EFFECTS OF AGRO-CHEMICALS ON THE PHYSIOLOGICAL STRESS ON TELEOST FISH

BHAVIKA N. DESAI

DIVISION OF ECOTOXICOLOGY

DEPARTMENT OF ZOOLOGY, FACULTY OF SCIENCE

THE MAHARAJA SYAJIRAO UNIVERSITY OF BARODA

VADODARA

PH.D. THESIS MARCH, 2013

The Maharaja Sayajirao University of Baroda

DEPARTMENT OF ZOOLOGY
FACULTY OF SCIENCE
VADODARA 390 002, INDIA

CERTIFICATE

This is to certify that the thesis "EFFECTS OF AGRO-CHEMICALS ON THE PHYSIOLOGICAL STRESS ON TELEOST FISH**"** incorporate the results of investigation carried out by the candidate herself in the Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara.

Candidate
(Bhavika Desai)

Guiding Teacher
Dr. Pragna Parikh

A THESIS SUBMITTED TO

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

VADODARA

FOR THE PARTIAL FULLFILLMENT OF THE REQUIREMENT OF

DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

CONTENTS

Acknowledgement

The completion of this thesis would not have been possible without the help of many people. I take this opportunity to acknowledge them for their contribution in my thesis.

At this very moment of accomplishment, I pay earnest homage to my guide, ***Dr. Pragna Hitesh Parikh, Associate Professor, Department of Zoology*****,** from the very bottom of my heart. Her faith in me and continuous showering of encouragement she offered have been catalyst in every leap I made for my Ph.D. Her enduring enthusiasm for research and distinguished guidance kept me buoyant and stimulating during difficult times in my Ph.D. pursuit. My words and thoughts fall short whenever I try to describe those always astonishing ideas and discussion she offered during tenure of Ph.D. Despite of her ill health, she worked late hours for series of days to review my thesis. I am very thankful to her for helping to secure financial support for my Ph.D. Her unflinching courage and conviction will always inspire me. She has been more than mentor and nothing less than my parents. Beyond this research, I must say that she has been quite well in nurturing emotional bonding with me. Nonetheless, I appreciate those serious co-operation and parental care offered on many occasions by ***Mr. Hitesh Parikh***, sparing his holidays for my thesis work. I am also thankful to ***Mr. Yashesh Parikh*** for creating light atmosphere in the stressing times of my thesis compilation.

I appreciate those handy ideas and insightful advice from ***Dr. B. Suresh*** for my research work. His pragmatic thinking transformed my approach towards difficulties coming across in my research. I am equally grateful to ***Dr. Dolly Kumar*** for her warmth and support.

I am thankful to Head, Department of Zoology for providing me facilities to conduct my research work. I am very thankful to all my teachers in Department of zoology for their valuable help. I am also grateful to Non teaching staff for their timely help in various situations. I am also thankful to my research colleagues for their help during the experimental tenure of my thesis.

I would be doing injustice to myself, if I forget to convey my gratitude to my senior, ***Dr. Ayaz Rangrez***. He always stood by me like my elder brother. He has been highly transparent in sharing knowledge as well equally vocal in discussion throughout the tenure of my work. My friend and a close lab mate ***Miss Shalaka Sadekarpawar***, deserves special thanks for her

immense support since the day she joined. It was her positive attitude and comforting nature made the work exciting for all of us. There is not a single part of my thesis which does not involve her help and support. Saying few words of thanks are not enough to describe her contribution in my work. I am also thankful to ***Mr. Ankur Upadhayay***, for his cool attitude and patience and helping me in the referencing. They have been like family members since ever I have known them. I am thankful to God for providing me a research team in the form of a family, where my guide nourished me like mother, and my fellow mates were like my brothers and sister, helping me day in and out. I am also thankful to ***Miss Khushali Pandya*** for being the part of the team and helping me during the final compilation of the thesis.

My endeavours without the love and support from my parents & siblings are beyond my imagination. My parents have always strived for providing us the education best in its class. I thank them for letting me pursue my interest, even when it seemed incomprehensible. I feel very proud to mention that my parents are very simple and they taught me how to lead a simple life. I would simply say, "Mummy, Pappa you are unique in this world!". Words cannot express how grateful I am to my mother-in law, father-in-law for their love, care and endless prayer helping me to sustain so far. I would also like to thank to my beloved husband, who has always been my pillar, my joy and my guiding light.

Last but not the least I would like to acknowledge and thank my loving and caring grandparents. It is their support that made possible for me to migrate to Vadodara City and achieve this huge and challenging task of Ph. D. I strongly believe that their prayers played a very important role in my life. They are the most important people in my world and I dedicate this thesis to them.

I thank them all and the others who genuinely offered their support. I appreciate those to whom I might have missed here in spite of their contribution in accomplishment of my Ph.D.

Last but not the least, I am thankful to The Maharaja Sayajirao University of Baroda, Vadodara, for providing financial support in the form of University Scholarship.

Bhavika Desai

LIST OF ABBREVIATIONS

ALP	Alakaline phosphatase
ALT	Alanine transaminase
APHA	American Public Health Association
ANOVA	Analysis of Variance
APVMA	Australian Pesticides and Veterinary Medicines Authority
AST	Aspartate transaminase
CAT	Catalase
CCME	Canadian Council of Ministers of the Environment
CSE	Center for Science and Environment
CSI	Cardiosomatic Index
CZ	Curzate M8
DDE	Dichlorodiphenyl- DIchloroethylene
EBDC	Ethylenebisdithiocarbamate
EDC	Endocrine disrupting chemicals
EDTA	Ethylene diamine tetra acetic acid
EPA	Environmental Protection Agency
ERA	Environmental Risk Assessment
ETU	Ethylenethiourea
FAO	Food and Agriculture of the United Nations
FRAC	Food Research and Action Centre
GDH	Glutamate Dehydrogenase
GPx	Glutathione Peroxidase
GR	Glutathione Reductase
GSH	Reduced Glutathione
GSI	Gonado-Somatic Index
Hb	Haemoglobin
HCA	Hypothalamic chromaffin axis
Hct	Haematocrit
HSI	Hepato-Somatic Index
HPI	Hypothalamic pituitary internal
IMI	Imidacloprod
LC	Lethal concentrations
LCL	Lower Confidential limits
LDH	Lactate dehydrogenase
LPO	Lipid Peroxidase
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
mg/L	Milligram per liter
mmole	Milli mole
µg/L	Microgram per liter
µmole	Microgram mole
MMC	Melamacrophage Center
MSDS	Material Sheet Datasheet Sheet
MT	Metallothioneiness
Na^+/K^+ ATPase	Sodium-Potassium Adenine tryphosphate
NIH	National Institute of Health

NOEL	NO Observable Effect Level
NRCC	National Research Council Committee
OBF	Opercular beat frequency
OSI	Organo-Somatic Index
PAH	Polyaromatic Hydrocarbons
PAN	Pesticide Action Network
PCBs	Polchlorinated Biphenyls
PCV	Packed Cell Volume
PMRA	Pest Management Regulatory Agency
PPB	Parts per billion
PPM	Parts per million
RBC	Red Blood Corpuscles
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
SSI	Spleno-Somatic Index
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid Reactive Substance
TBF	Tail beat frequency
TU	Toxic Units
UCL	Upper Confidential limits
US	United States
VSI	Viscerosomatic Index
WBC	White Blood Corpuscles
WHO	World Health Oraganization

INTRODUCTION

"Life originated in water, is thriving in water, water being its solvent and medium. It is the matrix of life." a quote from Szent-Gyorgyi (1958) clearly illustrates that: The unique physical and chemical properties of water have allowed life to evolve in it. Earth is known as the water planet in our Solar system and water is the absolute requirement of life. Its distribution, quantity, availability, and quality are the controls for the development of agriculture, industry, rural, urban, and municipal use. As a consequence study of water pollution is noteworthy as it influences living or biological systems either directly or indirectly.

There are several definitions of water pollution. In a report by the National Research Council Committee on pollution quoted by Warren (1971), water pollution was defined as "an undesirable change in the physiological, chemical, or biological characteristics of water that may or will harmfully affect human life or that of other desirable species, industrial processes, living conditions, and cultural assets, that may or will waste or deteriorate raw material resources". Heath (1995) quoted a definition of water pollution given by Lloyd (1992), as "the addition by humans of something to the water that alters its chemical composition, temperature, or microbial composition to such an extent that harm occurs to resident organisms or to humans". However, this study will follow the definition given by Mason (1981) who defined pollution as "the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structure or amenity, or interference with legitimate uses of the environment". Moriarty (1988) defined a pollutant as a substance that occurs in the environment at least in part as a result of man's activities, and which has a deleterious effect on living organisms. Thus, we can say that a serious threat to water is 'water pollution'and Water pollution means anything to add water which makes water dirty and unsafe not only for drinking and other human uses such as swimming, irrigation, and industrial use but it also makes water harmful for other creatures on the Earth such as marines, animals and plants.

There are several causes of water pollution. These causes can be classified into two categories: direct or point causes and indirect or nonpoint causes. Both direct and indirect causes can add number of strange substances in water. Examples of these substances are

organic, inorganic, radiological and biological. Direct or point contact is the contact in which the pollutant/contaminant directly enters into the water at its occurring place. On the other hand, the indirect (nonpoint) cause of water pollution is defines as the contact in which the impurity occurs elsewhere and then later on, gradually, enters into the water through natural means.

To meet the increasing demand for agricultural products, it is very common across the world to use more sophisticated cultivation methods and chemicals, such as fertilizers and pesticides, to get the higher yield. Although the use of chemicals and heavy machineries has increased the yield beyond over beliefs, the use of these chemicals is one of main causes of water pollution. Agrochemicals, such as, pesticides, fertilizers, organic manure, growth hormones, and nutrient solution, pollute water significantly when they enter into the water through rains (Kurian and Natarajan, 1997). Agricultural run-off affect groundwater and surface water sources as they contain pesticide and fertilizer residues. Fertilizers have an indirect adverse impact on water resources. Indeed, by increasing the nutritional content of water courses, fertilizers allow organisms to proliferate. These organisms may be disease vectors or algae. The proliferation of algae may slow the flow in water courses, thus increasing the proliferation of organisms and sedimentation. Pollution by agricultural run-offs has too main effects on the environment. Pesticides may be responsible for poisoning. They are especially difficult to remove from freshwater, and thus, can be found in municipal or bottled water, even after conventional treatment. A study from the Center for science and Environment (CSE) recently drew the alarm about the concentration in pesticides such as organochlorines and organophosphates that was exceeding the WHO standards in almost all the Indian brands of bottled water (Narain, 2003).

In spite of these well known adverse effects, and the worrying growth of fertilizer and pesticide use in the India agricultural sector, these products are still subsidized by the government. Fertilizer consumption in India has increased significantly in the last three decades. As per the record of FAO (2000) fertilizer use has increased from 7.7 MT (1984) - 13.4 MT (1996) - 113.40 MT (2002) - 194.57 MT (2008) - 166.29 MT (2009), almost an increase to a range of more than 2000%.

Water pollution, has been increasing at an alarming rate due to rapid industrialization, civilization and green revolution. Urban, agricultural and industrial activities release xenobiotic compounds that may pollute the aquatic habitat. Industrialization and growth of

human population have led to a progressive deterioration in the quality of the earth's environment. Schwarzenbach *et al.,* (2006) reported that about 300 million tons of synthetic compounds seep annually into water systems (rivers, lakes and sea) leading to water pollution. Pollution of water sources due to chemicals plays a primary role in the destruction of ecosystems. To improve the quality of aquatic ecosystems, it is necessary to know how the rivers and lakes are impaired and what factors caused the environmental deterioration.

Ecotoxicology is the science of the impact of toxic substances on living organisms, encompassing all levels of biological organization from single organisms to ecosystems (Fontanetti *et al.,* 2010). It is a multidisciplinary field which integrates environmental chemistry, biochemistry, toxicology and ecology. It studies the effects of toxic chemicals on biological organisms, especially at the population, community, ecosystem level. Ecotoxicological studies are obligatory to establish that there are no unacceptable risks to the environment as it aids in identifying the most efficient and effective action to prevent or remediate any detrimental effect. This research is the basis for the development of tools that can be used in environmental regulation (Escher *et al.,* 1997).

To assess pollutant or a group of pollutants, ecotoxicologists and environmentalists have focused on the pollutant's fate, persistence and toxic properties to the environment and man (Kroes 1988). Organic pollutants in the aquatic environment comprise a vast and ever-increasing range of compounds, which includes polyaromatic hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs), dioxins, nitroaromatics, aromatic amines, organophosphate and organochlorine pesticides and phthalate ester plasticizers. A major theme in ecotoxicology today is finding the best way of achieving scientific, technical, and practical goals while organizing a congruent body of knowledge around rigorously tested explanations (Braunbeck, 1994). Considerable attention has been devoted in identifying various chemicals among which are commonly used pesticides and heavy metals, and assessing their effects on aquatic animals (Kroes, 1988). Because ecotoxicology is an applied science, ecotoxicologists take on different roles that are also not fully integrated. Some ecotoxicologists are concerned chiefly with scientific goals, that is, organizing facts around explanatory principles. Others focus on the technical goals, that is, developing and applying tools to generate high-quality information about ecotoxicological phenomena. Still others focus closely on resolving specific, practical problems such as assessing ecological risk due to a chemical exposure or the effectiveness of a proposed remediation action. The long-lasting debate on the extrapolation of laboratory-based conclusions to natural

conditions may never be resolved (Kimball and Levin, 1985; Seitz and Ratte, 1991; Selck *et al.,* 2002), but the importance of toxicant-orientated, single or multiple compound tests is overwhelming within environmental risk assessment (Chapman, 2000; Breitholtz *et al.,* 2006).

In order to verify whether a biological response does indeed occur when organisms are exposed, bioassays must be developed and thoroughly tested. Ultimately, inter-calibration and standardization of new bioassays can be performed in order to validate their integration in regular surveillance monitoring and/or situation-specific Environmental Risk Assessment (ERA). The goal of practical ecotoxicology is the use of existing science and technology to document or solve specific problems such as remediating harm done by a chemical spill. Much of practical ecotoxicology is currently done within the ERA framework. A retroactive ERA estimates the risk from an existing situation such as a contaminated site, whereas a predictive ERA predicts the same for a future situation such as the proposed licensing of a new agrochemical (Newman, 2008).

PESTICIDES

In the last 50 years, there has been a steady growth in the use of synthetic organic chemicals such as pesticides. These have considerable advantages over the natural products in that they are potent, selective and comparatively cheap (Connell and Miller, 1984). Among the pollutants, pesticides rank a very important position, since pesticides and technical organic chemicals comprise the most dangerous group of pollutants. It is realized that these substances are totally alien to aquatic organisms. Today, the use of pesticide is widespread on agricultural crops, rangelands, forests and wetlands and this undoubtedly exposed many wildlife species to chemical hazards. Many pesticides need to be resistant to environmental degradation so that they persist in treated areas and thus their effectiveness is enhanced. This property also promoted long-term effects in natural ecosystems. The excess amounts of these pesticides and chemicals produce unwanted and unwarranted residues, which pose a great threat to aquatic organisms (Ramasamy *et al.,* 2007). They find their ways into the fresh water bodies and have produced unexpected consequences on aquatic fauna. Generally water bodies of croplands are mostly often polluted. The pesticide concentration of water bodies can reach the magnitude of dozens of milligrams per liter. The levels of water pesticide pollution can be ranked as: cropland water > field ditch water > runoff > pond water > groundwater > river water > deep groundwater > sea water (Lin *et al.,* 2000).

Pesticides include many specific chemical substances that can be grouped according to the type of pest they are intended to control. They represent artificial man-made materials, which are largely or entirely foreign to environment. A bewildering variety of pesticides, bought easily and used carelessly by farmers, are contaminating foodstuffs and posing health hazards, according to several surveys. Pesticides are poisons and would be expected to have adverse effects on any non-target organism having physiological functions common with those of the target that are attacked or inhibited by the pesticide. Physical, chemical and biological processes affect the distribution and fate of these substances in the environment. Such compounds are fat-soluble and are therefore readily taken up from the water, sediment and food sources into the tissues of aquatic organisms (Walker and Livingstone, 1992; Gil and Pla, 2001; Farah *et al.,* 2004). Lipophilic nature of water-insoluble pesticides enhances its ability to cross the plasma membrane, when the pesticides come in contact with the aquatic organism. Due to their widespread distribution they have been shown to exert adverse effects on the associated organisms (Singh and Reddy, 1990).

Pesticides have the potential to enter aquatic habitats from direct application, terrestrial runoff or wind-borne drift. Because there are thousands of different pesticides used around the world, data on aquatic contamination for any particular pesticide is usually quite limited. However, studies conducted in lentic and lotic systems have detected a variety of pesticides including the insecticides malathion, endosulfan and diazinon as well as the herbicides atrazine and glyphosate (Le Noir *et al.,* 1999, Hayes *et al.,* 2002; Kolpin *et al.,* 2002 and Thompson *et al.,* 2004). Interestingly, many pesticides found in aquatic systems are not intended or legally registered for application to aquatic systems, but they still appear (e.g. Thompson *et al.,* 2004). The concentrations found in surveys of natural habitats are often lower than the concentrations used in experimental tests, although these surveys are typically snapshots in time that are not always designed to detect peak concentrations. In most cases, we simply lack extensive data on natural pesticide concentrations to properly evaluate the validity of concentrations used in experiments. Given that pesticides find their way into aquatic systems, the relevant question is whether they affect the species in these systems. It is therefore important to study a range of different biological variables in exposed and non-exposed organisms, as well as a suit of additional environmental parameters, so that cause-effect relationships between contamination and ecosystem response can be correctly established. A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc.),

including insecticide, herbicide, fungicide, and various other substances used to control pests (EPA, 2009). The environmental pollution caused by pesticides in Asia, Africa, Latin America, the Middle East and Eastern Europe are now serious (Zhang *et al.,* 2011), further the scientists have reported that globally 4.6 million tons of chemical pesticides are annually sprayed into the environment and that only 1% of the sprayed pesticides are effective; 99% of pesticides applied are released to non-target soils, water bodies and atmosphere, and finally absorbed by almost every organism.

We surveyed the literature for ecotoxicological studies of pesticides in freshwater systems to understand the types of studies that have been conducted. As noted by Fleeger *et al.,* (2003), the vast majority (80%) of aquatic toxicology studies are freshwater systems. The literature survey also exhibited a number of interesting patterns. For example, the number of ecotoxicological studies published annually has steadily increased from 1992 to 2006 with a large increase during the past few years. Across all years, 71% of studies have been conducted in lentic systems (lakes, ponds and wetlands) while 29% have been conducted in lotic systems (streams and rivers). Data from real-world applications of pesticides (Donaldson *et al.,* 2002) indicate that herbicides were actually the most commonly used pesticides (52%), followed by insecticides (35%) and fungicides (13%). Relyea and Hoverman, (2006) in their studies have reported that of the scientists (80%) have examined a single pesticide with considerably fewer studies that compared different pesticides applied separately (8%) or mixtures of pesticides (8%). Among the single-pesticide studies, the major research focus has been on insecticides (65%), with a moderate focus on herbicides (33%), and a minor focus on fungicides (2%).

INSECTICIDES

The insecticides constitute a group of pollutants, both synthetic and natural, which contribute to the environmental problems. Continuous use of chemical inputs such as pesticides has resulted in damage to the environment, caused human ill-health, negatively impacted on agricultural production and reduced agricultural sustainability (Pimentel *et al.,* 1992; Pimentel and Greiner, 1997). Fauna and flora have been adversely affected (Pimentel and Greiner, 1997). At present, it seems that the problem is more conspicuous in developing countries, where lately there has been an increase in the use of insecticides as a means of increasing agricultural productivity (Tilman, 2001; FAO, 2001), without much concern to the consequences of indiscriminate application and have been found to be highly toxic to non-target organisms that inhabit natural environments close to agricultural fields. The main

environmental impacts of agriculture come from the conversion of natural ecosystems to agriculture, from agricultural nutrients that pollute aquatic and terrestrial habitats and groundwater, and from pesticides, especially bioaccumulating or persistent organic agricultural pollutants. Small fractions of the pesticides used in agricultural or urban settings end up moving with surface runoff into streams, rivers, and lakes (Clark *et al.,* 1999); leaching to the groundwater systems (Kolpin *et al.,* 2000); or volatilizing to the atmosphere (Majewski *et al.,* 2000; Foreman *et al.,* 2000; Tilman *et al.,* 2002). The presence of insecticides in the environment, due to extensive use in agriculture and their low degradation capacity, are of potential toxicological concern because, once these pesticides leave their point of use they change from being "crop-protection" and "pest control" chemicals to being environmental contaminants that are suspected sources of stress to aquatic plants and animals (Battaglin and Fairchild, 2002).

In spite of these concerns, there has been relatively little research directed at determining the risk of pesticides to non-target aquatic organisms. Aquatic environments receive direct and indirect pesticide inputs, inevitably exposing aquatic organisms to pesticides. While pesticides elicit a variety of acute and chronic toxicity effects on these organisms, they also have the capability to accumulate, detoxify, or metabolize pesticides to some extent. Insecticides have been found to be highly toxic not only to fish, but also to organisms which constitute the food of fish (Anderson, 1960). According to WHO, the incorrect use of some insecticides is responsible for a great number of cases of acute poisoning, characterized by the development of cholinergic syndrome and multiple chronic complications, with neuropathy being one of the most presented symptoms. These complications are very important because their frequency is progressively increasing and they may go unnoticed (Sharom *et al.,* 2002). Study on the neonicotinoid insecticides would help in determining their fate in the environment and possible implications to biota. The neonicotinoids are a new insecticide class which include the commercial products imidacloprid, acetamiprid, nitenpyram and thiamethoxam and are important to agriculture because of their activity against sucking insects and some Heteroptera, Coleoptera and Lepidoptera (Stark *et al*. 1995; Yamamoto and Casida, 1999; Suchail *et al.,* 2000; Iwasa *et al.,* 2004). In 2006, the neonicotinoid family accounted for worldwide annual sales of around $US 1.56 billion, representing nearly 17% of the global insecticide market (Jeschke and Nauen, 2008). Toxicity data for these new group of insecticides for aquatic invertebrate are far from enough (Tomizawa and Casida, 2003; Beketov and Liess 2008; Pestana *et al.,* 2009; Barbee and

Stout, 2009; Stoughton *et al.,* 2008; *Lukancic et al.,* 2010; Azevedo-Pereira *et al.,* 2011; Malev *et al.,* 2012), however, very less data exists for these chemicals on non-target organisms, especially those inhabiting fresh water aquatic systems are either in sufficiently known or not reported yet.

IMIDACLOPRID (IMI)

Imidacloprid was first registered for use in the U.S. in 1992 and is possibly the most widely used insecticide of the group. It has a wide range of target pests and sites, including soil, seed, structural, pets, and foliar treatments in cotton, rice, cereals, peanuts, potatoes, vegetables, pome fruits, pecans, and turf. It is a systemic with long residual activity and particularly effective against sucking insects, soil insects, whiteflies, termites, turf insects, and Colorado potato beetle. Products are available in dusts, granules, seed dressings as flowable slurry concentrates, soluble concentrates, suspension concentrates, and wettable powders (Fishel, 2010).

IMI is a systemic chloronicotinyl insecticide with physical/chemical properties that allow residues to move into treated plants and then throughout the plant via xylem transport and translaminar movement (Elbert *et al.,* 2000 and Buchholz and Nauen, 2002). It enters the target pest via ingestion or direct contact. Being a neonicotinoid, it acts by disrupting nicotinic acetylcholine receptors in the insect central nervous system. The disruption of the nervous systems results in modified feeding behavior, paralysis, and subsequent death of the insect (Mullins, 1993; Tomizawa *et al.,* 1995; Yamamoto, 1988; Yamada *et al.,* 1999; Matsuda *et al.,* 2001; Tomizawa and Casida, 2003).

