanks of 5 different concentrations of each pesticide as (0.5mg/l, 0.8mg/l, 1.5mg/l, 1.8mg/l and control 0.00mg/l) for dimethoate and 0.2mg/l, 0.5mg, 0.8mg/l, 1.00mg/l and control 0.00,g/l in case of dichlorvos (DDVP) was used.

All experiments were conducted at room temperature and the tanks aerated. Fish were not fed during the experiment (Reish and Oshida 1986). Observations were recorded every 12 hours, number of dead fishes were removed. Experiment lasted for 96 hours for the different concentrations of dimethoate and dichlorovos (DDVP). The susceptibility of fish to pesticide was determined using probit log method of Finney and Stevens (1948) for LC_{50} at 96 hours. From the results of acute toxicity, sublethal concentration as 50%, 70% and 90% of the LC_{50} value were prepared for both pesticides.

Dimethoate (Rogor)

LC50 = 1.70mg/l

5o% of LC50 = 0.85 mg/l

70% of LC50 = 1.20mg/l

90% of LC50 = 1.53 mg/l

Dichlorvos (DDVP)

LC50 = 1.30mg/l

50% of LC50 = 0.65mg/l

70% of LC50 = 0.90mg/l

90% of LC50 = 1.17mg/l

Ten glass aquaria were used with 3 replicates per treatment and with same conditions as in acute toxicity. Percentage morality of *Cyprinus carpio communis* exposed for several hours of exposure to different sublethal concentrations of dimethoate and dicholorvos were recorded.

3.21 Method for the Determination of acetylcholinesterase activity in Brain and ovary of the Fish

Acetylcholinestrease (AChE) activity is a well established biomarker of exposure to organophosphate compounds in fish. AChE is present in cholinergic nerves and is responsible for the degradation of the neurotransmitter acetylcholine. Organophosphate binds irreversibly to the esteric site of AChE and thereby rapidly inhibits its activity. As AChE activity has a very high sensitivity to organophosphrous compounds, it has been considered to be a specific biomarker for these (Thomas, 1990)

The brain of the fish was selected for assay of cholinesterase (AChE) since it provided a relatively large amount of the specific AChE in a readily accessible tissue (Mendal and Rudnely, 1943).

The fish were divided into seven groups (7 in each) and kept in 36 liter glass aquaria containing chlorine free tap water of pH 7.2; hardness, 154 mg/l (as CaCo3); dissolved oxygen, 7.4 mg/l (APHA). The untreated group-I served as control. The group-II, group-III and group-IV were exposed to a toxicologically safe concentration 0.85, 1.20 and 1.53 mg/l respectively of commercial formulation of dimethoate (the

highest concentrations of pesticide) which does not produce any apparent harmful effect in 96 hour of exposure has been termed the toxicologically safe concentration (Mount and Stephan, 1967).

For the test of dichlorovos, four groups were established, group-I was untreated and served as control. Group-II, group-III and group-IV were exposed to commercial formulation of 0.65, 0.90 and1.17 of dichlorvos for 96 hours.

The fish brains were recurred by cutting off the top of the skill and shipping the brain loose at the optic nerves and base of the medulla. This permitted lifting the brain free of the skill the wet weights of the brains were determined by weighing on small pieces of tarred aluminum foil on sensitive balance. The weighed brain was then transferred to a tissue solutions of the following composition; 0.2M NaCl, 0.02M $MgCl_2$, 0.194M K_2HPO_4 and 0.006M KH_2PO_4 with the pH adjusted to 8.2.

After draining and rinsing from homogenizer, the brie was diluted with additional buffer to contain approximately 1-4 mg of brain tissue per ml of dilution.

One milliliter of the diluted brain brie was pipette into a test tube and incubated for 20 minutes at 25^0C with 1ml of 0.004 acetylcholine iodide (sigma chemicals) prepared in 0.001M sodium acetate of pH 4.5.

For each fish, (7 from each group), whole brain AChE activity was arrayed in triplicate according to procedure of Ellman *et al.* (1961).

(A spectrophotometer method for determining acetylcholinesterase activity of tissue extracts homogenates, cell suspensions etc. the activity is measured by following the increase of yellow color produced when the thiodmion produced by enzymatic hydrolysis of the substrate (acetylthiocholine) reacts with DTNB).

Arrays were conducted at 25^0C change in absorbance at 405 nm was measured at 30-8 intervals for 2-3 min. using a Gilfor spectrophotometer. Acetylchilinesterase activities are expressed as µmol acetylcholinecodide hydrolysed/min/g of tissue.

Acetycholinesterase Activity in Ovary of *Cyprinus carpio communis*

The specimens were sacrificed by decapitation from both treated and untreated groups separately to test the effect of dimethoate and dichilorvos on acetylcholinnesterase activity in ovary of fish. The ovaries were removed and weight was determined on small pieces of tared aluminum foil using sensitive balance.

The weighed ovary was then transferred to a tissue homogenser and brie prepared in 8.12 ml of buffer solution of the following composition; 0.2M NaCl, 0.2M $MgCl_2$, 0.14M K_2HPO_4 and 0.006M $KH_2\ PO_4$ with the pH adjusted to 8.2. After draining and rinsing from homogenizer the brie was diluted with additional buffer to contain approximately 4-10mg pf ovary tissue per ml of solution.

Acetycholine esterase activity was expressed as:

µmol acetylcholinecodide hydrolyzed/min/g of tissue.

3.22 EFFECT ON BODY WEIGHT

Immature fish, *Cyprinus carpio communis* (10±2g, size, 6±0.2) were collected from the Hazratbal market, Kashmir from a local fisherman and brought to the ichthyology laboratory of P.G. Department. Of Zoology, University of Kashmir, in plastic buckets with sufficient air. The plastic buckets were opened and the fish specimens were shifted to the glass aquaria for about 15 days to be acclimatized and to eliminate transport induced stress and allow for capture induced mortalities prior to pesticide exposure (OECD recommendation).

The fish were divided into seven groups and were kept in seven glass aquaria each containing 36 liter of chlorine free water with pH 7.2; hardness 154mg/l (as CaCo3); dissolved oxygen 7.4mg/l measured after (APHA). The untreated group-I served as control the group-II, III and IV were exposed to three different concentrations of stock solution of dimethoate as 0.85mg/l,1.20mg/l and 1.53mg/l. while as groups V, VI and VII were treated with three different concentrations as 0.65mg/l,0.90mg/l and 1.17mg/l of DDVP (Dichlorvos) respectively.

The water of aquaria with the pesticides were changed every alternate day. The duration of the exposure of fish to pesticide was in group II, III, IV, V, VI and VII was from 5th March to10th May 2009.

For the determination of changes in body weight of the fish, each fish was taken out from all the groups separates and body weight was taken at different intervals according to the method adopted by Singh (1987) and others. Body weight was measured by putting individual specimens from all the groups one by one in wide mouthed jar containing half filled water. The reading was taken on digital electronic balance as follows:

Weight of fish = weight of Jar with water - weight of jar with water containing fish.

Mean, S.D and ANOVA was used to determine the statistical significance of the data.

3.23 EFFECT OF TOXICANTS ON GSI: (Gonado somatic Index)

Healthy adult female *cyprinus carpio communis,* weighing 90 ±1.6gm and measuring 15 ±1.2cm in length, used in this investigation, were brought from the local fish market at Hazratbal, Kashmir during the months of November, December (2008) and from January to July (2009) and acclimatized to laboratory conditions for 15 days before starting the experiments every month.

The fish lots were divided into seven equal groups and kept in seven 36 liter glass aquaria containing chlorine free tap water of pH 7.2 hardness 154mg/l (as $CaCO_3$); dissolved oxygen, 7.4mg/liters and temperature 8-14^{0}C.

The untreated group-I served as control. The group-II, III and IV were treated with different concentration of test substance dimethoate as (o.85mg/l, 1.20mg/l and 1.53mg/l). The groups V, VI, and VII were treated with different concentration of test substance dichlorvos (0.65mg/l, 0.90mg/l and 1.17mg/l). The aquaria water with the pesticides was changed every alternate day after feeding the fish with commercial fish feed.

The experiment was started in the month of December when the fishes were in resting phase and ended after continuous exposure up to the month of July, when the gonads of the experimental fish were in spawning phase. The aquaria were kept in natural light and temperature conditions. The approximate average monthly water temperatures from November to July were 7±1.0^{0}C, 6±0.5^{0}C, 6.5±1.6^{0}C, 7±2.4^{0}C, 14±2.9^{0}C, 18±1.9^{0}C, 22±1.5^{0}C, 24±2.1^{0}C and 24±1.40C respectively. At the end of the

experiment, specimens were sacrificed by decapitation and the required tissues were removed and processed for the following investigations.

GSI (Ganado Somatic Index)

The gonado somatic index was calculated using the formula.

GSI = (weight of gonad /weight of fish) ×100

ANOVA was used to determine the statistical significance of the data.

3.24 OVARY WEIGHT

The ovaries were made free from the adjoining tissues and traces of extraneous fluid and then weighed on a balance sensitive to 0.1mg. The observed results were recorded separately for the control as well for dimethoate and dichlorovos exposed groups for all concentration and presented in the tables 4 and 5 respectively for both the test substances.

3.25 OVA DIAMETER

Ova diameter is the diameter of the eggs from the three different (Anterior, middle and posterior) parts of the ovary. The diameter of 100 ova from the samples of each ovary of the fish species from different groups(Control and pesticide treated groups) was measured using stercospic microscope fitted within an ocular micrometer 100 and 400 magnification. The means of the measurements from the three regions was taken as the standard diameter of the ova of the particular fish.

Chapter - 4

RESULTS

The effects of pesticidal contamination of wildlife habitats may be expected to be proportional to the toxicity of the compounds, the rate and manner of application, persistence of the basic chemical and/ or any toxic metabolites, and the extent to which these substances are stored in animal tissues or concentrated by successive elements of wildlife food chain. Measurement of these effects under field conditions is difficult, but the need for field studies may be reduced or eliminated by controlled laboratory tests. An attempt has been made in the present investigation to demonstrate influences of organophosphate pesticides (Dimethoate and Dichlorvos) on the reproductive organs of female common carp, *Cyprinus carpio communis.*

4.1 PHYSIO-CHEMICAL PARAMETERS

Environmental parameters have a great role in effecting the toxicity of different pesticides. The major parameters are temperature, dissolved oxygen, pH, hardness, alkalinity etc.

The water quality parameters (Temperature, Dissolved oxygen, pH and Total Hardness) monitored during the exposure period did not differ within various concentrations of test substances (Dimethoate and Dichlorvos) as well as with control. pH and alkalinity values tended to increase with increasing concentrations of

the test chemicals, however, values between treatments were not significantly different (ANOVA, P> 0.05). Temperature and dissolved oxygen values were almost uniform all through the study irrespective of the treatment. Similarly, total hardness values exhibited minimal variation in values, which were statistically not significant within and between treatments (ANOVA, P> 0.05). The limited variation in the physicochemical variables is similar to the trend observed in the wild which is tolerated by the test organism even in the wild. Table 1 and Table 2 depict mean concentration (mg/litter), range and standard deviation of physicochemical parameters for control, Dimethoate and dichlorvos treated tanks.

Table 1: Mean concentration (mg/l), range and standard deviation of physicochemical parameters for dimethoate treated tanks.

Conc. of dimethoate mg/l.	**pH**	**Temp. (^{o}C)**	**DO(mg/l)**	**Alkalinity**
0	6.73±0.15 (6.7-7.0)	24±0.20 (23.9-24.10)	4.15±0.32 (3.5-4.1)	70.5±7.3 (64.8-78.8)
0.85	6.78±0.15 (6.9-7.1)	24±0.15 (24.0-24.50)	3.98±0.32 (3.75-4.42)	73.7±5.9 (64.05-79.16)
1.20	6.80±0.12 (6.6-7.0)	23±0.19 (24.10-24.18)	3.96±0.45 (3.59-4.62)	76.4±7.2 (66.91-82.15)
1.53	7.02±0.06 (7.0-7.1)	24±0.15 (24.15-24.10)	3.94±0.40 (3.62-4.61)	80±8.5 (70.18-80.20)

Table 2: Mean concentration (mg/l), range and standard deviation of physicochemical parameters for Dichlorvos treated tanks.

Conc. of dichlorvos mg/l.	pH	Temp. (^{o}C)	DO(mg/l)	Alkalinity
0	6.63±0.12 (6.5-7.2)	24±0.20 (23.9-24.10)	4.14±0.30 (3.2-4.5)	72.5±7.3 (66.8-78.8)
0.65	5.98±0.15 (6.5-6.9)	24±0.15 (24.0-24.50)	4.0±0.32 (3.85-4.50)	75.7±5.9 (69.05-79.16)
0.90	6.85±0.15 (7.0-7.5)	23±0.19 (24.10-24.18)	3.90±0.45 (3.70-4.62)	78.4±7.2 (68.91-82.15)
1.17	7.0±0.17 (7.0-7.1)	24±0.15 (24.15-24.10)	3.98±0.40 (3.72-4.61)	80.5±8.5 (71.18-80.20)

4.2 Effects of different concentrations test substances, Dimethoate and Dichlorvos (Organophosphate pesticides) on survivability of test animal, *Cyprinus carpio communis*

In the present experiment, no mortality (100% survivability) was observed in the control group; however mortality increased as concentration and treatment time increased. Data on survivability of *Cyprinus carpio communis* to Dimethoate (Ragor) and dichlorvos (Neuon) is shown in the table-3 and table-4 respectively. 2.5% mortality was recorded in 0.85 mg/l (50% of LC_{50}) concentration tank at 24 hours, this value increased to 15% in 48 hours, and 35% in 96 hours.

In 1.20 mg/l (70% of LC_{50}) concentration tank 10% mortality at 24 hours, 24% in 48 hours and 40% in 96 hours exposure was reported in group-II and 16% mortality was recorded in1.53 mg/l (90% of LC_{50}) for 96 hours concentration tank (group-III) at 24 hours. This value increased to 30% in 48 hours and 47% in 96 hours exposure.

Similarly, 8% mortality was recorded in 0.65mg/l (50% of LC_{50}) concentration of Dichlorvos (DDVP) at 24 hours in group-VI, 19% at 48 hours and 30% at 96 hours exposure of the same concentration

This value increased to 15% mortality in0.90mg/l (70% of LC_{50}) DDVP at 24 hours, 30% at 48 hours and 43% at 96 hours in group-IV and mortality rate was observed to increase 18% in 1.17mg/l (90% of LC_{50}) at 24 hours, 32% at 48 hours and 47% at 96 hours exposure in group-VII. Thus the mortality was dose and pesticide treatment time dependant.

Table 3: Effects of different sub lethal concentrations of Dimethoate on survivability of *Cyprinus carpio communis*.

Percentage of Mortality in hours								
Name of group	**Name of pesticide used**	**Concentration used (mg/l)**	**Number of fishes treated**	**Time duration (Hours)**			**Percentage of fishes survived after 96 hours**	**Percentage of mortality after 96 hours**
				24	48	96		
Control		0.00	10	0	0	0	100%	0%
Group-I	Dimethoate	0.85	10	5%	15%	25%	75%	25%
Group-II	Dimethoate	1.20	10	10%	24%	40%	60%	40%
Group-III	Dimethoate	1.53	10	16%	30%	47%	53%	47%

Table 4: Effects of different sub lethal concentrations of Dichlorvos on survivability of *Cyprinus carpio communis*.

Percentage of Mortality in hours								
Name of group	**Name of pesticide used**	**Concentration used (mg/l)**	**Number of fishes treated**	**Time duration (Hours)**			**Percentage of fishes survived after 96 hours**	**Percentage of mortality after 96 hours**
				24	**48**	**96**		
Control		0.00	10	0	0	0	100%	0%
Group-IV	Dichlorvos (DDVP)	0.65	10	8%	19%	30%	70%	30%
Group-V	Dichlorvos (DDVP)	0.90	10	15%	30%	43%	57%	43%
Group-VI	Dichlorvos (DDVP)	1.17	10	18%	32%	47%	53%	47%

Table 3 and Table 4: Percentage mortality of *Cyprinus carpio communis* exposed for several hours of exposure to different sub lethal concentrations of Dimethoate and Dichlorvos.

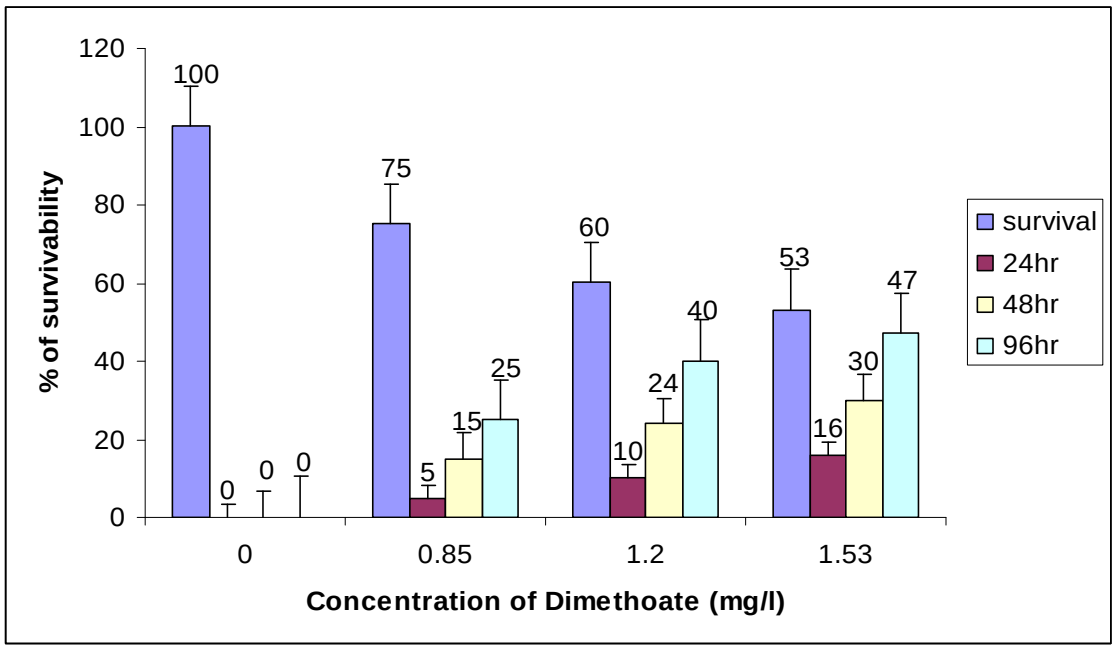


Fig. 1: The histogram shows the effects of different sub lethal concentrations of Dimethoate on survivability rate (percent) of *Cyprinus carpio communis* at different time intervals.

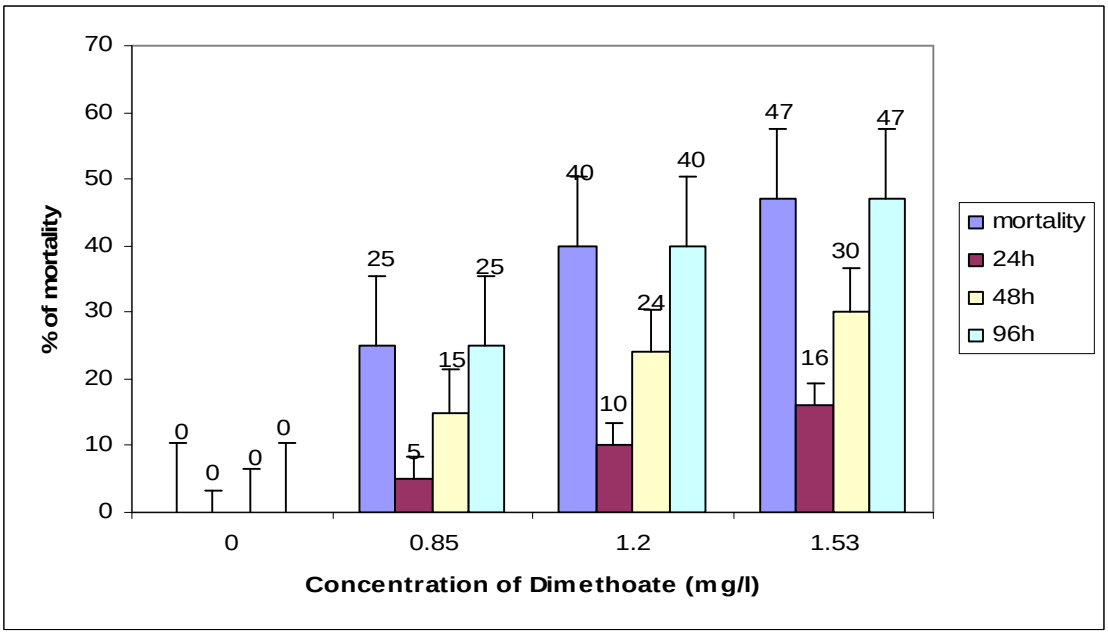


Fig. 2: The histogram shows the effects of different sub lethal concentrations of Dimethoate on mortality rate (percent) of *Cyprinus carpio communis* at different time intervals.

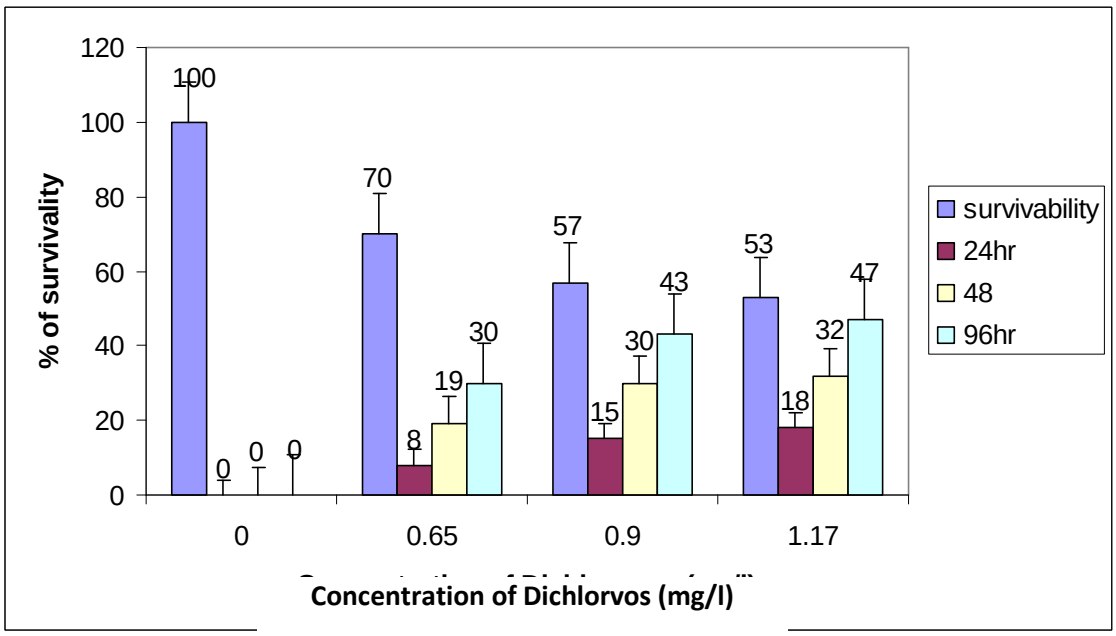


Fig. 3: The histogram shows the effects of different sub lethal concentrations of Dichlorvos on survivability rate (percent) of *Cyprinus carpio communis* at different time intervals.

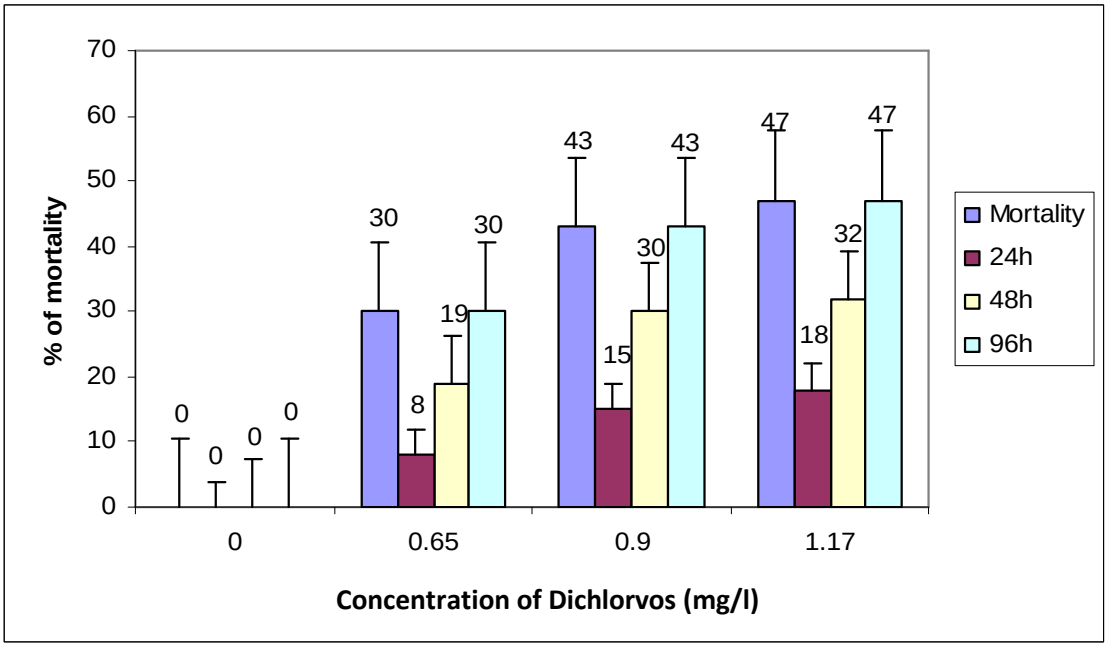


Fig. 4: The histogram shows the effects of different sub lethal concentrations of Dichlorvos on mortality rate (percent) of *Cyprinus carpio communis* at different time intervals.

4.3 Effects of different concentrations test substances, Dimethoate and Dichlorvos (Organophosphate pesticides) body weight of test animal, *Cyprinus carpio communis*

The body weight of Dimethoate and dichlorvos (organophosphates) exposed fish showed a slight but progressive decrease in time course when compared with normal fish. The tables 5 & 6 show the data on effects of test substances Dimethoate and dichlorvos on changes in the body weight of the control and exposed fish respectively. In the present experiment net weight gain was observed in control fish (untreated) as compared to the pesticide treated fish, and increased weight loss in increased pesticide concentration which is depicted in fig.5& fig.6 for different concentrations Dimethoate and dichlorvos respectively.

Table 5: Analysis by mean and standard deviation showing body weight in grams in control and Dimethoate treated groups of *Cyprinus carpio communis.*

Test Animal Groups	**Body Weight in Grams**	
	Initial Weight	**Final Weight**
Control	50±0.45	55.84±0.93
Dimethoate (0.85mg/l) Group II	50±0.45	48.37±0.78
Dimethoate (1.20mg/l) Group III	50±0.45	47.32±0.75
Dimethoate (1.53mg/l) Group IV	50±0.45	45±0.70

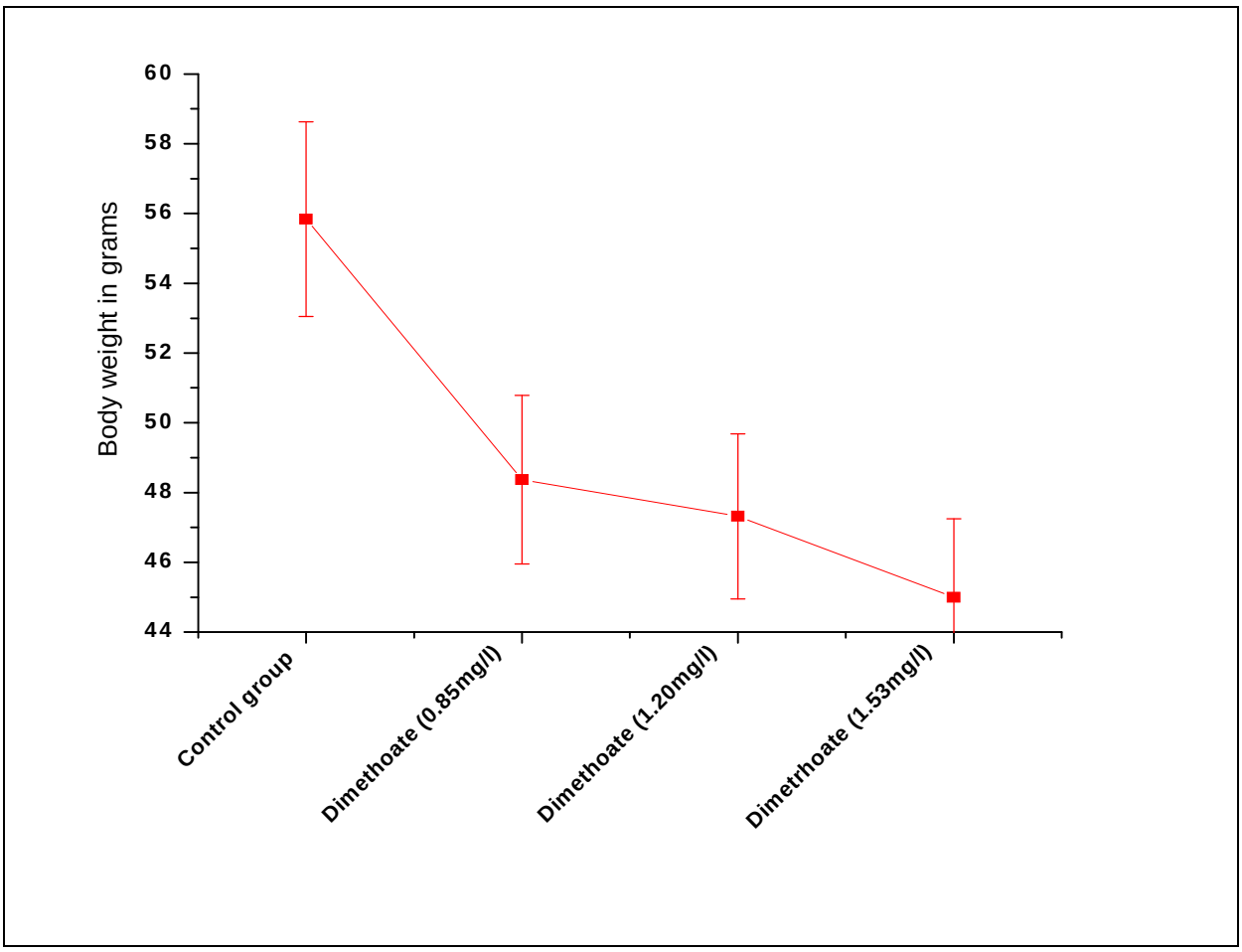


Fig. 5: Diagrammatic representation of the quantitative values (mean±SD in graphs) of changes in Body weight of female common carp after exposure of different concentrations of dimethoate, an organophosphate pesticide.

Table 6: Analysis by mean and standard deviation showing body weight in grams in control and Dichlorvos treated groups of *Cyprinus carpio communis.*

Test Animal Groups	**Body Weight in Grams**	
	Initial Weight	**Final Weight**
Control	50±0.45	55.84±0.93
Dichlorvos (0.65mg/l) Group V	50±0.45	48.44±0.61
Dichlorvos (0.90mg/l) Group VI	50±0.45	45.78±1.03
Dichlorvos (1.17mg/l) Group VII	50±0.45	44.21±1.12

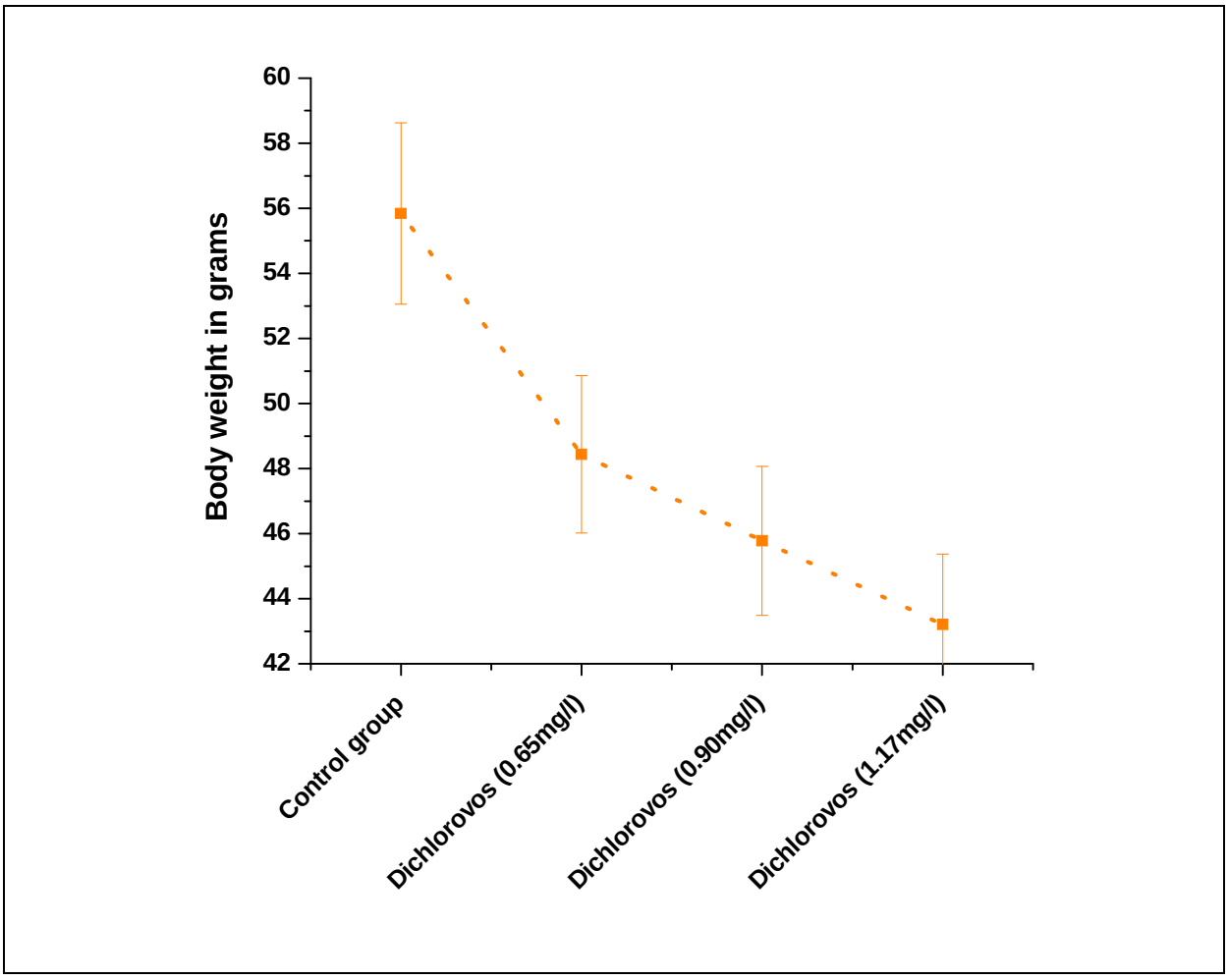


Fig. 6: Diagrammatic representation of the quantitative values (mean±SD in graphs) of changes in body weight of female common carp after exposure of different concentrations of dichlorvos.

4.4 Effects of different concentrations test substances, Dimethoate and Dichlorvos (Organophosphate pesticides) on ovary weight of test animal, *Cyprinus carpio communis*

In the present investigation there was found no significant change in the ovarian weight until 10 days of the pesticide treatment. The decrease in the weight of ovary was observed only on day 20 which continue until the end of the exposure period. The decline in the ovarian weight was highest on the 60^{th} day of exposure which coincided with the prespawning phase of the experimental fish. With the dimethoate treatment the ovarian weight did not deviate from the control value until after about 15 days of exposure. However, from 20^{th} day onwards until the termination of the experiment ovarian weight varied significantly from the respective control value and weight decreased with increased concentration of the pesticide and exposure time.

Similarly, with dichlorvos treatment there was significant deviation from the control value on increasing the exposure time and concentration of the pesticide. The following tables 5 &6, shows the deviation of ovary weight from control values in pesticide treated fish.

Table 7: Analysis by mean and standard deviation showing ovary weight in grams in control and dimethoate treated groups of *Cyprinus carpio communis.*

Ovary weight in grams				
Duration of pesticide exposure (Days)	**Control group**	**Dimethoate 0.85 mg/l**	**Dimethoate 1.20 mg/l**	**Dimethoate 1.53 mg/l**
5	15.51±2.34	14.00±2.9	13.5±2.7	11.5±1.2
10	17.34±3.5	12.53±2.9	10.01±2.4	7.2±1.9
15	20.48±2.5	15.24±2.5	12.17±2.4	8.54±2.9
30	25.75±4.5	20.98±2.8	14.74±2.7	9.73±3.01
45	28.34±3.5	22.39±1.7	18.49±2.4	12.82±2.1
60	32.94±2.5	24.63±5.2	20.73±1.6	16.74±3.4

Table 8: Analysis by mean and standard deviation showing ovary weight in grams in control and dichlorvos treated groups of *Cyprinus carpio communis.*

Ovary weight in grams				
Duration of pesticide exposure (Days)	**Control group**	**Dichlorvos 0.65 mg/l**	**Dichlorvos 0.90 mg/l**	**Dichlorvos 1.17 mg/l**
5	15.51±2.34	13.5±2.3	11.05±1.9	9.57±2.4
10	17.34±3.5	12.01±1.3	7.06±1.09	4.07±0.4
15	20.48±2.5	13.26±1.7	9.35±1.1	7.16±1.9
30	25.75±4.5	18.72±1.5	12.24±1.7	8.24±1.7
45	28.34±3.5	19.47±1.2	15.74±3.5	9.23±0.9
60	32.94±2.5	21.7±1.7	16.25±1.9	9.65±1.8

4.5 Effects of different concentrations test substances, Dimethoate and Dichlorvos (Organophosphate pesticides) on ova diameter of test animal, *Cyprinus carpio communis*

In the present investigation sub lethal doses of the Dimethoate and dichlorvos organophosphate pesticides produced various effects on the oocytes of different types. The diameter of the oocytes decreased with progressive duration of the pesticide exposure as compared to controls. In 5 days exposure no significant change in ova diameter was observed in the immature oocytes but subsequent exposure produced significant changes. The percentage of immature oocytes increased while that of mature oocytes decreased from 0 to 60 days of exposure. The maturing oocytes underwent slight reduction in size and deformity in shape in all treated groups. The yolk vesicles where damaged and interfollicular spaces increased.

Table 9: Analysis by mean and standard deviation showing the egg diameter in control and Dimethoate treated ovaries of *Cyprinus carpio communis*.

Duration of pesticide exposure (Days)	Oocyte maturity	Egg diameter in microns			
		Control	Dimethoate 0.85 mg/l	Dimethoate 1.20 mg/l	Dimethoate 1.53 mg/l
5	Immature	66.75±5.30	40.30±6.35	41.2±7.23	48±8.25
10	Immature	70.87±7.80	52.88±4.50	37.4±6.61	30.25±5.51
	Maturing	157.57±25.5	110.61±12.0	105.25±9.25	100.75±6.78
15	Immature	78.68±7.24	50.74±8.24	43.28±5.44	39.64±3.45
	Maturing	160.05±45.36	115.64±20.65	110.25±13.5	105.26±8.25
	Mature	268.53±36.78	150.78±24.6	145.24±23.7	130.78±16.5
30	Immature	75.62±26.27	60.78±21.02	55.74±28.3	48.24±12.6
	Maturing	125.24±22.4	116.24±22.4	110.24±2.54	95.24±22.4
	Mature	305.30±24.5	280.78±22.4	265.24±21.4	240.5±16.50
45	Maturing	140.70±3.45	135.68±22.5	125.5±14.50	105.28±22.5
	Mature	380.70±22.4	330.78±22.0	310.75±22.5	305.5±15.5
60	Maturing	170.78±22.5	150.5±22.5	130.78±22.5	120.78±21.5
	Mature	480.5±45.4	450.78±24.5	430.64±9.08	405.74±6.05

Table 10: Analysis by mean and standard deviation showing the egg diameter in control and DDVP treated ovaries of *Cyprinus carpio communis*.

Duration of pesticide exposure (Days)	Oocyte maturity	Egg Diameter in Microns			
		Control	Dichlorvos (DDVP) 0.65 mg/l	(Dichlorvos) DDVP 0.90 mg/l	(Dichlorvos) DDVP 1.17 mg/l
5	Immature	66.75±5.30	39.10±4.31	43.35±5.32	49.65±8.25
10	Immature	70.87±7.80	48.78±3.75	40.66±8.89	35.64±7.70
	Maturing	157.57±25.5	105.78±22.5	100.68±13.69	90.59±6.39
15	Immature	78.68±7.24	47.24±5.39	43.24±6.8	39.22±7.80
	Maturing	160.05±45.36	110.78±24.5	102.78±13.6	98.34±12.5
	Mature	268.53±36.78	145.24±6.25	140.78±3.45	130.24±6.65
30	Immature	75.62±26.27	55.24±16.5	50.24±22.4	47.34±10.25
	Maturing	125.24±22.4	100.24±24.5	90.24±16.50	75.22±41.00
	Mature	305.30±24.5	270.45±22.4	250.40±16.50	225.45±25.5
45	Maturing	140.70±3.45	130.33±22.4	120.34±21.50	100.25±21.5
	Mature	380.70±22.4	320.5±25.4	300.76±33.5	280.5±2.20
60	Maturing	170.78±22.5	140.6±22.5	120.50±22.1	100.5±22.5
	Mature	480.5±45.4	440.24±2.5	405.78±24.5	380.74±22.5

4.6 Effects of different concentrations test substances, Dimethoate and Dichlorvos (Organophosphate pesticides) on Gonado Somatic Index of test animal, *Cyprinus carpio communis*

Cyprinus carpio communis breeds almost throughout the year with peak periods from January to April and again from July to August. Gonado somatic index (GSI) of species has been widely used to indicate the maturity and periodicity of spawning of the fish. The GSI increases with the maturation of the fish and is maximum during the peak period of maturity. It decreases abruptly after spawning.

Gonado somatic indices were calculated for control group and pesticide treated groups separately and is tabulated in tables 10 and 11.

In control group fishes GSI was found to increase gradually from 1.34± 0.35 (November), 1.67±0.36 (December), 1.84±0.24 (January), 2.02±0.41 (February), 2.26±0.29 (March), 2.70±0.42 (April), 3.13±0.35 (May), 2.47±0.37 (June) and 0.80±0.25 (July). A sudden increase of GSI during April and May period was indicative of onset of spawning activity. In the month of June to July GSI values decreased from 2.47±0.37 to 0.80±0.25 was observed and thus showing cessation of I^{ST} spawning act.

In Dimethoate treated groups GSI was found to increase gradually, but the increase was comparatively less than control groups. Also there was found variation in increase with different concentrations of the same pesticide. In these exposed groups the GSI was found to range from 1.28±0.31 (November), 1.58±0.39 (December), 1.69±0.27 (January), 1.86±0.32 (February), 2.10±0.36 (March), 2.58±0.44 (April), 3.06±0.36 (May), 2.32±0.37 (June), 0.73±0.37 (July) for the Dimethoate concentration of0.85mg/l, 1.24±0.32 (November), 1.43±0.26 (December), 1.83±0.12 (January), 2.07±0.14 (February), 2.30±0.17 (March), 2.28±0.13 (April), 3.04±0.5 (May), 2.71±0.29 (June), 0.20±0.15 (July) for the Dimethoate concentration of 1.20mg/l, and 1.19±0.35 (November), 1.35±0.35 (December), 1.41±0.35 (January), 1.53±0.33 (February), 1.85±0.12 (March), 2.81±0.22 (April), 2.59±0.37 (May), 2.43±0.37 (June),

0.17±0.10 (July) for the Dimethoate concentration of 1.53mg/l. Thus in case of all the Dimethoate treated groups there was increase in the GSI value but increase was less than the control groups. GSI values in exposed. Similarly, in the dichlorvos treated groups the decrease in GSI values was found as compared to control groups. There was slight increase observed from 1.19±0.32 (November), 1.28±0.29 (December), 1.46±0.28 (January), 1.66±0.29 (February), 1.80±0.35 (March), 1.96±0.35 (April), 2.25±0.40 (May), 2.15±0.41 (June), 0.60±0.41 (July) for dichlorvos concentration of 0.65mg/l, 1.15±0.33 (November), 1.23±0.31 (December), 1.41±0.25 (January), 1.69±0.18 (February), 1.89±0.19 (March), 2.21±0.26 (April), 2.64±0.31 (May), 2.48±0.31 (June), 0.52±0.41 (July) for dichlorvos concentration of 0.90mg/l, and 1.07±0.38 (November), 1.26±0.31 (December), 1.81±0.34 (January), 1.96±0.08 (February), 2.19±0.06 (March), 2.45±0.23 (April), 2.81±0.27 (May), 2.50±0.25 (June), 0.43±0.33 (July) for the dichlorvos concentration of1.17mg/l. It may be also noted that GSI did not deviate from the control value significantly until after 15 days of exposure for both the test substances every month. However, 20 days onwards until the termination of the experiment GSI values varied significantly form the respective control values. The tables 9 and 10 show the deviation of GSI from control values in pesticide treated fish for Dimethoate and dichlorvos respectively.