Many non-target beneficial arthropods such as honeybees, parasitic wasps, and predaceous ground beetles are sensitive to IMI (Guez *et al.,* 2001; Greitti *et al.,* 2006; Iwasa *et al*. 2004; Rortais *et al.,* 2005; Greatti, 2006; Chauzat *et al.,* 2006; Kreutzweiser *et al.,* 2008; Kindemba, 2009). These organisms may be adversely affected by sub-lethal doses of the insecticide, but the effects vary widely depending on application method and route of intake (Iwaya and Kagabu, 1998; Tomlin, 2002; Ding *et al.,* 2004; Feng *et al.,* 2004; Tan *et al*. 2007; Jemec *et al.,* 2007; Chen *et al.,* 2010). There is a potential for stress-related sub-lethal effects on fish in water contaminated with IMI. Since several IMI metabolites have been shown to be equal or greater in toxicity than the parent compound, (Tokieda *et al.,* 1997; Suchail *et al*. 2000) their presence in the environment should be studied.

The chemical is very persistent in soils and has a half-life of approximately 1,000 days, depending on the soil type and environmental conditions (Rouchaud *et al.,* 1994; Baskaran *et al.,* 1999). In water IMI has a half life of more than a year, which is also dependent on environmental conditions (Moza *et al.,* 1998; Wamhoff and Schneider, 1999; Malato *et al.,* 2001; Liu *et al.,* 2006). Research has shown that IMI has a high mobility in plants, and when used as a seed dressing becomes mobile and will migrate from the stem to the leaf tips and eventually into male flowers (Bonmatin *et al.,* 2005); as a result IMI residues have been detected in the pollen (Bonmatin *et al.,* 2005) and nectar (Scientific and Technical Committee, 2004) of a number of flowering crop plants, leading to prolonged exposure of non-target invertebrates to IMI. It gets degraded stepwise to the primary metabolite 6-chloronicotinic acid, which eventually breaks down into carbon dioxide. The most important steps were loss of the nitro group; hydroxylation at the imidazolidine ring, hydrolysis to 6-chloronicotinic acid and formation of conjugates (Kagabu and Medej, 1995; Elbert *et al.,* 1998; Suchail *et al.,* 2000; Schmuck, 2004; Tomlin, 2002; Kindemba, 2009).

IMI has the molecular formula $C_9H_{10}C_lN_5O_2$, with a molecular weight of 255.7 g/mol. In appearance, it consists of colorless crystals. The insecticide is quite water soluble even at the lowest solubility value (Krohn, 1989) and can potentially leach to groundwater (Cohen *et al.* 1984) or be transported in runoff (Mulye, 1995; Kagabu, 2003). Comparative bioassay studies of different neonicotenoids by Yu *et al.,* (2010), have proved that if some of the chemical groups are replaced then their insecticidal efficacy gets altered. Furthermore, CCME (2007) have also reported that as a result of differences in the formulation of the imidacloprid-containing product variation occurs in their toxicity. Based on the high water solubility of imidacloprid and its persistence, PMRA (2001) considers imidacloprid to have 'high' leaching potential. However, there is evidence to suggest that, if used correctly (e.g., at recommended rates, without irrigation, and when heavy rainfall is not predicted), imidacloprid does not characteristically leach into the deeper soil layers despite its high water solubility (Rouchaud *et al.,* 1994; Tomlin, 2000; Krohn and Hellpointner 2002).

Toxicological studies on rats and mice and dogs have proved IMI to be moderately toxic (Tomizawa and Casida, 2003)**.** Pathological studies of IMI toxicity in rats by Jain and Punia (2006) have reported no change in the body as well as organ weight; however hisopathological changes were very prominent. Response to IMI toxicity in birds has shown varied behavioural changes in birds like upland game birds, bobwhite quail, Japanese quail, red- winged blackbirds and brown-headed cowbirds Gastrointestinal distress and ataxia has

also been reported. Exposure to IMI has led to histopathological changes as reported by Kammon *et al.,* (2010). A Hematological and biochemical change due to short-tem oral administration of IMI has been reported by Balani *et al.,* (2011). Short term exposure of IMI has produced stress in poulty birds (Siddiqui *et al.,* 2007). The effect of vitamin C and glutathione as a protective agent against the action of IMI on liver and testis has been reported by Omiama (2004). Micronucleus test and comet assay performed by Li-tao and his co-workers (2006) and Feng *et al.,* (2004) for assessing the risks of novel pesticide IMI on amphibians, have proved that IMI is Genotoxic to tadpoles and frogs. IMI has been proved to be moderately toxic to fish. Toxic responses of IMI has been studied by Rajput *et al.,* (2012) on fresh water fish, C*larias batrachus* and have reported the adverse effect of these toxicant on the protein profile of the fish. IMI has also been found to have profound influence in serum biochemical profile of fresh water fish *Channa punctatus* (Padma priya *et al.,* 2012). In tests with the aquatic invertebrate *Daphnia magna* (Jemec *et al.,* 2007) and burrowing shrimp (Felsot and Ruppert, 2002) have assessed the chronic IMI on biochemical, reproductive and survival parameter in these non target organisms.

A review of toxicity data of IMI toxicity for terrestrial non-target organisms such as Mammals, birds, and amphibians as well as aquatic organisms such as fish, amphibians and various invertebrates presented here thereby suggests that they too are very sensitive to broad-spectrum neurotoxic insecticide IMI. Unfortunately, in spite of all the technical knowledge gathered in this area of science in recent decades, little effort has been made to study the toxicity of IMI insecticides to the non-target taxa particularly fresh water teleosts. **Thus, it is important to assess the concentration at which these chemicals are toxic to non-target aquatic organisms. It is rational thus to select imidacloprid for the present study.**

FUNGICIDE

Fungicides are either chemicals or biological agents that inhibit the growth of fungi or fungal spores; they also inhibit or kill fungi underlying diseases important to man. As reported by Lorgue *et al.,* (1996) in France pesticides are the most common cause of animal poisoning (45.5%), with fungicides accounting for 6.1% of all pesticides. The two most commonly involved species are dogs and cattle. In 2003, 992 cases involving dogs and cats were confirmed as poisoning in France, and fungicides caused 2.8% of all poisonings (Barbier, 2005). Further, as stated by Berny *et al.,* (2009) acute fungicide poisonings was 4.4% in 129 poisoning cases in Greece. In Italy, poisoning related with fungicides account was 8.1% of

pesticides in pet poisonings (Caloni *et al.,* 2004). However, Latijnhouwers *et al.,* (2000) is of the view that modern fungicides do not kill fungi; they simply inhibit growth for a period of days or weeks. In agriculture, fungicides are used to protect tubers, fruits and vegetables during storage or are applied directly to ornamental plants, trees, field crops, cereals and turf grasses (Ortolani *et al.,* 2004; Gupta and Aggarwal, 2007).

The types of fungicides used in agriculture and food processing and storage range from those of relatively low toxicity to those, which can be lethal to animals (Oruc *et al.,* 2009). Understanding mechanisms of fungicide action and toxicity is important because humans and domesticated animals encounter these pesticides through a wide variety of applications. Each year, livestock are accidentally poisoned by fungicides applied to grains, potatoes, or other agricultural materials. Unfortunately, most toxicity data are from model laboratory animal's i.e rats, mice, and rabbits and offer little information on fresh water organisms. Therefore, it is valuable to be aware of several generalizations for fungicide toxicity, at the same time as these generalizations serves as useful guidelines, it is better to obtain detailed information for a specific fungicide wherever possible. Because mechanisms of action and metabolic clearance differ among fungicides, specific reproductive, teratogenic, mutagenic, carcinogenic effects or patterns of organ toxicity may manifest according to the poison ingested (Hayes and Laws, 1990 and U.S. Environmental Protection Agency, 1999). Moreover, some animals may be more susceptible to poisoning than others due to their physiology or behaviour. As proved by several scientists fungicides (e.g., copper sulphate, thiram, chlorothalonil and captan) have especially toxic effects on fish (Pimentel, 1971; Lorgue *et al.,* 1996; Tomlin, 2000), and bees (Hartley and Kidd, 1983), whereas, wild birds were poisoned by mercurial fungicides in fields sown with treated seeds (Bartik and Piskac, 1981). Fungicides are often used in combinations with other pesticides and carriers or solvents which, in combination, may be more toxic than estimated for any one of the compounds (Osweiler *et al.,* 1985). Various school of thoughts have opined the importance of using multispecies toxicity evaluation of environmentally available compounds in particular when they are introduced to environment as complex mixture (Pagano, *et al.,* 2001; Fernandez-Alba *et al.,* 2002; Guidaa *et al.,* 2008).

CURZATE M8 (CZ)

CZ fungicide was discovered by Dupont and is primarily used on grapes, potatoes and tomatoes. It is currently registered for commercial use in over 50 countries on more than 15

crops. It is formulated as a 72% wettable powder: 8% cymoxanil and 64% Mancozeb.It is yellow coloured and odourless. Chemical name of the substance: Mancozeb is Manganese ethylenebisdithiocarbamate polymeric complex with zinc salt and that of Cymoxanil is 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea. Cymoxanil belongs to the class of aliphatic nitrogen fungicides. It acts as a foliar fungicide with protective and curative action. It has contact and local systemic activity, and also inhibits sporulation (FAO, 2005). It was first introduced in 1977, it is a compound used as both a curative and preventative foliar fungicide, as per FRAC (2012) it belongs to a chemical group cyanoacetamideoxime and has categorised it to be Low to medium risk as far as toxicity is concerned and have also suggested that resistance management is required. Cymoxanil provides effective control of economically important fungal plant pathogens, which cause downy mildew and blight in a wide range of crops. U.S.Environmental protection agency (1998) has classified Cymoxanil into Toxicity Category III for oral and dermal toxicity and Toxicity Category IV for inhalation toxicity with skin and eye irritation potential. The studies on cymoxanil have shown low oral acute toxicity on rabbits (Palmer *et al.,* 1981; Feussner *et al.,* 1982 and Ponnana, 1999). The no- observable-effect-levels (NOEL) for chronic toxicity are reported in dogs, however, gross or histopathological effects were observed (Venugopala, 1999; Teunissen, 2003). The potential neurotoxicity of cymoxanil was evaluated in rats (Malleshappa, 2003) and mice (Krishnappa, 1999 a, b and 2002).

Data reported in the pesticide fact sheet (EPA, 1999) cymoxanil exposure leads to myelin degeneration in the sciatic nerve axon of rodents, a finding that may explain the neurotoxic effect. It is considered to be moderate toxic to mammals. The chronic toxicity elicited by this compound is highly variable, since it depends on the species ranges of tested concentrations and the exposure period. Experimental evidence has proved the signs of intoxications in rats and mice bodyweight and organ alterations, reduction in food consumption, testicular perturbation and histopathological variations. Cymoxanil compound has showed potential mutagenic activity, since it induced chromosomal aberrations in Chinese hamster ovary cells and in human lymphocytes (Lages *et al.,* 2009). Cymoxanil is toxic to aquatic organisms, such as fish and Crutaceans. Chronic ecotoxicity of this compound has been proved in Daphnia magna. In spite of the absence of indications that cymoxanil is environmentally persistant, in high partition coefficient can lead to moderate bioaccumulation by living organisms including humans (Soares and Calow, 1993; Grandjean *et al.,* 1999). As reported by saturn agrochemicals Inc. Cymoxanil has been reported to be low toxic to birds.

Cymoxanil is slightly toxic to fish and other estuarine and marine organisms on an acute basis (Baer, 1993 a and b; Kraemer, 1996). Cymoxanil is found to be moderately toxic to insects (Sharma and Krishnamurthy, 1998; Turkar *et al.,* 1998).

Mancozeb, another constituent of CZ M8 is an Inorganic-Zinc dithiocarbamate, is a typical fungicide with a carbamate structure where sulphurs replace both oxygens in the amide functional group. It is chemically identified as ethylenebisdithiocarbamate (EBDC). It is available in the form of powder with yellowcolour and musty odor The poisoning caused with EBDC compounds cause symptoms of irritation of skin, eyes and respiratory tract, skin sensitization; chronic skin disease has also been observed in occupationally exposed workers. Mancozeb is "moderately to highly toxic to fish and aquatic invertebrate animals," (Dupoint de Nemours, 1983). Mancozeb 80% WP is a fungicide that inhibits the production of thyroid hormones (Cocco, 2002). Mancozeb exposure is associated with pathomorphological changes in liver, brain and kidney. It has produced significant enzymatic changes in the activities of various enzymes (Kackar *et al.,* 1999). Inhibition of implantation by Mancozeb due to hormonal imbalance or its toxic effects has been studied (Bindali and Kaliwal, 2002). **Hence, in the present study fungicide curzate, a combination of Cymoxanil and mancozeb was selected.**

Thus, from the foregoing literature survey one can be summarize that CZ is a unique cyanoacetamide, chemically unrelated to any other commercial disease control agent and the biochemical mode of action is also different. The chemical has got systemic action for cymoxanil and moderate persistancey for mancozeb (Roy *et al.,* 2010). Because of its major metabolite ethylenethiourea , recently it has come underclose scrutiny of health protection agencies due to its carcinogenic, teratogenic and goitrogenic effects in mammals (Ulland *et al.,* 1972; Keppel, 1971; Das *et al.,* 2009). These studies suggest that Mancozeb and Cymoxanil have been individually studied in various animal models and found to be mild to moderately toxic. However no studies have been recorded on CZ which is a mixture of Mancozeb and Cymoxanil particular with reference to fresh water teleost fish.

Although reliable data for extrapolating toxicant effects to humans are obtained through laboratory rodent studies, these are expensive, time consuming, and more restricted by law (Hill *et al.,* 2005). Studies involving fish in toxicology currently use these models either as surrogates for human health problems or as indicators of environmental health. There are numerous advantages for the use of fish as a toxicological model species (Spitsbergen and

Kent, 2003; Teraoka *et al.,* 2003) as well as for other disciplines. This is evident by the increasing number of publications which have used this organism in the recent past. Perhaps because of their fecundity, small size, and economical maintenance and use, fish models are becoming well established in many laboratories (Law, 2003). A number of recent reviews have pointed out the advantages of fish models for laboratory-based testing (Powers, 1989; Bailey et.al., 1996; Bunton, 1996; Law 2001 and 2003; Winn, 2001; Kazianis and Walter 2002).

The presence and potential hazards of agrochemicals in the aquatic environment have received increased attention recently (Daughton and Ternes 1999; Kummerer 2001). It is widely recognized that aquatic ecosystems serve as the final sink for many chemicals, and that water serves as the ultimate vehicle for exposure to many toxic agents. Relatively few methods exist to precisely and practically assess health risks from exposure to agrochemicals in the aquatic environment (Winn, 2001). In fact, it was historical observations of tumors in wild fishes that prompted the development of carcinogenicity testing utilizing fish species in the laboratory (Law, 2003). Fish are generally one of the most long lived organisms in aquatic ecosystems. There are an increasing number of studies using fish as ecological sentinel species, and specific responses of these organisms as integrators of past and existing environmental conditions, through multimarker and multilevel of organisation approaches (Hill *et al.,* 2005).

Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of the pollutants (Clarkson, 1998; Dickman and Leung, 1998; Olaifa *et al.,* 2004). Easy to capture and fairly easy to maintain and rear in captivity, fresh water fishes are remarkable indicators of aquatic ecosystem health status (Chin Sue, 2002; Maheshwari, 2005; Chellappa *et al.,* 2008). In many ways, fish are not that biochemically different from mammals. Aquatic vertebrates appear to have very similar enzyme and receptor systems as humans (Evans, 1998). The National Institutes of Health recognize the zebrafish (*D. reno*) as a biomedical model to elucidate an understanding of vertebrate development and disease (NIH, 2002). They are the richest source of an essentially healthy diet but they are, endangered by diet-borne pollutants transferred along the food chain (Yanaguchi and Brenner 1997; Nickerson *et al.,* 2001; Andersen *et al*. 2000). Fish are responding to the toxicant for the period of acute as well as the chronic stress. The stress result in to the physiological changes in the body of fishes and not only that but also the behavioral and the cellular changes are present in the tissues of the exposed fish.

Fish physiology is now becoming an integral part of aquatic toxicology. The pollutants in the environment at sub-lethal concentrations are an important variable to which a fish respond physiologically. Although the amount of environmental information collected to date is impressive, there are still gaps in our knowledge of agrochemicals, affected organisms and the related environmental conditions. The qualitative and quantitative description of harmful toxic effects is essential for an evaluation of the potential hazard posed by a particular chemical. It is also valuable to understand the mechanisms responsible for the manifestations of toxicity, i.e. how a toxicant enters the organism, how it interacts with target molecules, how it exerts its effects, and how the organism deals with the exposure as this has led to a better understanding of fundamental physiological processes (Gregus and Klaassen, 1996). Such information provides the basis for interpreting the effect of a toxic substance, estimating the probability that a chemical will cause harmful effects, establishing procedures to prevent or antagonize the toxic effects, designing drugs and industrial chemicals that are less hazardous, and developing pesticides that are selectively toxic for their target organisms.

Since fish have many similar enzyme and receptor systems, this potentially makes them susceptible to similar biochemical and physiological mechanisms of activation/inactivation. Because of the conservation of enzyme and receptor systems between mammals and these fish, chronic and target organ toxicity identified in mammalian safety assessments is likely to be useful in predicting the need for additional toxicity evaluation in teleosts.

Oreochromis mossambicus (Tilapia) (Susan *et al.,* 2010) are the most popular fish species which are economically important for fisheries, aquaculture, game fishing, as recreational aquarium fish and are also used extensively in biological, physiological and behavioural research (Skelton, 2001). They are a good biological model for toxicological (Casas-Solis *et al.,* 2007; Giron-Perez *et al.,* 2007 and 2008; Parthesarathy and Joseph, 2011) studies due to diverse characteristics, namely their high growth rates, efficiency in adapting to diverse diets, great resistance to diseases and handling practices, easy reproduction in capacity at prolific rate and finally and good tolerance to a wide range of environmental conditions (Fontainhas-Fernandes, 1998; Kumar *et al*, 2011). Particularly, *O.mossambicus* is found in abundance in the rivers, lakes and have been described as a 'miracle fish' owing to their bio-economic advantage such as quick growth, fewer bones, tasty flesh, good market acceptance, ease of reproduction and adaptability to wide range of environmental features, ready acceptance to artificial feed, direct assimilation of blue green algae (Jhingran, 1984). It is also considered to

be future of aquaculture, and is nicknamed “the aquatic chicken” due to its ability to grow quickly with poor-quality input.

Labeo rohita (Rohu) is an herbivorous cyprinid fish that inhabits the tropical lowland river systems of Pakistan, northern India, Nepal, Bangladesh and Myanmar. Rohu is considered to be the most important of the Indian 'major carps' and is the world's 10th highest cultured finfish by production volume. Approximately 1.2 million tonnes were produced in 2005 valued at more than US$1.6 billion. The major producing countries are India, Bangladesh and Myanmar. Being the prime cultured species in poly-culture practices in India, it occupies a prominent position in the aquatic system (Das and Mukherjee, 2000; Das *et al.,* 2009), hence the impact of agrochemicals on its physiological stress response of this candidate species was chosen for the study. Moreover, presently no work is available on the tolerance limits with particular reference to physiological stress of *Labeo rohita.* Therefore, present study was planned to study the sub lethal toxicity of the agrochemicals IMI and CZ M8.

Acute toxicities have been measured for many species in variety of ecological systems and most of the commonly used pesticides against Rainbow trout, Blue gills, Sun fish and the gaps are being filled for other species, such as channel fish, some cyprinids and salmons (Chichester, 1965; Lockhart *et al.,* 1973; Pandey *et al.,* 1976; Koundinya and Ramamurthi, 1979; Johnson and Finley, 1980; Joshi and Reg, 1980; Tilak et.al., 1980; Bakthavallhasalam and Reddy 1982; Sharma et.al., 1983; Nebeker *et al*. 1983; Haider and Imbaray 1986; Sunderam *et al*. 1992; Kumar and Gupta, 1997; Berrill *et al*. 1998; Santhakumar *et al.,* 2000; You *et al*. 2004; Wan *et al*. 2005; Koprucu *et al*. 2006; USEPA 2002, 2007; German Federal Environment Agency 2007; Nwania *et al.,* 2010; Singh *et al.,* 2010; Zhang *et al.,* 2010; Srivastava *et al.,* 2010; Kamble *et al.,* 2011; Carriger *et al.,* 2011; Barbieri and Ferreira, 2011; Maniyar *et al.,* 2011; Nikam *et al.,* 2011). Alterations in the chemical composition of the natural aquatic environment usually affect behavioural responses of aquatic organisms (Radhaiah *et al.,* 1987; Brewer *et al.,* 2001; Lucas *et al.,* 2002; Rao *et al.,* 2005). Doses of agrochemicals that are not high enough to kill fish are associated with subtle changes in behaviour and physiology that impair both survival and reproduction (Thirugnanam and Forgash, 1977; Subburaju and Selvarajan, 1988; Kelly *et al.,* 1998; Scott and Sloman, 2004). In aquatic toxicology however, the nexus of behavioral sciences with the study of toxicants has only become prominent recently. Altered behavioural changes includes erratic swimming, fast jerky movements and convulsions which is again dose dependent (Singh and Srivastava, 1982; Mustafa and Murad 1984; Haider and Imbaray, 1986). ***Hence, the first aim of the***

present study was to have an insight for the comparative acute toxicological profile of IMI and CZ M8 on O.mossambicus and L.rohita along with altered behavioural patterns (Chapter I).

Agrochemicals can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity (Banaee *et al.,* 2008; Al-Kahtani, 2011). Blood biochemistry test indicates what is happening in the body of fish exposed to insecticides. When different tissues are injured, the damaged cells release specific enzymes into plasma and we can recognize their abnormality levels in blood. Agrochemical induced changes have been observed in *Channa punctatus* (Agrahari *et al.,* 2007), *Oncorhynchus mykiss* (Velisek *et al.,* 2006 a and b; 2007), *O.mossambicus* (Arockia and Mitton, 2006; Matos *et al.,* 2007; Remia *et al.,* 2008; Ali and Rani, 2009), *Heteropneustes fossilis* (Saha and Kaviraj, 2009), *Cirrhinus mrigala* (Prashanth and Neelagund, 2008) *Clarias batrachus* (Begum, 2005; Ptnaik, 2010), *Cyprinus carpio* (Banaee *et al.,* 2008), *Oncorhynchus mykiss* (Banaee *et al.,* 2011), *Colisa fasciatus* (Singh *et al.,* 2004) exposed to monocrotophos, bifenthrin, carbaryl, dimethoate, cypermethrin, sevin, diazinon, and malathion respectively.

Changes brought about by a stressor could be metabolic in nature, affecting molecular and cellular components such as enzymes or impairing functions such as metabolism, immune response, osmoregulation and hormonal regulation (Barton and Iwama, 1991). Biomarkers are defined as changes in biological responses (ranging from molecular through cellular and physiological responses to behavioural responses) which can be related to exposure to or toxic effect of environmental chemicals (Peakall, 1994). Since the interaction between toxicants and biomolecules is the first step in the generation of toxic effects, the understanding of biochemical alterations induced by the exposure of Stressor/pesticide contributes to the prediction of toxic effects that may occur later at higher levels of biological organization. Moreover, the use of biochemical biomarkers allow early interventions with the objective of protecting wild populations exposed to chemical agents (George, 1994; Newman, 1998; van der Oost *et al.,* 2003; Sanchez *et al.,* 2008; Dong *et al.,* 2009; Haluzova *et al.,* 2011).

Several studies have shown that changes in fish energy metabolism may occur to overcome toxic stress. In fact, under chemical stress the attempt to enhance the supply of energy from anaerobic sources may be essential (Begum and Vijayaraghavan, 1999). Moreover, organic

compounds that interfere with the aerobic metabolic pathway altering the mitochondrial structure and causing disturbances on enzymatic activities and metabolites may lead to impaired levels of energy metabolism (Nath, 2000). The response is characterized by a switch from an anabolic to a catabolic state. Thereby providing the fish with the necessary resources to avoid or overcome the immediate threat, and has evolved as an adaptive response to short-term or acute, stresses. Biochemical constituents and certain enzymes have been explored as potential biomarkers for a variety of different organism because these parameters are highly sensitive and conserved between species and are less variable (Yang and Chen, 2003; Barata *et al.,* 2004; Kalender *et al.,* 2005; Gupta and Aggarwal, 2007; Sreenivasan *et al*. 2011; Narra *et al*. 2011).