Table 11: Monthly changes in the mean gonado somatic indices in control as well as pesticide (Dimethoate) treated groups of the female, *Cyprinus carpio communis.*

Months	Gonado Somatic Index in Different Groups			
	Control	**Dimethoate (0.85mg/l) Group I**	**Dimethoate (1.20mg/l) Group II**	**Dimethoate (1.53mg/l) Group III**
November	1.34±0.35	1.28±0.3	1.24±0.32	1.19±0.35
December	1.67±0.36	1.58±0.39	1.43±0.26	1.35±0.35
January	1.84±0.24	1.69±0.27	1.83±0.12	1.41±0.35
February	2.02±0.41	1.86±0.32	2.07±0.14	1.53±0.33
March	2.26±0.29	2.10±0.36	2.30±0.17	1.85±0.12
April	2.70±0.42	2.58±0.44	2.28±0.13	2.81±0.22
May	3.13±0.35	3.06±0.36	3.04±0.5	2.59±0.37
June	2.47±0.37	2.32±0.37	2.71±0.29	2.43±0.37
July	0.80±0.25	0.73±0.37	0.20±0.15	0.17±0.10

Table 12: Monthly changes in the mean gonado somatic indices in control as well as pesticide (dichlorvos) treated groups of the female, *Cyprinus carpio communis.*

Months	Gonado Somatic Index in Different Groups			
	Control	**Dichlorvos (0.65mg/l) Group IV**	**Dichlorvos (0.90mg/l) Group V**	**Dichlorvos (1.17mg/l) Group VI**
November	1.34±0.35	1.19±0.32	1.15±0.33	1.07±0.38
December	1.67±0.36	1.28±0.29	1.23±0.31	1.21±0.31
January	1.84±0.24	1.46±0.28	1.41±0.25	1.37±0.34
February	2.02±0.41	1.66±0.29	1.60±0.18	1.52±0.08
March	2.26±0.29	1.80±0.35	1.76±0.19	1.70±0.06
April	2.70±0.42	2.26±0.35	2.10±0.23	1.98±0.23
May	3.13±0.35	3.05±0.40	2.90±0.31	2.58±0.27
June	2.47±0.37	2.38±0.41	2.30±0.31	2.15±0.25
July	0.80±0.25	0.75±0.41	0.68±0.41	0.51±0.33

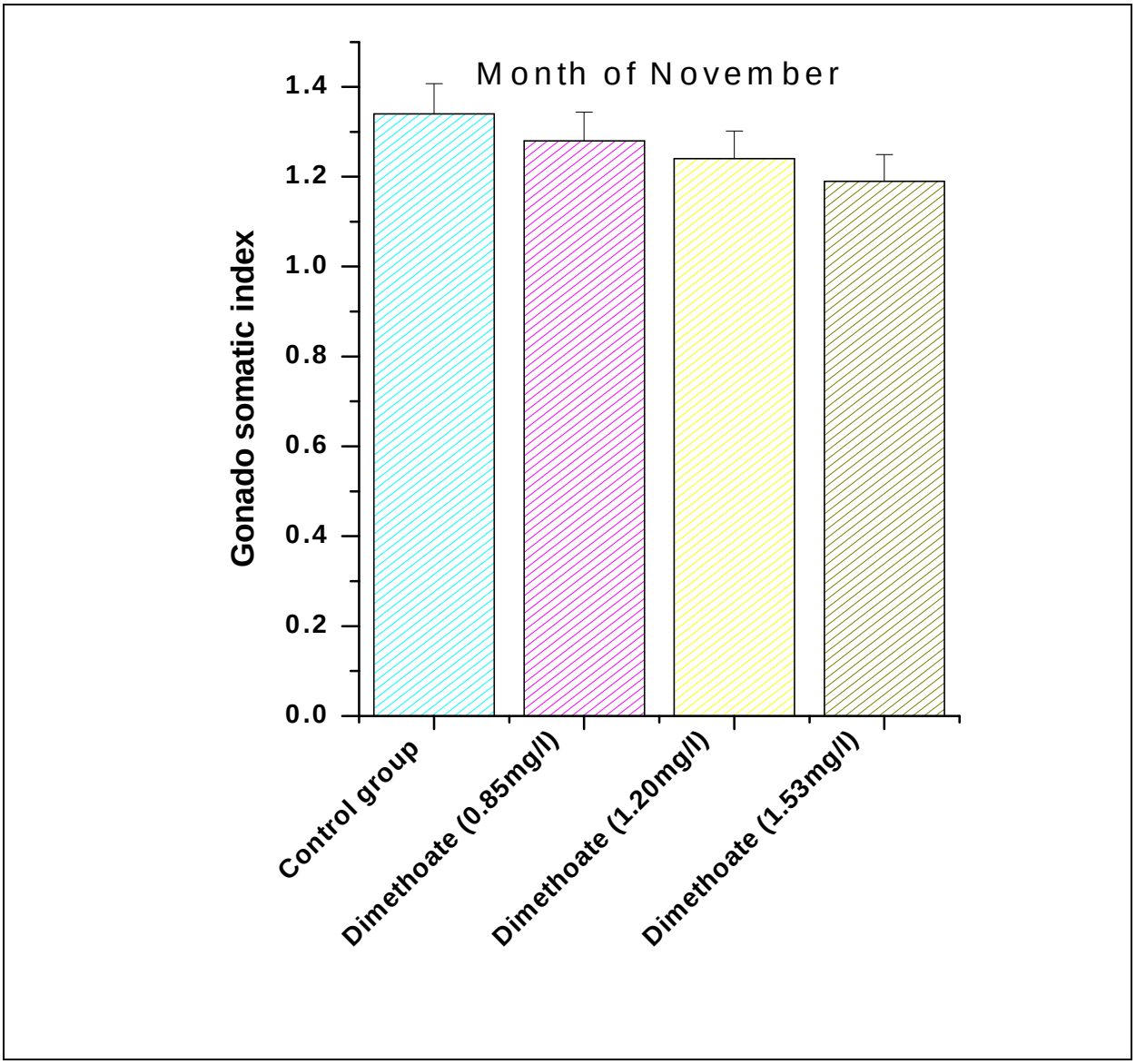


Fig. 7: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of dimethoate in the month of November.

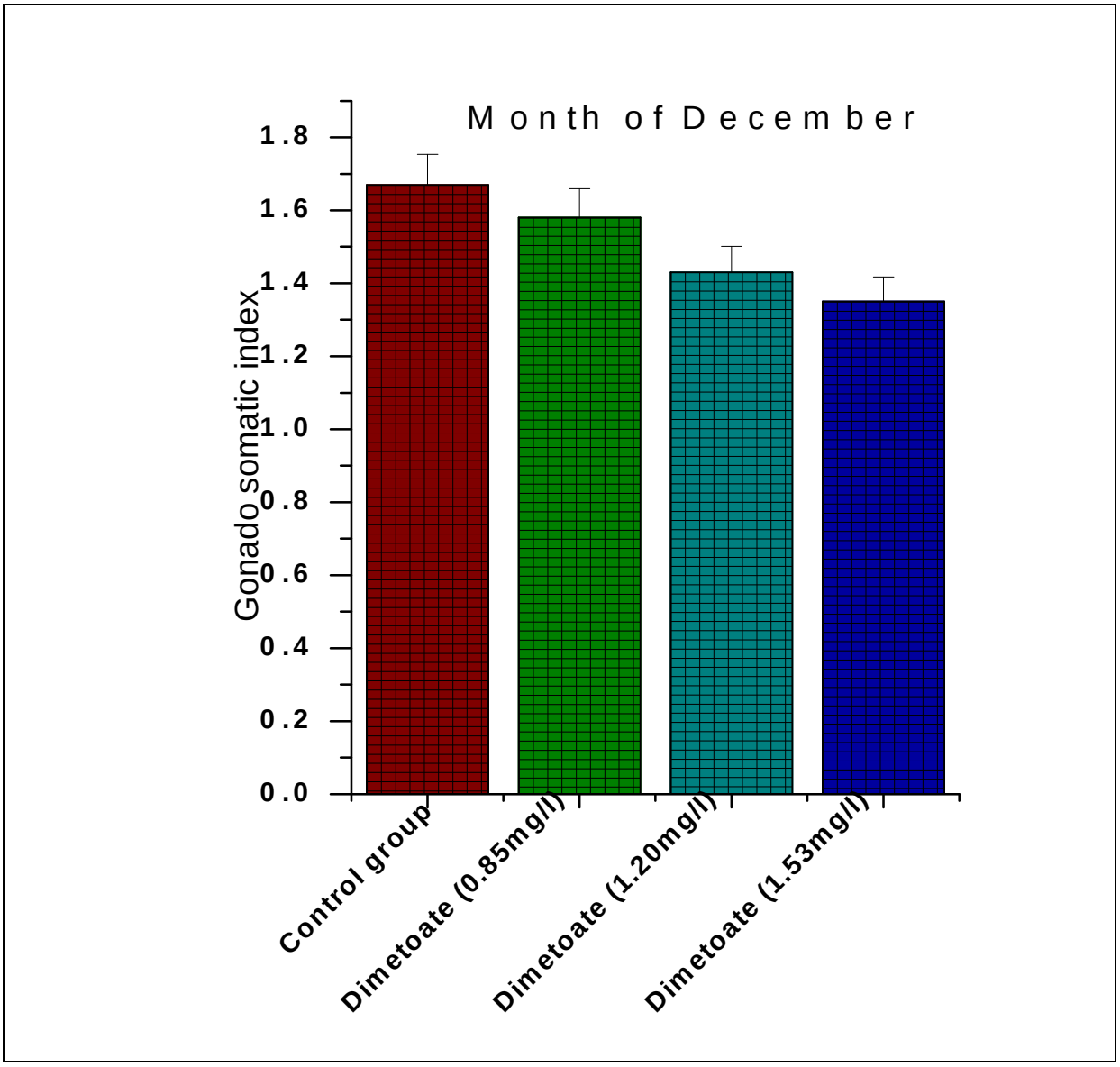


Fig. 8: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of dimethoate in the month of December.

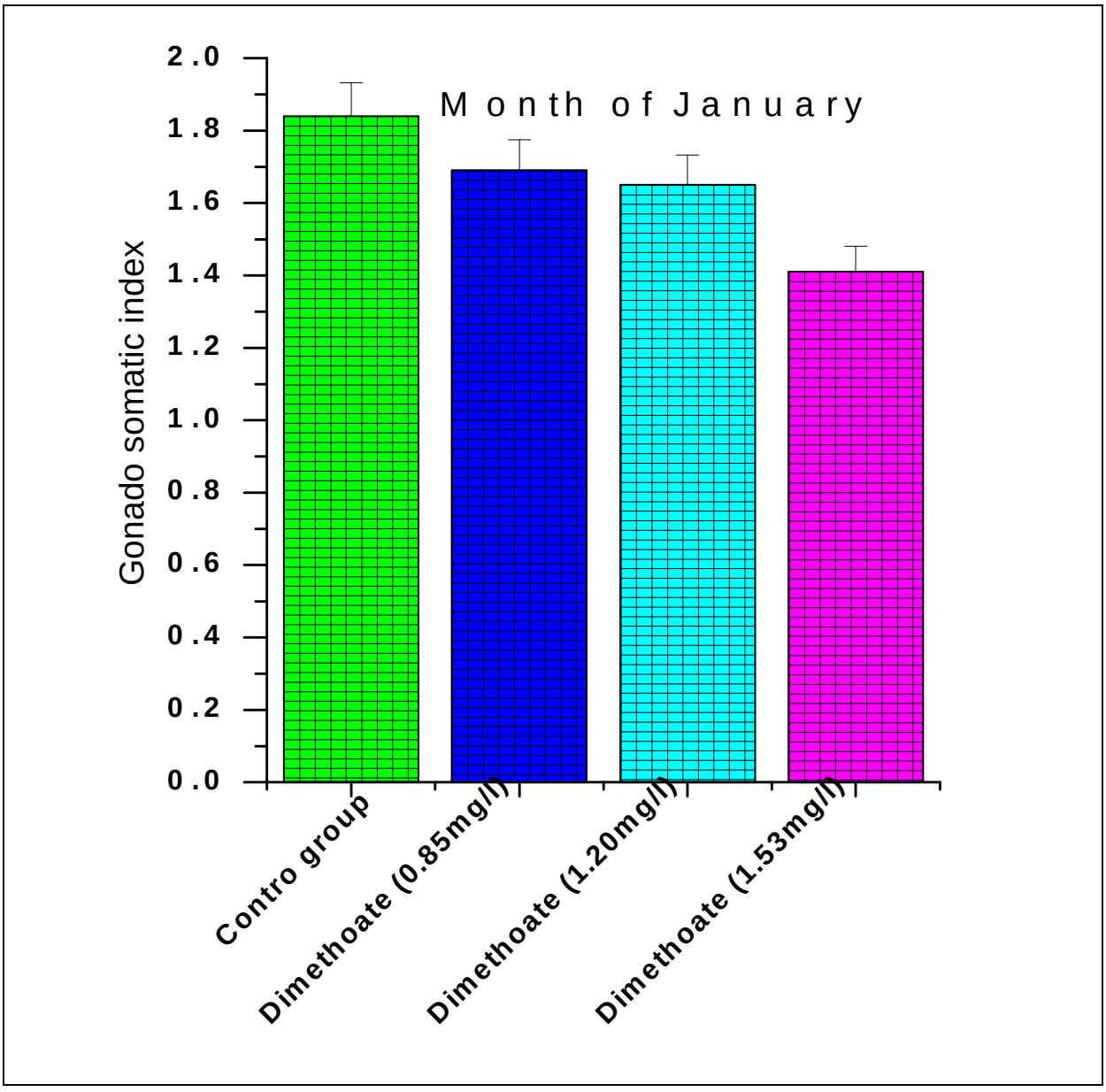


Fig. 9: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of dimethoate in the month of January.

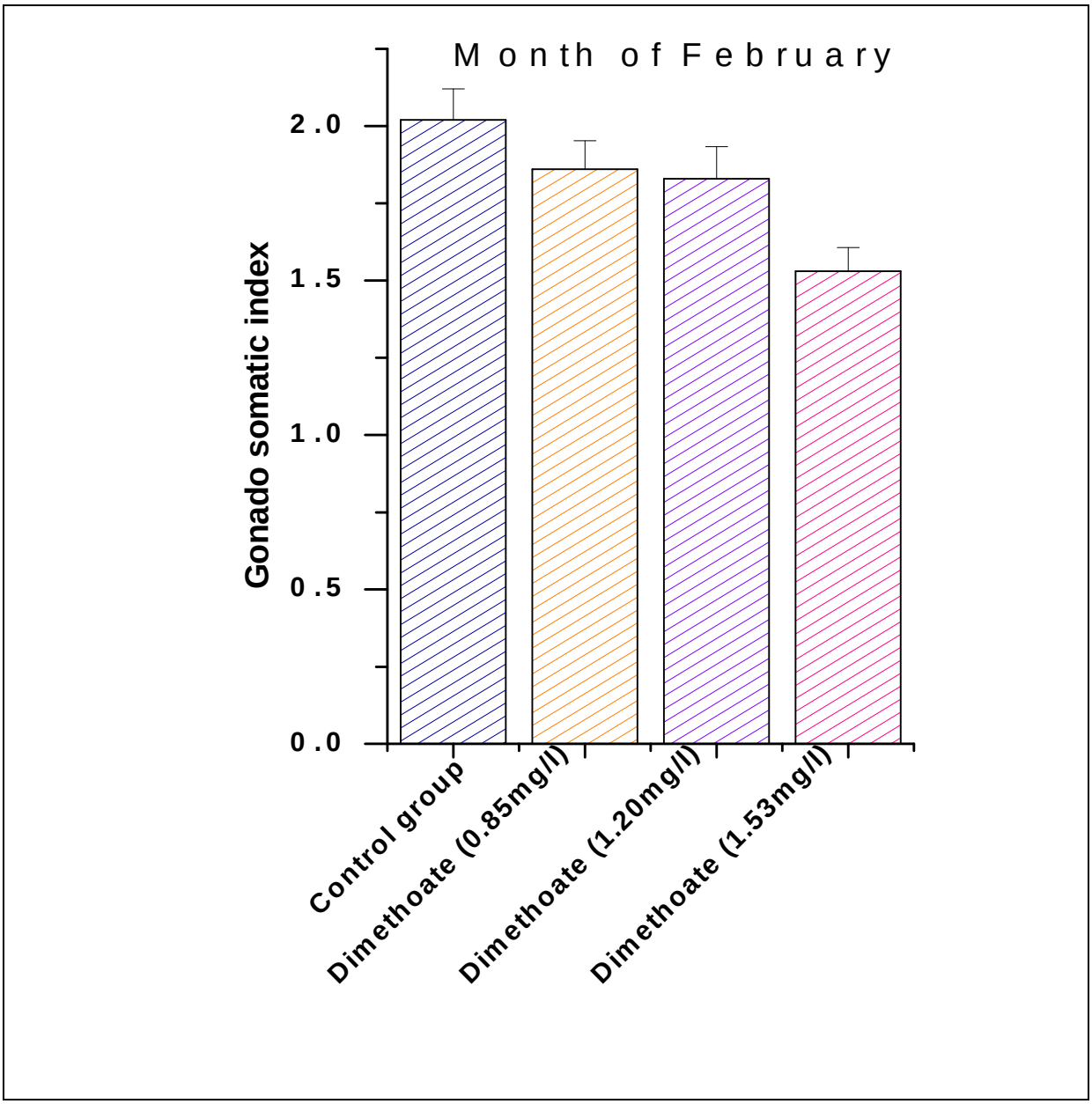


Fig. 10: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of dimethoate in the month of February.

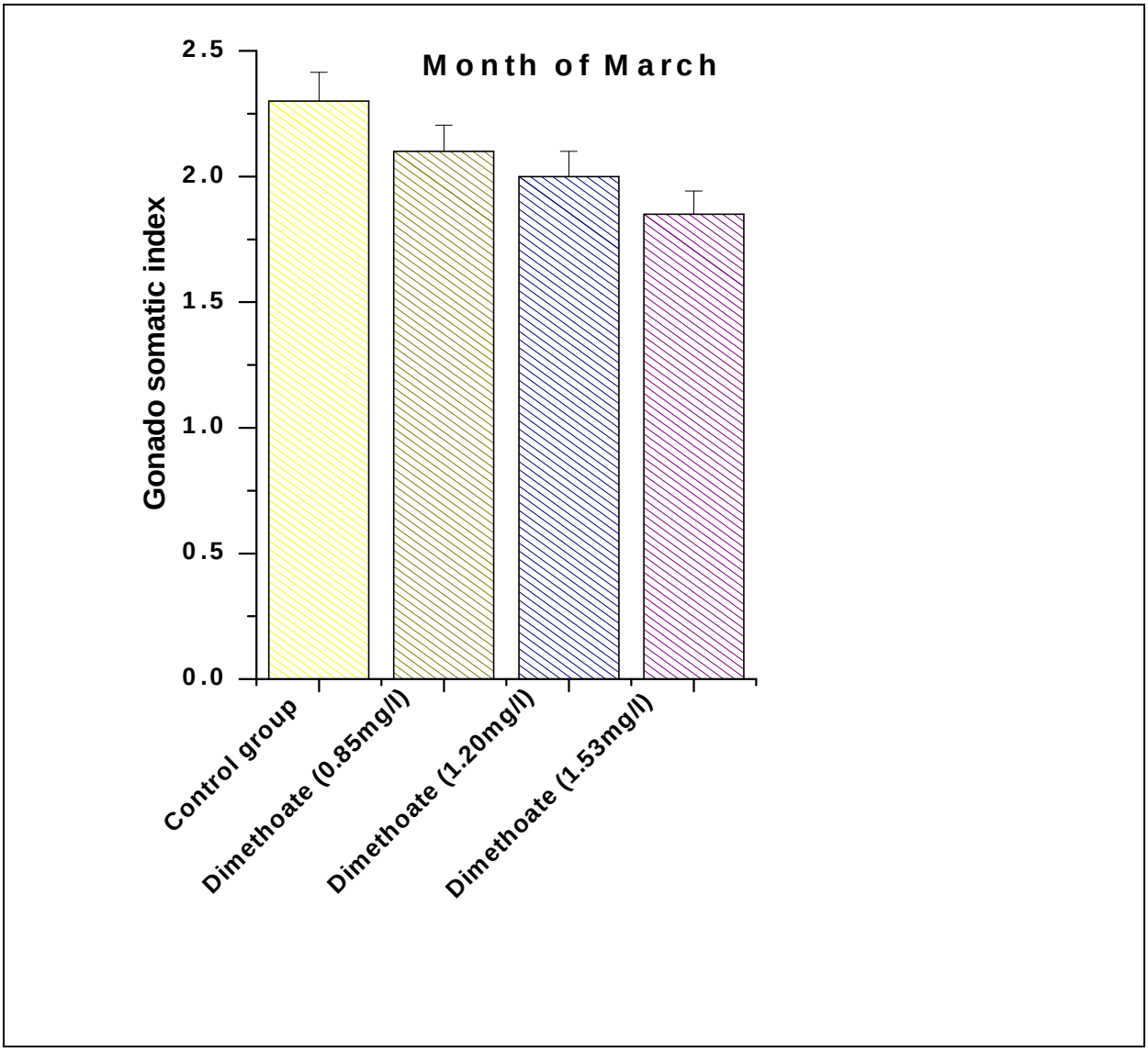


Fig. 11: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of dimethoate in the month of March.

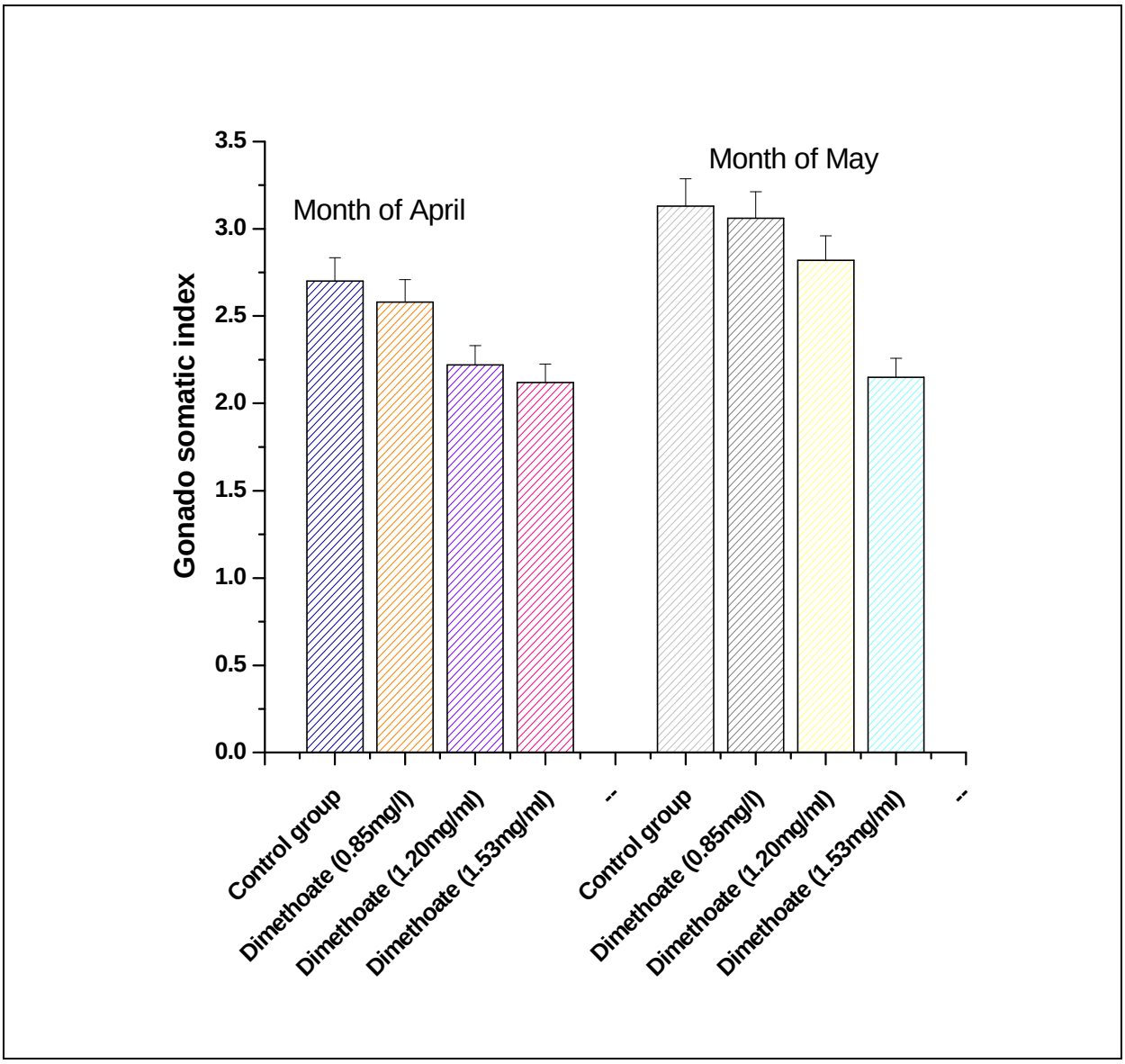


Fig. 12: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of dimethoate in the months of April and May.

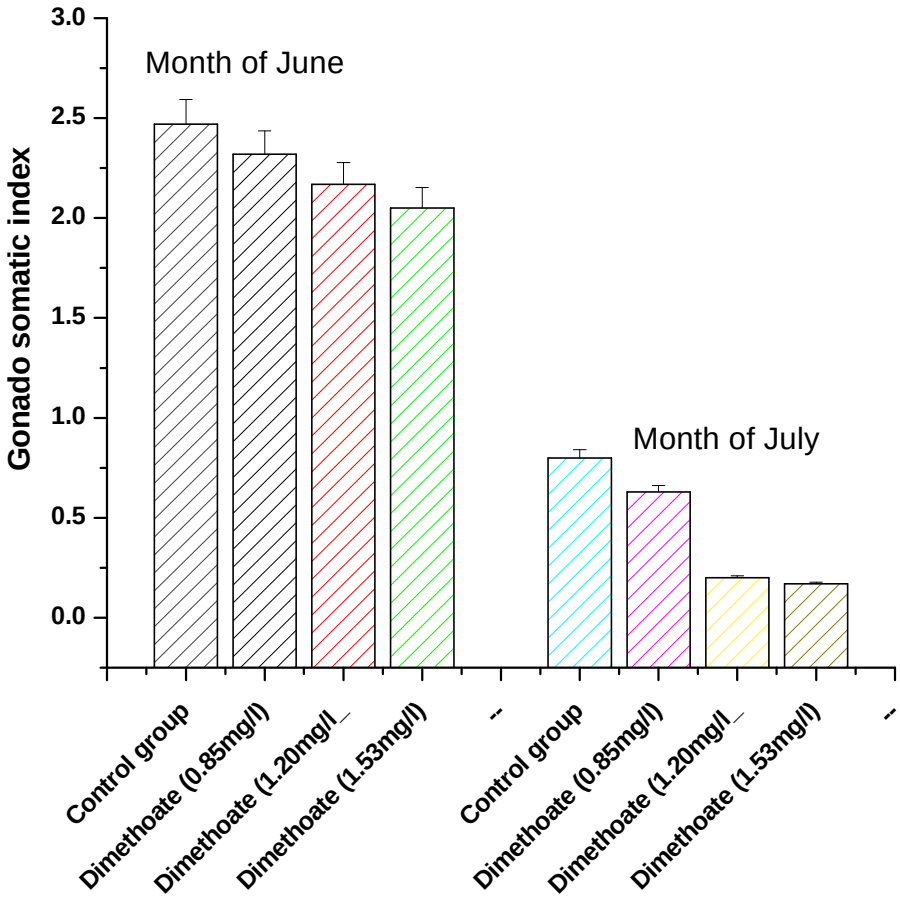


Fig. 13: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of dimethoate in the months of June and July.

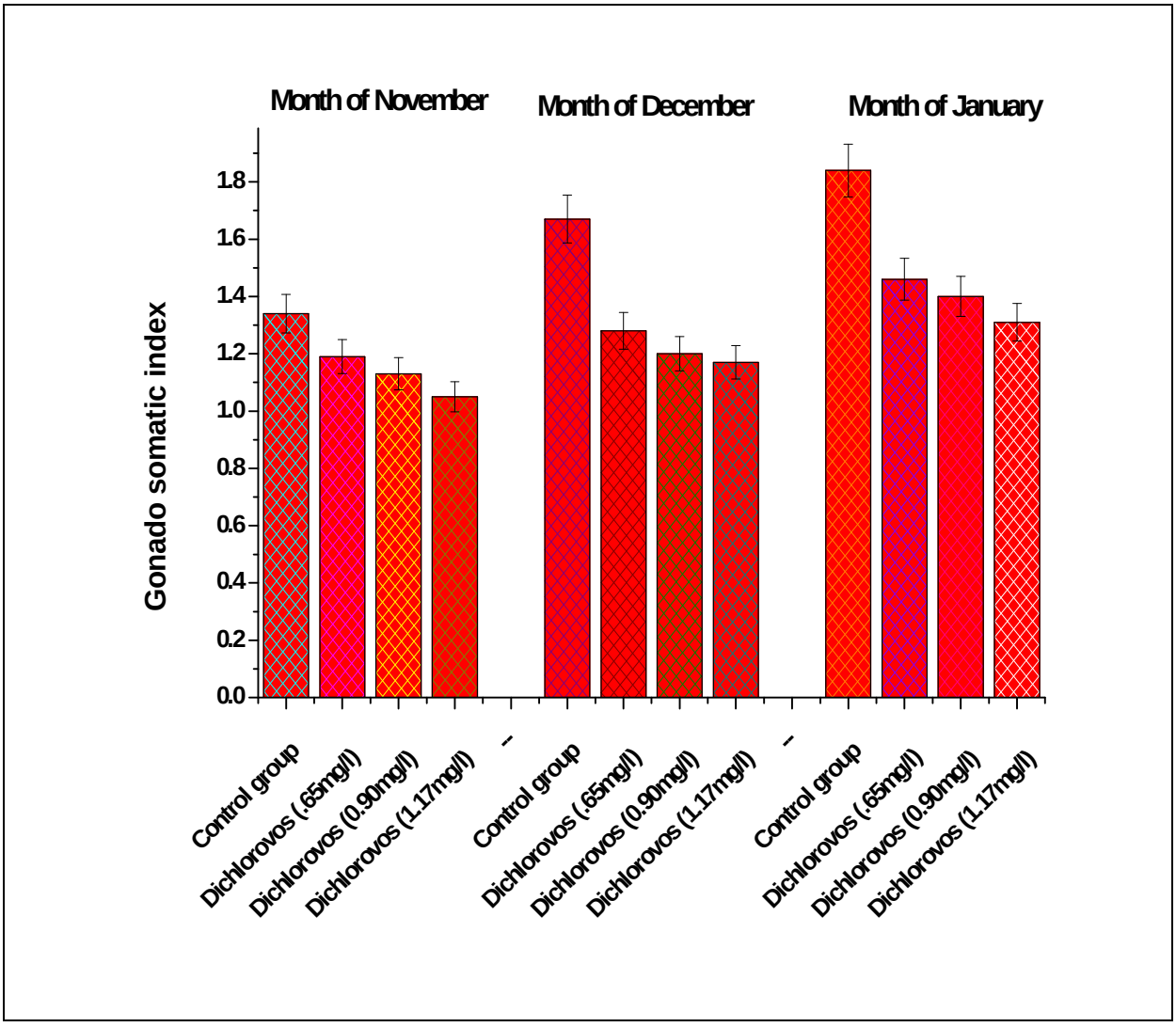


Fig. 14: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of Dichlorvos in the months of November, December and January.

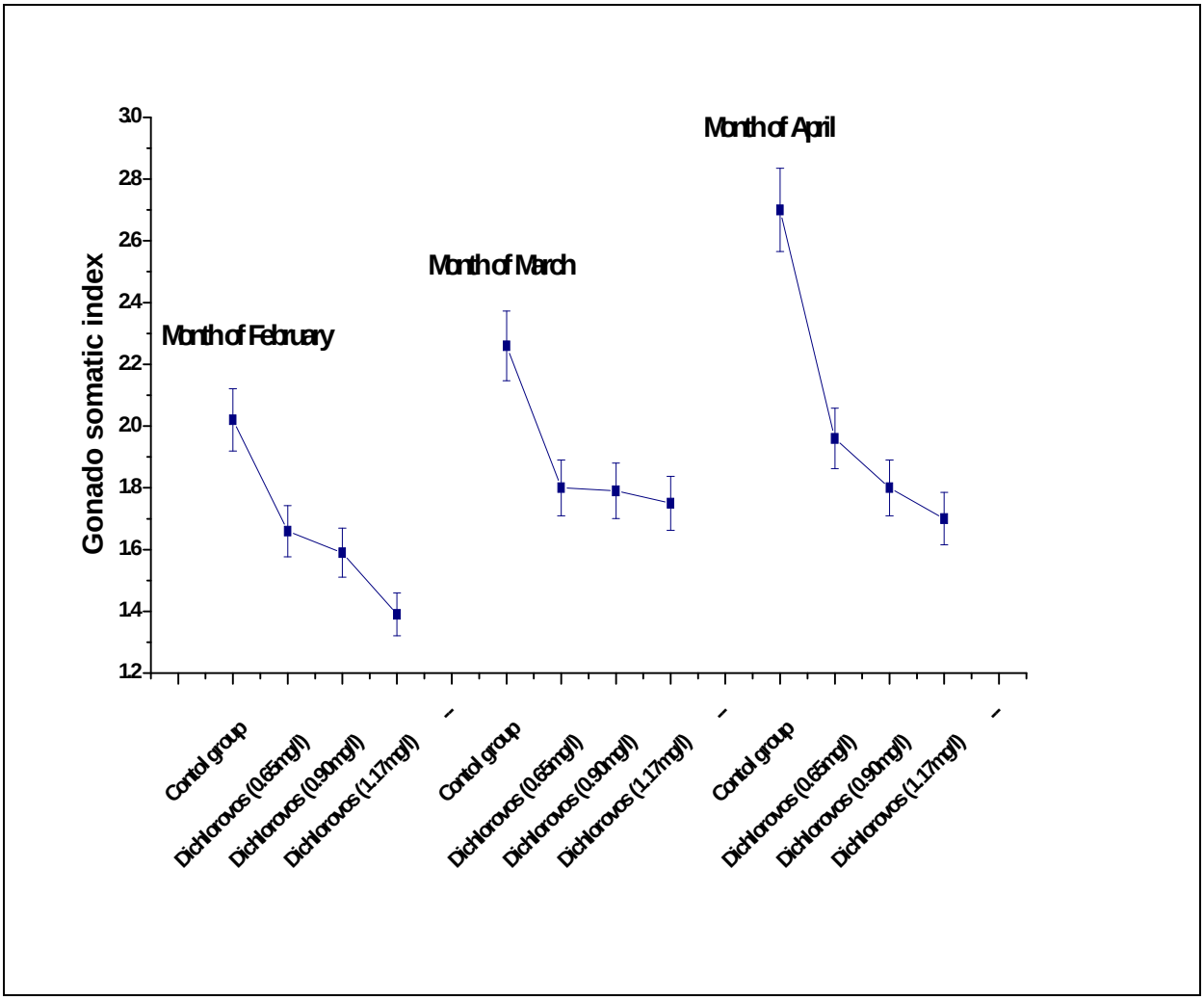


Fig. 15: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of Gonado somatic index of female common carp after exposure of different concentrations of Dichlorvos in the months of February, March and April.

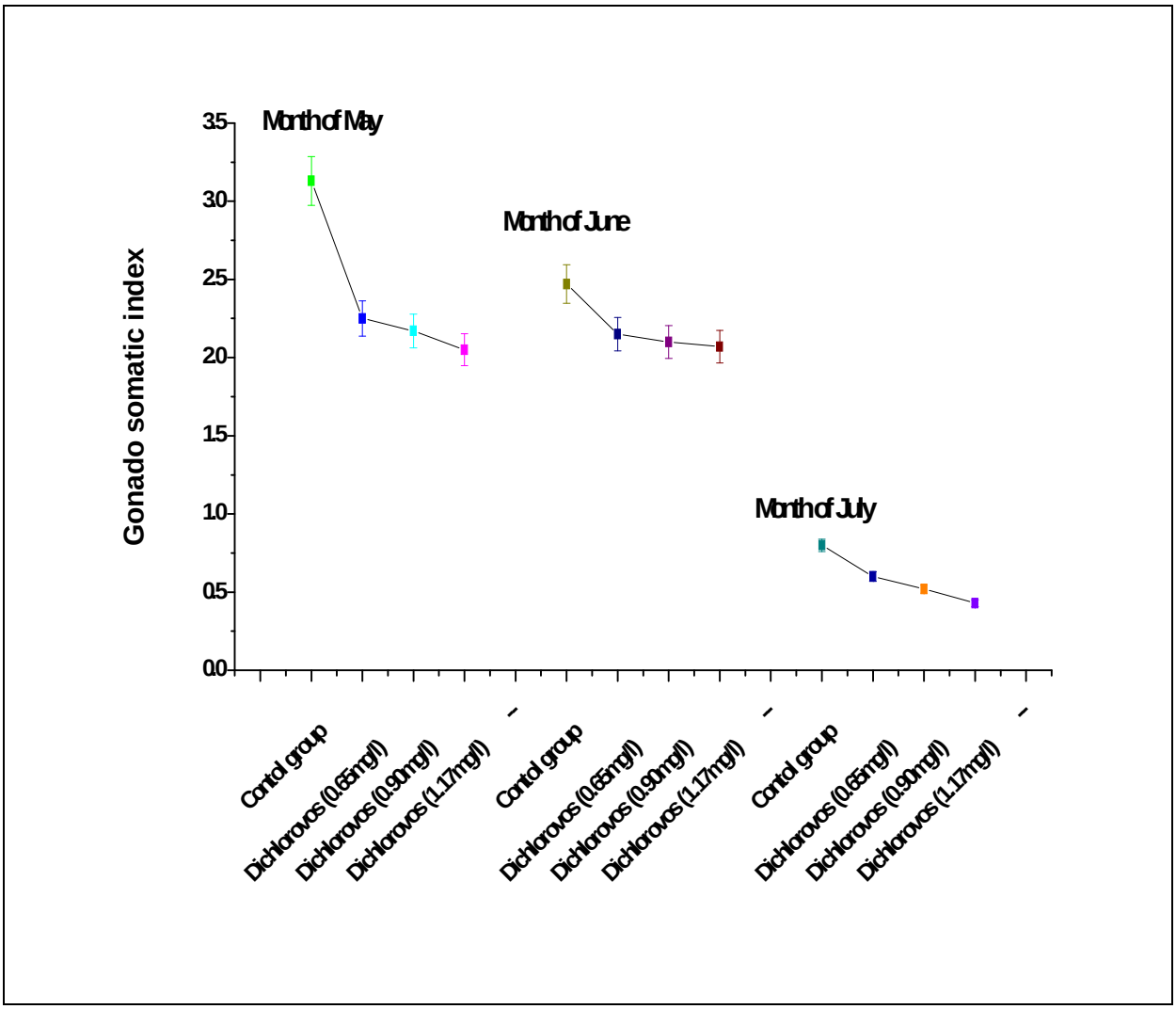


Fig. 16: Diagrammatic representation of the quantitative values (mean±SD in graphs) of Gonado somatic index of female common carp after exposure of different concentrations of Dichlorvos in the months of May, June and July.

4.6 Effects of different concentrations test substances, Dimethoate and Dichlorvos (Organophosphate pesticides) on acetylcholinestrease activity in brain of test animal, *Cyprinus carpio communis*

The AChE activity in brain of the experimental animal *Cyprinus carpio communis* was found to be inhabited at different rates in organophosphate treated fishes. The rate of inhibition of this enzyme at a given dose and duration of treatment was more in increased concentration of both the test substances (Dimethoate and dichlorvos). Brain acetylcholine esterase activity in dichlorvos treated *Cyprinus carpio communis* decrease gradually with the progress of treatment and increased concentration of the test substance. The rate of inhibition in AChE activity in the brain was about 30%, 42% and 49% following treatment of dichlorvos concentrations 0.65 mg/l, 0.90 mg/l and 1.17 mg/l respectively for 96 hours. Acetylcholine esterase activity in the brain of Dimethoate treated *Cyprinus carpio communis* was observed significantly inhibited as 20%, 29% and 35% for dimethoate concentrations of 0.85 mg/l, 1.20 mg/l and 1.53 mg/l respectively for 96 hours.

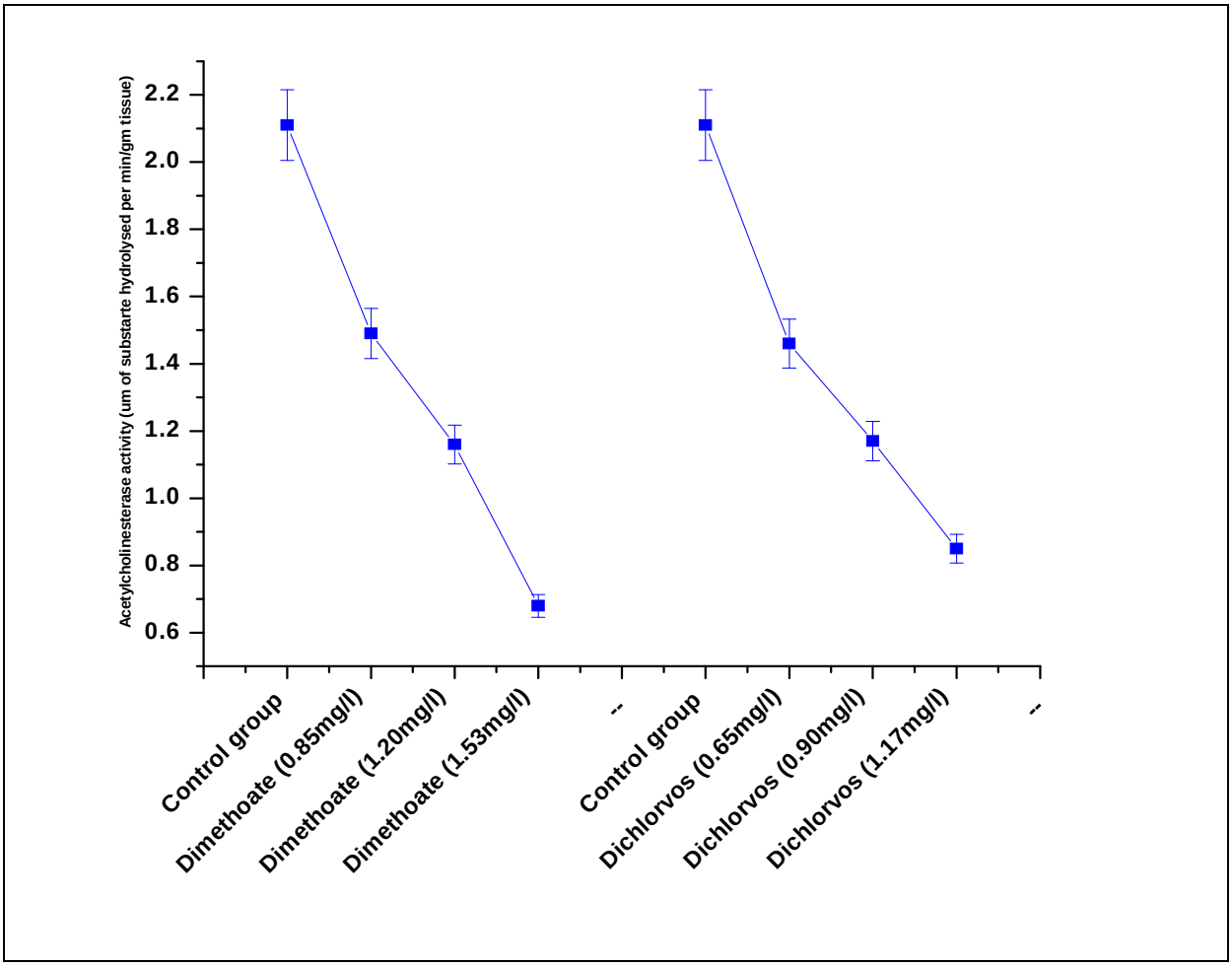


Fig. 17: Schematic representation of the quantitative values (mean±SD in graphs) of AChE activity in the brain of female common carp after exposure of different concentrations of Dimethoate and Dichlorvos organophosphate pesticides for 96hr.

4.7 Effects of different concentrations test substances, Dimethoate and Dichlorvos (Organophosphate pesticides) on acetylcholinestrease activity in ovary of test animal, *Cyprinus carpio communis*

Exposure of *Cyprinus carpio communis* to different sub lethal concentrations of dimethoate and dichlorvos organophosphates resulted in significant inhibition of ovarian AChE activity. The rate of inhibition of the enzyme was progressively increased on increasing the concentration and exposure time of the test substance. Dimethoate concentration of 0.85 mg/l, 1.20 mg/l and 1.53 mg/l resulted in 30%, 42% and 56% rate of inhibition of the AChE activity. The dichlorvos concentrations of 0.65 mg/l, 0.90 mg/l and 1.17 mg/l resulted in 35%, 45% and 50% inhibition of AChE activity in ovary respectively.

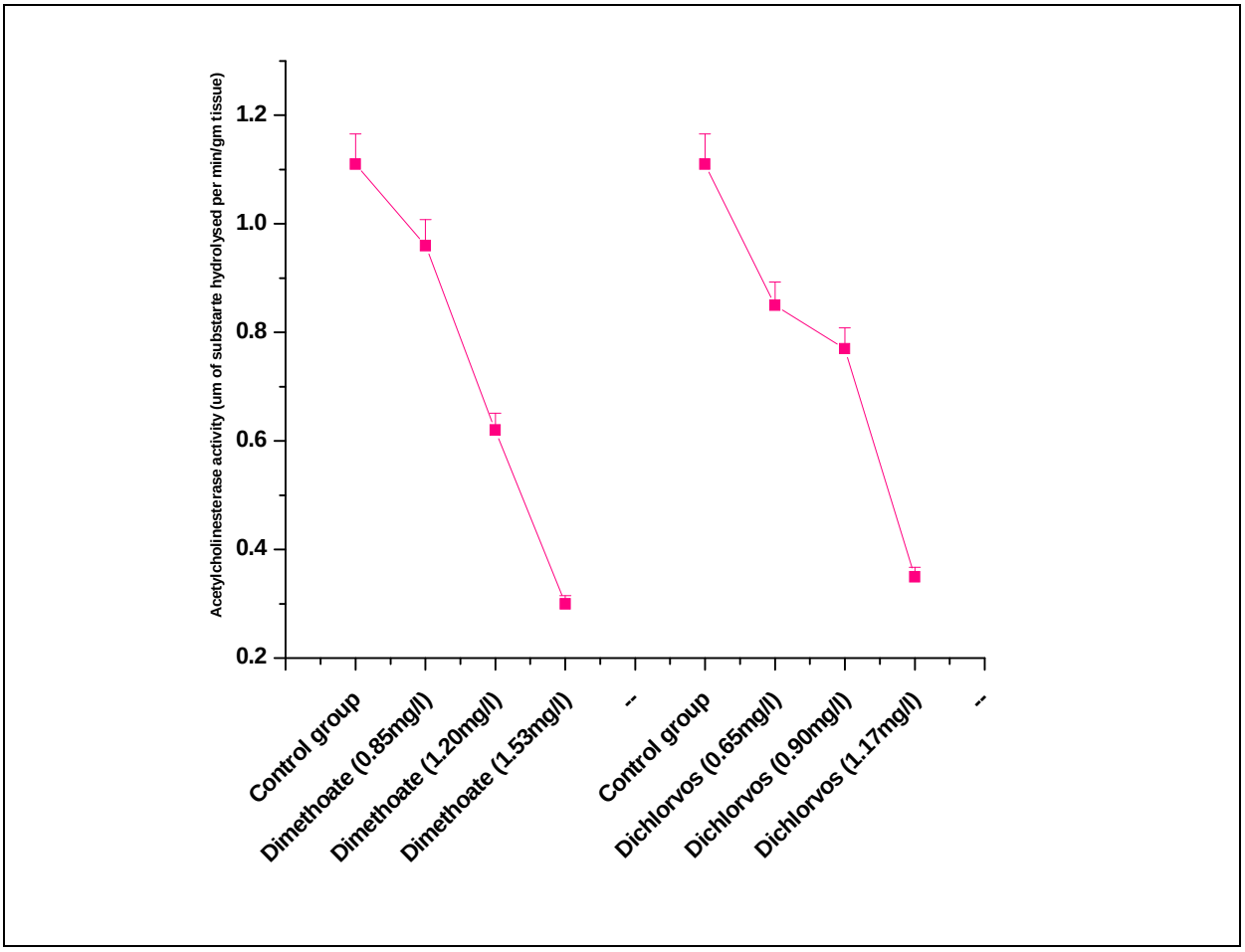


Fig. 18: Schematic representation of the quantitative values (mean±SD in vertical bar) of AChE activity in the ovary of female common carp after exposure of different concentrations of Dimethoate and Dichlorvos organophosphate pesticides for 96hr.