Biomarkers using aquatic species are important for detecting stressor components such as the presence of pollutants and changes in environmental factors. Enzymes activities are considered as sensitive biochemical indicators before hazardous effect occur in fish and are important parameters for testing water and the presence of toxicants. Such a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish (Patil and David, 2008; Montagna and Collins, 2007 and 2008; Narra *et al.,* 2011). Enzymes are attractive as indicators because they are more easily qualified than other indicators, such as changes in behaviour. The tissue specific response depends upon the metabolic requirements of the tissue in question.

The analysis of metabolites and marker enzymes such as protein, lipid, glycogen, cholesterol, lactate dehydrogenase (LDH), transaminases (AST and ALT) and phosphatises (ALP) serves as specific indications of water-pollution-induced changes activity of fish (Ramachandra 2000; Tilak *et al*, 2001; Prashanth *et al.,* 2005; Sarkar *et al*. 2005; Dobsikova *et al.,* 2006; Velisek *et al*. 2006a, b, 2007; Reddy *et al.,* 2011 a and b). ***In view of the above, and considering the lack of knowledge about the toxic potential of the insecticide IMI and the Fungicide CZ on fresh water fishes O.mossambicus and L.rohita and the growing use of this agrochemicals, the second objective of the present work was to evaluate its effects on hematological and biochemical parameters of teleosts (Chapter II and III).***

Homeostasis refers to the state of an organism in which its internal environment is maintained in a stable and constant condition. The physiological processes that maintain this equilibrium form a complex and dynamic system. The maintenance of homeostasis is critical to sustain life and changes in the environment can represent a threat to this equilibrium (Charmandari *et*

al., 2005), and can lead to an array of physiological responses often referred to as stress response. Stress responses occur in all animals when regulated physiological systems are extended beyond their normal range by external stressors. Failure of all or part of the integrated homeostatic response may lead to increasing physiological disturbance and ultimately death. Indicators of such stress responses are therefore useful in assessing the short-term well-being or long-term health status of an animal and such indicators have received considerable attention in commercially important aquatic organisms (Roche and Boge, 1996; Paterson and Spanoghe, 1997; Vijayan *et al.,* 1997; Barton, 2002; Iwama *et al.,* 2004; Urbiati *et al.,* 2004).

A "stressor" is a stimulus that acts on a biological system and a "stress response" is the animal's reaction to the stimulus (Pickering, 1981 and Barton, 2002). According to the general adaptation syndrome, a stress response consists of three stages: alarm, resistance, and exhaustion (Pickering, 1981). A lethal stressor exhausts an animal's ability to resist and adapt (Wedemeyer and McLeay, 1981). Sub-lethal stressors do not exhaust, but the energy used for resistance decreases energy availability for growth, immune function, and adaptation to other stressors (Wedemeyer and McLeay, 1981). An acute stress response occurs when the stress is removed before the animal has time to compensate and develop a resistance mechanism (Maule and Schreck, 1990). The stress response is a suite of physiological adjustments that allows an organism to regain homeostasis when it is challenged or threatened by stressors, including toxic compounds (Chrousos, 1998).

The responses to stressors are divided into primary, secondary and tertiary responses. primary response is neuroendocrine and is the result of a stimulation of the hypothalamic-chromaffin axis and the hypothalamic-pituitary interrenal (HPI) axis (Hontela 1997 and 1998; Stocco, 2000; Walsh *et al*. 2000). In response to stress two main classes of hormones, catecholamines and corticosteroids are released by the chromaffin and interrenal cells respectively (Wendelaar-Bonga, 1997), these primary responses may stimulate secondary responses such as increase in circulating red blood cell number (Perry *et al.,* 2005.) and in plasma glucose concentration (Alwan et. al., 2009). These responses are typically of short duration (Wendelaar-Bonga, 1997). However, the stress response may persist, such as during extended contaminant exposures (Bennett and Wolke, 2004).With extended contaminant exposures, secondary stress responses may give rise to tertiary stress responses that will be detrimental to the organism's survival and reproduction. Tertiary responses extend to the level of the organism and population leading to inhibition of growth, reproduction, immune response and

reduce capacity to tolerate subsequent or additional stressors. Of the three stages of stress, the primary and secondary stages are perhaps the easiest to monitor in the laboratory.

The hypothalamic–pituitary interrenal (HPI) axis is responsible for releasing corticosteroids and catecholamines in response to a stressor. Cortisol is the major corticosteroid in teleost fish and most mammals (Hontela, 1997). Cortisol regulates its own production through a negative feedback loop by altering ATCH secretion at the pituitary and hypothalamus (Hontela, 1998). Cortisol affects a variety of systems that regulate homeostasis. It can induce metallothioneines to sequester metals (Hyliner *et al.,* 1989), stimulate protein degradation (Freeman and Idler, 1973), increase mobilization of liver glycogen reserves, increase plasma glucose, increase Na+/K+ATPase activity (Shrimpton and McCormick, 1999), suppress the immune system, suppress maturation, and suppress sex steroid secretion (Carragher and Sumpter, 1990; Hontela, 1997). The stress response can also be affected by a number of other factors, including diurnal secretion cycles, temperature, background color, light wavelength, nutritional state, and disease (Gilham and Baker, 1985; Barton, 2002). Finally, multiple stressors can act in a synergistic fashion and a previous stressor may influence the response to a new stressor (Barton, 2002). Plethora of investigations has proved the fact that the HPI axis gets altered due to stressor either in the form of a pollutant or a pesticide (Donaldson, 1981; Shrimpton and Randall 1994; Wood *et al.,* 1996; Hontela, 1997; McCormick, 1998; Bisson and Hontela, 2002; Levesque *et al.,* 2002).

Oxidative stress, or the cellular damage from reactive oxygen species (ROS), occurs when ROS production exceeds cellular defense mechanisms. This happens if there is a sudden increase in ROS production, impairment of cellular defenses, or a failure to repair ROS damage (Halliwell, 1987; Packer, 1991; Dorval and Hontela, 2003; Oakes *et al.,* 2004; Palace, *et al.,* 2004). Parameters associated with oxidative stress are being investigated as early oxidative stress biomarkers allowing contaminant effects to be documented before population declines are observed. ROS are generated in several different ways. They are intermediates in the cellular respiration pathway and approximately 5% escape (Kelly *et al.,* 1998), they are the byproducts of oxidizing enzymes (Livingstone, 2001) and are involved in phagocytosis (Winston and Di Giulio, 1991). Additionally, ROS production may be enhanced by redox cycling of xenobiotics. ROS have many useful roles in biological systems as they act as cytotoxic agents against pathogens, as neurotransmitters, and transcription factors (Kelly *et al.,* 1998); however, when ROS accumulate they cause serious damage to cell

components and subsequently cell function (Matta, 1995; Wilhelm-Filho *et al.,* 2001; Elia *et al.,* 2003; Shen and Liu, 2006; Langiano *et al.,* 2008; Nwania *et al.,* 2010).

ROS damage lipids, proteins, and DNA. Reaction of ROS with lipids results in a process called lipid peroxidation (LPO). LPO causes structural damage affecting membrane permeability and fluidity (Kelly *et al.,* 1998). Tissues with more polyunsaturated fatty acids are more vulnerable to LPO (Livingstone, 2001; Oakes *et al.,* 2003; Oakes and Van Der Kraak, 2004; Parvez and Raisuddin, 2005; Puangkaew *et al.,* 2005; Atif *et al.,* 2005; Ganguly *et al.,* 2010; Farombi *et al.,* 2008)

There are various cellular mechanisms to remove excess ROS and avoid oxidative damage. Increased ROS production may be associated with the metabolism of a stressor/pesticide leading to the peroxidation of membrane lipids of the important metabolic or excretory organs. The liver is noted as site of multiple oxidative reactions and maximal free radical generation (Gul *et al.,* 2004; Avci *et al.,* 2005; Atli *et al.,* 2006)

To go over the main points the oxidative stress defenses is taken care by the markers which includes enzymes and scavengers. Enzymatic defenses include glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) (Jakoby and Habig, 1980; George, 1994; Yang *et al.,* 2000; Roberts and Oris, 2004; Sanchez *et al.,* 2008; Haluzova *et al.,* 2011). Reduced glutathione (GSH) and ascorbic acid is the most important antioxidant found in the extracellular fluid (Sies and Stahl, 1995). It is water soluble and scavenges a number of different ROS including thiol radicals generated during xenobiotic reduction (Palozza and Krinsky, 1992; Kelly *et al.,* 1998; Palace *et al.,* 1999). The scavenging and enzymatic antioxidants are linked in many ways. Often if one is deficient, others increase to compensate (Puangkaew *et al.,* 2005).

Recently there has been an explosion of research linking oxidative stress parameters and exposure to contaminants. Pesticidese induced lipid peroxidation has already been described for various fish species (Orbea *et al.,* 2002; Sevgiler *et al.,* 2005; Glusczak *et al.,* 2006 and 2007; Modesto and Martinez, 2010). Therefore, both the activity of antioxidant enzymes and the occurrence of oxidative damage have been proposed as indicators of pollutant-mediated oxidative stress (Ahmad *et al.,* 2000; Li *et al.,* 2003). ***All the antioxidant enzymes and scavengers work together to protect the fish from oxidative stress, hence, after having the altered biochemical profile on the exposure of the agrochemicals on the fishes the next***

target was to investigate the probable mechanism involved in the defense response of O.mossambicus and L.rohita on exposure of the Agro-chemicals.(Chapter IV).

Other than biochemical biomarker, Organo-somatic indices are useful indicators of general organ and fish health; these indices have been used extensively in fish health and population assessment as a first level screen to determine possible contaminant exposures (Slooff *et al.,* 1983; Larsson *et al.,* 1984; Goede and Brton, 1990; Schmitt and Dethloff, 2000; Ghosha *et al.,* 2006). These parameters are sensitive of specific, and are affected by non-pollutant factors (Van der Oost *et al.,* 2003). Organo-somatic indices thus serve as an initial screening biomarker to indicate exposure and effects (Mayer *et al.,* 1992). ***The condition factor and organo-somatic indices used in the present study are: hepato-somatic index (HSI), spleno-somatic index (SSI) and gonadal-somatic index (GSI) along with their histomorphological alterations.(Chapter V)***

The application of ecotoxicological studies on non-mammalian vertebrates is rapidly expanding; and for aquatic system, fish have become valuable indicator for the evaluation of the effects of noxious compounds (Khidr and Mekkawy, 2008). Histology and histopathology can be used as biomonitoring tools for health in toxicity studies (Schwaiger *et al.,* 1997; Meyers and Hendricks, 1985). Histopathological alterations are biomarkers of effect exposure to environmental stressors, revealing alterations in physiological and biochemical function (Hinton *et al.,* 1992). Histopathology, the study of lesions or abnormalities on cellular and tissue levels is useful tool for assessing the degree of pollution, particularly for sub lethal and chronic effects (Heath, 1995 and Teh *et al.,* 1997; Bernet *et al.,* 1999; Das and Mokherjee, 2000; Cengiz *et al.,* 2001; Cengiz and Unlu, 2002; Adeyemo, 2008). Due to residual effects of pesticides, important organs like the kidney, liver, gill are the first organs to be damaged (Rahman *et al.,* 2002). Many authors have recorded and observed histological abnormalities in gills, liver and kidney for fishes contaminated by pesticides. (Hinton and lauren, 1990; Ahel *et al.,* 1993; Alazemi *et al.,* 1996; Lewis and Lech, 1996; Coldham *et al.,* 1998; Visoottiseth *et al.,* 1999; Das and Mukherjee, 2000; Malik and Hodgson, 2002; Parashar and Banerjee, 2002; Velmurugan *et al.,* 2007; Camargo *et al.,* 2007; Garcia-Santos *et al.,* 2007; Marchand *et al.,* 2008; Mohamed, 2009). ***From the above reported studies one can understand that the organosomatic alteration in the agrochemical exposed fish can supplement and help in understanding and co-relating the results of the histomorphological alterations in the agrochemical exposed teleost fish (Chapter VI).***

Chapter I

Behavioural responses to acute exposure of Imidacloprid and Curzate on *Oeochromis mossambicus (peters, 1852)* and *Labeo rohita (Hamilton, 1822)*

Agrochemicals, such as, pesticides, fertilizers, organic manure, growth hormones, and nutrient solution, pollute water significantly when they enter into the water through run off (Kurian and Natarajan, 1997). Agricultural run-off affect groundwater and surface water sources as they contain pesticide and fertilizer residues. Pollution by agricultural run-offs has too many effects on the environment. Most of the agrochemicals/Pesticides are not readily degradable and remain in water for a considerable period adversely affecting fishes and other aquatic animals (Ramaswamy *et al.,* 2007). They have the potential to enter aquatic habitats from direct application, terrestrial runoff or wind-borne drift. The aquatic pollution caused by agrochemicals/pesticides in Asia, Africa, Latin America, the Middle East and Eastern Europe are now serious, further the scientists have reported that globally 4.6 million tons of chemical pesticides are annually sprayed into the environment and that only 1% of the sprayed pesticides are effective; 99% of pesticides applied are released to non-target soils, water bodies and atmosphere, and finally absorbed by almost every organism (Zhang *et al.,* 2011). Since there are thousands of different pesticides used around the world, data on aquatic contamination for any particular pesticide is usually quite limited. Studies conducted in lentic and lotic systems have detected a variety of pesticides including the insecticides malathion, endosulfan and diazinon as well as the herbicides atrazine and glyphosate (LeNoir *et al.,* 1999; Hayes *et al.,* 2002; Kolpin *et al.,* 2002; Thompson *et al.,* 2004). Considerable information is available on the toxicity of these compounds and chemicals to various aquatic organisms worldwide (Henderson *et al.,* 1959, Verma *et al.,* 1980; Nebeker *et al*., 1983; Haider and Imbaray, 1986; Sunderam *et al.,* 1992; Berrill *et al.* 1998; Wan *et al.,* 2005; USEPA, 2002b, 2007) but very little work has been done in India to evaluate the acute toxicity by bioassay on freshwater fishes.

Acute aquatic toxicity represents the intrinsic property of a substance to be injurious to an organism in a short-term exposure to that substance. Static acute toxicity tests provide rapid

and reproducible concentration-response curves for estimating toxic effects of chemicals on aquatic organisms (Casarez, 2001; Yuill and Miller, 2008). With the help of these tests the relative toxicity of large number of chemicals present in the natural aquatic systems due to variety of chemical spills can be determined. There is a vigorous documentation of the use of acute toxicity tests for assessing the potential hazard of chemical contaminants to aquatic organisms (Boyd, 1957; Henderson *et al.,* 1960; Sanders and Cope, 1966; Macek and McAllister, 1970; Brack *et al.,* 2002; Diez *et al.,* 2002).

Nikam *et al.,* (2011) in their studies have mentioned that the acute toxicity study is essential to find out toxicants limit and safe concentration, so that there will be minimum harm to aquatic fauna. Among the several aspects of toxicity studies, the bioassay constitutes one of the most commonly used methods in aquatic environmental studies with suitable organisms. The necessity of determining the toxicity of substances to commercially aquatic forms at the lower level of the food chain has been useful and accepted for water quality management. Several studies have been conducted in assessing the toxicity of pesticide to the aquatic biota especially fishes (Verma *et al.,* 1982; Ravikrishnan *et al.,* 1997; Vasait and Patil, 2005; Susan *et al.,* 2010).

Acute toxicity is expressed as the median lethal concentration (LC_{50}) that is the concentration in water which kills 50% of a test batch of fish within a continuous period of exposure which must be stated (Amweg and Weston, 2005).The application of the LC_{50} has gained acceptance among toxicologists and is generally the most highly rated test of assessing potential adverse effects of chemical contaminants to aquatic life (Brando *et al.,* 1992; Kumar, 2004; Fagr *et al.,* 2008; Gad and Saad, 2008; Khayatzadeh and Abbasi, 2010). The use of 96-h, LC_{50} has been widely recommended as a preliminary step in toxicological studies on fishes (McLeay, 1976; Whittle and Flood, 1977; Reish and Oshida, 1987; Ardali, 1990; Chapman, 2000; Ali and SreeKrishnan, 2001; ASTM, 2002; USEPA, 2005; APHA, 1998, 2005; Parrott *et al.,* 2006; Moreira-Santos *et al.,* 2008). LC_{50} is customary to represent the lethality of a toxicant to a test species in terms of lethal concentration (for aquatic animals) and lethal dose (for terrestrial animal). It is always expressed in terms of g or mg/kg body weight of the animal and lethal concentrations (LC) in terms of Parts/million (ppm) or parts/billion (ppb) or milligram/liter (mg/L). The relationship between the concentration of an environmental toxicant and its lethal effects on living organisms is often a sigmoid curve.

Probit analysis is a parametric statistical procedure for making the sigmoidal response curve into a straight line so that an LC_{50} can be calculated and the associated 95% confidence interval can be calculated (Finney, 1978; Hahn and Soyer, 2008).

Mortality is obviously not the only end point to consider and there is growing interest in the development of behavioural markers to assess the lethal effects of toxicants. Abnormal behaviour is one of the most conspicuous endpoints produced by these toxicants, but until recently it has been under used by ecotoxicologists (Little and Brewer, 2001; Dell'Omo, 2002; Gerhardt, 2007; Hellou, 2011). Amiard-Triquet (2009) states that "bridging the gap between early, sensitive responses to stress at the infra-organismal levels and the long-term, ecologically relevant responses at the supra-organismal levels is a challenge for a better assessment of the ecological status of our environment." Behavioral ecotoxicology provides, according to her, an approach that clearly links disturbances at the biochemical level to effects at the population level either in a direct or indirect way. She notes that "Because behavioural disturbances may be observed in aquatic biota at concentrations of contaminants that can exist in the field, the sensitivity of these responses can allow improving environmental risk assessment." Therefore, she recommends "to use behavioural biomarkers, associated to biochemical and physiological markers in carefully selected species that are key-species in the structure and functioning of ecosystems because impairments of their responses, used as biomarkers, will reveal a risk of cascading deleterious effects at the community and ecosystem levels."

Thus, behaviour can be considered as a promising tool in ecotoxicology (Little and Brewer, 2001). Behaviour is both a sequence of quantifiable actions, operating through the central and peripheral nervous systems (Baatrup, 2009; Gravato and Guilhermino, 2009), and the cumulative manifestation of genetic, biochemical, and physiologic processes essential to life, such as feeding, reproduction and predator avoidance (Smith and Weis, 1997; Grue *et al.,* 2002; Perez and Wallace, 2004; Riddell *et al.,* 2005; Moreira *et al.,* 2006). It allows an organism to adjust to external and internal stimuli in order to best meet the challenge of surviving in a changing environment.

Fish are ideal sentinels for behavioral assays of various stressors and toxic chemical exposure due to their constant, direct contact with the aquatic environment where chemical exposure

occurs over the entire body surface, ecological relevance in any natural systems *(*Little *et al.,* 1993; Velmurugan *et al.,* 2006; Omitoyin, 2007), ease of culture, ability to come into reproductive readiness *(*Henry and Atchison, 1986*)*, and long history of use in behavioral toxicology. The behavioural patterns vary widely with different species of fish and exposure conditions. Fishes exposed to toxicants undergo stress, which is a state of re-established homeostasis, a complex suite of mal-adaptive responses (Chrousos, 1998). Fishes in a contaminated environment show some altered behavioural patterns which may include avoidance, locomotor activity and aggression and these may be attempts by the fish to escape or adjust to the stress condition (Morgan *et al.,* 199 and Gormley *et al.,* 2003). Avoidance and attractance behaviour in fish has proven to be an easy and realistic behavioural endpoint of exposure.

Moreover from the past 50 years the utility of avoidance behavior has been demonstrated an indicator of sublethal toxic exposure (Tiwari and Singh, 2004; Chindah *et al.,* 2004; Bobmanuel *et al.,* 2006; Gabriel *et al.,* 2009). Avoidance behaviour is a fusion of many behaviours that may culminate in a single endpoint (Little and Brewer, 2001). A group of scientists (Kleerekoper *et al.,* 1972; Brewer *et al.,* 1999; Allin and Wilson, 2000; Kwak *et al.,* 2002) have demonstrated alterations in swimming behaviours due to sublethal metal and pesticide exposures.These alterations in the swimming behaviours results in an increase in the expenditure of energy (Venkata Rathnamma *et al*., 2008), which may result in hyperactivity. Similar behaviour had been observed in *Channa striatus* (Yadav, *et al.,* 2007), and in *Clarias gariepinus, Heterobranchus bidorsalis* and their hybrid (Ekweozor *et al.,* 2001; Bobmanuel *et al.,* 2006; Inodi *et al.,* 2010) exposed to toxicant showed hyperactivity characterized by linear movement, jumping, opercular and tail beat frequencies, distance movements and somersaulting depending on the concentrations.

A change in respiration rate is one of the common physiological responses to toxicants and is easily detectable through opercular beat frequency. Respiration is a rhythmic neuromuscular sequence regulated by an endogenous biofeedback loop as well as by external environmental stimuli which in turn will induce reflexive cough and gill purge responses to clear the opercular chamber leading to increase in rate and amplitude of the respiratory cycle (Magare and Patil, 2000; Katja *et al.,* 2005; Patil and David, 2008). Thus the relative changes in the respiratory frequency and cough frequency can be measured to study the effect of the external

change in the environment on restrained sentinel fish. These systems have the great advantage of sensitivity since many waterborne stressors, even at low environmental concentrations; affect gill tissue and respiratory function.

In the behavioural study various scientists have studied the effects of different toxicants on the fishes taking into consideration opercular beat frequency (OBF) and tail beat frequency (TBF). Alterations in TBF and OBF may be associated with sudden response of the fish to the shock of exposure to the agro-chemical (Chindah *et al.,* 2004). This behaviour may be an adjustment of the internal homeostatic of the fish to the stress imposed by the toxicant (Perkins and Schlenk, 2005; Ujagwung *et al.,* 2010).

Series of studies has been conducted on fingerlings (Ugwemorudong and Sunday. (2010) and adult fish (Kidd and James, 1991; Santhakumar *et al.,* 2000; Battaglin and Fairchild, 2002; Chindah *et al.,* 2004; Prasanth *et al.,* 2005; Ujagwung *et al*., 2010; Parikh *et al.,* 2010; Singh *et al.,* 2010; Srivastava *et al.,* 2010; Zhang *et al.,* 2010; Barbieri and Ferreira, 2011; Maniyar *et al.,* 2011) with a variety of pesticides. Perusal of literature reveals paucity of information on acute toxicity of IMI and CZ on freshwater fish, *Oreochromis mossambicus* and *Labeo rohita*. Hence, keeping in mind the importance of the acute toxicity as well as the behavioral responses, the present study has been focused to evaluate the acute toxic effects on mortality and behaviour of freshwater teleosts fish.

Materials and Methods:

Collection and maintenance of experimental animals:

Two freshwater teleosts, *O. mossambicus* and *L. rohita* of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies

Preparation of the Agrochemicals: Two agrochemicals were selected for the present study which were procured from DuPont™, Vadodara.

1. Imidacloprid (IMI) with the chemical name 1-(6-chloro-3-pyridil methyl)–N-Nitroimidazolidin-2-ylideneamine-Triazole-1-yl–2–butanone) as an active ingredient, a systemic insecticide which is a water and fat soluble. Solution of IMI was made by dissolving in the preheated (20 ºC) water.

2. Curzate® M8 (CZ) a mixture of Cymoxanil 8% with a chemical name 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea + Mancozeb 64% with a chemical name Manganese ethylenebisdithiocarbamate polymeric complex with zinc salt is a fungicide available in the form of wettable powder hence easily dispersible in water. Solution of CZ was prepared by directly dissolving it in water.