Chapter - 5

DISCUSSION

The ever growing need for pesticides to meet the increasing demands to prevent resistance of target organisms and to obtain maximum response with minimum quantity contemplated the use of different pesticides. The aim is to overcome the effects or to see if material applied conjointly for the purpose enhances or decreases the individual effects. Though it is found to be useful in the control of target species its effect on non target organism, particularly of aquatic habitat proved to be more hazardous (Macek, 1975). From this it can be concluded that there could be a progressive addition of pesticides into the freshwater ecosystem as time progress (Hiltibran, 1974; Chambers and Yarbrough, 1975; Kabeer and Ramana, 1980, Murthy *et al*., 1983). The pesticide magnification in terms of quantity to dimensions becomes more evident when the animal usage of pesticide of a region is considered. Therefore, a polluted ecosystem represents a pool where all the types of pesticides used in the vicinity will ultimately conglomerate resulting in complex effect on biota. Thus, the effects resulting from various combinations of pesticides are often as complicated as they are unpredictable and are commonly referred to as interactions.

5.1 PHYSICOCHEMICAL PARAMETERS OF TEST SUBSTANCE

Environmental parameters have a great role in effecting the toxicity of different pesticides. The major parameters are temperature, dissolved oxygen, pH, hardness, alkalinity etc.

Among all the environmental parameters, the effect of temperature on the toxicity of pesticides has been studied a lot and it is very important because it directly influence factors like enzyme activity, metabolic rate, oxygen uptake etc. Generally, toxicity increases with the high temperature, Macek (1969) studied the effects of 10 pesticides to the rainbow trout and 11 pesticides to the bluegills at different temperatures and found that the toxicity increased with increasing temperature. Similar results have been reported by Singh *et al.* (1982) on endosulfan to *Heteropneuestes fossilis*; Schoettger (1970) on endosulfan to rainbow trout, and Duangsawasdi *et al.* (1979) on fenitrothion and acephate to rainbow trout. But a completely opposite results have been found by DDT and some pyrethroids where toxicity is less at higher temperature. Ogilvie and Anderson (1965) showed that DDT exposed to Atlantic salmon from control. Mauck *et al.* (1976) described that natural pyrethrum and some synthetic pyrethroids (dimethrin, RU-11679, resmethrin) was higher at 12^{o}C than 17^{o}C and dimethrin were more toxic on 17^{o}C than 22^{o}C.

pH has an excellent role in influencing various physicochemical properties of pesticides like hydrolysis, volatilization and in balancing the dissociated and undissociated forms (Weber, 1972). With few exceptions, the toxicity of organochlorine (OC) and organophosphate (OP) compounds are not influenced by pH. The toxic effects of 2, 4-D were reduced when the pH was raised by the addition of sodium chloride (Holcombe *et al*., 1980). Malathion is toxic bellow pH 7, and loses its activity in alkaline pH (Bender, 1969). In the case of carbamate compounds, generally toxicity was reported to increase with pH. Mexacarbate was 38 times more toxic to bluegills at pH 9.5 than at 7.5 (Mauck *et al*., 1977). The toxicity of picloram to cutthroat trout and lake trout increased with a rise pH from 6.5 to 8.5. (Marking *et al.,* 1981). Generally, the activity of synthetic Pyrethroids was not altered by a change in the pH; the biological activity of natural pyrethrums was influenced by pH, its toxicity being higher at lower pH (Mauck *et al*., 1976). Hardness of water does not seem to

influence the toxicity of OC and OP compounds (Pickering *et.al.,* 1962). The toxicity of three formulations of 2, 4-D, three formulations of endothall, fenoprop., PCP and dichlorobenil was not affected by hardness. (Inglis *et al.,* 1972). Lioyd and Jordan (1964) found that survival time of Rainbow trout in rapidly lethal pH value shortened with decrease of calcium carbonate content of water. Hardness of water had no effect on toxicity of maxacarbate (Mauck *et al*., 1976) and cararyl (Johnson *et al.,* 1980). While water hardness had no effect on toxicity of synthetic pyrethroids, natural pyrethrums was more toxic in hard water (Mauck *et al.,* 1976). Not much work seems to have been carried out on the influence of low oxygen on the toxicity of pesticides to fish. Davies (1975) attempted to formulate the criteria for minimum dissolved oxygen requirement of fish. His approach was on examining the threshold levels of dissolved oxygen that cause changes in some physiological lesions. Channel Catfish exposed for 72h to an oxygen content of 1.5ppm showed anomalies in gill, liver, kidney and spleen (Scott and Rogers, 1980).

In the present investigation the water quality parameters (Temperature, Dissolved oxygen, pH and Alkalinity) monitored during the exposure period did not differ within various concentrations of pesticides as well as with the control. The limited variation in the physicochemical variables is similar to the trend observed in the wild, which is tolerated by the test organism even in the wild.

5.2 SURVIVABILITY OF THE TEST ANIMAL

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. The LC_{50} values of dimethoate for certain teleosts are reported to be very high, as in *Clarias batrachus* (Begum and Vijayaraghavan, 1995), it is 65 mg/l for 96 hours, in *Channa punctatus* (Srivastava and Singh, 2001), it is 17.9 mg/l for 96 hours, whereas in *Heteropneustes fossils* (Pandey *et al*., 2009). Very low LC_{50} value for 24, 48, 72 and 96 hour dimethoate exposure is recorded as 3.38, 3.12, 3.08 and 2.98 mg/l respectively. In

contrast, the carps are very sensitive to dimethoate and record very low LC_{50} values. In *Caltla catla* (Kumar and Singh, 2000) the LC_{50} value for 96 hours is reported as 0.07 ppm.

In the present investigation the 24, 48 and 96 hours LC_{50} values of dimethoate for *Cyprinus carpio communis* was found to be 1.45, 1.55 and 1.70 mg/l respectively. Similarly, for dichlorvos it was observed to be 1.15, 1.23 and 1.30 mg/l for 24, 48 and 96 hours respectively. An increase in number of mortalities with an increase in percentage concentration of LC_{50} values of the test substances was observed and is summarized.

The differential toxic potential of dimethoate and dichlorvos like organophosphate pesticides for different fish species can be attributed to the differences in susceptibility and tolerance related to their accumulation, biotransformation and excretion. Different metabolic pathways occuring among fish species may result in different pattern of biotransformation, leading to more or less toxic metabolites (Johnsson and Toledo, 1993). Magnitude of toxic effects of pesticides also depends on length and weight, corporal surface/body weight ratio and breathing rate (Singh and Narain, 1982; Murty, 1986). This is in agreement with Sprague (1969) who observed variation in LC_{50} values for the same species and toxicant depending on size, age and condition of test species along with experimental factors. Behavioral changes observed in exposed carp, *Cyprinus carpio communis* appear to be manifestation of dimethoate and dichlorvos toxicity. Upon exposure to these pesticides, increase in surfacing and gulping of surface waters appears to be an attempt by the fish to avoid breathing in poisoned water. Similar observations have been reported in *Anabas testudineus* after exposure to monocrotophos (Santha and Balaji, 2000). Moreover, hypoxic condition also contributes to increase surfacing as reported by Radha *et al*. (1998). Hypoxic condition arises primarily due to damage of gills of pesticide exposed fish which hampers oxygen uptake (Velmurugan *et al.,* 2007). Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and muscular junctions (Rao *et al.,* 2005).

It is concluded that carps (*Cyprinus carpio communis*) are more sensitive to Dimethoate and dichlorvos (DDVP) as compared to other fish species such as air breathing fish. They exhibit Behavioral, physiological, hormonal and histological changes which reflect the rate of survivability or mortality at a specific pesticide concentration and exposure time.

5.3 BODY WEIGHT OF THE TEST ORGANISM

The present work also showed that the prolonged exposure of *Cyprinus carpio communis* to different concentrations of Dimethoate and Dichlorvos organophosphate pesticides in water induces a variety of anomalies in feeding behavior, food utilization and body weight of the fish. The significant dose dependant reduction in the body weight in experimental animal, *Cyprinus carpio communis* exposed for Dimethoate and Dichlorvos indicates that the fish were severely stressed in both the cases. The suppressive effect on food consumption due to toxicant cannot be ruled out. Studies with other organophosphorus compounds like methylparathion on the fish *Tilipia mossambica* (Siva Prasada, 1980) and malathion, Sevin and lindane on the same species (Basha, 1980) showed a decrease in the body weight. Ghatak and Konar, 1991, also reported the feeding rate of *Tilapia* was reduced significantly when exposed to pesticide at various concentrations. Similarly, Ponmani *et al.,* 1997, observed significant reduction in feeding rates and body weight of *Cyprinus carpio* exposed to sub-lethal concentrations of monocrotophos. Also O' Brien, R.D. (1976) reported that the acetylcholinesterase inhibition decreased the feeding rate due to impairment of impulse transmission. Pal and Konar, 1987, reported that methylparathion of various digestive enzymes. A reduced body weight may also be attributed to an increased activity associated with attempt to avoid the contaminated waters, or an increased expenditure of energy on chemical detoxification and tissue repair. The variations in body weight of fish exposed to different concentrations of organophosphate pesticides (dimethoate and dichlorvos) should be due to loss of some constituents other than water. Similar observations in relation with weight changes were made in *Tilapia mossambica* treated with different pesticides like, Malathion, methyl parathion and parathion (Kabeer *et al*., 1981). They observed that

decrease in the body weight can partly be attributed to the loss of ions from the body. In the present context also, the same situation can be expected. However, changes in body weight are more in fish exposed to different concentration of dichlorvos than in fish exposed to different concentration organophosphate pesticide dimethoate.

5.4 REPRODUCTIVE PARAMETERS OF THE TEST ANIMAL

I. Ovarian Weight

The teleost ovary undergoes a seasonal reproductive cycle which may, for convenience, be divided into four main phases:

1. Vitellogenisis, involving the major growth phase of the ovary during which ovarian secretion of oestradiol stimulates hepatic synthesis of vitellogenin which in turn, is incorporated into the developing oocytes.
2. Oocyte maturation, during which the germinal vesicle migrate to the periphery of the oocytes and breaks down under control of pituitary gonadotrophins and ovarian progestrogens.
3. Ovulation and Spawning and
4. Postspawning in which the gonads regress in preparation for the next reproductive cycle.

Clearly the stage at which the fish are exposed to the pesticides and the duration of such exposure will determine to a large extent the effect on the ovary. Results from the previous investigations have suggested that the long term exposure of fish to pesticides invariably lead to the antigonadal influences such as decrease in ovarian weight, smaller, less developed oocytes and fewer large mature oocytes and an increase in the numbers of the atretic follicles and oocytes frequently contained less yolk granules (Sukumar and Krpagaganapathy, 1992). Kulshrestha *et al*., 1984 worked on the exposure of endosulfan and carbaryl pesticides on the ovaries of *Channa straitus* (bloch) and observed reduction in the number of oocytes, increased number of damaged oocytes, development of interfollicular spaces and decreased

gonado somatic index. Shukla *et al.,* 1984, noted decreased ovarian activity and atretic oocytes in *Sarotherodon mossambica* exposed to melathion, and Ghosh *et al*., 1985 has described on damage in ovary viz., degeneration of follicular wall, ooplasm and connective tissue due to melathion toxicity on *Heteropneustes fossils*. In the present investigations a significant reduction in the ovarian weight was observed following exposure of different concentrations of dimethoate and dichlorvos organophosphate compounds to experimental animal for 60 days. According to Kiling (1986) total follicular atresia, inhibition of vitellogenisis and disruption of reproductive endocrine functions due to the pesticide exposure could have caused significant decrease in ovarian weight. Thus it appears logical to summarize that the decrease in ovarian weight in *Cyprinus carpio communis* may be due to the decrease in number of mature follicle, increase in number of atretic follicles and decreased levels vitellogenesis (Plate 2 and 3).

II. Oocyte Diameter

Oocyte maturation in teleost fish is a necessary condition for successful ovulation. This phase of oocytes development (Plate 4) is initiated by gonadotrophin, which induces both migration of the germinal vesicles to the periphery of the oocyte and the follicular synthesis of a maturation inducing steroid (which is often considered to be 17, 20β-dihydroxy-4-pregnen-3-one). The maturation inducing steroids then causes germinal vesicle breakdown (GVBD) which is usually followed by ovulation. Unlike prolonged period of vitellogenesis oocytes maturation is very short and is potentially very susceptible to pollutants such as pesticides. The eggs of most fish species vary in size (Bagenal, 1971) and chemical composition, and some of these variations will be important from the point of view of fish production. A relationship between the fat content of the female parent and the egg size and quantity of the yolk has been shown in a number of species, for example in Herring (Anokhina, 1963). Results from the previous investigations have suggested that organophosphate pesticide exposure to fish caused necrosis and fibrosis of ovarian connective tissue, dilation of blood vessels, decrease in oocytes diameter and increase in intrafollicular space in *Rasbora deniconius* and *Channa straitus* (Kulshrestha and Arora, 1984;

Rastogi and Kulshrestha, 1990). Vaculisation and granulation of oocytes (Shukla and Pandey, 1985). Increased oocytes attresia was reported in aldrin treated (0.05mg/l) *Puntius conchonius* (Kumar and Pant, 1988). Treatment with organophosphate pesticides such as monocrotofos, methylparathion, phenthoate, melathion and phenetrothion also resulted in follicular attresia and other degenerative changes in ovary (Pawar and Katdare, 1983). Organophosphate pesticide treated ovary contained only stage I oocytes while as control showed a large number of stage I and stage II oocytes (Haider and Upadhyaya, 1985). Stage I primary oocytes were predominantly higher in carbofuran treated *Heteropneustes fossils* than the prevalent stage II and III oocytes in the control animals (Chatterjee *et al*., 1997), Pandey (1988) suggested that the edosulfan treatment caused effect on dynamics of oocytes developments in the teleostean fish *Colisa faciatus* and described that ovarian activity was retarded greatly, the diameter of oogonia and stage I oocytes was greatly reduced and reduction in the number of oocytes, increased number of damaged oocytes, reduction in egg diameter was observed in *Channa straitus* on treatment with endosulfan and carbaryl pesticides (Kulshrestha *et al*., 1984). In the present investigation the exposure of fish to sublethal doses of dimethoate and dichlorovos produced various effects on the oocytes of different types in experimental animal, *Cyprinus carpio communis*. The diameter of the oocytes decreased with progressive duration of the exposure as compared to controls. In first few days of exposure no significant change in diameter was observed in the immature oocytes but subsequent exposure produced significant changes. The percentage of immature oocytes increased while that of mature ones decreased from 0 to 75 days of exposure. Collectively our results demonstrate an inhibitory influence of organophosphate pesticides on the female gonads, thus it appears logical to summarize that the decrease in ova diameter may be due to effect of organophosphate pesticides on vitellogenesis, gonadotrophin formation like physiological processes. Hence our observations are supported by the similar results demonstrated in previous investigations.

III. Gonado Somatic Index

Seasonal cycles in the gonadal development and the breeding behavior have been conclusively shown to be regulated through several external factors including photoperiod, temperature and other physical and chemical factors. These are known to serve as proximate factors and act through brain, pituitary and gonadal axis to control the reproductive behavior of the fish (Nikolsky, 1963; Hoar, 1965a; Love, 1970 and de Vlaming, 1972) Earlier reports have indicated that *Cyprinus carpio*, undergoes changes with respect to its breeding behavior (Raina, 1978). In *Cyprinus carpio*, the ovaries are typically cystovarian and ova develop within the ovarian sac being liberated directly to the exterior through a small oviduct. The ovaries are paired and situated in the posterior-dorsal part of the body cavity covered by a thin walled peritoneum and attached by a mesovarium. Posteriorly the ovaries are fused but the paired nature can still be recognized. The shape ,size and color of the ovaries varies with the stages of maturation .During their immature stages they occupy about 3/4th of the length of the abdominal cavity but as they grow, they become distended and on maturation occupy the entire abdominal cavity. Histologically each ovary is made up of large number ova within the ovarian sac. The walls of the sac present a number of folds the ovigerous lamellae. These were seen to be lined by germinal epithelium where the oocytes developed and were budded off into the cavity of the ovarian sac.

The breeding season of the carp extends from March and early April to the middle of June and may therefore be regarded as a spring breeder. The ovaries show a series of cyclic changes in the morphology and histology which represents the various maturation stages and are related to the gonad somatic index of the experimental fish. The various recognizable seasonal changes are:

- During September, October and November the ovaries are in the maturing stage. The ovigerous lamellae are full of small rounded microscopic oocytes. The cytoplasm at this is deeply staining. The nuclear membrane is smooth and peripheral vacuoles are present in some oocytes. The blood supply becomes conspicuous and increases considerably. Along with these visible changes in the histomorphology (Plates 1 and 2) of the ovaries, the gonad somatic index also exhibits a linear increase. It was found to gradually increase from

1.34±0.35 in November to 3.13±0.35 in April showing a three fold increase. The eggs appeared to be fairly advancing.

- During the period of December, January and February the ovaries does not show an active histomorphological transformation, the ovaries remain rather quiescent and rate of maturity is slow. The oocytes attain a large size and the ovaries look highly packed. The yolk attains the granular form and both granular and non-granular yolk is present in oocytes. Two zones of the yolk are distinguishable; outer with large yolk plates the inner with smaller ones. The vitellogenesis is also very slow during this period. During the post-spawning or spent period (July- August) the ovaries are shrunken flaccid and blood shot, occupying very little space in the body cavity of the fish. The ovaries are marked with dark red spots, which are due to degenerating ova on the surface of the ovary and also show a high degree of vascularity.

The exposure doses of the two organophosphate pesticides (Dimethoate and Dichlorvos) caused less significant mortality of the experimental fish, female of *Cyprinus carpio communis* but did manifest signs of physiological distress. However, they were both potent enough to cause significant reproductive impairment in terms of specific damage to ovarian tissue. As reported by Pandey and Shukla (1982), GSI (Gonado somatic index) is greatly affected by DDT, BHC, Endosulfan, Chlordane and Toxaphane. Dey and Bhattacharya (1989) observed the preponderance of stage –I and destruction of stage-II and stage-III oocytes in association with decreased ovarian weight in Phenthoate exposed *Channa punctatus.*

The Ganado somatic index increased in all control and pesticide treated groups during the investigation but reduction in Gonadosomatic index was observed on exposure to the test substances, Dimethoate and Dichlorvos as compared to control groups. It may be also noted that the reduction in GSI values was maximum at highest concentrations of both the organophosphate pesticides in series and reduction was treatment time dependant also.

Deleterious effects of pesticides have been observed in earlier studies such as delayed maturity (Crandall and Goodnight,1962),abortion in *Gambusia* (Boyd, 1964), reduction in reproductive efficiency (Burdick *et al*., 1972) and decrease in the percentage of different stages of oocytes along with reduction in GSI (Kulshretha and Arora,1984; Pandey and Shukla 1984; Singh and Shai, 1985). Chandra *et al.* (2004) observed retardation in the onset of first ovarian maturity in carbofuran treated common carp, *Cyprinus carpio communis.*

5.5 ACETYLCHOLINE ESTERASE ACTIVITY IN BRAIN AND OVARY OF THE TEST ANIMAL

Organophosphate pesticides are competitive inhibitors of acetylecholineesterase (AChE), the key enzyme in the transmission of nerve impulse. AChE is readily phosphorylated by the organophosphate pesticides at the active site serine (Aldrige and Reiner, 1972; Taylor, 1990) the selectivity of action of organophosphates is that it causes inhibition of AChE and accumulation of acetylecholine at the synapse (Loskowsky and Dettbam, 1975) over stimulating the postsynaptic cells (Pope *et al*., 1995). Reports also demonstrated that the organophosphate pesticide agents can bind to the acetylecholine receptors and this direct interaction is responsible for the manifestation of stress (Pope *et al*., 1995). Therefore, the AChE activity in different tissues (Brain and Ovary) in experimental animal *Cyprinus carpio communis* has been used in the present investigation as the neurophysiological marker or early signs of organophosphate neurotoxicity. Results of previous studies have suggested that even the undetectable quantity of organophosphate pesticides will affect the enzymatic activity. Several authors have reported that enzymes of the same tissue of different species show difference in the sensitive to various organophosphate insecticides (Pan and Dutta, 1998; Monserret and Bianchini, 1998). In the present work all the groups treated with Dimethoate and dichlorvos organophosphate pesticides revealed significant ($P>0.01$) inhibition of AChE activity in the brain as well as ovary of exposed fish. The inhibition of enzyme was more significant at higher doses of pesticides to fish in both the cases. The time, dose and species related differences in enzyme susceptibility to organophosphate

pesticides can primarily be attributed to dissimilar enzyme amount and inhibitor affinity degree to cholinesterase receptor. Although 50% or more depletion is supposed to be life threatening, available investigatrion shows that some fish are capable to tolerate over 90% inhibition in AChE activity (Day and Scott, 1990). More than 90% depletion was also reported by Balint *et al.* (1995); Pan and Dutta, (1998) in fish exposed to various insecticides. The highest reduction in the present study 72% which is considerably low. Oruce and Usta, (2007) reported that *Cyprinus carpio* showed to be more resistant to diazinon, this may be because of its low rate of bioactivation and relatively high activity of detoxicating enzymes (Keizer *et al*., 1991). Rath and Misra, (1981) and Ansari and Kumar, (1984) reported that inhibition of acetylecholine activity has relation with age of fish, concentration of pesticide and time of exposure. Their findings extended a considerable support to our observations. Therefore, the present study demonstrates that both the organophosphate pesticides (dimethoate and dichlorvos) are potent inhibitors of brain and ovarian AChE activity, but under identical dose the rate of enzyme inhibition was different for different pesticides.

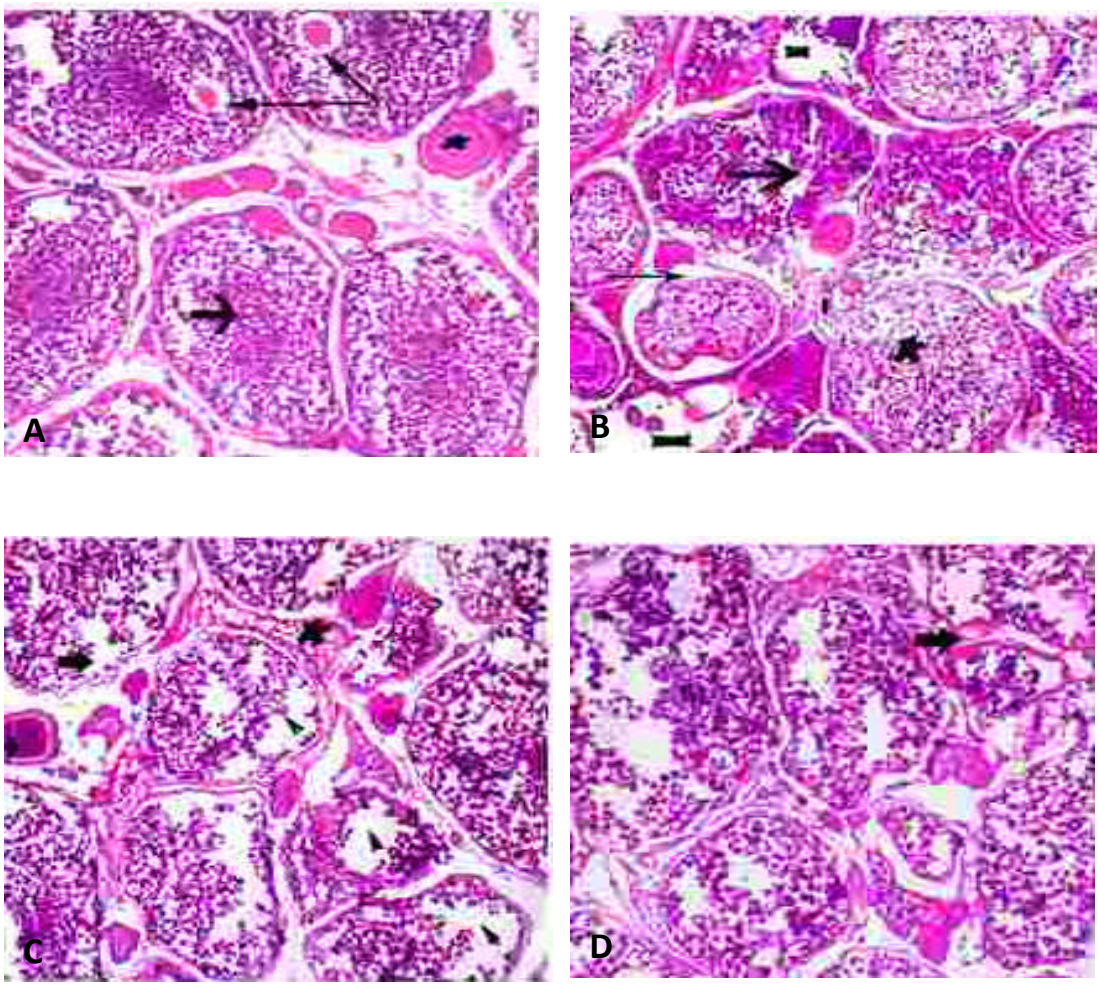


Plate-2 (a-d): Sections of ovaries of *Cyprinus carpio communis,* fish stained with H &E(a) ovary of control fish showing normal ovary tissue (x100); (b-d) ovaries of *Cyprinus carpio communis* exposed to different concentrations of *dimethoate*, (b) showed coagulation necrosis in yolk granules, and atresia of ripe oocytes (x100), (c) showed depletion in yolk granules, and atresia of oocytes (x100), (d) showed ovary in ripe oocytes stage with some atretic state (x100).

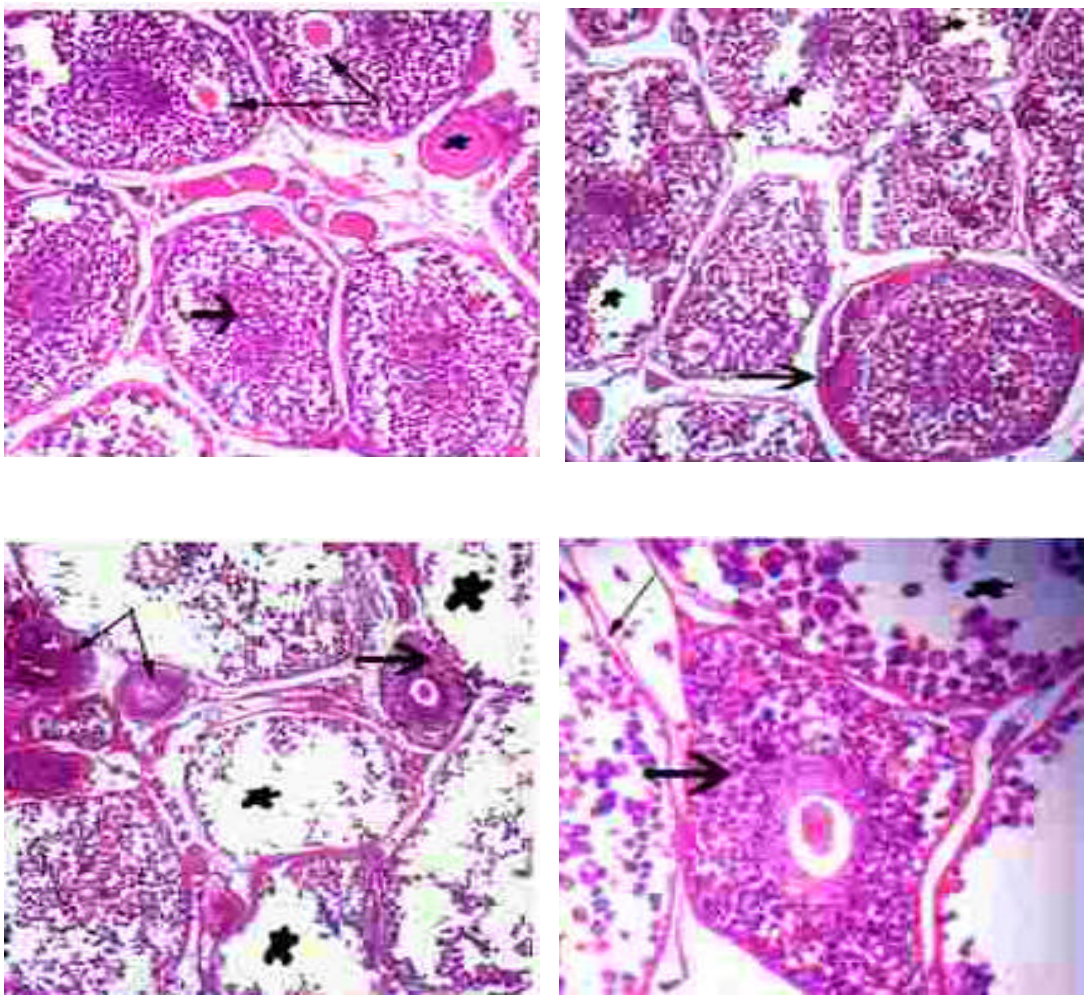
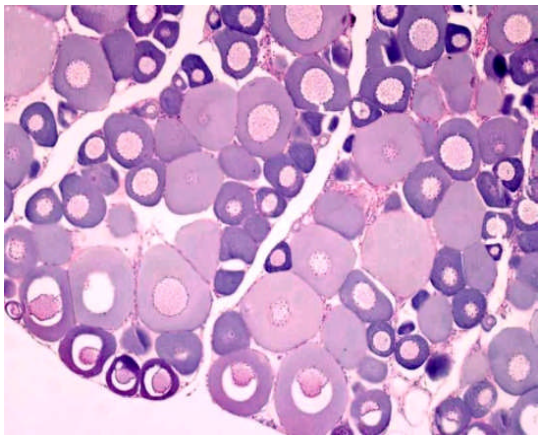
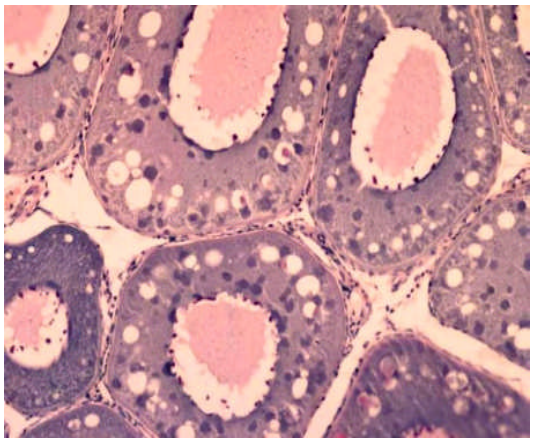


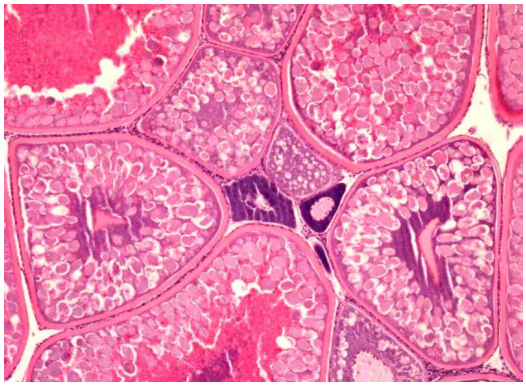
Plate-3(a-d): Sections of ovary of *Cyprinus carpio communis* fish exposed to different concentrations of Dichlorvos(DDVP),(a) ovary of control fish showing normal ovary tissue (x100), (b) showed ovary in ripe stage with slight histological changes of oocytes as liquefaction of cytoplasm of oocyte with nucleus loses its circularity and degeneration in wall of oocyte (x100). (c) showed severe of pathological alterations ovary; as lysis with atresia of oocytes (x100), (d) showed abnormal shape of oocyte in Vitellogenic stage(x100) respectively.



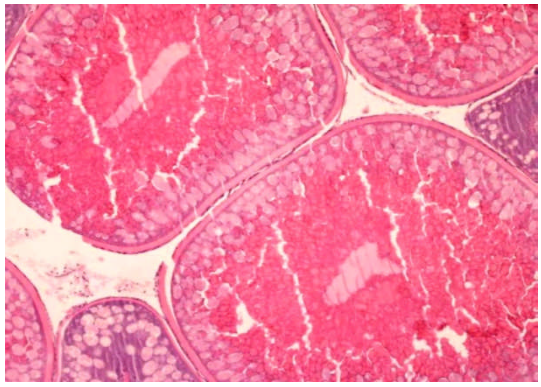
Stage 1(Previtellogenic stage), x40, HE



Stage 2(Cortical alveolar stage), x100, HE



Stage 3(Vitellogenic stage), x100, HE



Stage 4(Ovum stage), x100, HE

Plate 4: Developmental Stage of Ovary of common carp *Cyprinus carpio communis*

Chapter - 6

CONCLUSION

The general conclusion which we can draw is the obvious one that pesticides have an inhibitory effect on reproduction. Pesticide exposure of both dimethoate (Rogor) and dichlorovos (Neuon) leads to impairment in reproductive functions of female carp, *Cyprinus carpio communis*. The quality of the natural surroundings of fish has an important role in their development and reproduction. Even slight changes in concentration of certain chemical compounds can negatively affect the reproductive properties of fish. As seen in this study, marked deterioration and damage occurs even after a 48-hr exposure to very lower concentrations of pesticides. In a recent study, Pandey (1988) observed that when ovaries of the freshwater fish, *Colisa fasciatus* was treated with a 1ppm concentration of endosulfan ovarian activity was retarded and the diameter of oocytes was severely reduced. Reproductive toxicity in general, indicates changes on the pattern of breeding response, on ovary, on gonad somatic index, on fecundity, on fertilization rate, hatchability of larvae and above all survivability of larvae, fry etc. Though, it is a prime subject of research because it directly relates to productivity of fishes. Very less research has been conducted in this field. There are, surprisingly, little differences between the effects of different classes of pesticides. Evidence has been presented to show that these effects may occur at multiple sites of reproduction. They may cause lesions, hemorrhage, or malformation in the gonads and other body tissues. Production and secretion of hormones of the hypothalamus, pituitary, and gonads is usually inhibited and their metabolism by the liver can be altered. It may also be noted that the brain and ovary acetylcholine esterase activity dynamics after the test animal had been exposed to sub lethal concentrations of the test substances dimethoate and dichlorovos revealed an inhibitory effect, which was more intensive and of longer duration in case of higher concentrations. Little work has been reported on their effects on the binding of hormones to their cellular receptor sites, to plasma binding proteins, or on the production and activity of such receptors. Effects might also be expected at the level of gene transcription or translation though these are

not, perhaps, likely to be specific to reproduction. Gametes have been shown to be particularly sensitive to pesticides, both in their development, particularly the production and growth of oocytes involving vitellogenin synthesis, and in their fertility. There is also a considerable literature on the survival of eggs, larvae and fry, which are particularly susceptible to pesticides, and may have major impact on population dynamics. The scope of the review has, however, been limited to reproductive events prior to fertilization, although the boundary is not clear cut since absorption of pesticides into the yolk during vitellogenesis may result in death or malformation of the embryos or larvae at much later stages of development.

Pesticide effects on reproduction are often, of necessity, considered in isolation from other effects on the whole fish. We must, however, realize that its lifespan may be considerably shortened as a result of such exposure and that it will therefore experience significantly fewer reproductive cycles which will also affect the population dynamics. The overall impact of long term pesticide exposure can, therefore, decrease population by decreasing fecundity, decreasing the numbers of reproductive cycles in the life time of each fish, and decreasing the survival of the offspring at early stages of their life cycles. Where heavy pesticide is combined with intensive fishing, the resulting decline in fisheries catch might expected to be catastrophic.

In cases where pesticides completely suppress reproduction, rapid extinction will occur. In other cases the levels of pesticides may be such that fecundity or fertility is significantly depressed. This leads to the possibility that those fish which are, or whose gametes are, more resistant to the pesticide will produce more off springs, leading eventually to a more pesticide resistant strain of the species. There is evidence that this might occur in the killifish (*Fundulus heteroclitus,* Cyprindontidae) in which eggs from a pesticide exposed creek showed a higher fertilization rate when mixed with sperm in the presence of methyl mercury than eggs taken from a clean area (Khan and Weis, 1987c). Such adaptation to pollution may be much more widespread and could be useful in providing resistant stocks for sport fishing in recently pesticide polluted areas. Adaptation of this type, however, poses

considerable dangers since such species no longer act as biological indicators of dangers to human health and their flesh may well contain unacceptable levels of pesticides. Stocks in which such selection has occurred, and the extent of such an occurrence, might be recognizable by the lower genetic diversity in the surviving fish population than in comparable areas. In addition to such adaptation, we know little about whether fish sense low level pesticide and avoid contaminated areas or whether the toxicity of the pesticide is affected by other factors, such as pH, calcium or oxygen content etc.

Fish are prime food source for both humans and their domestic animals, and the effect of pesticides described in this review might be expected to have similar disturbances on these consumers. Pesticides can thus act both indirectly to decrease the food supply, and directly by concentration in the food chain itself on the ultimate predator, ourselves. Our understanding of their effects on fish may enable us to limit such harmful effects by monitoring and limiting their release in to the aquatic environment.

The literature covered in this review leaves no doubt that all types of pesticides have a serious inhibitory effect on fish reproduction, even when present in minute quantities. Fish thus make excellent bioindicators of the harmful effects that might be expected in mammals in general, and human populations in particular. The longer period of exposure of human populations to pesticides before completion of their reproductive activity compared with fish, and their position at the top of the food chain, together with their concentration in high population densities in close proximity to the major sources of pesticide pollution, suggests that humans may be particularly susceptible to the effects observed in fish. Recent studies (Sharpe and Skakkebak`, 1993) suggest that such a predicted decrease in human fecundity is already occurring and, as a species, we ignore such early warnings at our peril.

Though reports on the adverse impact of pesticides on the reproductive processes and endocrinology of reproduction of Indian fishes are available, studies focusing on the underlying cellular and molecular mechanisms are lacking. The nature of degenerative changes in reproductive tissues and gametogenesis in fish exposed to

pesticides are generally similar, with a few exceptions. The degree of impact varies with the nature, dose, duration of exposure, and the physiological state of fish at the time of exposure. Organophosphates appear to be more toxic as they are potent at relatively low concentration, perhaps due to their rapid accumulation and long persistence in the lipid rich gonads during the active phase of reproduction, while organochlorines and carbamates induce changes at relatively higher concentrations. Very little information is available on the reversibility of the adverse effects of pesticides after withdrawal of treatment. The majority of studies show the effects of a single dose of pesticides. The use of graded doses of pesticides could help in identifying in the minimum pesticide dose that will affect fish reproduction and other targets. Adequate field studies have not been carried out in India. There is practically no basic information on the reproductive status of fishes collected from pesticide polluted natural water ecosystems. The effects of pesticides on fishes discussed herein are based on experimental laboratory conditions. Most Indian workers have carried out their studies in static systems in which fishes are exposed to pesticide solutions, which are replaced with the same concentration at definite intervals, while in nature fishes usually live in flow-through water bodies. Moreover, a good amount of pesticides undergo degradation, absorption, and transformation depending on the physio-chemical and biological factors of the static or natural water ecosystem. These laboratory data therefore have to be validated with field data. Studies on the effects of pesticides on reproduction have also ignored the impact of changing environmental factors such as photoperiod, temperature, salinity, pH, nutritional status, etc., since these factors not only synchronize and influence reproduction , but also influence the metabolism of pesticides and thereby the overall nature and degree of their impact.

Hence, on the basis of this study we can compare toxicity of these selected pesticides to other pesticides and can also use common carp as a model for other fish species. The reported results would be useful contribution in ecotoxicity risk assessment studies of these organophosphate pesticides on fish species.

REFERENCES

Abbot, O. C., Harrison, R.B., Tandon, J. O. G. and Thomson, J. (1965). Organochlorine pesticides in the atmospheric environment, *Nature*, **208**(5017): 1317-1318.

Abdus, S. B., Badrun N. and Quamrun, N. (2001). Effects of sumithion on the Histological changes of spotted Murrel, *Channa punctatus* (Bloch). *Pak. J. Bio. Sci.* **4**(10): 1288-1290.

Abu, T. K., Judith S. W. (1987). Toxic Effects of Mercuric chloride on sperm and egg viability of two populations of Mummichog, *Fundulus hetroclitus*. *Environmental pollution* **48**: 263-273.

Adhikari, S., Sarkar, B., Chattopadhyay, A., Chattopadhyay, D.N., Sarkar, S. K. and Ayyappan, S. (2006). Effect of cypermethrin on breeding performances of a fresh water fish, *Labeo rohita* (Hamilton). *Chemistry and Ecology* vol. **22**(3): 211-218.

Akobundu, I. O., Sweet, R. D. and Duke, W. B. (1975). A method of evaluating herbicide combinations and determining herbicide cynergism. *Weed science*. **25**(1): 20-25

Aldridge, W. N. and Reiner, E. (1972). Enzyme Inhibitors as substrates. *North Holland publishing Co*., *Amsterdam*.

American Public Health Association (APHA), American water works association (AWWA) and water pollution control Federation (WPCF) (1985). Standard methods for Examination of water and waste water. *16th Edn*., *APHA New York*, pp: 1193.

Anam, K. K. and Maitra, S. K. (1995). Impact of quinalphos on blood glucose and acetylcholinesterase activity in brain and pancreas in a rsoseringed parakee, *Psittacula Kramesi* borealis; Newmann. *Arch. Environ. Contem. Toxicol*, **29**: 20-23.

Anderson, D.G., (1969).Trans of the 1959 seminar on biological problems in water pollution ,*R.A. Tabt. Samitary Eng. Cant. Tech.Rept*. W60-3.

Anjali, R., and Kulshrestha, S.K. (1990). Effect of sublethal Doses of three pesticides on the ovary of a carp Minnow *Rasbora daniconeus*. *Bull. Environ. Contam-toxicol*. **45**: 742-747.

Anokhina, L. E. (1963).Some aspects of the fecundity of the herring in the White sea.*Rapp. P.V. Revn. Perm. Cons-Explor.Mcr*.**154**: 123-127.

Ansari, B. A. and Kumar, K. (1984). Malathion toxicity: In vivo Inhibition of acctylcholinesterase in the fish *Brachydanio rerio* (Cyprinidae). *Toxicol .lett*. **20**: 283-287.

APVMA (2008): Australian pesticides and veterinary Medicines Authority Dichlorvos: *Environmental Assessment APVMA*. P.O. Box E240 KINGSTON Act 2604 Australia pp: 1-71.

Aston, L. and Seiber, J. (1996). Exchange of airborne organophosphorus pesticides with pine needles. *Journal of Environmental Science and Health*, Part B, Pesticides, Food Contamination and Agricultural Wastes, **31**: 671-698.

ATSDR (Agency for toxic subethal and Disease Registry) (1997). Toxicological profile for Diclorvos, U.S. Department of Health and Human Services public health service 1600 Clifton Road NE, F-29, Atlanta, Georgia 30333, p: 1-119.

Auqustinson, K. (1948). A study in comparative enzymology*. Acta physical*. Second-15 supplementum **52**.

Auta, J. and Ogueji, E. O. (2006). Sublethal Effect of Dimethoate on growth and Food utilization of *Oreochromis niloticus* (Trewavas) Med. *J.Fish-Int*. **1**(4-2): 163-166.

Bagenal, T. B. (1971). The Interrelationship of the size of fish eggs, the data of spawning and reproductive cycle. *Journal of fish Biology* **3**: 207-219.

Bahner, L. H. and Nimmo, D. R. (1976) metals, pesticides and PCB's; toxicity to shrink singly and in combination In: Esturine processes (Ed Wilely, M.), 1: 523-532, *Academic Press New York*.

Balint, T., Szegletes, T., Szegletes, Z. S., Halasy, K. and Nemesok, J. (1995). Bochemical and Subcelluar changes in carp exposed to the organophosphorus methidathion and the pyrethroid deltamethrin. *Aquat. toxicol*. **333**: 279-295.

Bandi, A., Spandrof, A. and Calmi, R. (1957). The nutritive value of various fodder plants of carp. *Bamidgeh* **9**(1): 13-18.

Basha, S.M. (1980). A comparative study of the three types of the fresh water teleost, *Tilapia mosambica* (Peters) under subacute exposure, M.Phil-Thesis-Sri Venkateswara University, Tirupati.

Bauman, F. (1981).Draft environmental report on India SA/ToA 1-77.074, *PN-AAH-752, ARDA* vol.**9**(2): 31-32

Begum, G. and Vijayaraghavon, S. (1995). In vivo toxicity of dimethoate on protein and transminase in the liver tissue of freshwater fish *Clarias batrachus* (Linn.) *Bull Environ-contam. Toxicol*. **54**: 370-375.

Bender, M.E.(1969). The toxicity of the hydrolysis breakdown product of malathion to the fathead minnow,*Pimephales promelas* Raf. Wat. Res.**3** : 571-582.