Experimental protocol for LC_{50} determination:

Acute 96-h static bioassay was conducted in the laboratory following the methods of Sprague (1975) and APHA (1985). The acute fish bioassay experiments for 24, 48, 72 and 96 hours were conducted. Concentrations of the test compounds used in short term definitive tests were between the lowest concentration for IMI (0.55 mg/L for *O. mossambicus* and 0.79 for *L. rohita*) and for CZ (36.0 mg/L for *O. mossambicus* and 46.0 for *L. rohita*) at which there was no mortality , and the highest concentration for IMI (0.95 mg/L for *O. mossambicus* and

0.88 for *L. rohita*) and for CZ (46.0 mg/L for *O. mossambicus* and 55.0 for *L. rohita*) at which there was 100% mortality in the range finding tests.

To determine the 96-h LC_{50}, for each concentration, ten fishes were used in 50-L containers. Three replicates were used for each concentration. During experimentation fishes were kept deprived of the feed. The aquaria were kept closed to avoid the effect from sunlight. The mortality of the fishes at 96- hr were recorded and the behavioural response to each dose of each test chemical was also observed twice in the day.

Statistical Analysis:

Probit analysis (Finney, 1971) was used to calculate the median lethal concentration and time with their upper and lower confident limits.

Toxicity data obtained as the 50% mortality endpoint were converted into toxic units (Tu) by the following formula: Tu = [1/LC50] × 100 (Michniewicz *et al.,* 2000) and were characterized according to the categorization proposed by Isidori *et al.,* (2000).

Data of Behavioural changes for OBF and TBF were subjected to analysis of variance (ANOVA) for difference between means of both the groups using statistical programme (Biostat 2009 Professional 5.8.1 and Graphpad Prism 5). Other abnormal behaviours were noted and the extent of mucus production on the skin and gills of exposed fish was assessed by feeling with the fingers. Opercular beat frequency (OBF), tail beat frequency (TBF) and cumulative mortality was recorded. A fish was considered dead when it failed to respond to simple prodding with a glass rod. Death was defined as complete immobility with no flexion of the abdomen upon forced extensions (Lockwood, 1976).

Results

The mortality of fish increased with the increase in the concentration of the toxicant, depicting a direct correlation between the mortality and the concentration (Fig. 1.1 and 1.2). The 96 hrs LC_{50} values along with its 95% lower and upper confidential limits (LCL and UCL) for IMI and CZ agrochemical are presented in Table 1.1 and 1.2.

The probit analysis (Fig. 1.1 and 1.2) revealed the fact that the LC_{50} value for *L. rohita* (0.8536 – IMI, 51.2689 – CZ) was much higher than *O. mossambicus* (0.7319 – IMI, 39.84 – CZ) for both the agrochemicals. The corresponding toxic units are shown in Table 1.1 and 1.2 for *O.mossambicus* and *L.rohita.* Toxic unit results for the IMI and CZ raveled that *O.mossambicus* possess more sensitivity than *L.rohita.*

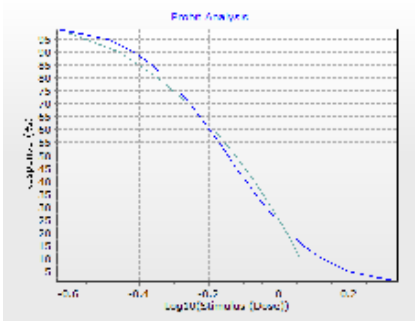
Behavioural responses were found changed on exposure to the agrochemicals, IMI and CZ. In control group, fishes showed a tight school covering the part of bottom of the tank. They were found in well-coordinated manner and were alert to the slightest disturbances. When exposed to pesticides, the shoal was observed as disturbed. Fishes were initially surfaced, followed by vigorous and erratic swimming showing agitation. Quick opercular and fin movements were observed initially and gradually became feeble and often showed gulping of air. Excess secretion of mucus was a prominent observation. Opercular opening became wider and exhibited respiratory distress. As the period of exposure increased, fishes were found to settle down to bottom and towards the final phase of exposure, fishes showed barrel-rolling indicating loss of equilibrium. Swimming with belly upwards and gradually became lethargic. Excess mucous was produced during intoxication.

Table 1.1 LC_{50} values (mg/L) of acute toxicity tests and corresponding Toxic unit value for *Oreochromis mossambicus*

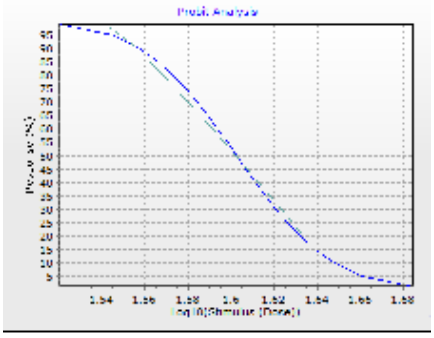
Agrochemical	Application	Duration	LCL	LC_{50}	UCL	Tu
Imidacloprid	Insecticide	48 hrs	0.6896	0.7319	0.7742	136.63
Curzate	Fungicide	48 hrs	38.67	39.84	40.78	2.51

Note:
LCL = Lower Confidence Limit
UCL = Upper Confidence Limit
LC_{50} = Lethal Concentration for 50 percent of the exposed fish
Tu = Toxic unit mg/L

Fig. 1.1 Plot of adjusted probits and predicted regression line for three agro-chemicals to *Oreochromismossambicus*



Imidacloprid



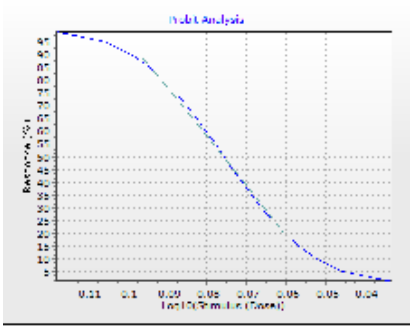
Curzate

Table 1.2 LC_{50} values (mg/L) with their fiducial limits used in acute toxicity tests and corresponding Toxic unit value for *Labeo rohita*

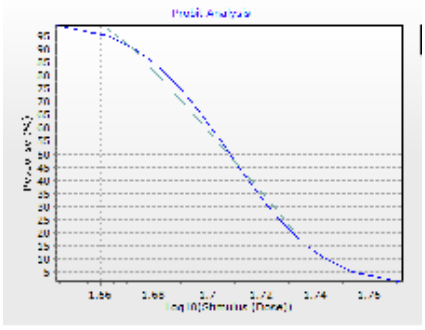
Agrochemical	Application	Duration	LCL	LC_{50}	UCL	Tu
Imidacloprid	Insecticide	48 hrs	0.8536	0.840	0.8292	119.05
Curzate	Fungicide	48 hrs	52.2689	51.048	50.0976	1.96

Note:
LCL = Lower Confidence Limit
UCL = Upper Confidence Limit
LC_{50} = Lethal Concentration for 50 percent of the exposed fish
Tu = Toxic unit mg/L

Fig. 1.2 Plot of adjusted probits and predicted regression line for three agro-chemicals to *Labeo rohita*

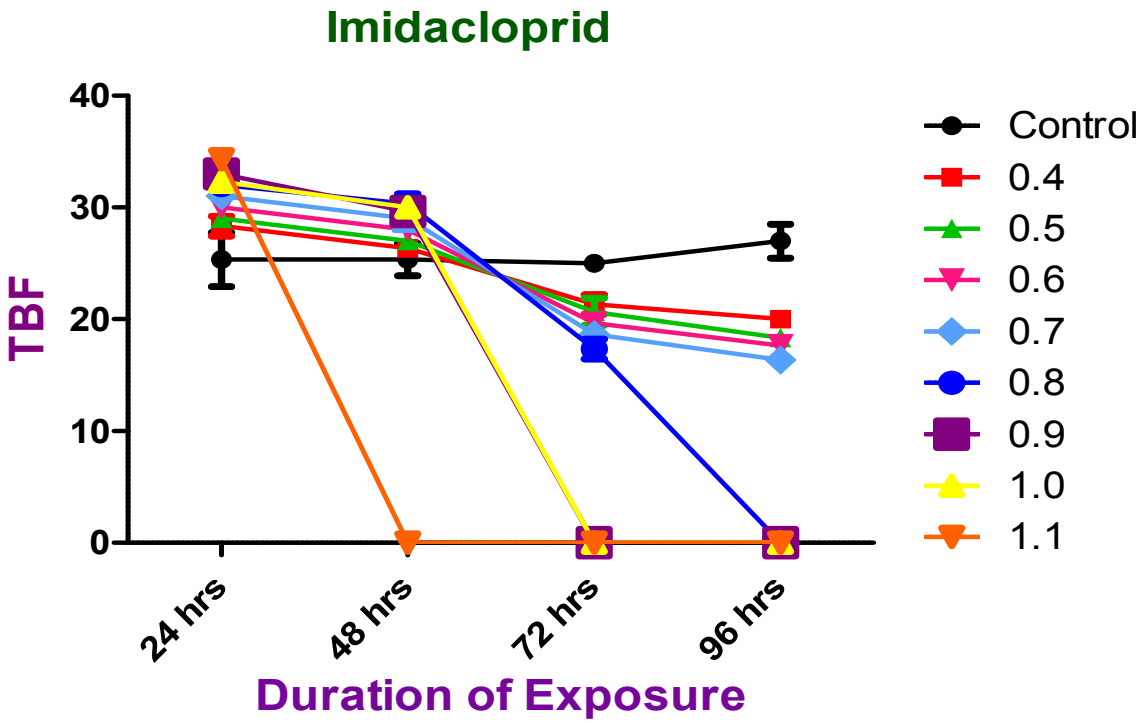


Imidacloprid



Curzate

Fig. 1.4 Graphs showing alterations in TBF of *Oreochromis mossabicus* on exposure of IMI and CZ



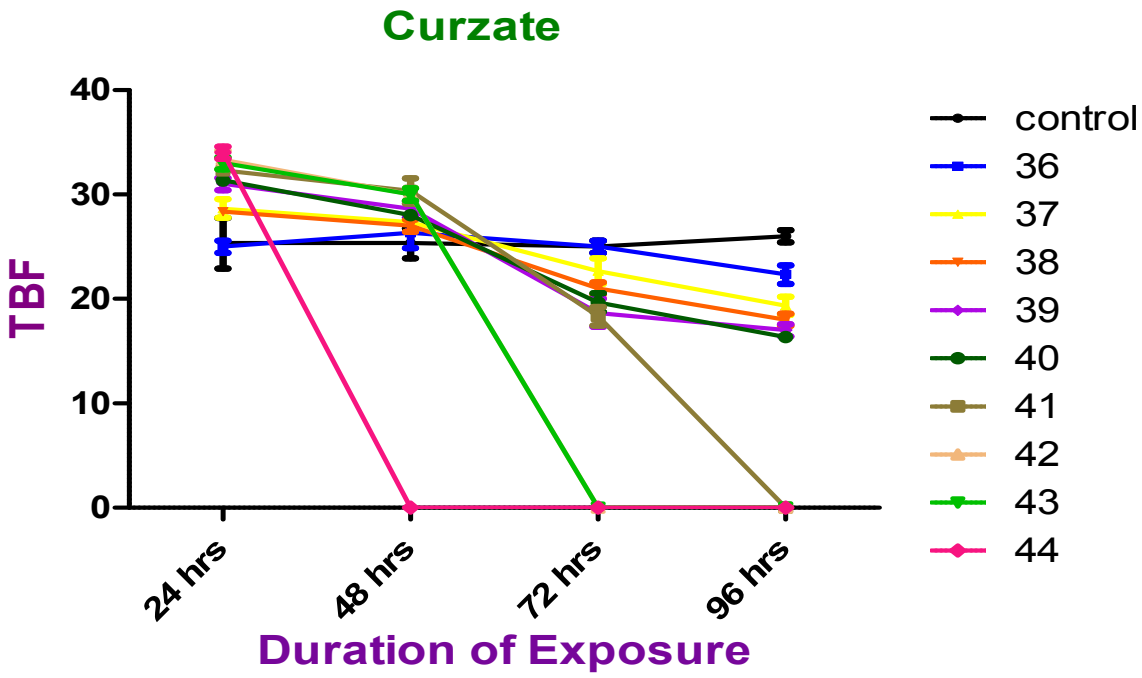
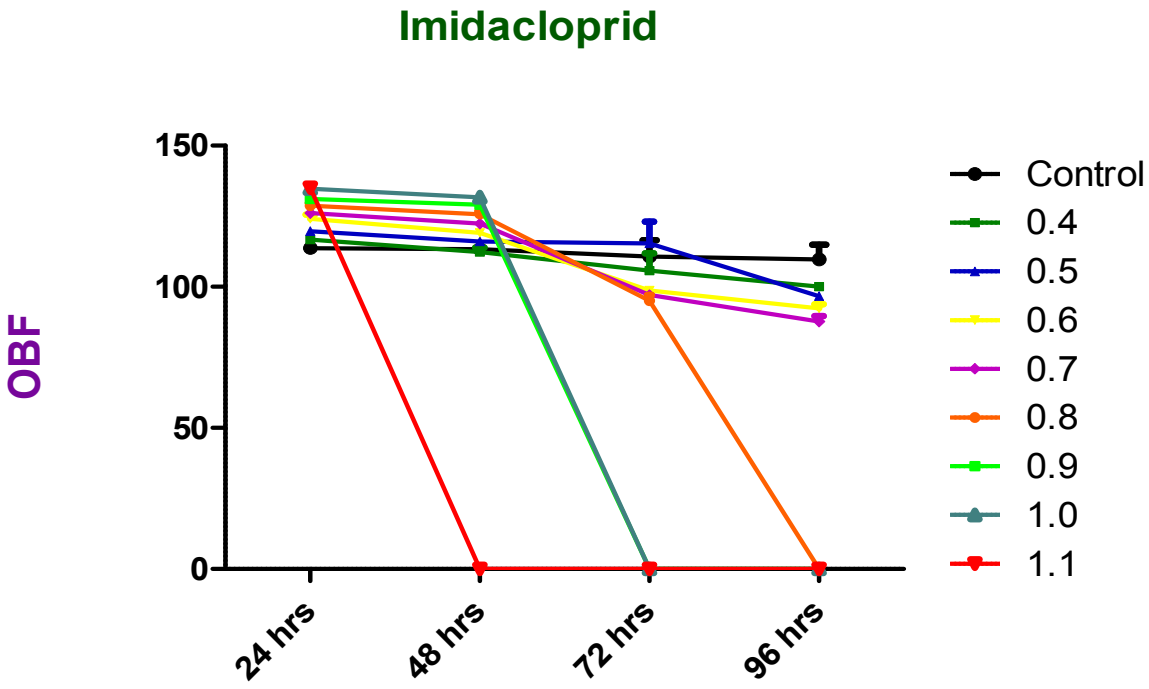


Fig. 1.5 Graphs showing alterations in OBF of *Oreochromis mossabicus* on exposure of IMI and CZ



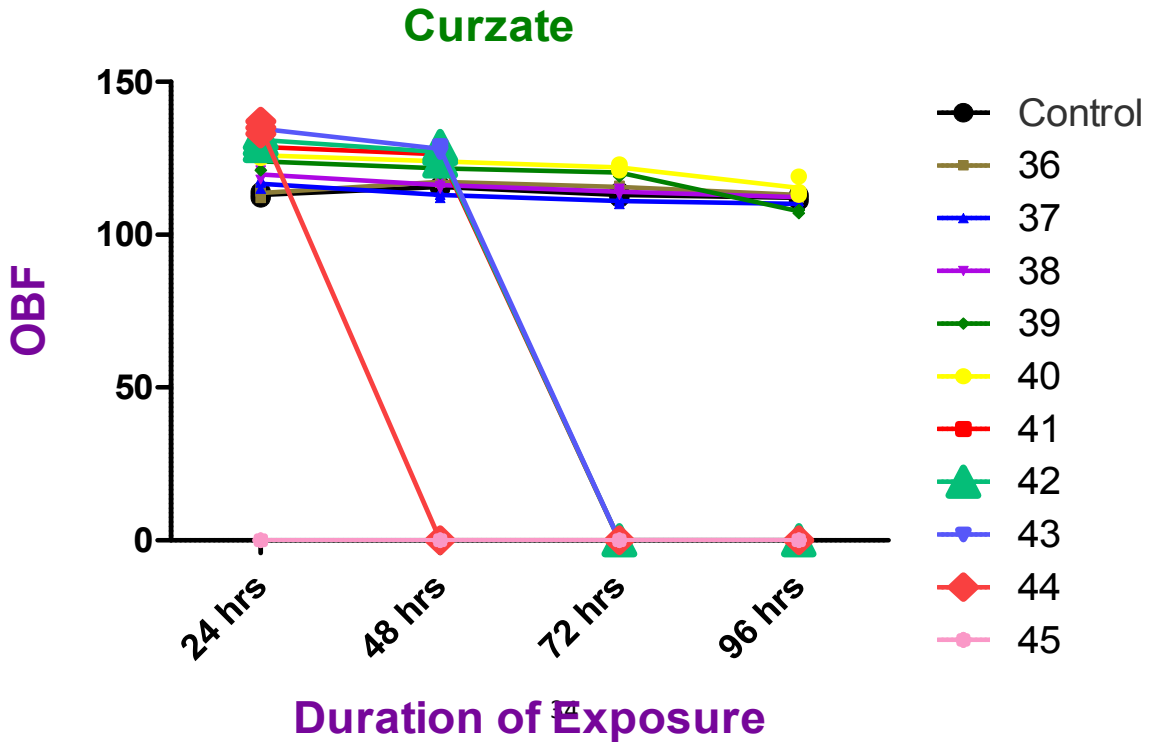
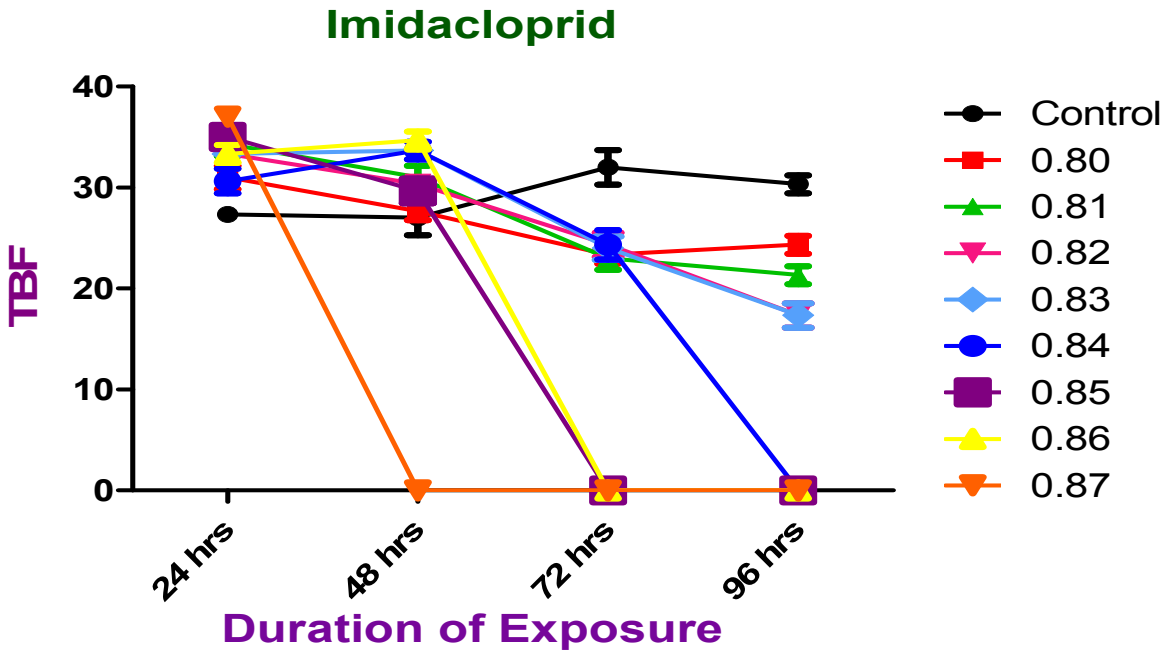


Fig. 1.6 Graphs showing alterations in TBF of *L.rohita* on exposure of IMI and CZ



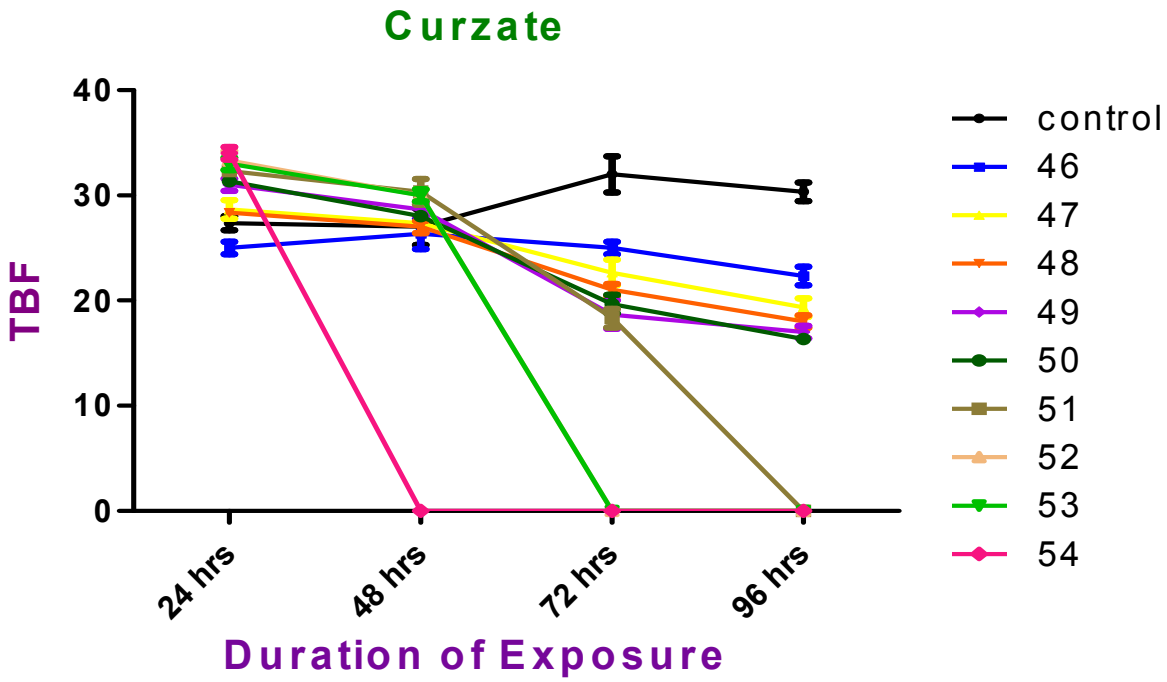
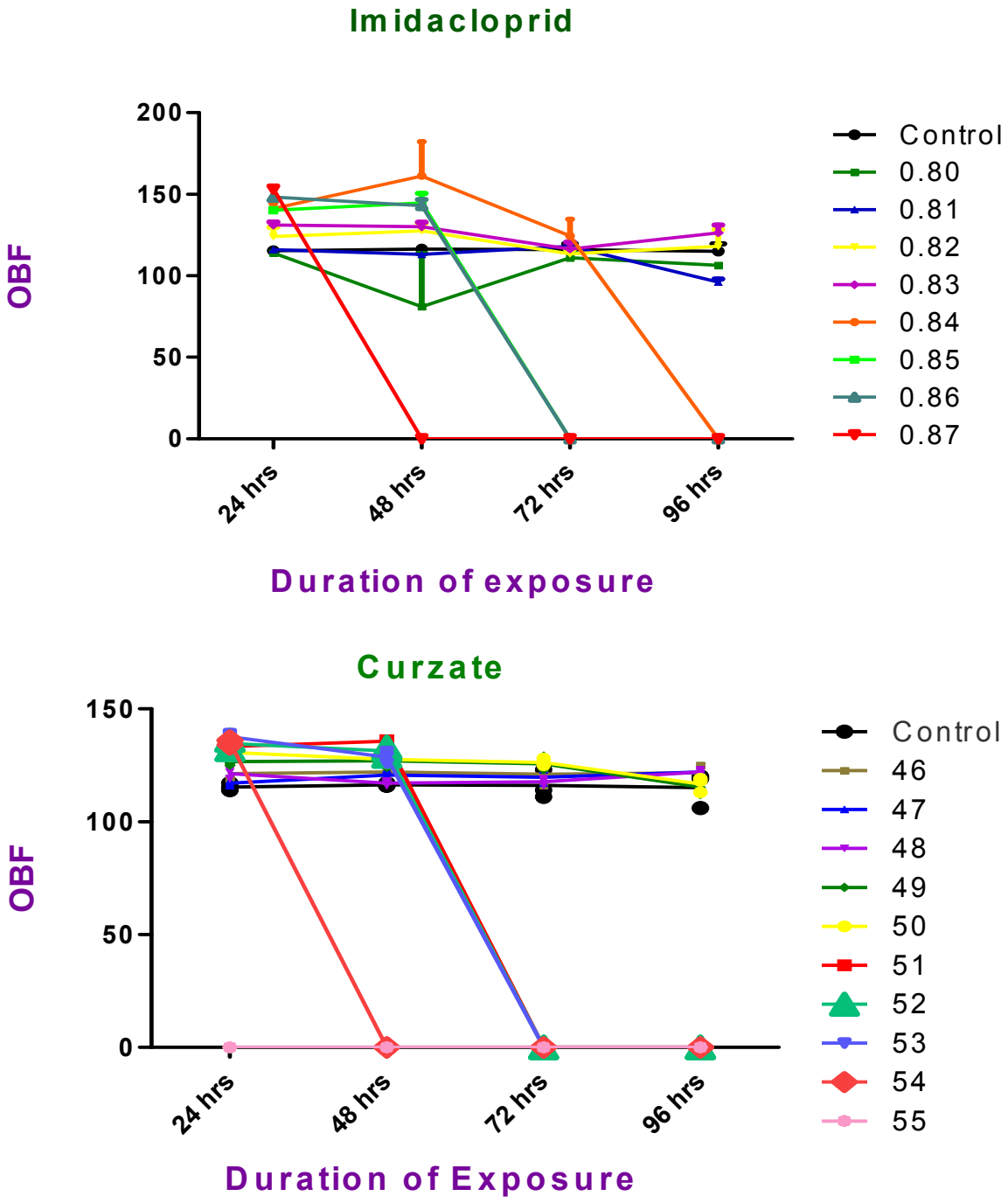


Fig. 1.7 Graphs showing alterations in OBF of *Labeo rohita* on exposure of IMI and CZ



Discussion:

Fish mortality due to pesticide exposure mainly depends upon its sensitivity to the toxicants, its concentration and duration of exposure (Kamble *et al.,* 2011). The evaluation of LC_{50} concentration of pollutants is an important step before carrying out further studies on physiological changes in animals. The percent survival rate of the fish decreased with increasing concentration and period of exposure. In present probe, acute toxicity test shows a relationship between the length of exposure period and concentration of pesticide. The LC_{50} values of the fish decreased gradually as the exposure period goes on increasing.