Boetius, J. (1961). Lethal action of mercuric chloride and phenyle mercuric acetate on fish. *Medd. Danm. Fisherki-OgHavinders N. S*. **3**:93-115.

Boyd, C. E (1964). Insecticides cause mosquito fish to abort. *progr.fish.cult*. **26**:138.

Boyd, C. E. (1963). Insecticides cause mosquito fish to abort. *Prog. Fish. Culturist*. **26**: 138.

Buckler, D. R., Witt, A. M. and Huckins, J. N. (1981). Acute and chronic effects of kepone and mirex on the fathead minnow. *Trans. Am. Fish. Soc*. **11**: 270.

Burdick, G. C., Dean, H. J., Harris, E. J. and Karcher, R.(1972). Feeding DDT on the reproduction of salmonid fishes reared and held under controlled condition, *N.Y Fish Game*.J. **19**:97-115.

Butler, P.A.(1969).In: Chemical fall out,(ed.miller, M.W. and BERG. G.g.,), *Thoms, C.C., Springfield, iilinois*, pp. 531.

Butler, P.a., Chidress, R. and Wilson, A.J. Jr.(1970).*FAO Tech. conference* on marine pollution and its effect on living resources and fishing. *Rome,Italy*, ,Dec. 9-18, p.13.

Caio, R. D. A., Ian, P. G. A., Patricia, F. C., Luiz, B. C. and Ramilson, S. B. (2007). Effect of dicholorovos on the acetylcholinesterase from tambaqui, *Colorsoma macropomum* brain. *Environ. Toxicol*. **22**(2): 194-202.

Campagna, A. F., Eler, M. N., Espindola, E. L. G., Senhorini, J.A., Rego, R. F. and Silva, L. O. L. (2006). Dimethoate 40% organophosphorus pesticide toxicity in *Prochilodus lineatus* eggs and larvae.

Cairns, J., Jr., Health, A.G. and Parker, B.C. (1975). The effects of temperature upon the toxicity of chemicals to aquatic organisms. *Hydrobiologia*, **47** : 135.

Carlson, A. R. (1972). Effects of long term exposure to carbaryl (Sevin) on survival, growth and reproduction of fathead minnow, *Pimephales promdelas*. *J. Fish Res*. *Beard Can*. **29**: 583-587.

CEPA (1996): California Environmental Protection agency (1996)-Dichlorvos (DDVP) Risk characterization document, medical toxicology and worker Health and safety Department of pesticide regulation CEPA, USA. pp: 1-119.

Chambers, J. E. and Yarbrough, J. D. (1975). Parathion and Methyparathion toxicity to insecticide resistant and susceptible mosquito fish, *Gambusia affinis. Bull. Environ. Contam. Toxicol*. **14**: 315-320.

Chandra, S. and Ram, R.N. (2004). First ovarian maturity and recovery response in common carp, *Cyprinus carpio* after exposure to carbofuran. *J. Environ. Biol*. **25**: 239-249.

Charler, M. W. (1958). The Determination of choline-starase in the Brain tissue of three species of fresh water fish and its Inactivation in Vivo. *Bull Environ Contam toxicol*. **82**: 569-573.

Chatterjee, S., Dutt , A. B. and Ghosh, R. (1997). Impact of carbofuran in the oocyte maturation of the cat fish *Heteropneustes fossilis* (block). Arch. *Environ contam toxicol*. **32**: 426-430.

Chciko, G. M., Podgornaya, V. A., Zhelnin, Y. Y. (2000). Acetylcholinesterase and butrylcholinesterase activities in brain and plasma of fresh water teleosts: cross-species and cross family differences*. Comp Biochem physiol C. toxicol pharmacol*. **127**(3): 233-42.

Chindall, Ac; Sikoki; F.D. Vincent-Akpu, IJEOMA (2004).

Chirashree, C., Arun K. R., Samir B. and Shelly B. (1992). Non lethal concentrations of pesticides impair ovarian function in the fresh water perch, *Anabas testudinenus*. *Environmental biology of fishes.* **36**: 319-324, 1993.

Chirashree, C., Ray, A. K. and Bhattacharya, S. (1993). Non lethal concentration of pesticides impair overian function in the freshwater perch, *Anabas testudineus*. *Environ. Boil. Fishes*. **36**: 319-324.

Chowdhury, S.R.D., Ghose,J.L., Lonsane, B.K. and Sandhu, D.S. (1981).Pollution through pesticide plants- treatment and control, *J.Sci Ind.Res.,* **40**(2):125-131.

Chuiko, G. M. (1999). Comparative study of acetylcholinesterase in brain and serum of several fresh water fish: specific activities and invitro inhibition by DDVP, an organophosphate pesticide. *Comp Brochem physiol C pharnacol toxicol Endocrinol*. **122**(1) 21-5.

Chuiko, G. M., Slynko, Y. V. (1994). Relation of allozyme genotype to survivorship of juvenile bream, *Abramis brama* L., acutely exposed to DDVP, an organophosphorus pesticide. *Bull Envirn. Contam. Toxicol*. **52**(3): 338-45.

Claudia, A. D. B., Mana, D. F. M. V. and Erica P. G.C. (2003). Ovary maturation stages and Oocyte features in three species of the Neotropical fish *Aemiodus* (Muller, 1842) *Brazilian archives of Biology and technology* vol. **46**: on pp 433-441.

Cobioch, L. (1973). The fight against pollution by oil on the coasts of Brittainy, In: Water pollution by oil (Ed. Hepple, P.). *Great Britain*. 245-249.

Cohen, S.D. and Murphy,S.D.(1974).A simplified bioassay for OP detoxification and interactions, *Toxicol, and Appl. Pharmacol*.,**27**,537.

Cope, O. B., Wood, E. M. and Watten, G. H. (1970). Some chronic effect of 2, 4-D on the bluegill, *Lepomis macrochirus Trans. Am. Fish. Soc*. **99**: 1.

Cowman, D. and Mazanti, L. (2000). Ecotonicology of "new generation" pesticides to amphibians. In. C. Bishop, G. Linder and D. sparling (Eds). *Ecotoxicology of Amphibrans, Reptiles*. pp. 233-268. Pensacola, FL: SETAC.

Crandall, C.A. and Goodnight, C.J.(1962).Effects of sub lethal concentrations of several toxicants on growth of common guppy, *Lebistes reticulates.Limmon .Oceanog*,**7**:233-239.

Curtis, L. R., Beyers, R. J. (1978). Inhibition of ovipositions in the teleost *Oryzias latipes*, induced by subacute kepone exposure. *Comp. biochem. Physio*. **61**: 15.

Davies, J.S. (1975). Minimal dissolved oxygen requirement of aquatic life with emphasis on Canadian species: *a review. J. Fish. Res. Bd. Can*. **32**(12): 2295-2332.

Day, K. E. and Scott, I. M. (1990). Use of acetylcholinesterase activity to detect sublethal toxicity in stream invertebrates exposed to low concentration of organophosphates insecticides. *Aqual. Toxical*. **18**: 101-104.

Dey, S. and Bhattacharya, S. (1989). Ovarian damage to *Channa punctatus* after chronic exposure to low concentration of elsan, mercury and ammonia. *Ecological Environ safety*. **17**: 247-257.

Dikshith, T. S. S., Nath, G. and Datta, K. K. (1978). Combined cytogenic effects of Endosulphan and Metapa in male rats, *Ind. J. Exp. Biol*., **16**(9): 1000-1002.

Doudoroff, P. (1952). Some recent developments in the study of toxic industries wates. *Proc. 4th Pacific Northwest Industr. Waste conf., State Coll of Washington, Pullman, Wash*. 21-25.

Duangswawasdi, M. and Klaverkamp, J.F.(1979). Acephate and fenitrothion toxicity in rainbow trout : effects of temperature stream and investigations on the sites of action. *Aquat. Toxicol*.**35.**

Edwards, C. A. (1973).In: Environmental Pollution by pesticides, *Plenum Press ,London and New York.*

Ellman, G. L., Courtney, K. D. and Andreas, V. (1961). A new and rapid colorimetric determinatim of acetylcholinestearse activity. *Biochem pharmacol*. **7**: 88-95.

Environmental protection Agency EPA. (1975). Methods for the acute toxicity tests with fish, macroinvertebrates and amphibians. The committee on Methods for toxicity tests with aquatic organisms, *Ecological Research Series EPA*. **9**: 660-75.

EPA, Toxic substance control, Part IV 16 March (1979).

Erwin, W. M. R., Rineke, K., Cornelis, A. M. and Van, G. (2003). Acetylcholinesterase inhibition and increased food consumption rate in the zebrafish, *Danio rerio*, after chronic exposure to parathion *Environ Res*. **91**(3): 157-62.

Frawley, J.P. (1965).Synergism and antagonism ,In:Chichester,C.O.ed., Research in pesticides,*Academic Press, New York*,PP.69-83

Freeman, H. C. and Idler, D. R. (1975). The effect of polychlorinated biphenyl (PCB) on steroidogensis and reproduction in the brook trout, *Salvelinus fontinalis*. *Can. J. Biochem*. **53**: 660-670.

Frumin, G. T., chuiko, G. M., Pavlov, D. F. and Menzykova, V. (2009). New rapid method to evaluate the median effect concentrations of xenobiotics in hydrobiouts. *Hum Exp toxicol*. **21**: 20.

Ghatak, D.B. and Konar, S.K.(1991).Chronic effects of mixture of pesticides, heavy metal, detergent and petroleum hydrocarbon in various combinations on fish.*Ecol*.**9**:829-836.

Ghosh, C., Ray, A. K., Bhattacharya, S., and Bhattacharya, S.H. (1999). Effect of an organophosphate pesticide in the induced ovulation of maturity air breathing

fish *Anabas testudineus* (Bloch) with hypothalamic gonadotropin releasing hormone fraction from *Channa punctates* (bloch) poll Res. **18**: 121-127.

Ghosh, R., Dutta, J. (1985). Malathion toxicity in air breathing catfish Singhi, *Heteropneustes fossilis*. *Bulletin ind. J. Fish*. **32**: 447-452.

Ghosh, T. K. and Konar, S. K. (1976). Safe disposal of pesticides; a new method of estimation tested on ambithion. *Proc Natt Acad sci India sect*. B. **46**: 373-387.

Ghosh, S. K. and Ponniah, A.G. (2001). Freshwater Fish Habitat Science and Management in India. *Aquat. Ecosyst. Health Manage*. **4**(4): 367-380.

Golovanova, I. L., Chuiko, G. M., Pavlov, D. F. (1992). Effects of cadmium, naphthalene, and DDVP on gut carbdydrases activity in bream (*A bramis brama L*.) and *Mozanbique tilapia* (*Oreochromis morsambicus* Peters). *Bull environ. Contam toxicol*. **49**: 361-7.

Golovanova, L., Kuz'mina, V.V., Gobzhelian, T. E., Pavlov, D. F. and Chuiko, G. M. (1999). In vitro effects of cadmium and DDVP (dichlorvos) on intestinal carbohydrase and protease activities in fresh water telecost. *Bull envirn. Contam Toxicol*; **62**(2): 193-8.

Goodman, L. R, Hansen. D. J., Manning, C. S. and Favas, L. F. (1982). Effects of kepine on the sheeps head minnow in an entire life cycle toxicity test. *Arch. Environ. Contam. Toxicol*. 11-335.

Graham, R. S. and Katherine A. S. (2003). The effects of Environmental pollutants on complex fish behaviour. Integrating behavioural and physiological indicators of toxicity. *Aquat toxicol* 10: **64**(4): 451-60.

Grazenda, A. R., Paris, D. P. and Taylor, W. J. (1970). The uptake, metabolism and elimination of chlorinated residues by Gold Fish, *Carassius auratus* fed a C^{14} DDT contaminated diet. *Trans. Am. Fish. Soc*. 101: 686-690.

Gruber, S. J. and Munn, M. D. (1998). Organophosphate and carbamate insecticides in Agricultural waters and cholinesterase (ChE) inhibition in common carp, *cyprinus carpio*. *Arch. Environ. Contam Toxicol.* **35**: 391-396.

Gunther, A. (1868). *Cat. Fish. Brit. Mus. Lond* **7**: 25.

Gupta, R. C. (2006). Toxicology of organophosphate and carbamate compounds Amsterdam Boston Elsevier Academic Press 763.

Haider, S. and Upadhyaya, N. (1985). Effect of commercial formulations of four organphosphorus insecticides on the ovaries of a fresh water teleost. *Mystus vittatus* (Bloch)-A histological and histochemical study *J. Environ Sci. Health B*. **20**: 231-340.

Haider, S. and Upadhyaya, N. (1986). Effect of commercial formulation of four organophosphorus insecticides on the LH-induced germinal vesicle breakdown in the oocytes of a fresh water teleost. *Mystus vitatus*-A preliminary in vitro study. *Ecotoxicol Envion safety*. **12**: 161-165.

Halter, M. T. and Johnson, H. E. (1974). Acetetoxicities of a polychlorinated biphenyl (PCB) and DDT alone and in combination to early life stage of coho salmon, *Oncorynchus kisutch*. *J. App. Ecol*. **5**:48.

Hanson, R., DoDoo, D. K., Essumang, D. K. (2007). Uptake and toxicity of some pesticides on three freshwater fishes, *Oreochromis niloticus*, *Clarias gariepirnus* and *Chrysicthys nigrodigitatus*. *Toxicological and Environmental chemistry*. **89**(1): 111-123

Hazarika, R. and Das, M. (1998). Toxicological impact of BHC on the ovary of the air breathing cat fish, *Hetropnenstes fossilis* (bloch). *Bull. Environ. Contam and Toxicol.* **60**(1): 16-21.

Hiltibran, R. C. (1974). Oxygen and phosphate metabolism of bluegill liver mitochondria in the presence of some insecticides, *Trans. ILLs. State Acad. Sci*. **67**(2): 228-237.

Hiran, M. D. and Dane, A. (2000). Effects of Endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish. *Environ toxicol chem*. **21**(7): 1469-74.

Holcombe, G.W., Flandt, J.T. and Phipps, G.L.(1980). Effect of pH increases and sodium chloride addition on the acute toxicity of 2-4 dichlorophenol to the fathead minnow. *Water Res*.**14**: 1073.

Holden, A. K. (1972). The effects of pesticides on life in freshwater, *proc. R. soc. London ser. B*. **180**: 383.

Holden, A. V. (1962). The absorption of C^{14} labelled DDT from water by fish .*Ann .Appl. Biol*., **50**:467-477.

Hoss, D. E., Klements, L. C. and Kolby, D. R. (1977). Synergistic effects of exposure of temperature and chlorine on survival of young of the year esturine fishs. In: "Biological responses of marine biota to pollutants". *Academic Press, Inc*., London. 344-376.

Howells, G. and Bath, D. (1969). Trace elements in the Hudson River. Development of a biological monitoring system and a survey of trace metels, madionucleids and pesticide residues in the Hudson River, *New York, Univ. Medical Centre, Instt. Of Environ. Medicine.*

Hunt, E.G., and Bischoff, A.I. (1960). Immical effects on wildlife of pesticide DDT applications to clear lake, *Calif. Fish Game***, 46**:91.

Hynes, H. B. N. and Roberts, F. W. (1962). The biological effects of synthetic detergents in the river Lee, Hertfordshire, *Ann. Appl. Biol*. **50**: 779-790.

Inglis, A, and Davis, E.L. (1972). Effects of water hardness on the toxicity of several organic and inorganic herbicides to fish.

Ismail, M., Ali, R., Ali, T., Usman, W. and Qaiser, M. K. (2009). Evaluation of the acute toxicity of profenofos and its effects on the behavioural pattern of fingerling common carp, *Cyprinus carpio (L., 1758*). Bull Environ. Contam toxicol. **82**: 569-573.

Jenkins, J. T. (1961). The fishes of the British Isles 2nd ed. London: Fredrick Warne and Co.

Johnson, W.W. and Julin, A.M. (1980). Acute toxicity of toxaphene to fathead minnows. *EPA-600/3-80. 005. U.S. Environmental Protection Agency, Duluth: inn.*

Johnson, W.W. and Finley, M.T.(1980). Handbook of acute toxicity of chemicals to fish and Aquatic invertebrates. *Resource publ. 137, US Department of Interior Fish and wildlife Service Washigton, DC.*

Johnsson, C. M. and Toledo, M. C. F. (1993). Acute toxicity of endossulfan to the fish *Hyphessobrycon bijassciatus* and *Brachydanio rario*. *Archiv environ. Cuta Toxicol*. **24**: 151-155.

Jones, S. B., king, L. B., Sappington, L. C., Dwyer, F. J., Ellersieck, M. and Buckler, D. R. (2003). Effects of carbaryl, permethrin, 4-nonylphenol, and copper on muscarinic cholinergic receptors in brain of surrogate and listed fish species. *Biochem physiol B Biochem Mol Biol*. **135**(1): 55-61.

Kabeer, A. S., Sailatha, D. and Ramana, R. K. V. (1980). Impact of melathion on acetycholinesterase in the tissues of the fish, *Tilapia mossambica* (Peters). A time course study. *J. Biosie*. **2**(1): 37-41.

Kabeer, A. S., Jagannatha, R. K.S, and Ramana, R. K.V. (1981). Effect of malathion exposure on some physical parameters of whole body and on tissue cations of telost *Tilapia mossambica* (peters). J*. Biosco*. Vol. **3**, No.1 pp. 17-21.

Kaur, K. and Toor, H. S. (1977). Toxicity of pesticides to embryornic stages of *cyprinus carpio communis*. *Indian J. Exp Bio.* **15**: 193-196.

Kaur, K. and Dhawan, A. (1993). Variable sensitivity of *Cyprinus carpio's* eggs. Larvae and fry to pesticides. *Bull. Environ. Contam toxicol*. **50**: 539-597.

Keizer, J., Agostino, G. and vittori, L. (1991). The Importance of biotransformation in the toxicity of lenobiotics to fish. Toxicity and bioaccumulation of diazinon in guppy, *Poecillia reticulate* and zebra fish, *Brachydanio rerio*. *Aquat.toxicol*. **21**: 239-254.

Kerr, S.R. and Vass, W.P. (1973). Pesticde residues in aquatic invertebrates,In:Environmental Pollution by Pesticides *(Ed.Edwards*, *C.A*),*Plenum Press, London* ,134-180.

Khalid, A., Ghanion, A., Hmoud, F., Kahem, A., Balawi, A., Akel, A. A., Fahad., A. M., Zubair, A. and Annazri, H. (2008). Ethological response and haematological and biochemical profiles of carp, *Cyprimus carpio* exposed to trichlorfon*. J. food, Agri. and Environ*. Vol. **6** (3edn.) 473-479.

Khan, A.T. and Weis, J.S.(1987c). Effect of methyl mercury on egg and juvenile viability in two populations of killifish, *Fundulus heteroclitus*.*Env.Res*.**44**: 272-8

Kirubagaran, R. and Joy, K. P. (1990). Changes in brain monoamine levels and monoamine oxides activity in the catfish *clarias batrachus* (L.) during chronic treatments with mercurials*. Bull Environ. Contam. Toxicol*. **45**: 88-93.

Kling, D. B. (1986). Toxic effects of sublethal concentrations of Lebaycid upon the ovaries of *Sarotherodon leucostictus*. *Z. Angero. Zool.* **73**(1): 75-92.

Koenig, C. C. (1975). The effects of DDT and mirex alone and in combination on the reproduction of a Salt Marsh Cyrinodont fish, *Adinix xenica*, Phd *Thesis, Florida University, Tallahassee, Florida, USA*.

Krishnan, M. and Chockalingam, S. (1989). Toxic and subethal effects of endosulfan and carbaryl on growth and egg production of *Monia micrura* kurz. Enviro. Pollut. 0269-749. *Elsevier sci. pub ltd, England.* 319-326.

Kristen, M. G., Aaron, P. R., Norman, E., Anthony, D. S. and Stephen, J. K. (2005). Biochemical and behavioral effects of Diazinon Exposure in Hybrid striped Bass. *Bull Environ. Contam Toxicol*. **75**(2): 368-73.

Kristensen, P. (1994). Sensitivity of embryos and larvae in relation to other stages in the life cycle of fish, a literature review, pp. 339-352. In: R, Muller and R., Lloyd (eds). sublethal and chronic effects of pollutants on freshwater fish. United nation organization: Fishing News Books.

Kulshretha, S. K., and Arora, N. (1984). Impairments induced by sublethal doses of two pesticides in the overies of fresh water telecast *Channa Striatus* (bloch). *Toxicol. Lett*. **20**(1): 93-98.

Kumar, K. and Ansari, B. A. (1984). Malathion Toxicity. Skeletal deformities in Zebra fishes, *Brachydanio rerio*, *Cyprimidae pestic. Sci* **15**: 107-111.

Kumar, S. and Pant, S. C. (1988). Comparative sublethal ovarian pathology of some pesticides in the teleost *Puntius chonchonuis* (Hamilton). *Bull Environ. Contain toxicol*. **41**: 227-232.

Kumar, S. and Singh, M. (2000). Toxocity of dinethoate to a fresh water teleost, *Catta catta. J. Exp. 2001. India.* **3**(1): 83-88.

Kuz' mina, V. V. *et al*. (2003). Effects of adrenaline and piracetam on fish feeding behaviour. *J. Evol. Biochem. Physol*. **39**(4): 451-456.

Kuz'mina, V. V., Chuiko, G. M. and Pavlov, D. F. (1995). Effects of DDVP, naphthalene, and cadmium an intestinal proteolytic activity in Mozambique tilapia *Oreochromis mossambicus* (Peters). *Bull envirn. Contam Toxicol*. **55**(5): 738-45.

Lal, B. (2007). Pesticide-Induced reproductive dysfunction in Indian fishes. *Fish physiol Biochem*-**33**: 455-462.

Lal, B. and Singh, T. P. (1987a). Changes in tissue lipid levels in the freshwater catfish, *Clarias batrachus* associated with the reproductive cycle. *Fish physiol Biochem*. **3**: 191-120.

Lal, B. and Singh, T. P. (1987b). The effect of malthion and gamma BHC on the lipid metabolism in relation to reproduction in the tropical teleost, *Clarias batrachus. Environ poll.* **48**: 37-47.

Lal, B. and Singh, T. P. (1987c). Gamma BHC and cythion-induced alteration in lipid metabolism in a fresh water catfish, *Clarias batrachus* during different reproductive phases of its annual reproductive cycle. *Ecotxicol Environ safety*. **13**: 56-63.

Lal, B. and Singh, T. P. (1987d). Impact of pesticides on lipid metabolism in the freshwater catfish, *Clarias batrachus*, during the vitellogenic phase of annual reproductive cycle. *Ecotoxicol Environ safety*. **13**: 13-23.

Ligia, M. S., Benjamim, E. and Metry, B. (2008). Effects of endosulfan sublethal concentrations on carp, *Cyprinus carpio*, (Linnaeus, 1758). Morphometric, hystologic, ultrastructural analyses and Cholinesterase activity evaluation. *Braz. J. Vet. Res. Anim. Sci. Sao Paulo*, V. **45**, n. 2, p. 87-94.

Lioyd, R. and Jordan, D.H.M.(1964). Some factors affecting the resistance of rainbow trout, *Salmo gairdneri* Richardson to acid waters. *Ind. J. Air. Wat*. *Pollut.* **8**: 393-403.