The acute toxicity data provides useful information to identify the mode of action of a substance and also help to comparison of dose response among different chemicals. In the present studies no fish died during the acclimation period before exposure, and there was no dead fish in control aquaria during acute toxicity tests. The 96-h LC_{50} tests are conducted to measure the vulnerability and survival potential of organisms to particular toxic chemical. The mortality of fish increased with the increase in the concentration of the toxicant, depicting a direct correlation between the mortality and the concentration (Fig. I and II). The 96 hrs LC_{50} values along with its 95% lower and upper confidential limits (LCL and UCL) for IMI and CZ agrochemical are presented in Table - I and II.

The probit analysis (Fig. - I and II) revealed the fact that the LC_{50} value for *L. rohita* (0.8536 – IMI, 51.2689 – CZ) was much higher than *O. mossambicus* (0.7319 – IMI, 39.84 – CZ) for both the agrochemicals. It is evident from the result that CZ is less toxic than IMI. The toxicity of IMI and CZ LC_{50} for freshwater fishes when compared, revealed the fact that *O. mossambicus* was more sensitive to both the agrochemicals than *L. rohita*.

According to the toxic units, the substances are characterized from "very toxic" to "extremely toxic" (Isidori *et al.,* 2000). In the present study the categorization of the agro-chemicals revealed that *O.mossambicus* was more responsive compared to *L. rohita*.

In order to categorize the samples according to the results from the toxicity tests, the values of LC_{50} were converted to toxic units (TU). Table 1.1 and 1.2 indicates high toxicity levels of IMI than that of CZ, which reveals the high sensitivity of *O. mossambicus* compared to *L. rohita*. Substances with lower LC_{50} values are more toxic because lower concentrations results 50% of mortality in organisms which points to the fact that *O. mossambicus* was more

responsive compared to *L. rohita*. These results are in line with those from a previous related work on hazardous wastes (Clement *et al.,* 1996; Isidori *et al.,* 2003; Pablos *et al.,* 2009; Bortolotto *et al.,* 2009). The toxicity categorization was established using toxic unit ranges (highly toxic (TU > 100); very toxic (10 < TU < 100); toxic (1 < TU < 10); and no toxic (TU < 1) (Pablos *et al.,* 2011). Accordingly IMI can be categorized to be highly toxic and curza to be toxic. The results of the present work appear to agree with previous acute studies for IMI performed by Bowman and Bucksath (1990) and Grau (1988) on Bluegill (*Lepomis machrochirus*), and Rainbow Trout (*Ochorhynchus mykiss*), where the effective substance of IMI has been found to be moderately to highly toxic to fish, depending on the sensitivity of the species (Howard, 1991; Kidd and James, 1991; Methomyl, 1996; Tomlin, 2003).

CZ is a mixture of Cymoxanil (8%) and Mancozeb (64%). There are immense literatures for acute studies on cymoxanil (Baer, 1993a, b; Kraemer, 1996; Boeri *et al.,* 1996a and b, 1997). Cymoxanil is slightly toxic to fish on an acute basis. The 96-hour LC_{50} for various species (in mg/L) were: 91 for common carp; 61 for rainbow trout; 29 for bluegill sunfish; >47.5 for sheeps head minnow (MSDS). On the other hand mancozeb is moderately to highly toxic to fish (48-hour LC_{50} are 9 mg/L in goldfish, 2.2 mg/L in rainbow trout, 5.2 mg/L in catfish, and 4.0 mg/L in carp) as reported by (Bisson and Hontela, 2002; Muhmmed and Telat, 2013; Knauer *et al.,* 2007; Gopi *et al.,* 2012). However, the acute studies on mixture are lacking. In the present studies CZ was found to be moderate to slightly toxic for both the teleost fishes and as proposed by (U.S. EPA, 2004).

Acute toxicity involves the damage to the organism by fastest acting mechanism. Our results are in agreement with the comparative studies of John (2007) on *Heteropneustes fossilis* and *Ophiocephalus striatus*; Vasait and Patil (2005) and Nikam *et al.,* (2011) on freshwater fish, *Nemacheilus botia*. Hedayati et al., 2012 in their investigation of acute toxicity for two systemic pesticides have reported variation in the response for blue gourami, *Trichogaster trichopterus.* Kreutz *et al.,* (2008) have also reported degree of difference for the mortality of two pesticides on silver catfish (*Rhamdia quelen*) fingerlings. Comparative Study on the acute toxicity of synthetic pesticides by Boateng *et al.,* (2006); Srivastava *et al.,* (2010); Nikam *et al.,* (2011) have also observed the different response of the pesticides. Selective toxicity of the agrochemical may due to differential physiological response of the individual fish i.e detoxification, absorption and or excretion capacities (Viran *et al.,* 2003). As

Thompson and Schuster (1968) noted, the study of toxic effects on the behavioural level offers ecologists and environment a lists two major advantages. First, chemical agents that produce only behavioural changes and have serious and possibly irreversible deleterious effects on the animals' ability to adapt can be identified and controlled. Second, the behaviourally toxic effects of chemical agents can be considered as an early warning system for the detection of the toxicity before irreversible structural and biochemical damage are caused by them. Most of the fish which died during the experiment exhibited symptoms of poisoning such as change in color as well as behavior. Initially their color darkened and they swam erratically with their body inclined downwards. Matsumura (1975) reported that hyperactivity is a primary and principal sign of nervous system failure due to pesticide poisoning, which affects physiological and biochemical activities.

Behaviour is a visible reaction of an organism to a stimulus on the whole-organism organization level. However, being based on biochemical reactions and exerting consequences on the population and biocoenosis levels, behaviour can be regarded as highly integrative (Little, 1993; Janssen, 1997; Dell'Omo, 2002). Behavior can be classified in different ways, such as: internal biochemical, physiological processes and external ecological consequences e.g., avoidance, mating. Behavioural tests have a high potential to be applied in ecotoxicological research as well as in biomonitoring, in addition to other biological and chemical methods. They offer ecologically relevant, sensitive, fast and non-destructive tests, which can be quantified and automated in order to achieve time- and cost-effective test systems.

The control fish behaved in a natural manner, they were active with well-coordinated movements and they were alert to the slightest disturbance, but in the toxic environment relatively reduced activity was exhibited during early hours of pesticide exposure. The intensity of the behavioural activities of the fish decreased with increasing concentration and duration of exposure. The fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium due to exposure of IMI and CZ. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over their bodies, was more pronounced at higher concentrations, suggesting sensitivity to the agrochemicals (Wu and Chen, 2004; Shwetha and Hosetti, 2009).

After induction of agrochemicals, the intoxicated fishes were seen to be getting aggregated at the corner of the aquarium resting at the bottom and frequently coming to the surface followed by heavy breathing with stronger opercular movements and loss of equilibrium. Also the over secretion of mucus was observed on the fishes treated to pesticide. Gabriel and his co-workers (2009) reported that the accumulation of mucus in the gills surface of the exposed fish may have contributed immensely to the death of the fish in this study. Lebedeva *et al.,* (1998) reported that external mucus reflects metabolic processes that take place in the fish organs, which may serve also as a criterion of the physiological status of the fish leading to the establishment of specific effects that different factors such as toxicant and the environment produce on it. The accumulation of mucus on the gills reduces respiratory activity which prevents the gill surface from carrying out active gaseous exchange and thereby causing death of the fish (Jones *et al.,* 1974; Davis, 1975; Omitoyin *et al.,* 1999; 2006; Magare and Patil, 2000; David *et al.,* 2002). Besides, the mortality in the exposed fish may have resulted from the distortion of gill architecture by the agrochemical (Lebedeva *et al.,* 1998; Hartl *et al.,* 2001; Kalavathy *et al.* 2001; David *et al.,* 2002, 2003; Obomanu *et al.,* 2007; Srivastava *et al.,* 2007, 2010; Kaoud; 2011). According to Srivastava and coworkers (2010) accumulation of mucus on the gills and distortion of gill architecture a common effect of toxicants on the gills may impair gill functions resulting in an internal toxic environment from the accumulation of nitrogenous wastes in the body leading to death.

On comparative basis this altered behaviour was more conspicuous in *O. mossambicus* than *L. rohita.* In case of IMI, fishes appeared to be excited within few minutes of exposure at higher concentrations however, they were seen to be calm down and gathered at the corner of aquarium. Such behaviour has been observed in fishes treated with other pesticides also (Bradbury and Coats, 1989; Jee et al., 2005; Dobsikova *et al*., 2006; Velisek *et al*., 2009).

A dose dependent decrease in OBF and TBF activities observed for both the fishes exposed to IMI and CZ are shown in fig. III and IV. The behavioural response to agrocheimcals with marked deviation in the rate of OBF and TBF in control group fishes, imputes an adjustment in physical fitness as a result of the stress condition (Edwards and Fusher, 1991, Leight and Van Dolah, 1999; Chindah *et al.,* 2000; Chindah *et al.,* 2001; Ekweozor *et al.,* 2001; Chindah *et al.,* 2004; Bobmanuel *et al.,* 2006; Gibson and Mathias, 2006). The OBF in fish exposed to the agrochemicals was least variable at the 24^{th} and 48^{th} hrs however; it was depressed and

less variable at 48th and 96th hours. This may be due to the gill damage, where the toxicant acts as respiratory poison possibly affecting the gills, impairing respiration and leading to various abnormal behaviour and eventually death (Kane *et. al.,* 2005). The route of entry of the pesticide in toxicity tests is generally through the gill and hence the respiratory process may be adversely affected. Changes in behavioural patterns exhibited by fish were possibly to counteract aquatic hypoxia condition (Kind *et al*., 2002) caused by the agrochemical. When there is impossibility of escape from hypoxic stress, physiological alterations may be evoked to compensate for low oxygen supply (Graham, 1997; Val *et al*., 1998).

Similarly the fishes in control experiment showed limited variation in the TBF, whereas in the treated animals the TBF showed a gradual decrease with increase in time. Steinhausen *et al.,* (2005) and Herskin and Steffensen (1998) have proved the TBF as a predictor of swimming speed and oxygen consumption. The sudden change in behavior may be due to shock, the rise and subsequent decrease in OBF and TBF may be due to fatigue resulting from suppressed metabolic rate which finally result in low oxygen demand (Jensen *et al.,* 1993 and Fafioye *et al*., 2004). The stressful behaviours of exposed fish such as erratic swimming reflected increased OBF and TBF, regular visit to the surface to gulp in air, loss of balance, restlessness and finally death of fish in this study agree with the findings of Shah (2002); Oti, (2002); Chindah *et al.,* (2004); Chukwu and Okpe, (2006); Omitoyin *et al*., (2006).

Thus, on the exposure to both the agrochemicals fresh water fishes *O. mossambicus* and *L. rohita* showed immediate behavioral changes such as surfacing, followed by vigorous and erratic swimming associated with agitation. The present studies also indicate that these abnormal changes in the fish exposed to lethal concentration of IMI and CZ are time dependant. The LC_{50} values were found to decrease constantly with increasing of exposure periods, signifying that even at very low concentration the agrochemical particularly IMI was fatal for the fish compared to CZ. Furthermore, the results also provide evidence that IMI is highly toxic and had a detrimental impact on the behavioral responses of *O. mossambicus* and *L. rohita* and that the freshwater fish *L. rohita* is more susceptible to the agrochemicals compared to *O. mossambicus*. Hence, from the present studies one can conclude that the acute response of the both the agrochemicals demonstrated variation perhaps due to their physiological status and this reflected the change in their behavior.

Chapter II

Haemogram of freshwater fish Tilapia (*Oreochromis mossambicus*) and Rohu (*Labeo rohita*) exposed to repeated doses of Imidacloprid and Curzate

Blood is most important and abundant body fluid. Its composition often reflects the total physiological condition (Venkatesan *et al.,* 2012). Blood of living organisms are very sensitive to changes and are widely used in Ichthyology research, aquaculture research as well as toxicology and biological monitoring (Svoboda *et al*., 2001; Adedeji *et al.,* 2007; Adeyemo, 2008). As blood being the medium of intercellular and intracellular transport, which comes in direct contact with various organ and tissues of the body, the physiological state of an animal at a particular time is reflected in its blood. Thus, blood provides an ideal medium for toxicity studies. The blood parameters have been considered as diagnostic indices of pathological condition, findings are important for the assessment of systemic functions and overall health of animals. Furthermore, the findings also helps in diagnosing the structural and functional status of animals exposed to the toxicant (Atamanalp and Yanik, 2003; Talas and Gulhan, 2009; Suvetha *et al.,* 2010). It is important in toxicological research because a haematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound. A thin epithelial membrane separates fish blood from the water and any unfavourable changes in the water body is reflected in the blood (Shahi and Singh, 2011; Kori-Siakpere and Ubogu, 2008).

The use of haematological parameters in assessment of fish physiology was proposed by Hesser (1960), since then haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful conditions such as handling, pollutants, metals, hypoxia, anaestehtics and acclimation (Blaxhall, 1972; Duthie and Tort, 1985; Bakthavathsalam, 1991; Ogbulie and Okpowasili, 1999; Jhosi and Bose, 2003; Hori *et al.,* 2008; Alwan *et al.,* 2009).

Further, fishes are known to be in close relationship with the aqueous environment, hence, the blood will reveal conditions within the body of the fish long before there is any visible manifestation of disease (Musa and Omoregre, 1999; Okechukwu *et al.,* 2007), haematological indices are therefore widely used by fish biologists and researchers the world over (Svoboda *et al.,* 2001; Saxena and Seth, 2002; Atamanalp and Yanik, 2003; Cakmak and Gorgon, 2003; Adhikari *et al.,* 2004; Cazenave *et al.,* 2005; Kori-Siakpere *et al.,* 2007; Greaves, 2007; Ali and Rani, 2009; Sharaf *et al.,* 2010; Nte *et al.,* 2011; Khan *et al.,* 2012).

Consequences of pesticides on hematological factors of a number of fish species have been investigated in several studies: in *Cyprinus carpio* (Gluth and Hanke, 1985; Satyanarayan *et al.,* 2004; Salvo *et al.,* 2008) and *Clarias batrachus* (Banerji and Rajendranath, 1990; Patnaik and Patra, 2006; Kharat and Kothavade, 2012; Summarwar and Verma, 2012) in *Oreochromis mossambicus* (Sampath *et al.,* 1993; Ali and Rani, 2009; Desai and Parikh, 2012), in *Heteropneustes fossilis* (Singh and Srivastava, 1994; Nath and Banerjee, 1996 and Deka and Dutta, 2012), in *Cyprinion wabsoni* (Khattak and Hafeez, 1996) and in *Piaractus mesopotamicus* (Travares *et al.,* 1999; Saxena and Seth, 2002; Carraschi *et al.,* 2012).

Hematocrit, haemoglobin, number of red blood cells and white blood cells as well as haematological indices such as MCV, MCH and MCHC are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Sancho *et al.,* 2000; Barcellos *et al.,* 2003). Erythrocytes and leucocytes being the essential cellular components of fish blood, their concentration is maintained within well-defined limits in different fish species unless the balance between production and elimination is disturbed by pathological process. Generally, the erythrocytes not only pump out sodium and pump in potassium against electrochemical gradient but also reduce methaemoglobin to haemoglobin (Hb) to transport oxygen to the body tissues. Packed cell volume (PCV) or haematocrit (Hct) play an important role as an index of anaemia, hypoproteinemia and leukocytosis (Bell *et al.,* 1972; Houston, 1997; Adam and Agab, 2008; Zaki *et al.,* 2010; Ighwela *et al.,* 2012). White blood corpuscles (WBC) play a major role in defence mechanism (Jurd, 1985; Bebarji and Rajendranth 1990; Golovina, 1996; Hart *et al.,* 1997; Hrubec *et al.,* 2000; Rehulka, 2002a, b; Adedeji *et al.,* 2009).

The response of the leucocytes to the changes in water quality and chemicals is variable (Nussey *et al.,* 1995). The effect of pesticides on WBC is not very consistent as seen in some fishes when exposed to pesticides expressed Lecucocytosis (Santhakumar *et al.,* 1999 and Mgbenka *et al.,* 2003) increased leucocyte count (Oluah and Nwosu, 2003), leucocytopenia (Mcleay, 1975), lymphocytosis accompanied by neutropenia (Krutzmann, 1977; Scott and Rogers, 1981; Oluah and Mgbenka, 2004).

Thus from the foregoing literature survey one can see that the haematological parameters are an important bioindicator. However, limited information is available on the effect of IMI and CZ, particularly with reference to the sub-lethal concentration on the haematological modulation. ***Hence, the present study was undertaken to assess* and *contribute to knowledge on the haematological alterations in fresh water teleost fishes at different concentration of IMI* and *CZ.***

MATERIALS AND METHODS:

Experimetnal design:

Freshwater teleosts, O. mossambicus and L. rohita of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies

Based on the result of the 48 h LC50, 30 tilapia fish were divided in 3 groups, 10 fish for each group:

- Group 1 served as control without any treatment of Agro-chemicals.
- Group 2 were treated with low dose of IMI and CZ (LC 50 / 10).
- Group 3 were treated with high dose of IMI and CZ (LC 50 / 20)

for a period of 21 days. Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests.

Haematological estimation of fish:

Test organism was removed, from each tank for blood analysis. About 4 - 5ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant; properly mixed and stored at -20°C for haematological analysis. The blood was stored in -4°C in deep freezer prior to analysis.

Blood Cell Count:

The red blood corpuscles (RBC) and White blood corpuscles (WBC) were counted using haemocytometer crystalline chamber using "Hayem's" and "Turch's" diluting fluid, respectively.

Haemoglobin Estimation (HB) and Pack Cell Volume (PCV):

They were analyzed in NIHON KOHDEN Automated Hematology Analyzer (Celtics α, Japan).

Mean Cell Haemoglobin Concentration (MCHC):

This refers to the percentage of haemoglobin in 100 ml of red blood cell. This was calculated by dividing the haemoglobin content in g/dL by the PCV % of red blood according to the formulae:

MCHC = HB/PCV*1000 g/dL

Mean Corpuscular Volume (MCV):

The value of the corpuscular volume was calculated from the haematocrit value (PCV %) and the erythrocyte count (106/ μL) using the formula

MCV =PCV*1000/ RBCs fL

Mean Corpuscular Haemoglobin (MCH):

Mean corpuscular Haemoglobin concentration expresses the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from the haemogobin value (HB) and from the erythrocyte count according to the following formulae

MCH = HB/RBCs pg

Leukocyte differential count:

Leukocyte differential count was done using auto-analyzer NIHON KOHDEN (Celtics α, Japan).

Statistical analysis:

Statistical analysis was performed using Graph pad prism 5 software. The data was analyzed using two-way ANOVA test followed by multiple comparison test (Tukey's). Results were presented as mean ± SEM. The level of significance was set as $P<0.05$, $P<0.01$ and $P<0.001$.

Results:

Haematological changes in the selected haematological parameters of the control group and those exposed to IMI and CZ for the period of 21 days to *O. mossambicus* and *L. rohita* have been tabulates (Tables 2.1 and 2.2) and plotted in Fig: 2.1 to 2.3. Significant decrease was observed in RBCs, Hb and PCV values in *O. mossambicus* exposed to IMI and CZ. While, in *L. rohita,* a significant elevated RBCs, Hb and PCV values was observed in a dose dependent manner as compared to control (Table 2.1).

From the study it is obvious that the total number of RBCs in Tilapia (Table I) decreased from 1.95 x 10^6/µL ± 0.04 to 1.59 x 10^6/µL ± 0.03 at low dose and to 0.83 x 10^6/µL ± 0.04 at high dose when exposed to IMI while on the CZ the RBCs decreased to 1.52 x 10^6/µL ± 0.01 at low dose and to 0.938 x 10^6/µL ± 0.014 at high dose. On the contrary in Rohu (Table 2.2) there was an increase in the total number of RBCs from 0.7 x 10^6/µL ± 0.003 to 0.82 x 10^6/µL ± 0.003 at low dose and to 0.91 x 10^6/µL ± 0.004 at high dose when exposed to IMI while on the exposure to CZ the RBCs increased to 0.75 x 10^6/µL ± 0.026 at low dose and to 0.8 x 10^6/µL ± 0.002 at high dose. There were considerable alterations in MCV, MCH and MCHC in both the fishes when exposed to agrochemicals. MCV and MCH increased significantly in both fishes while MCHC decreased in *O. mossambicus* and increased in *L. rohita*. Also the percentage of PCV showed the contradictory response in both species showing a decrease in *O. mossambicus* and increase in *L. rohita.*

The total number of WBC in *O. mossambicus* increased from 10.47 x 10^3/µL ± 0.09 to 12.53 x 10^3/µL ± 0.39 at low dose and to 16.3 x 10^3/µL ± 0.29 at high dose when exposed to IMI while it increased to 13.09 x 10^3/µL ± 0.65 at low dose and to 15.48 x 10^3/µL ± 0.21 at high dose. Similarly in *L. rohita* it increased from 62.300 x 10^3/µL ± 0.564 to 68.90 x 10^3/µL ± 0.64 at low dose and to 75.60 x 10^3/µL ± 0.74 at high dose when exposed to IMI while it increased to 64.30 x 10^3/µL ± 0.61 at low dose and to 67.9 x 10^3/µL ± 0.689 at high dose when exposed to CZ. Also the percentage of small lymphocyte and neutrophils increased reaching maximum percentage of (128% and 95%) respectively.

Table 2.1: Haemogram of *O. mossambicus* subjected to sub-acute concentrations of IMI and CZ

Oreochromis mossambicus					
Parameters	**Control**	**IMI**		**CZ**	
		LD	**HD**	**LD**	**HD**
RBCs 10^6 /µL	1.95 ± 0.04	1.59 ± 0.03***	0.83 ± 0.04***	1.52 ± 0.01***	0.938 ± 0.014***
HB g/dL	7.34 ± 0.19	4.41 ± 0.178***	3.31 ± 0.16***	5.92 ± 0.11***	4.457 ± 0.287***
PCV (Htc) %	23.3 ± 0.21	21.49 ± 0.3***	15.5 ± 0.37***	20.47± 0.069***	16.04± 0.505***
MCV fL	137.2 ± 0.42	149.18 ± 0.30***	164.6 ± 0.40***	148.2± 0.881***	170.2± 2.557***
MCHC g/dL	29.53 ± 0.29	30.43 ± 0.16***	28.8 ± 0.17**	30.27± 0.08	29.37± 0.074***
MCH pg	43.43 ± 0.46	45.41 ± 0.26	52.3 ± 0.19***	45.67± 0.346***	51.15± 1.011***
Total WBC 10^3/µL	10.47 ± 0.09	12.53 ± 0.39*	16.3 ± 0.29***	13.09 ± 0.65	15.48 ± 0.21***
Small Lymphocytes %	65.49 ± 0.18	66.34 ± 0.16*	67.4 ± 0.21***	67.42 ± 0.14***	68.88 ± 0.17***
Large Lymphocytes %	10.17 ± 0.20	9.69 ± 0.22	7.79 ± 0.30***	10.20 ± 0.15**	7.798 ±0.17***
Neutrophils %	17.85 ± 0.18	20.85 ± 0.32***	21.9 ± 0.31***	19.01 ± 0.12	21.46 ± 0.15***

- Values are vary significantly between treatment groups.
- * indicates P<0.05
- ** indicate P<0.01
- *** indicate P<0.01
- Each value represents the mean ± SEM of six separate experiments.

Table 2.2: Haemogram of *L. rohita* subjected to sub-acute concentrations of IMI and CZ

Parameters	*Labeo rohita*				
	Control	IMI		CZ	
		LD	HD	LD	HD
RBCs 10^6 /µL	0.7 ± 0.003	0.82 ± 0.003**	0.91 ± 0.004**	0.75 ± 0.026	0.8 ± 0.002*
HB g/dL	3.5 ± 0.029	5.1 ± 0.042***	6.2 ± 0.041***	4.1 ± 0.037**	4.8 ± 0.034***
PCV (Htc) %	9.8 ± 0.038	12.2 ± 0.054***	15.5 ± 0.058***	10.6 ± 0.042	11.9 ± 0.050***
MCV fL	139.9 ± 1.34	144.6 ± 1.12*	149.9 ± 1.30***	141.3 ± 0.800	143.5 ± 0.90
MCHC g/dL	50,00 ± 0.056	63.44 ± 0.09***	75.4 ± 0.08***	54.6 ± 0.084***	61.5 ± 0.071***
MCH pg	35.7 ± 0.69	42.68 ± 0.70***	49.3 ± 0.79***	38.68 ± 0.58*	41.3 ± 0.765***
Total WBC 10^3/µL	62.3 ± 0.564	68.90 ± 0.64***	75.60 ± 0.74***	64.30 ± 0.61*	67.9 ± 0.689***
Small Lymphocytes %	102.4 ± 0.11	131.2 ± 0.12***	156.9 ± 0.15***	113.8 ± 0.13***	128.3 ± 0.13***
Large Lymphocytes %	62.2 ± 0.030	49.27 ± 0.03***	41.28 ± 0.02***	52.2 ± 0.036***	46.9 ± 0.027***
Neutrophils %	79.1 ± 0.365	91.23 ± 0.32***	102.2 ± 0.50***	79.1 ± 0.365***	87.3 ± 0.414***

- Values are vary significantly between treatment groups.
- * indicates P<0.05
- ** indicate P<0.01
- *** indicate P<0.01
- Each value represents the mean ± SEM of six separate experiments.

Figure 2.1: RBCs, Hb and PCV values of *O. mossambicus* and *L. rohita* subjected to subacute doses of IMI and CZ (T=Tilapia and R=Rohu)

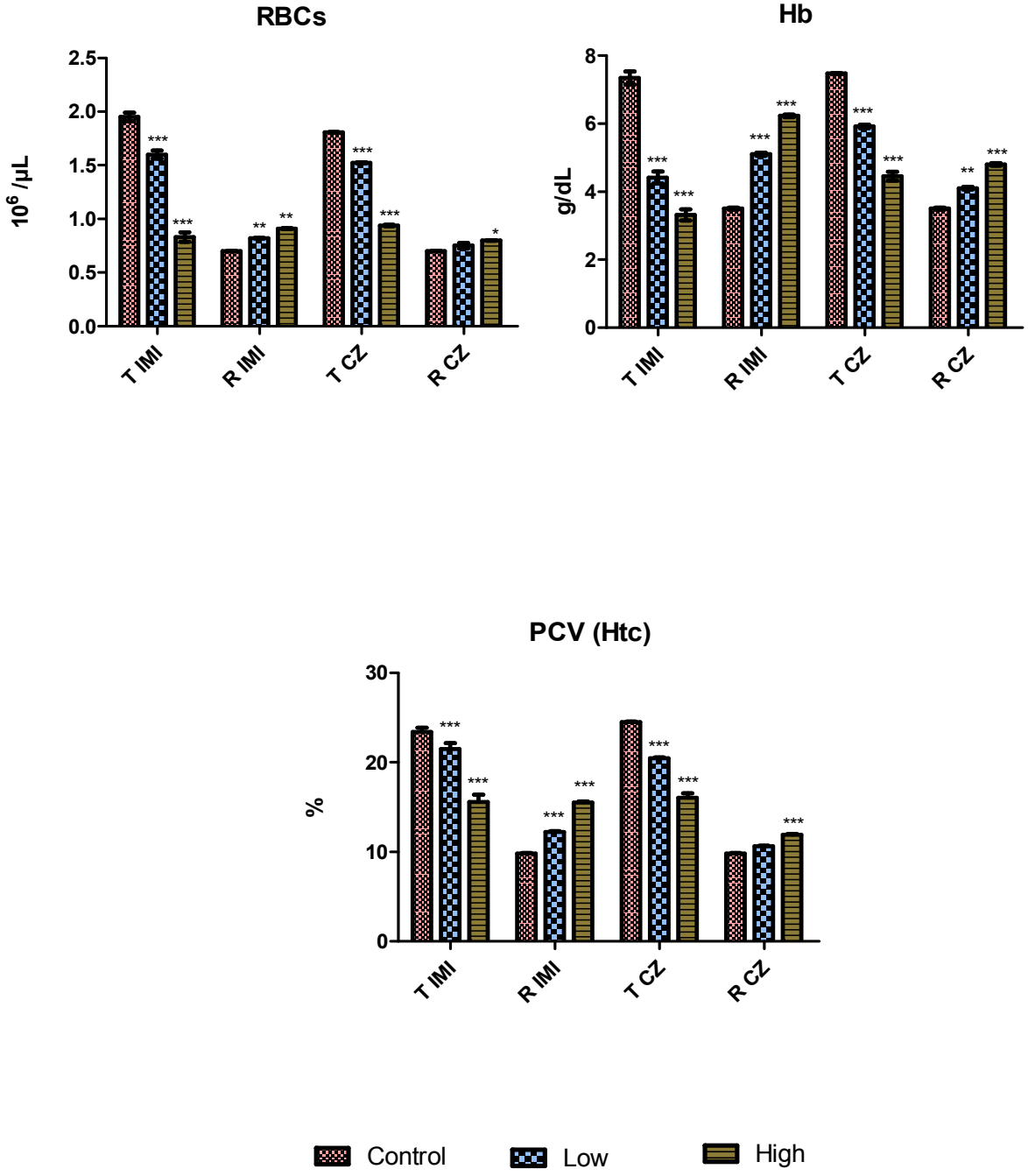


Figure 2.2: MCV, MCH and MCHC values of *O. mossambicus* and *L. rohita* subjected to subacute doses of IMI and CZ (T=Tilapia and R=Rohu)

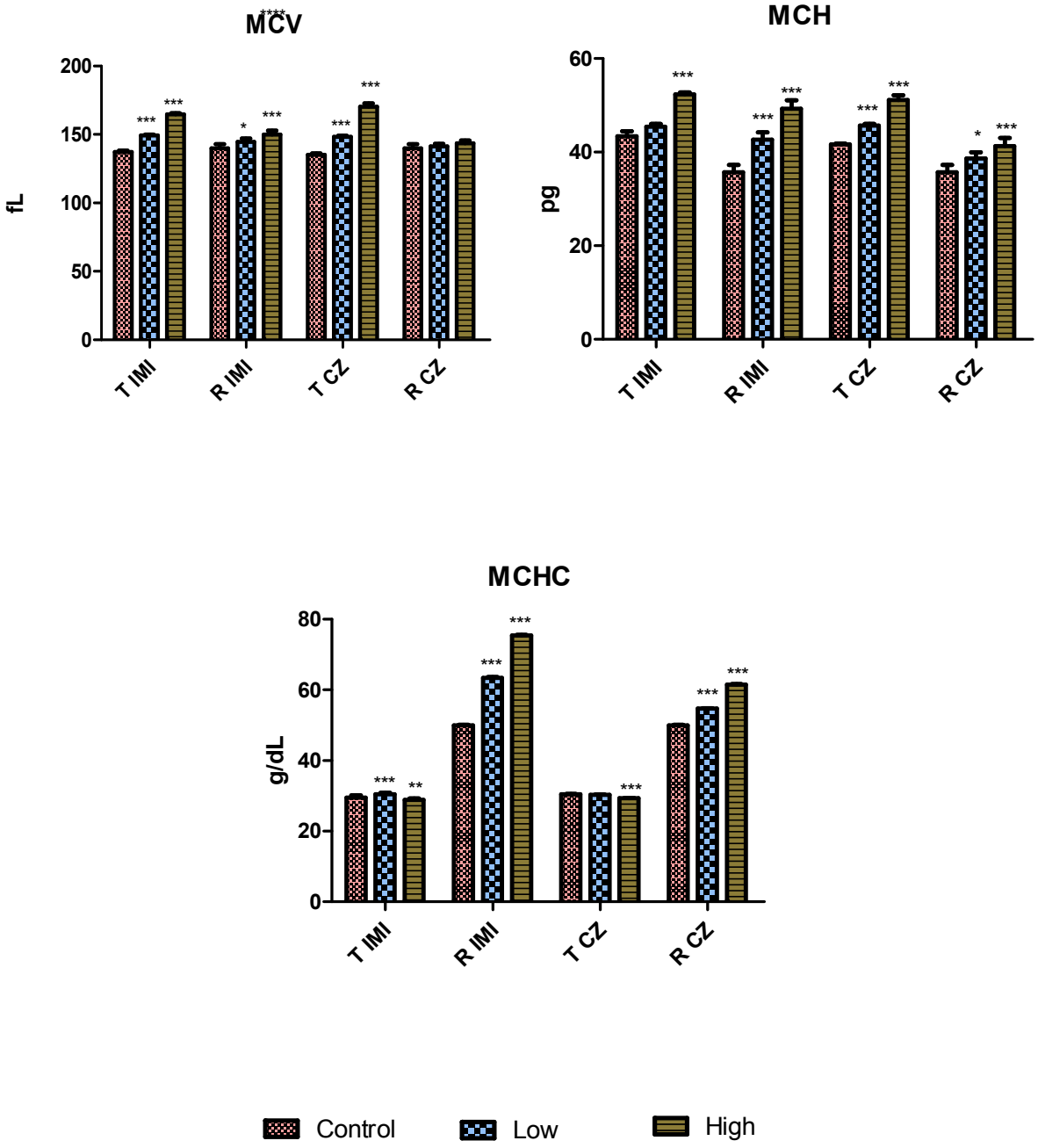
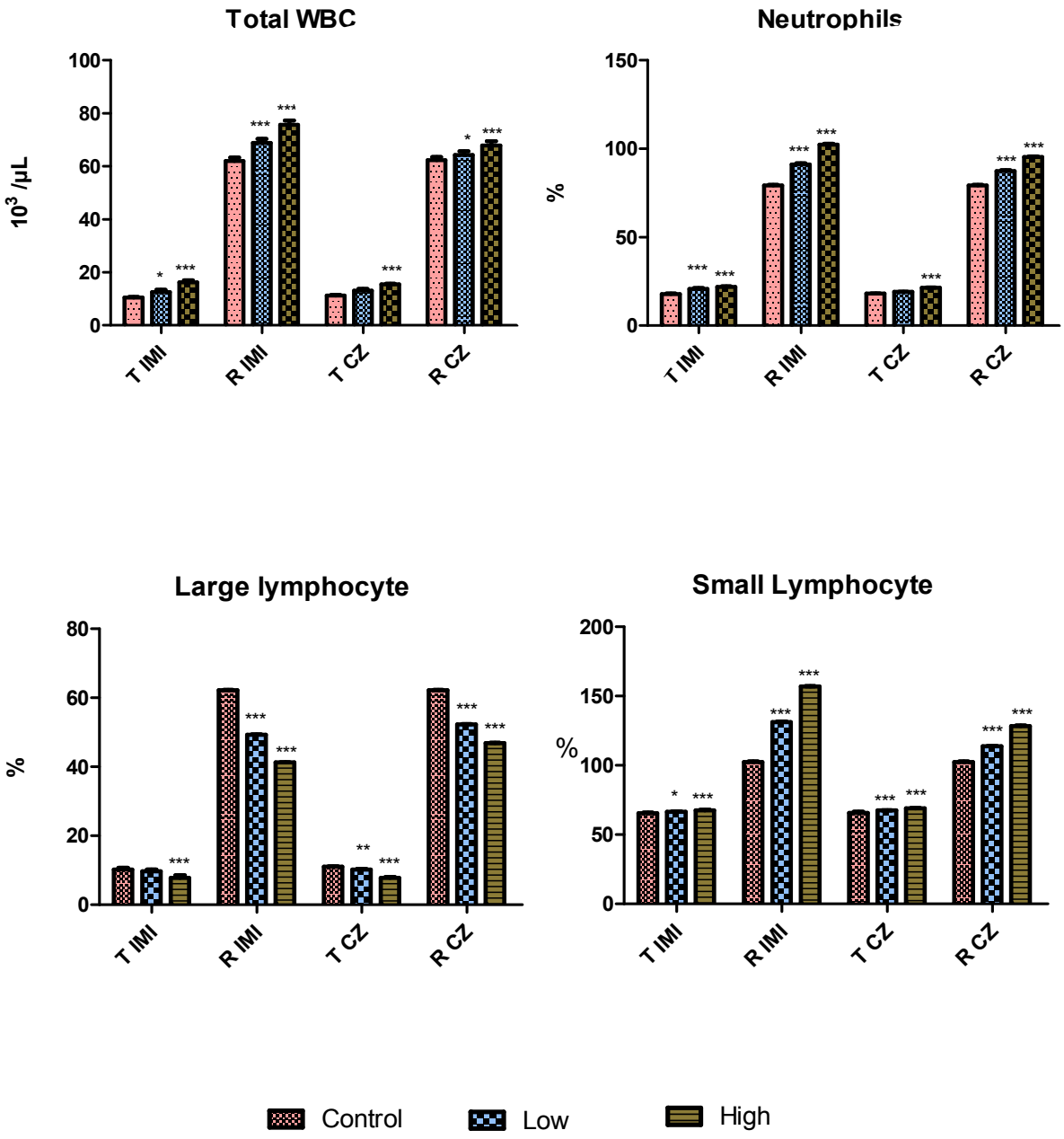


Figure 2.3: Total WBCs count and Differential count of *O. mossambicus* and *L. rohita* subjected to subacute doses of IMI and CZ (T=Tilapia and R=Rohu)



Discussion:

Alteration of haematological parameters of fish has been associated with their physiological state and may be induced directly by pesticides. Clinical chemistry analyses are faster and cheaper than analytical chemistry. Since the variations of these parameters have been demonstrated to be sensitive to sub-lethal concentration of different toxic agents, they can be used for detecting pollutants exposure in the environment (National Research Council, 1989; Parma *et al*., 2007)

Haematological parameters are reported to be affected by a range of factors which includes, size, age, physiological status, environmental conditions and species (Ighwela *et al.,* 2012).

In the present study the species specific differences in haematological indices were evident. As far as values of RBC, Hb and PCV are concerned *O. Mossambicus* showed a significant decrease and *L. rohita* showed a significant increase in proportion to concentration of the pesticide exposure compared to control (Table 2.1 and 2.2; Fig 2.1). Such species-specific differences are common among fish and hence, our results are parallel with the earlier reported species specific changes in the heamatoogical parameters of the fish. (Ainsworth, 1992; Hine, 1992; Suzuki and Iida, 1992; Erickson *et al.,* 1992; Anderson and Zeeman. 1995; Adedeji *et al.,* 2000; Orun *et al*., 2003; van Ginneken *et al.,* 2005; Reavill and Roberts, 2007; Clauss *et al.,* 2008; Velisek *et al.,* 2009 a and b; Adedeji and Adegbile, 2011).

The low levels of Hb indicated anaemic conditions in fish due to stress-caused hemolysis (Panigrahi and Mishra, 1978; Wahbi, 1992) and inhibition of aerobic glycolysis curtailing denovo synthesis of hemoglobin (Lewis, 1970; Koundinya and Ramamurthi, 1979; Bielinska, 1987; Wahbi, 1998; Desai and Parikh, 2012). Furthermore, the lower hemoglobin levels of treated fish in the present study might be due to the distruption of the iron synthesizing (Beena and Vishwarajan, 1987; El-Ezaby, 1994; Sastry and Sachdeva, 1994; Nounou *et al.,* 1997). The reduction in haemoglobin content in fish exposed to toxicant could also be due to the inhibitory effect of the toxic substance on the enzyme system responsible for synthesis of haemoglobin (Pamila *et al.,* 1991; Singh *et al.,* 2010). IMI and CZ exposure to *O. mossambicus* led to a significant decrease in Hb thereby suggesting proposes that the fish was under stress of anaemia. Moreover, the reduction in haemoglobin and haematocrit content might have

resulted from anaemia, hemoglobinisation or shrinkage of red blood cells due to toxic action of agrochemicals on the erythropoietic tissue. A decrease in RBC, Hb content and PCV has been observed earlier in fishes exposed to different pesticides (Svobodova *et al.,* 1997; Park *et al*., 2004; Kori-Siakpere and Oghoghene, 2008; Palanisamy *et al*., 2011; Saravanan *et al.,* 2011).

The observed significant reduction in the value of packed cell volume (PCV) on exposure of IMI and CZ on *O.* mossambicus is probably due to increased rate of erythropoieisis as well as haemolysis due to anaemia or hemodilution (Srivastava and Singh, 1979; Wedemeyer and Mcleay, 1984; Dorucu and Girgin, 2001; Adhikari and Sarkar, 2004; Ramesh and Sarvanan, 2008; Padma Priya *et al*., 2012).

Red blood cell mass as measured by packed cell volume (PCV) and haemoglobin content (Hb) of effluent exposed fish groups showed a progressive fall parallel to increased pesticide concentration. Decrease in the RBC to haemolytic crisis that results in severe anaemia in fish exposed to pollutant or due to reduction of haem synthesis affected by pollutant has been reported earlier (Wintrobe, 1978; Khangarot and Tripathi 1991; Chen *et al.,* 2004; Zaki *et al*., 2008). In the present study the observed decrease in RBCs, Hb and PCV in *O. mossambicus* indicates that pesticide exposed fish are anaemic. This results are in affirmative agreement with the finding of Sastry and Sachdeva, (1994); Nounou *et al*. (1997); Ezzat, (1998); Das and Mukherjee, (2000); Wahbi, (2004); Devi and Benerjee, (2007); Singh *et al.,* (2008).

Hematological parameters in fish can significantly change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. Some of these changes may be the result of the activation of protective mechanisms (Cazenave *et al.,* 2005) such as the results of the blood parameters observed in the present work. *L. rohita* exposed to sub-lethal concentration of the IMI and CZ showed an increase in PCV, Hb and in the number of erythrocytes, indicating the release of erythrocytes from blood deposits and/or from hemopoetic tissues into the blood stream (Svobodova *et al*., 1994).

The present findings are just opposite to the alterations observed in *O. mossambicus*. This difference in response may be explained by the fact that the effects of environmental toxicants on hematological characteristics of fish vary according to the target species (Glusczak *et al*., 2006; Elahee and Bhagwant, 2007). The significant

increase value of Hb could well have been to elevate the oxygen capacity of the blood in order to supply more oxygen to the tissues. This is, therefore, a mechanism by which the body attempts to absorb more oxygen from the surrounding medium to meet the increased oxygen demand (Cyriac *et al*., 1989). Increase in Hb, PCV and RBCs is suggestive of a strategy used by the fish to increased the ability of oxygen transportation in the blood during periods of metabolic break down (Montero *et al*., 1999; Gbore *et al*., 2006; Grigoras, 2008).

Along with the increase in Hb there was a significant increase in PCV as well as in the number of erythrocytes in *L. rohita* exposed to IMI and CZ, indicates the release of erythrocytes from blood deposits and/or from hemopoetic tissues into the blood stream (Svoboda *et al.,* 2001).

The PCV readings are valuable in determining the effect of stressors on the health of fish and are also used to determine the oxygen carrying capacity of blood (Larsson *et al.,* 1985). The recorded significant increase in PCV and MCHC *L. rohita* exposed to IMI and CZ may be attributed to swelling of RBCs due to increase CO_2 in blood or stressful condition. Zaki *et al.,* (2010) in their study on the impact of phenol on haematological profile have reported an increase in RBC, Hb and PCV and have opined that the conditions are similar to that of polycythemia. In the present study the elevated PCV, Hb and MCHC points to the fact of possibly expressing the similar condition. Similar finding were reported by Mckim *et al.,* (1970) and Hilmy *et al.,* (1979).

Pronounced increase in RBC count, points to the fact the fish has developed an oxygen deficiency. This decrease causes the build-up of oxygen debt, hypoxia, in the fish. As a result of the increased anaerobic respiration, the fish is subjected to a situation, to have a higher carbon dioxide concentration in their blood. During anaerobic respiration, lactic acid is produced and this, as well as the buffer action of the excess carbon dioxide, causes a rise in the acidity of the blood. This increase in acidity causes swelling of the red blood cells, as reflected by the significant increase in the mean corpuscular volume (MCV) (Soivio *et al.,* 1974). In the present study also a significant increase in MCV was well defined (Table 2.1 and 2.1; Fig 2.2). An increase of erythrocyte size (MCV) has been associated with several factors such as anaesthesia and hypoxia, but it is generally considered as a response to stress (Valicre and Stickney, 1972; Weber, 1982).

Various workers have demonstrated that rising total erythrocyte count and Hb content might be due to the reason that fish are compensating for impaired oxygen uptake by release of erythrocytes from the spleen (Yamamoto and Itazawa, 1983 and 1985; Lal *et al.,* 1986; Mustafa and Murad, 1984; Svobodova *et al.,* 1994; Tort *et al*., 2002). Another possible reason for the increased RBCs could be due to the release of large number of erythrocytes as a part of compensatory effect to minimize a threatening tissue hypoxia which improves the oxygen-carrying capacity of the blood (Erslev, 1977). The hypoxia may have resulted from hyper excitability and hyper mobility which, was a distinct behavioural observation (Chapter I), which could not have been sustained by normal supply of oxygen to the cells. Interference of pesticide with oxygen uptake by the cells cannot be ruled out but merits investigation before anything is said about this aspect with certainty.

The erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) seems to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with values of RBC count, hemoglobin concentration and packed cell volume. The values of erythrocyte indices were altered in *O. mossambicus* and *L. rohita* fishes after the exposure of sub-lethal concentrations of IMI and CZ (Table 2.1 ans 2.2; Fig 2.2).