Livingston,R.J., Koeing, C.C ., Lincer, J.L., Michel, A., MicAliffe, Nadeau, R.j., Sparks, R.E Thompson, N., and Vaughn, B.E.(1974).Synergism and modifying effects: Interacting factors in bioassay and field research,*In:Marine Bioassay Workshop Proceedings.marine Technological Society*,Washington D.C.225-304.

Loskowski, M. B. and Dettbam, W. D. (1975). Presynaptic effects of neuromuscular cholinesterase Inhibition. *J. Pharmac. exp. ther.* **194**: 351-361.

Macek, K. J. (1968). Reproduction in brook trout, *Salvenlinus fontinalis*, fed sublethal concentration of DDT. *J. Fish. Res. Board can*. **25**: 1787.

Macek,K .J., Hutchinson, C. and Cope, O.B.(1969). The effect of temperature on the susceptibility of blue gills and rainbow trout to selected pesticides. *Bull. Environ. Contam. Toxicol*. 4: 147.

Macek, K. J. and McAllister, W. A. (1970). Insecticide susceptibility of some common fish family representatives. *Trans. Am. Fish. Soc.* **99**: 20-27.

Macek, K. J. (1975). Acute toxicity of pesticide mixtures to Bluegills. *Bull. Environ. Contaim. Toxicol*. **14**: 648-652.

Martine, B., Christelle, E., Xavier C. and View, S. (2004). The use of Zebra fish mutants to identify secondary target Effects of acetyleholinesterase inhibitors. *Toxsci.* **77**, No. 2,325-333.

Matsumura, F., Boush, G. M. and Misato, T. (1972). Environmental toxicology of pesticides, *Academic Press, New york, London*.

Mauck, W.L., Olson, L.E. and Marking. L.L.(1976). Toxicity of natural pyretrins and five pyrethroids to fish. *Arch. Environ. Contam. Toxicol*. **4** :18.

Mauck, W.L., Olson, L.E. and Hogan, J.W.(1977). Effects of water quality on deactivation and toxicity of mexacarbate (zectran) on fish. *Arch. Environ*. *Contam. Toxicol*. **6** : 385.

Mendel, B. and Rudney, H. (1943). On the type of cholinesterase present in brain tissue. *Science.* **98**: 201-202.

Monserrat, J. M. and Bianchcini, A. (1998). Main kinetic characestics of thoracic cholinesterase of Chasmagnathus gramulate (Decapoda, Graspidae). Comp. Biochem. *Physiol. 120c*. 193-199.

Morse, D. I. (1978). Some comments on the assessments of joint action in the herbicide mixtures.*Weed Science*, **26**(1),58-71.

Mouat, D.I. and Stephan, C. E. (1967). A method for establishing acceptable toxicant limits for fish; malathion and the butoxyethnolesters of 2, 4-D Trans. *Amer. Fish. Soc*. **96**: 185-93.

Murphy, P. G. and Murphy, J. V. (1971). Corellations between respiration and direct uptake of DDT in the mosquito fish, *Gambusia affinis*. *Bull. Environ. Contam. Toxicol*. **6**:581-588.

Murphy, S. D. and DuBois, K. P. (1957).Quantitative measurement of inhibition of the enzymatic detoxification of malathion by EPN (Ethyl P-nitrophenyl).

Murthy, A. S., Rajabhushanam, B. R., Ramani, A. V. and Chritopher, K. (1983). Toxicology of fenitrothion to the fish, *Mystus cavasius* and *Labeo rohita*. *Environ, Pollution (Series A)*. **30**: 225-232.

Murty, A. S. (1986). Toxicity of pesticides to fish. *CRC Press Inc., Boca Raton, F.L*., 143p.

Naglrer, J. J., Aysola, P., and Ruby, S. M. (1984). The seasonal effect of sub-lethal pentachlorophenol exposure on ovarian development in rainbow trout, *Salmo gairdneri*. *11th annual aquatic toxicity workshop, Richmond, B.C. Nov* 13-15.

Nathaniel, L., Scholz, N. K. T., Barbara, L. F., Barry A. B., Thomas, P. Q., Edmundo, C. and Tracy, K. C. (2000). Diazinm disrupts antipredator and homing behaviours in chinook salmon, *Oncorhynchus tshawytsch)*. *Can. J. Fish. Aquat. Sci.* **57**: 1911-1918.

Nelson, J. S.1984. Fishes of the World. *John Wiley and Sons*, New York.

Nevin, U. and Flif, O. Y. S. Neuro-toxicity Evaluation of the organofluorine pesticide Ctoxazole in the Brain of *Oreochromis niloticus*. Ukv Biokhim : **76**(6); 39-43.

Nicholson, H. P. (1970). Occurrence and significance of pesticide residues in water. *J. Washington Acad. Sci*. **59**: 77-85.

Nimmo,D.R. and Bahnar,L.H.(1974).Some physical consequences of PCB's and salinity stress in penaeid shrimp,In: *pollution and physiology of Marine organisms* (Vernber,F.J. and Vernber, W.B.),*Academic Press,new York.*

O'Brein, R.D. (1976).Acetylcholinesterase and its inhibition. In:*Insecticide biochemistry and physiology(ed) C.F Wilkinson(new York:plenum press)* pp.271-286.

OECD, (1992). Draft OECD guidelines 210. Fish early life stage toxicity test, fish toxicity on egg and sac fry stages, fish juvenile growth test.

OECD, paris, (1981). Test guideline 203, Decision of the council C (81) 30 final and updates.

Oern, S., Andersson, P.L., Foerlin, L., Tysklind, M. and Norrgren, L. (1998). The impact on reproduction of orally administered mixture of selected PCBS, in zebra fish, *Danio rerio*. *Arch. Environ. Contam. Toxicol.* **35**(1): 52-57.

Ogilvie, D.M., and Anderson, J.M.(1965). Effect of DDT on temperature selection by young Atlantic salmon*, Salmo solar*, *L.Fish. Res. Board Can*. 22 : 503.

Okada, Y. (1960). Studies on freshwater fishes of Japan. *S. Fac. Fish. Univ. mie*. **4**(2) 267-588.

Oruc, E. O. and Usta, D. (2007). Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ. toxicol. Pharmacol*. **23**: 48-55.

Pal, A.K. and Konar, S.K. (1987).Long-term effects of organophosphorus insecticides methyl parathion on fish.*Environ.Ecol*.**5**: 564-571.

Palumbo, R.F (1961). Recent Advances IN Botany, Vol, **II**, *University of Toronto Press*, 1367.

Pan, G. and Dutta, H. M. (1998). The Inhibition of brain accetylcholinesterase activity of juvenile largemouth bass, *Micropterus salmoides* by sublethal concentration of diazinon. *Environ. res*. **79**: 133-137.

Pan, G. Dutta, H. M. (1998). The inhibition of brain acetylcholiesterase activity of juvenile large month bass *Micropterus salmoides* by sublethal concentrations of diazinon. *Comp Biochem physiol C. pharmacol toxicol Endorinol*. **120**(3): 405-14.

Pandey, A. K. and Shukla, L. (1980). Effect of beuzene hexachloride-an insecticide on the testicular histology in *Oreochromis mossambicus. Geobios* **7**: 251-253.

Pandy, A.K. and Shukla, L. (1982).Effect of an organophosphorus insecticide malathion on the testicular histology in *Sarotherodon mossambica*.*Nat.Acad. Sci.Lett*,**5**:141-3.

Pandey, A.C. (1988). Impact of endosulfan (thiodon) Ec 35 on behavior and dynamics of oocyte development in the teleostean fish, *Colisa, Trichogstter fasciatus*. *Ecotoxicol. Environ. Saf*. **15**(2): 221-225.

Pandey, R. K. Singh, R. N., Singh, S., Singh, N. N. and Das, V. K. (2009). Acute toxicity bioassay of Dimethoate on freshwater air breathing catfish *Hetropneustes fossilis* (Bloch). *J. Environ. Biol.* **30**(3): 437-440.

Pandian, T. J. and Bhaskaran, R. (1983). Food utilization in the fish *Channa striatus* exposed to sublethal concentrations of DDT and methyl parathion, *Pro. Indian Acad. Sci. (Amin. Sci.).* **92** (6): 475-481.

Paths and Misra, B. N. (1981). Toxicological effects of dichlorvos (DDVP) as brain and liver acetylcholine sterase (AChE) activity of *Tilepia morsmbica. peters. Tomicol*. **19**: 239-245.

Pavlov, D. D., Chuiko, G. M, Gerassimor, Y. V. and Tonkopiy, V. D. (2008). Feeding behaviour and brain accetylcholinesterase activity in bream *Abramis brama* (L.) as affected by DDVP, an organophosphorus insecticide. *Environ. Toxical Chem*. 13.

Pawar, K. R. and Katdare, M. (1983). Effect of sumithion on the ovaries of freshwater fish *Garra mullya* (Sykes). *Curr. Sci* **16**: 784-785.

Peezely, P. and Kovacs, K. (1998). Effects of a fungicide (VITAVAX 200 FF) on the reproductive endocrine function in mallards*. Environ*. *Toxicol. Water Qual.* **13**(2): 133-141.

Pickering, O.R., Henderson, C. and Lemke, A.E.(1962). The toxicity organic phosphorus insecticides to different species of warm fishes. *Am. Fish. Soc*. **91** : 175.

Ponmani, R. B., Dhakkodi and Logaswamy S. (1997).Sublethal effect of monocrotophos on food utilization of *Cyprinus carpio*. *J. Environ. Biol.***18**: 321-324.

Pope, C. N., Chaudhuri, J. and Cakrabati, T. K. (1995). Organophosphate sensitive cholinergic receptor in enzyme of the cholinesterase family pp. 305-312 *(Eds. Qunn, D. M., Balasubramanian, A. S., Dotor, B. p. and Taylor, P.), Plenum Press, new York.*

Price, P., Szeto, S. and Wan, M. (1994). Organo-phosphorus Insecticides residues in farm ditches of the lower Fraser valley of British Columbia. *Journal of Environmental Science and Health*. **29**: 917-949.

Ragnarsdottir, K. (2000). Environmental fate and toxicology of organophosphate pesticides. *Journal of the Geological Society of London*. 157: 859-876.

Raj, N. and Sathyaneran, A. G. (1987). Effects of long term exposure to cythion on the reproduction of the teleost, *Channa punctatus* (bloch). *Environ. Pollut. 0269-7491. Elsevier App. sci. pub. Ltd*. 49-60.

Ram, N. S., Rakesh, K. P., Narendra, N. S. and Viji, K. D. (2009). Acute toxicity and behavioral responses of common carp, *cyprinus carpio* (Linn.) to an organophosphate (Dimethoate). *World. Zoo*. **4**(2): 70-75

Ram, R. N. and Joy, K. P. (1988). Mercurial induced changes in the hypothalamo-neurohypophyseal complex in relation to reproduction in the releostean fish, *Channa punctatus* (Bloch). *Bull Environ. Contam toxicol.* **41**: 329-336.

Rand, G. M., Wells, P. G. and Mc Carty, L. S. (1995). Introduction to aquatic toxicology pp.3-67. In: G. M., Rand (eds.). Fundamentals of aquatic toxicology-Effects Environmental fate, and Risk Assessment. Washingon*. D.C. Taylor and Francis Publishers*, 1125p.

Rao, J. V., Begum, M., Pallela, M., Usman, P. K. and Rao, R.N. (2005). Chnages in behavior and brain acctylcholine starase activity in mosquito fish, *Gambusia affinis* in relation to sublethal exposure of chlorphyrifos. *Intl. j. Environ Res. Public health.* **2**(3-4): 478-483.

Rastogi, H. and Kulshrestha, S. K. (1990). Effect of sublethal doses of three pesticides on the ovary of a carp minnow, *Rarbora daniconius*. *Bull environ Contam Toxicol.* **45**: 742-747.

Rath, S. and Misra, B. N. (1981).Toxicological effects of dichlorovos (DDVP) on brain and liver acetylcholinesterase (AChE) activity of Tilapia mossambica,peters.*Toxicol*.**19**: 239-245.

Redhaiah, V. and Jayantha, K. R. (1998). Behavioral response of fish *Tilapia mossambica* exposed to fenvalerate. *Environ. Ecol*. **6**(2): 2-23.

Reish, D. J. and Oshida, P. S. (1986). Manual of methods in *aquatic environmental* research part 10 short term static bioassays FAO fish technical paper. (247) 62 pp.

Rodrignes, E. L. (1994). Alteracoes morfologias emhepatocitos de *Brachydanio rerio* (Hamilton Buchaman 1822; 1823) exposio a acao do organoforforado dimetoato 500 cm dose subletal. Dissertacao de mestrado, Universdade dederal do parana, curitiba, 76p.

Santhakumar, M. and Balaji, M. (2001). Acute toxicity of organophosphorus insectivide monorotophos and its effect on behaviour of an airbreathing fish, *Anabas testudineus* (Bloch). *J. Environ Biol*. **21**(2). 121-123.

Sastry, K. V. and Rao, D. R. (1981). Enzymological and biochemical changes by mercuric chloride in a teleost fish, *Channa punctatus*. *Toxicology letters*. **9**: 321-326.

Sastry, K. V. and Rao, D. R. (1983). Mercuric chloride induced alteration in the intestinal transport of glucose and fructose in the freshwater murrel, *Channa punctatus*. *Water, air, soil pollution*. **19**: 143-147.

Saxena, P.K. and Garg, M. (1978). Effect of Insecticidal pollution on ovarian recrudescence in the fresh water teleost, *Channa punctatus* (Bloch). *Ind. J. Exp. Biol*. **16**: 690-691.

Schaperclaus, W. (1933). Text book of pond culture; rearing and keeping of carp, trout and allied fishes (TV. F. Hund), *Fish leaflet. Wash.* (311): 260p.

Schmid, O. G. and Mann, H. (1961). Action of a detergent (Dodecylobenzenesulphonate) on the gills of the trout, *Nature*, London. **192:** 675.

Scott, A.L. and Rogers, W.A. (1980). Histological effects of prolonged sublethal hypoxia of channel catfish ,*Ictalurus punctuatus. J. Fish. Dis*. **3** : 305.

Sharmistha, C., Lal, B. and Singh, T. P. (1992). Effect of Endosulfon (Thiodon) on vitellogensis and its modulation by different hormones in the vitellogenic *Clarias batrachus*. *Toxicology*. **75**: 191-198.

Sharpe, R.M. and Skakkebak, N.E. (1993). Are estrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet.* **B 41**: 1392-5.

Shukla, L. and Pandey, A. K. (1985). Ovarian recrudescence in a fresh water teleost, *Sartherodon mossambicus* in DDT treatment and pattern of recoupment. *J. Environ Biol*. **6**: 195-204.

Shukla, L., Shrivastava, A., Merwani, D. and Pandey, A. K. (1984). Effect of Sublethal Malathion on ovarian histopathology in *Sarothrodon mossambicus*, comp. *physiol. Eco*. **9**: 13-17.

Singh, B. B. and Narain, A. S. (1982). Acute toxicity of thiodane to cat fish, *Heteropneustes fossilis*. *Bull Environ. Couta Toxicol*, **28**: 122-127.

Singh, H. (1989). Interaction of xenobiotics with reproductive endocrine functions in a protogynous teleost, *Monopterus albus. Mar Environ Res.* **28**: 285-289.

Singh, S. and Sahai, S. (1985). Pesticide metabolism in a freshwater teleost, Puntius ticto. In:Environment and Ecotoxicology.(Eds:R.C. Dalela and Saroj gupta).211-218.

Singh, S. Shai, S. (1985).Effect of malathion on the immature ovary of Rasbora caniconius. Ahistopathological study.*Intl. J. Acad. Ichthyol*,**6**:11-15.

Singh, H. and Singh, T. P. (1982a). Effect of some pesticides on hypothalamo-hypophyseal ovarian axis in the fresh water catfish, *Heteropneustes fossilis* (Bloch). *Environ Poll (Series A).* **27**: 283-288.

Singh, H. and Singh, T. P. (1982b). Effect of pesticides on fish reproduction. *Ichthyologia.* **15**: 71-81.

Singh, P. B. and Canario, A. V. M. (2004). Reproductive endocrine disruption in the fresh water catfish, *Heteropneustes fossilis*, in response to the pesticide: hexachlorocyclohexane. *Ecotoxcol Environ safety.* **58**: 77-83.

Singh, P. B., Kime, D. E., Singh, T. P. (1993). Modulatory actions of *Mystus* gonadotropin on gamma-BHC induced histological changes, cholesterol and sex steroid levels in *Heteropneustes fossilis*. *Ecotoxicol. Environ. Saf*. **25**(2): 141-153.

Singh, T. P., Lal, B. and Yadav, A. K. (1997). Pesticides and fish in pesticides, man and biosphere. Edited by board of Editors. *Hindustan Publishing corporation new Delhi*. Pp.197-248.

Sismeiro, V. J., Abrantes, N., Pereira, J. L., Castro, B. B. and Goncalves, F. (2006). Short term effects of Quirian (R) (chlorfenvinphos) on the behavior and acetylcholinesterase activity of *Gambnsia holbrooki*. *Turkiya parasitol Derg*. **30**(3): 233-238

Siva, P. R. S. (1980). Studies on some aspects of metabolic changes with emphasis of carbohydrate utility in the cell free systems of the fresh water teleost. *Tilapia mossambica* (peters) under methyl parathion exposure, Ph.D Thesis, Sri, Venkateswara University, Tisupati.

Siva, P. R. S. and Ramana, R. K. V. (1983). Regulation of phosphorylases and aldolases in tissues of the teleost, *Tilapia mossambica* under methyl parathion impact. *Bull. Environ. Contam. Toxicol*. **31**: 474-478.

Sprague, J. B. (1969). Measurement of pollutant toxicity to fish: *Bioassay methods for acute toxicity water Res*. **3**: 793-821.

Srivastava, A. K. (1994). Effect of chlordecone on gonads of freshwater catfish, *Hetropneustes fossilis*. *Bull Environ contam Toxicol.* **53**: 186-191.

Srivastva, V. K. and Singh, A. (2001). Studies of seasonal variation in toxicity of frequently used commercial organophosphate, carbonate and synthetic pyrethoid pesticides against fresh water fish, *Channa Punctatus* and behavioral responses to treated fish. *Malays. Appl. Biol*. **30**: 17-23.

Stickel, W. H. (1974). Effects on wildlife of newer pesticides and other pollutants. *Proc. Ann. Cani. West. Assoc.* Game and Fish Comm. **53**: 484-491.

Stockely,I.H.(1971).The practical significance of drug interaction,*Pharma.j.*,**207**,351.

Su. M.Q., Kinoshota, F.K. Frawley, J.P. and DuBois, K.P.(1971).Comparative inhibition of aliesterase and cholinesterase in rats fed eighteen OP insecticides,*Toxicol, and Appl. Pharmacol*.,**20**:241-249.

Sukumar, A. and Karpagaganapathy, P. R. (1992). Pesticide-Induced atresia in ovary of a freshwater fish*, Colisa lalia* (Hamiltion-Buchanan*). Bull. Environ contam toxicol.* **48**: 457-462.

Taylor, P. (1990). In: The pharmacological basis of therapecutics (8^{th} edn.), (Eds. Gulman, A. G., Rall, T. W., Nies A. S. and Taylor, P.) *Macunillan publishing Co., New York*. 131-149.

Thomas, P. (1990). Molecular and Biochemical Responses of fish to stressors and their potential use in Environmental Monitoring, edited by Adams S.M, and shuter BJ. Symposium 8 American Fisheries, society. 9-28.

Todd, A. P., Jigang, W., Robertc, S., Gary, J. A. (1998). Acute toxicity and cholinesterase inhibition in larval and early juvivile walleye exposed to chlorpyrifos. *Environ. Res*. **79**(2): 133-7.

Tomlin, C. D. S. (1997). The pesticide Manual, 11^{th} edition*. British crop protection council*.

Toor, H. S. and Kaur, K. (1974). Toxicity of pesticides to the fish, *cyprinus carpio communis*. *Indian J. Exp. Biol*. **12**: 334-337.

Toxicity of an organophosphate pesticide (chloropyrifos) on a common Niger Delta wetland Fish-*Tilapia guineensis* (Blecker 1862) *J. Appl. Sc. Environ. Mgt*. **8**(2): 11-17.

Tripathi, P. K.,. Srivastava, V. K. and Singh, A. (2003). Toxic effects of Dimethoate (organophosphate) on metabolism and Enzyme system of fresh water teleost fish, *Channa punctatus*. *Asian fisheries science* **16**: 349-359.

USEPA (1994). United States Environmental protection agency, integrated risk information system (IRIS) on Dichlorvos. Environmental criteria and assessment office, office of health and environmental assessment office of Research and development, Cincinnatic, OH.

USEPA (2007). Dichlorvos TEACH chemical summary U.S. EPA, toxicity and exposure assessment for children. Teach 1-13.

Velmurugan, B., Selvanayagam, M., Cangiz, E. and Unlu, E. (2007). The effects of monocrotophos to different tissues of freshwater fish *Cirrhnus mrigala. Bull. Environ. Contam toxicol*. **78**(6): 450-454.

Venkateswara, J. R., Ghousia, B., Jakka, N. M., Sriknath, K. and Nageswara, R. (2006). Sublethal effects of profenofos on locomotor behaviour and gill archtecture of the mosquitofish, *Gambusia aiffnis*. *Drug Chem toxicol*. **29**(2) 157-165.

Venugopalan, V. K. and Sasibhushana, P. R. (1979). Pesticides induced impairment in incubation and post embryonic development of planktonic eggs of eustrine fish of porto Novo (South India) waters. *Proc. Symp. Environ. Biol*. *Academy of Environmental Biology.* Muzzaffarnagar, India 397.

Vineet, K., Patil, K. and David, M. (2008). Behavoiur and Respiratory Dysfunction as an index of malathion toxicity in the Freshwater fish, *Labeo rohita* (Hemilton). *Turkish J. of Fisheries and aquatic sci*. **8**: 233-237.

Weber, J.B.(1972). Interaction of organic pesticides with particulate matter in aquatic soil system, in fate of organic pesticides in the Aquatic Environment (Advances in Chemistry Series III). *Faust, S.D., Eds. American Chemical Society Washington D.C.* pp.55.

Weis, J. S. and Weis, P. S. (1976). Optical malformations induced by insecticides in embryoes of the Atlantic silverside, *Menidia menidia*. *Fish. Bull*. **74**: 208.

Westlake, W.E. and Gunther, F.A.(1966).Organic pesticides in the environment,p. 110.Advances in the chemistry series 60, Ed. Gould, *R.F.,American Chemical society*, Washington, D.C.

WHO (1999). Technical Diclorvos Full specification WHO/Sit/16. R4, pp. 1-11.

Wilbur, R. L., Whitney, E. W. (1973). Toxicity of the herbicide kuron (Silvex) to bluegill eggs and fry. *Trans. Am. Fish. Soc.* 102-630.

Wilkinson, C. F. (1976). Insecticide synergism, In: Metcalf, R. L. and Mc Kelvey, J. J. Jr. (Ed) "The future of insecticide: needs and prospects. New York, *Wiley Publishers.*

Zagatto, O. A. (1999). "Mni-curso: *ecotoxcologia aquatia*." VII congresso Brasileiro de limnologia. Florianopolis, SBL, 124P.