In *O. mossambicus* there was significant decreased in PCV associated with significant increase in MCV and MCH. Our results are parallel with the experiments performed by Nte *et al.,* (2011) on fish hematology and have correlated the increase in MCV and MCH with decreased in PCV. The decrease in PCV indicates hypoxic condition of the fish due to anaemia on exposure of the pesticide. Furthermore, the observed low concentration of MCHC during the present work might have resulted from decrease in Hb synthesis consequent of effluent toxicity (Joshi *et al*., 2002; Shah, 2002; Parma *et al.,* 2007; Adam and Agab, 2008; Rao, 2010; Ada *et al.,* 2011; Desai and Parikh, 2012; Venkatesan *et al.,* 2012).

Another type of haematological response to the effect of IMI and CZ in *L. rohita* was a significant increment of MCV, MCH and MCHC associated with increment of PCV and Hb value (Table 2.1 and 2.2; Fig 2.2). PCV could be used to detect haemolysis and is used as a tool for checking anaemic condition in fishes and increase in PCV increased

Hb concentration in the blood of the fish. Oluah and Mgbenka, (2004) indicates that oxygen is carried in combination with haemoglobin and this is very important for the survival of the fish. The range of MCHC, MCV and MCH obtained in this study were similar to findings of Terry *et al.,* (2000); Nilza *et al.,* (2003); Gabriel *et al.,* (2004).

In the present study, elevated total leukocyte count was observed in both the fishes exposed to sub-lethal concentration of IMI and CZ relative to control. The total leukocyte count (WBCs) of the pesticide exposed and control fish can be seen in (Table 2.1 and 2.2; Fig 2.3).

With respect to the leukocyte count there was a significant increase in the total count associated with increase small lymphocyte and neutrophills in both the fishes as well as with both the pesticides. The WBCs in fish respond to various stressors including infection and chemical irritants (Svobodova *et al.,* 1994). Thus, altered number of WBCs is a normal reaction o the exposure of the toxicant (Kori-Siakpere *et al.,* 2006). In the present investigation the significant increase in WBCs count may have resulted from the excitation of defence mechanism of the fish to counter the effect of pesticide (Gabriel *et al.,* 2009). The increase in number of leucocytes is a defensive reaction against pesticide stress. These alterations are probably the result of the activation of the immune system in the presence of pesticide, which in turn may be an adaptive response of the fish resulting in a more effective immune defence (Barreto *et al*., 2005; Modesto and Martinez, 2010). Similar kind of actin have previously considered by other researchers for some pesticides such as, Diazinon (Svobodova *et al.,* 2003 and Padash-Barmchi *et al.,* 2010), Dichlorovos (Banerji and Rajendranath, 1990), Choloropyriphos (Aniladevi, 2008), Malathion (Khattak and Hafeez, 1996), Paraquet (Safahieh *et al.,* 2012) and Curzate (Desai and Parikh, 2012).

Thus from the present study it can be concluded that the exposure of fish to IMI and CZ pesticides resulted in significant alterations in haematological parameters. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment. And furthermore, the haematological studies provide a rapid and sensitive method for predicting the effects of sub-lethal exposure on general health and well being of fish.

Chapter III

Biochemical alterations on exposure of Imidacloprid and Curzate on fresh water fish *oreochromis mossambicus* and *Labeo rohita.*

The impacts of industrialization and exponentially growing population, contamination of air, water, soil and food have become a threat to the continued existence of the living communities of the ecosystem and may threaten the very survival of human race (Dorey and Thatheyus, 2012). Pesticides are occasionally used indiscriminately in large amount causing environmental pollution; therefore they are of great concern. Environment pollution by agro-chemicals has become one of the most important problems in the world (Chandrana *et al*., 2005). The pesticide contamination of aquatic system has attracted the attention of researcher all over the world (Dutta and Dalal, 2008) and has increased in the last decades due to extensive use of them in agriculture. Fishes are more frequently exposed to these pesticides because it is believed that regardless of where the pollution occurs, it eventually end up in the aquatic environment (Firat *et al*., 2011).

The insecticide IMI has been increasingly used since 1991 (Elbert *et al*., 1991) and belongs to the fastest growing group of insecticides introduced to the market, referred to as neonicotinoids (Tomizawa and Casida, 2003). Neonicotinoids – from zero to hero in insecticides has been proposed by Jeschke and Nauen (2008). It acts as an agonist of the postsynaptic nicotinic acetylcholine receptors (Matsuda *et al*., 2001), disrupting the normal neural processes and is used mainly to control sucking insects in crops (Tomlin, 1997; Tomizawa and Casida, 2005). IMI is a potential groundwater and surface water contaminant (PAN pesticides database, 2006), because it can leach and runoff from soil and crops (Felsot *et al*., 1998; Gonzalez-Pradas *et al*., 1999; Armbrust and Peeler, 2002; Gupta *et al*., 2002; Fossen, 2006). Additionally, it may enter water bodies from spray drift or accidental spills, leading to local point-source contaminations. IMI is considered a possible replacement for urban uses of diazinon (TDC Environmental, 2003), one of the most used insecticides in the last 50 years. Diazinon is currently

subject to phased revocation in USA (US EPA, 2004), European Union and Australia (APVMA, 2003), because unacceptable risk to agricultural workers and environment.

To regulate the impact of IMI on aquatic ecosystems, its toxicological profile needs to be thoroughly established. Until now, the toxicity of IMI to aquatic invertebrates has been assessed but very few monitoring studies of this insecticide have been performed in aquatic environments. This is due to the former belief that the compound is relatively immobile in soil and does not leach to ground water (APVMA, 2003; Krohn and Hellpointner, 2002). However, experimental evidence has now proved that there is a potential for IMI to enter streams and ponds via drift during application or in runoff water (Baskaran *et al*., 1999; Moza *et al*., 1998; Sarkar *et al*., 2006; Zheng and Liu, 1999; Nemeth-Konda, 2002; Capowiez *et al*., 2003).

Toxicological studies on rats and mice and dogs have proved IMI to be moderately toxic (Tomlin, 1997; Paul *et al*., 2004; Jain and Punia, 2006). Response to IMI toxicity in birds has been well explored and has shown varied behavioural and pathological changes in birds (Omiama, 2004; Aulakh *et al.,* 2005; Siddiqui *et al.,* 2007; Kammon *et al.,* 2010; Balani *et al.,* 2011). Micronucleus test and comet assay performed by Feng *et al.,* (2004) and by Li-tao and his co-workers (2006) for assessing the risks of novel pesticide IMI on amphibians, have proved that IMI is Genotoxic to tadpoles and frogs. IMI has been proved to be moderately toxic to fish (Kidd and James, 1991; Tomlin, 1997). Toxic responses of IMI has been studied by Rajput *et al.,* (2012) on fresh water fish, C*larias batrachus* and have reported the adverse effect of these toxicant on the protein profile of the fish. IMI has also been found to have profound influence in serum biochemical profile of fresh water fish *Channa punctatus* (Padma priya et al., 2012).

CZ a mixture of Cymoxanil and mancozeb, has got systemic action and is moderately toxic. Because of its major metabolite ethylenethiourea (ETU), recently it has come under close scrutiny of health protection agencies. ETU has carcinogenic, teratogenic and goitrogenic effects in mammals (Roy *et al.,* 2010; Ulland *et al.,* 1972; Keppel, 1971). Cymoxanil acts as a foliar fungicide with protective and curative action. It has contact and local systemic activity (FAO, 2005). Cymoxanil provides effective control of economically important fungal plant pathogens, which cause downy mildew and

blight in a wide range of crops. It is considered to be moderate toxic to mammals (Sarver, 1992; Panepinto, 1992; Tompkins, 1994; Hurtt, 1995). The chronic toxicity elicited by this compound is highly variable, since it depends on the species ranges of tested concentrations and the exposure period. Experimental evidence has proved the signs of intoxications in rats and mice bodyweight and organ alterations, reduction in food consumption, testicular perturbation and histopathological variations (Lages *et al*., 2009). Cymoxanil is toxic to aquatic organisms (Kreamer, 1996; Boeril *et al*., 1997; Derbalah *et al.,* 2008; Rekanovie *et al.,* 2012). At high partition coefficient it has been found to get bioaccumulated moderately by living organisms including humans (Soares and Calow, 1993; Grandjean *et al*., 1999). Mancozeb, despite its low acute toxicity, it has been shown to have significant toxic effects in mice (Mehrotra *et al*., 1990; Kackar *et al*., 1997; Baligar and Kaliwal, 2001; Calviello *et al*., 2006). Mancozeb exposure is associated with pathomorphological changes in liver, brain and kidney in rats (Bindali and Kaliwal, 2002; Joshi *et al*., 2005). Mancozeb is moderately to highly toxic to zebra fish (Cocco, 2002); rainbow trout (Bisson and Hontela, 2002; Atmanalp and Yanik, 2003; Ekinci and Beydemir, 2010).

The utility of biochemical approaches in environmental pollution monitoring and characterization of effect/exposure to stressor for the use in environmental risk assessment is based on the assumption that low concentrations of a toxicant will cause biochemical responses within individual organisms before these effects are observed at higher levels of biological organization (Sarkar *et al*., 2006). Such biochemical responses are considered to be rapidly responding endpoints (Adams, 2002), and thus most biochemical biomarkers in the laboratory studies are assessed after acute exposure to chemicals. Changes in the biochemical profile indicate alterations in metabolism of the organism resulting from the effect of the pesticide and they make it possible to study the mechanisms of the effects of these pesticides (Luskova *et al.,* 2002). **In the view of paucity of information available on IMI and CZ toxicity, the present work was under taken on fresh water teleosts, *O.mossambicus* and *L.rohita*, so as to have an insight regarding its biochemical alterations.**

Materials and Method:

Experimetnal design:

Freshwater teleosts, *O. mossambicus* and *L. rohita* of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5 - 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies

Based on the result of the 48 h LC_{50}, 30 tilapia fish were divided in 3 groups, 10 fish for each group:

- Group 1 served as control without any treatment of Agro-chemicals.
- Group 2 were treated with low dose of IMI and CZ (LC 50 / 10).
- Group 3 were treated with high dose of IMI and CZ (LC 50 / 20) for a period of 21 days.

Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests.

Preparation of the tissue samples for the study.

At the end of the experiment (21 days) the fish were carefully netted to minimize stress, and weighed. Prior to sacrificing the fish, about 1 - 2ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes. The blood was stored in -4°C in deep freezer prior to analysis. Fishes were sacrificed by pithing (damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle). Tissues such as liver, kidney, gills and muscle were carefully removed, wiped

thoroughly, using blotting paper to remove blood and other body fluids. Then they were washed in chilled PBS and again blotted dry. After noting the total weight of the tissues, the desired amount of the tissues were weighed and used.

Parameters investigated:

Assay of Glucose-6-phosphatase (EC. 3.1.3.9)

Glucose-6-phosphatase was ssayed according to the method of King (1965b)

Reagents

a. Citrate Buffer: 0.1 M, pH 6.5

b. Substrate: Glucose-6-phosphate, 0.1 M in distilled water.

c. Ammonium molybdate reagent: added 25 g of ammonium molybdate to 200 ml distilled water. To 300 ml 10 N H_2SO_4, added molybdate solution and diluted to 1 litre with distilled water.

d. Amino naphthol sulphonic acid (ANSA): Ground 0.2 g of ANSA with 1.2 g of Na_2SO_3 and 1.2 g of sodium bisulphite ($NaHSO_3$). Kept the mixture in the freezer. At the time of use, dissolved 0.25 g in 10 ml distilled water.

e. TCA: 10%

f. phosphate standard: 35.1 mg of KH_2PO_4 was dissolved in 100 ml double distilled water. Working standard was prepared by taking 1ml of the stock and diluted to 10ml with distilled water.

Procedure:

10% homogenate of liver tissue was prepared in 0.33 M sucrose solution and centrifuged at 11.000 g for 30 minutes in a refrigerated centrifuge. The supernatant obtained was again centrifuged for 60 minutes at 10,500 g and the supernatant was discarded. The pellet was suspended in ice-cold 0.33 M sucrose solution and homogenized in a glass-Teflon homogenizer. The homogenate obtained was used as the enzyme source. The incubation mixture in a total volume of 1 ml contained 0.3 ml of buffer, 0.5 ml of substrate and 0.2 ml of enzyme preparation. The incubation was carried out at 37°C for 60 minutes. Arrested the reaction by the addition of 1 ml of 10% TCA and centrifuged. The phosphorus content of the supernatant was estimated by the

method of Fiske and Subbarow (1925). The enzyme activity was expressed by as µg of inorganic phosphorus liberated / min / mg protein.

Estimation of Blood Glucose

Blood Glucose was estimated by the method of Sasaki and Matsubs, (1972).

Reagent:

a. Ortho toluidine boric acid reagent: this reagent consists of 2.5 g of thiourea and 2.4 g of boric acid in 100 mi solvent, consisting of a mixture of water, acetic acid (AR) and ortho toluidine in the ration of 10:75:15.

b. Standard glucose: 1oo mg of glucose was dissolved in 0.1 % benzoic acid. 10 ml of the above solution was diluted to 100 ml to give 100 µg of glucose per ml.

Procedure:

To 0.2 mi of blood, 0.8 ml of 10% TCA was added. The contents were mixed well. The tubes were centrifuges at 1000 g for 5 minutes. 0.5 ml of supernatant was taken. To this 2.0 ml of ortho toluidine reagent was added. The tubes were then heated in a boiling water bath for 15 minutes. The standards were also treated in the same manner along with the reagent blank. The values were expresses as mg glucose / dl.

Estimation of Total protein

Protein was estimated by the method of Lowry et al., (1951).

Principle:

Protein reacts with folin-phenol reagent to develop a blue colored complex due to reduction of phosphomolybdic and phosotungustic component in folin reagent.This reaction is given by the amino acid tyrosine and tryptophan present in the protein, color develops by biuret reaction, of the proteins with alkaline cupric tartarate. The intensity of the color deepens on the amount of these aromatic amino acid residues present and thus varies for different proteins.

Reagents:

a. Alkaline Copper Reagent.

Reagent A: 2 % Na_2CO_3 in 0.1 N NaOH.

Reagent B: 0.5% $CuSO_4.5H_2O$ in sodium potassium tartrate.

The alkaline copper reagent was made by mixing 50 ml of reagent A and 1 ml of reagent B.

b. Folin – Ciocalteu Phenol Reagent

Folin – Ciocalteu Phenol Reagent is commerciaaly available which is diluted with distilled water in the ratio 1:2.

c. 0.1 N NaOH.

d. 10% TCA.

e. protein (stock) standard solution: 100 mg % Bovine serum albumin in 0.1 N NaOH.

Working standard: 10 ml of the stock was diluted to 100 ml with distilled water.

Procedure:

Pipette out 0.2 ml of tissue homogenate to the test tube and added 1 ml of 10 % TCA. The tubes were centrifuged at 5000 g for 10 minutes. The supernatant was discarded and the precipitate was dissolved in 1 ml of 0.1 N NaOH. Added 5 ml of alkaline copper reagent and kept for 10 minutes at room temperature. After 10 minutes added 0.5 ml Foline-Ciocalteu phenol reagent kept in dark for 30 minutes. The absorbance was read at 620 nm against a reagent blank. A set of graded volumes of protein standard were also run simultaneously. The values are expressed as mg of protein / g wet wt. of tissue.

Estimation of Glycogen

Glycogen was estimated by the method of Seifter et al., (1950)

Principle:-

Glycogen present in tissue is first hydrolyzed to glucose and then is estimated per known weight of tissue using conversion factor 1.11(1 gm glycogen yield 1.11 gm of glucose on hydrolysis). Fresh tissue is digested in hot KOH solution and glycogen precipitation are suspended in water. This suspension is treated with Anthrone reagent prepared in sulfuric acid. Sulfuric acid hydrolyses glycogen to glucose which in turn reduces Anthrone to develop green color. The color intensity is proportional to amount of glucose present in solution. This solution is read at 620 nm (red filter.)

Reagents:

a.30% KOH: 30 gm KOH pellets dissolved and made up to 100 ml in distilled water.

b.95% ethyl alcohol: 95 ml ethyl alcohols made up to volume 100 ml with distilled water.

c. (Freshly prepared) Anthrone reagent (2 mg/ml): prepare in 95% pure sulfuric acid.

d. std. glucose solution:

stock solution: - 30 mg glucose dissolved in 100ml distilled water.

working solution:-1 ml stock solution dissolved and made up to 100 ml distilled water.

Procedure:-

Remove fresh tissue from animal's body and blot it free of blood and body fluids. Take known weight of tissue and add it to test tube containing 2 ml KOH solution. Digest tissue by placing test tube in boiling water bath. Allow test tube to cool and then add 2.5 ml ethyl alcohol. Place test tube in boiling water bath until few bubbles appears in solution. Centrifuged at 3000 RPM for 40 minutes. Discard the supernatant and allow tubes to drain off dissolved precipitation in known quantity of distilled water and note the dilution factors. Label three test tubes as standard, blank and sample. Add 1ml sample solution, 1ml working standard solution and 1ml distilled water in these tubes respectively.Place tubes in ice bath and add 4 ml Anthrone reagent in each tubes. Shake tubes in ice bath and take reading at 620 nm on a spectrophotometer.

Estimation of Lipid

Lipid was estimated by the method of Folch *et al.,* (1957)

Principle:

Lipid content present in tissue is measured gravimetrically by this method

Reagents:

a. chloroform methanol solution: Chloroform methanol mixture is made in 2:1 ratio.

b.1% calcium chloride solution: 1 gm calcium chloride dissolved in 100 ml of distilled water

Procedure:-

Take known amount of tissue and crush it in test tube with the help of sand. Add 5 ml chloroform-methanol mixture (2:1) and 2 ml of 1 % calcium chlorides. Allow the two layers to separate and leave the sample overnight. Next day remove the upper layer and

decant the remaining fluid in another test tube. this is done using watt man filter paper no. 1 to remove tissue debris from this solution take 2 ml in pre weighted lipid tubes and keep it in over at 60 ºc till the tubes dry. After tubes dry weight tube again and note the difference in weight.

Estimation of Cholesterol

Cholesterol was estimated by the method of Crawford *et al.,* (1958)

Principle:

This method depends on interaction of ferric chlorides and sulfuric acid with cholesterol in CH_3COOH solution. The resulting red purple color is measured spectrophotometrically and compared with std. the content of lipid cholesterol in dry lipid sample determines intensity of color but exact reaction is however not known.

Reagents:

a. Ferric chloride stock solution: 2.5 gm ferric chloride dissolved in 50 ml glacial acetic acid.

b. Ferric chloride working solution: 1 ml of stock solution diluted to 50 ml with glacial acetic acid.

c. Standard cholesterol stock solution: Weight exactly 20 mg of cholesterol and make up to 50 ml in volumetric flask with glacial acetic acid. This will serve as stock solution.

d. Working std solution: Take 5 ml of stock and dissolve it to 100 ml with glacial acetic acid to form working std solution.

Procedure:

Take fresh tissue and grind it with sand. Add chloroform: methanol mixture and extract total lipid in given tissue. Dissolved lipid in known quantity f chloroform: methanol mixture. Add 3 ml of ferric chloride colored reagent in sample, standard and blank. Boil it for 5 minutes and cool it in ice-bath. Mix it by swirling slowly. Read after 30 minutes at 540 nm.

Assay of Lactate Dehyrogenase (LDH) (E.C.1.1.1.27)

Lactate Dehydrogenase was assayed according to the method of king (1965a).

Principle:

LDH catalyzes the oxidation of lactate to pyruvate accompanied by the simultaneous reduction of NAD to NADH. LDH activity in serum is proportional to the increase in absorbance due to the reduction of NAD.

Reagents:

a. 0.1 M glycine buffer.

b. Buffered substrate: Dissolved 2.76 g of lithium lactate in 125 ml of glycine buffer containing 75 ml of 0.1 N NaOH to adjust the pH to 10. Prepare this just prior to use.

c. 0.4 N NaOH.

d. Dissolved 5.0 mg of NAD^+ in 1.0 ml of distilled water. Prepared this just before use.

e. 2. 4 – dinitrophenyl hydrazine (DNPH) reagent: dissolved 200 mg of DNPH in 85 ml of concentrated HCl and made up to 1 litre with distilled water and prepared fresh each time.

Procedure:

To 1.0 ml of the buffered substrate, added 0.2 ml of sample and incubated at 37º C for 15 minutes. After adding 0.2 ml of NAD^+ solution, continues the incubation for another 30 minutes and then added 1.0 ml of DNPH reagent. Incubated the mixture for a period of 15 minutes at 37º C. Then added 7.0 ml of 0.4 N NaOH solution and measured the colour developed at 520 nm in a spectrophotometer. Treated the standards also in the same manner along with blank. The enzyme activity was expressed as µ moles of puruvate liberated / h / mg protein.

Estimation of Pyruvate

Pyruvate was estimated by the method of Friedman and Haugen (1943).

Reagents:

a. TCA: 10%

b. 2,4 – Dinitrophenyl hydrazine reagent (DNPH): 0.2%

c. 2.5 N NaOH

d. Pyruvate standard: Dissolved 125 mg of sodium pyruvate in 10 ml of 0.1 N H_2SO_4 and diluted to 100 ml with 0.1 N H_2SO_4.

Procedure:

5 % homogenate of liver and 10% homogenate of gills, kidney and muscle tissues were prepared in 10% TCA and centrifuged at 1000 g for 15 minutes. To 2.0 ml of supernatant, 0.5 ml of 0.1% 2,4 – DNPH reagent was added and the tubes were kept at room temperature for 5 minutes and 3.0 ml of 2.5 N NaOH solution was added. After 10 minutes the absorbance was read at 540 nm in a spectrophotometer against a reagent blank. The blank consisted of 2.0 ml of 10% TCA, 0.5 ml of 0.1% 2,4 – DNPH and 3.0 ml of 2.5 N NaOH solutions. Treated the standards also in the same manner. The values were expressed as μ moles of pyruvate / g wet wt. of tissue.

Assay of Alanine aminotransferase (ALT) (EC 2.6.1.2)

Aspartate aminotrasferase was assayed by the method of Mohun and Cook (1957).

Principle:

In this reaction, L-Alanine and alpha-ketoglutarate react in the presence of GPT in the sample to yield pyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340 nm.The rate of reduction in absorbance is proportional to GPT activity in sample

Reagent:

a. buffered substrate (0.1 M phosphate buffer, pH 7.4; 0.2 M DL – alanine; 2mM 2 – oxoglutarate).

b. 2,4 – Dinitro phenyl hydrazine (DNPH).

c. Standard pyruvate: Dissolved 11.01 mg of sodium pyruvate in 100 ml of distilled water.

e. 0.33 M sucrose.

Procedure:

10 % homogenate fo gills, liver, kidney and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant obtained was used as the enzyme source. Pipette out 1 ml buffered substrate into test and control. Added 0.2 ml of the enzyme source into the test and incubated the tunes at 37^{o}C for 60 minutes.

After incubation, 0.2 ml enzyme was added to the control. 1 ml of 2,4 – DNPH reagent was added and kept at room temperature or 20 minutes. The reaction was stopped by the addition of 10 ml of 0.4 N NaOH, vortexed and kept at room temperature for 5 minutes. The absorbance was measured at 540 nm in a spectrophotometer against a reagent blank. The AST activity were expresses as μ moles of pyruvate liberated / h / mg protein.

Assay of Aspartate aminotransferse (AST) (EC 2.6.1.1)

Alanine aminotranferase was assayed by the method of Mohun and Cook (1957).

Principle:

In this reaction L-Aspartate and Alpha-Ketoglutarate react in the presence of GOT in the sample to yield oxaloacetate and L-glutamate. The Oxaloacetate is reduced by malate dehydrogenase (MDH) to yield L-malate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340 nm. The rate of reduction in absorbance is proportional to GOT activity in sample.

Reagents:

a. Buffered substrate (0.1 M phosphate buffer, pH 7.4; 0.2 M DL – alanine; 2mM 2 – oxoglutarate).

b. 2,4 – Dinitro phenyl hydrazine (DNPH).

c. 0.4 N NaOH.

d. Standard pyruvate: Dissolved 11.01 mg of sodium pyruvate in 100 ml of distilled water.

e. 0.33 M sucrose.

Procedure:

10% homogenate of gills, liver, heart, kidney and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant obtained was used as the enzyme source, pipette out 1 ml buffered substrate into test and control. Added 0.2 ml the enzyme source into the test and incubated the tubes at 37°C for 60 minutes. After incubation, 0.2 ml enzyme was added to the control. 1 ml of 2,4 – DNPH reagent was added and kept at room temperature or 20 minutes. The reaction was stopped by the addition of 10 ml of 0.4 N NaOH, vortexed and kept at room

temperature for 5 minutes. The absorbance was measured at 540 nm in a spectrophotometer against a reagent blank. The AST activity were expresses as μ moles of pyruvate liberated / h / mg protein.

Assay of Alkaline phosphatase (ALP) (EC 3.1.3.1)

Alkaline phosphatase was assayed by the method of king and king (1954).

Principle:

Alkaline Phosphatase in a sample, hydrolyses para-nitrophenyl phosphate into paranitrophenol and phosphate, in the presence of magnesium ions. The rate of increase in absorbance of the reaction mixture at 405nm due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity.

Reagent:

a. Substrate: Disodium phenyl phosphate (10 mmol/L).

b. Buffer: Sodium carbonate – bicarbonate buffer (100 mmol/L).

c. Buffered substrate: Mixed equal volumes of substrate and buffer, this had a pH of 10.

d. Stock phenol standard: 100 mg % in 0.1 N HCl. Working standard: 1 mg %

e. Sodium Hydroxide (NaOH): 0.5 N.

f. Sodium Bicarbonate (NaHCO3): 0.5 N.

g. 4 – Aminoantipyrine: 6 g/L in water.

h. Potassium ferricyanide: 24 g/L in water.

Procedure:

10% homogenate of gills, liver, kidney and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant into test and control, and incubated for a few minutes at 37°C. Then added 50 µl of enzyme source to the control. This was followed by the addition of 1 ml of 4 – aminoantipyrine and 1 ml of potassium ferricyanide to both the tubes. Read the absorbance at 520 nm. Treat the blank and standard also similarly. The values were expressed as mg of phenol liberated / min/ mg protein.

Assay of Glutamate dehydrogenase (GDH) (EC 1.4.1.3)

Glutathione dehydrogenase was assyed by the method of Plummer (1987).

Reagents

a. sodium phosphate buffer (0.1 M, pH 7.4)

b. 2 – oxoglutarate (0.15 m), prepared in phosphate buffer and pH adjusted to 7.4.

c. Ammonium acetate (0.75 M), prepared in phosphate buffer and pH adjusted to 7.4.

d. EDTA (30mM), prepared in phosphate buffer and pH adjusted to 7.4.

e. NADH (2.5 mg/ml in phosphate buffer, prepaed fresh).

f. Triton X-100.

Procedure:

10% homogenate of gills and 5% homogenate of liver, kidney, gills and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant obtained was used as the enzyme source, o.1 ml NADH, 0.2 ml Ammonium acetate, 0.2 ml EDTA and 0.1 ml Triton X-100. The about mixture was equilibrated at room temperature for 10 minutes. Started the reaction by adding 0.1 ml of 2.oxoglutarate and the rate of change of extinction at 340 nm with time were noted. Molar Extinction Coefficient of NADH is 6.3 x 10^3 litres /mol/cm. The enzyme activity was calculated as micromoles of NADH oxidized / minute / mg protein.

Statistical Analysis:

The statistical analysis was carried out using the software Graph pad prism 5 package. For determining the significant difference between different treatments in biochemical parameters, Two-way ANOVA followed by Tukey's test for multiple comparisons between different concentration of IMI and CZ was done. Significance level (P value) was set at 0.05 in all tests.

RESULTS:

PROTEIN:

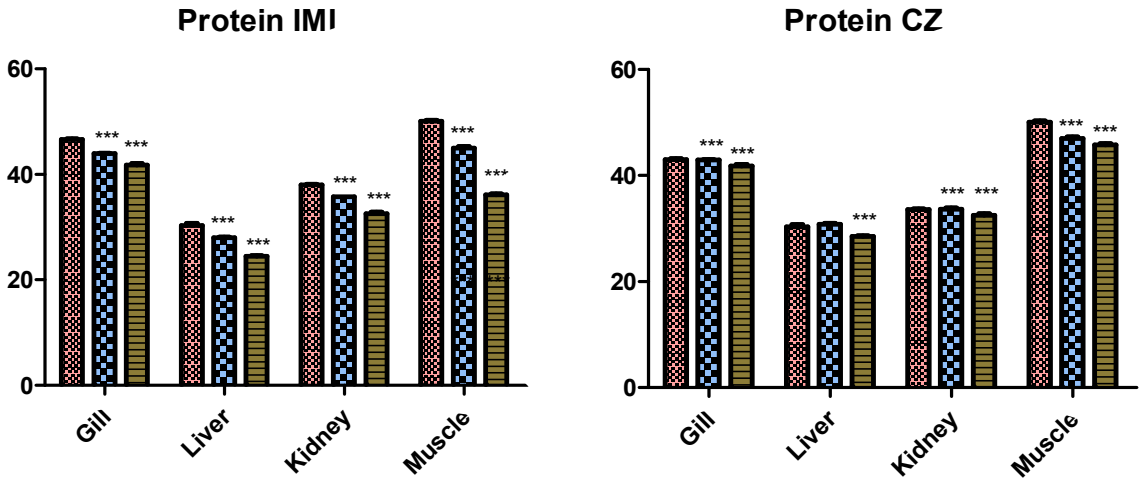
Two-way ANOVA followed by Tukey's test showed that there was significant decrease in protein level in all the tissues of both the fishes exposed to IMI and CZ compared to control (Table 3.1 and Fig 3.1). Among the treated group CZ treated group showed the least values compared to control.

Table: 3.1 Effect of IMI and CZ on total protein level (mean ± SEM) in *O.mossambicus* and *L.rohita*.

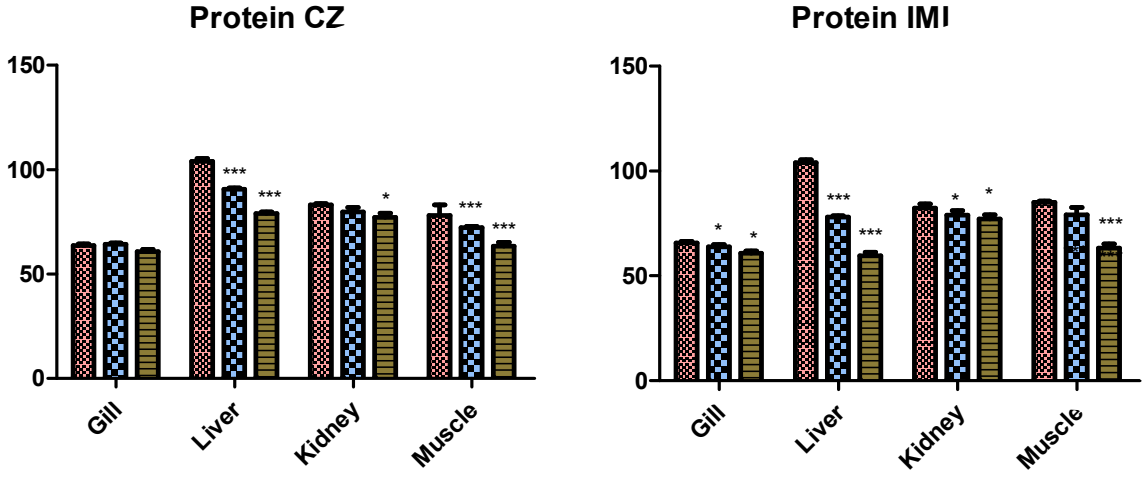
	Total Protein						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	41.76 ±0.29	43.99 ±0.05***	46.61 ±0.16***	41.76 ±0.29	42.91 ±0.14***	42.99 ±0.20***
	Liver	30.32 ±0.38	27.98 ±0.15***	24.49 ±0.12***	30.32 ±0.38	30.84 ±0.1***5	28.54 ±0.10***
	Kidney	32.53 ±0.29	35.76 ±0.01***	38.03 ±0.09***	32.53 ±0.29	33.69 ±0.20***	33.56 ±0.15***
	Muscle	50.08 ±0.20	44.97 ±0.29***	36.12 ±0.20***	50.08 ±0.20	46.99 ±0.29***	45.76 ±0.25***
L.rohita	Gills	60.81 ±0.95	63.89 ±1.04	65.71 ±0.70	60.81 ±0.95	64.32 ±0.60	62.71 ±1.01
	Liver	103.98 ±1.39	78.00 ±0.70***	59.57 ±1.77***	103.98 ±1.39	90.67 ±0.70***	79.00 ±0.72***
	Kidney	77.13 ±1.98	78.98 ±2.08*	82.31 ±2.02*	77.13 ±1.98	79.83 ±2.13	83.24 ±0.58*
	Muscle	63.26 ±1.98	79.00 ±3.69	85.00 ±0.75***	63.26 ±1.98	72.17 ±0.62***	78.00 ±5.17***

- Value are expressed as mg protein / g wet wt of tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.1 Effect of IMI and CZ on total protein level (mean ± SEM) in *O.mossambicus* and *L.rohita*.



O.mossambicus



L.rohita

GLYCOGEN:

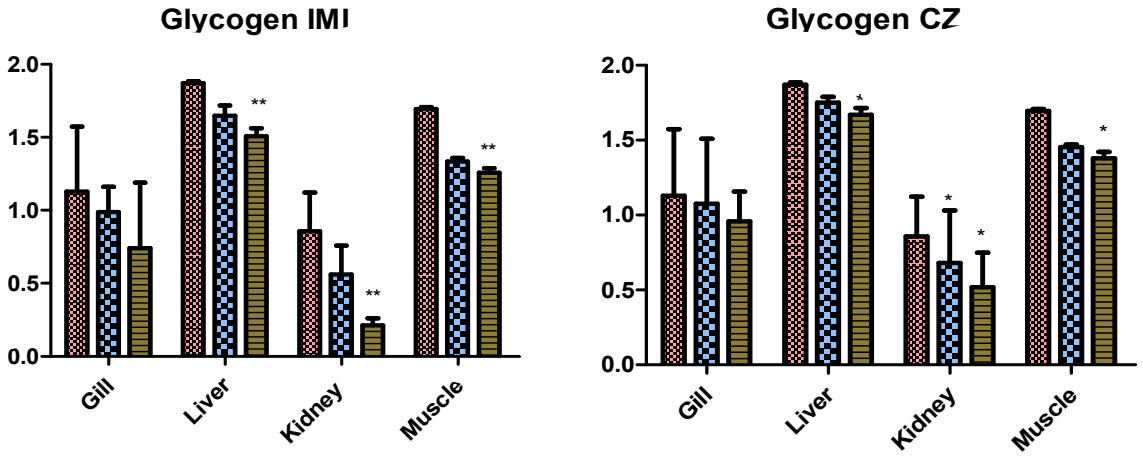
Two-way ANOVA followed by Tukey's test showed that there was significant decrease in glycogen level in liver, muscle and gills tissues of both the fishes exposed to IMI and CZ compared to control (Table 3.2 and Fig 3.2). Whereas, muscle tissue of *L.rohita* did not show any significance difference expose to both IMI and CZ.

Table: 3.2 Effect of IMI and CZ on level of glycogen (mean ± SEM) in *O.mossambicus* and *L.rohita.*

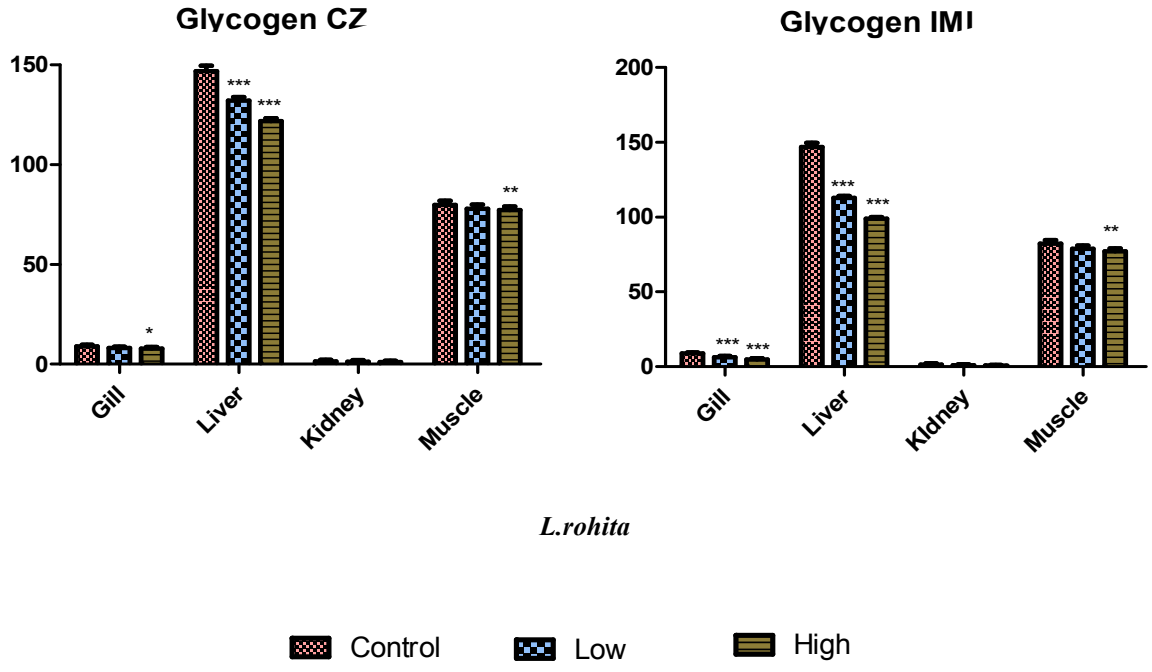
Glycogen							
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	1.130 ±0.443	0.990 ±0.171	0.740 ±0.451	1.130 ±0.443	1.076 ±0.433	0.960 ±0.197
	Liver	1.870 ±0.015	1.648 ±0.070	1.508 ±0.054	1.870 ±0.015	1.750 ±0.040	1.670 ±0.045*
	Kidney	0.858 ±0.264	0.560 ±0.197	0.212 ±0.049	0.858 ±0.264	0.680 ±0.350	0.520 ±0.229
	Muscle	1.694 ±0.014	1.334 ±0.025	1.258 ±0.030	1.694 ±0.014	1.454 ±0.017	1.380 ±0.040
L.rohita	Gills	8.990 ±0.66	6.370 ±0.83	4.81 ±0.88	8.99 ±0.66	8.08 ±0.70	7.78 ±0.75
	Liver	146.99 ±2.54	112.83 ±1.32	98.93 ±1.07	146.99 ±2.54	132.18 ±1.77	121.83 ±1.49
	Kidney	77.13 ±1.98	78.83 ±2.07	82.31 ±2.26	77.13 ±1.98	77.93 ±2.02	79.83 ±2.13
	Muscle	1.380 ±0.85	0.970 ±0.60	0.79 ±0.49	1.38 ±0.85	1.21 ±0.75	1.07 ±0.66

- Values are expressed as mg protein / g wet wt of tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Figure: 3.2 Effect of IMI and CZ on level of glycogen (mean ± SEM) in *O.mossambicus* and *L.rohita.*



O.mossambicus



L.rohita

BLOOD GLUCOSE:

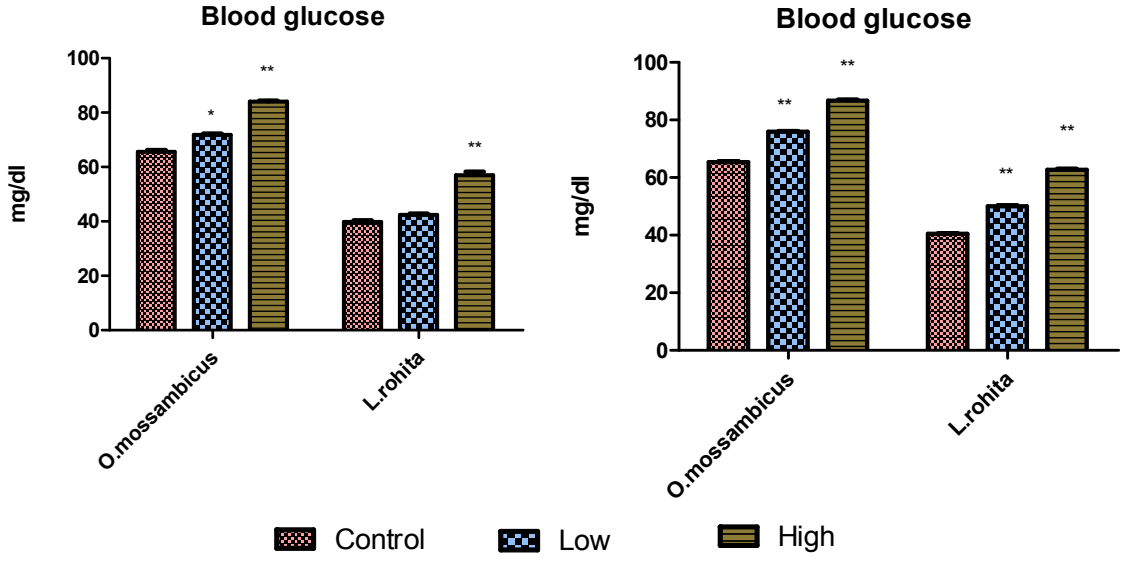
Two-way ANOVA followed by Tukey's test showed that there was significant increase in blood glucose in both the fishes exposed to IMI and CZ compared to control (Table 3.3 and Fig 3.3).

Table:3.3 Effect of IMI and CZ on blood glucose level (mean ± SEM) in *O.mossambicus* and *L.rohita.*

	Blood Glucose					
	IMI			CZ		
	C	LD	HD	C	LD	HD
O.mossaobicus	65.549 ±0.698	71.79 ±0.45**	84.038 ±0.306**	65.356 ±0.335	75.86 ±0.245	86.728 ±0.215**
L.rohita	39.7952 ±0.593	42.3492 ±0.407**	56.8999 ±1.26**	40.49 ±0.213	50.08 ±0.245**	62.702 ±0.345**

- Values are expressed as mg/dl.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure :3.3 Effect of IMI and CZ on blood glucose level (mean ± SEM) in *O.mossambicus* and *L.rohita.*



Lipid:

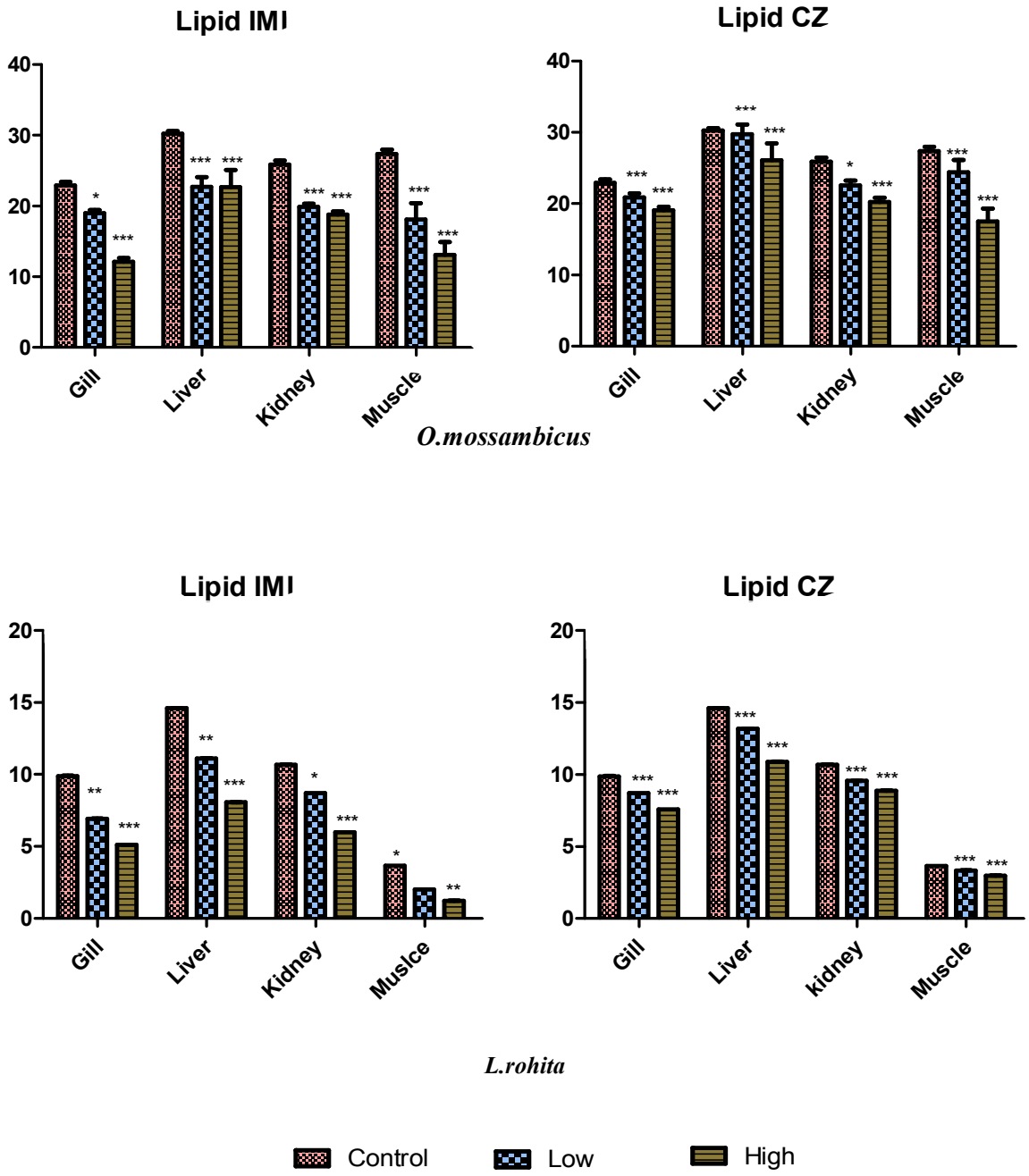
Two-factor ANOVA followed by Tukey's test showed that there was significant dose dependent decrease in all the tissue of both the fishes exposed to both the chemicals as compared to control. (Table 3.4 and Fig 3.4).

Table: 3.4 Effect of IMI and CZ on level of Lipid (mean ± SEM) in *O.mossambicus* and *L.rohita.*

LIPID							
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	22.91 ±0.48	18.99 ±0.47*	12.11 ±0.51***	22.91 ±0.48	20.87 ±0.54***	19.05 ±0.50***
	Liver	30.25 ±0.34	22.71 ±1.38***	22.70 ±2.37***	30.25 ±0.34	29.76 ±1.33***	26.09 ±2.37***
	Kidney	25.87 ±0.57	19.87 ±0.44***	18.79 ±0.44***	25.87 ±0.57	22.56 ±0.69*	20.21 ±0.60***
	Muscle	27.40 ±0.57	18.07 ±2.34***	13.07 ±1.81***	27.40 ±0.57	24.43 ±1.68***	17.49 ±1.81***
L.rohita	Gills	9.870 ±0.039	6.910 ±0.004**	5.100 ±0.005***	9.870 ±0.039	8.710 ±0.005***	7.590 ±0.003***
	Liver	14.600 ±0.015	11.100 ±0.018**	8.070 ±0.004***	14.60 ±0.015	13.180 ±0.015***	10.880 ±0.005***
	Kidney	10.680 ±0.004	8.710 ±0.004*	5.990 ±0.006***	10.68 ±0.004	9.570 ±0.004***	8.880 ±0.004***
	Muscle	3.660 ±0.005	2.010 ±0.005*	1.210 ±0.009**	3.660 ±0.005	3.320 ±0.044***	2.980 ±0.005***

- Values are expressed as mg / g wet wt of tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.4 Effect of IMI and CZ on level of Lipid (mean ± SEM) in *O.mossambicus* and *L.rohita.*



CHOLESTEROL:

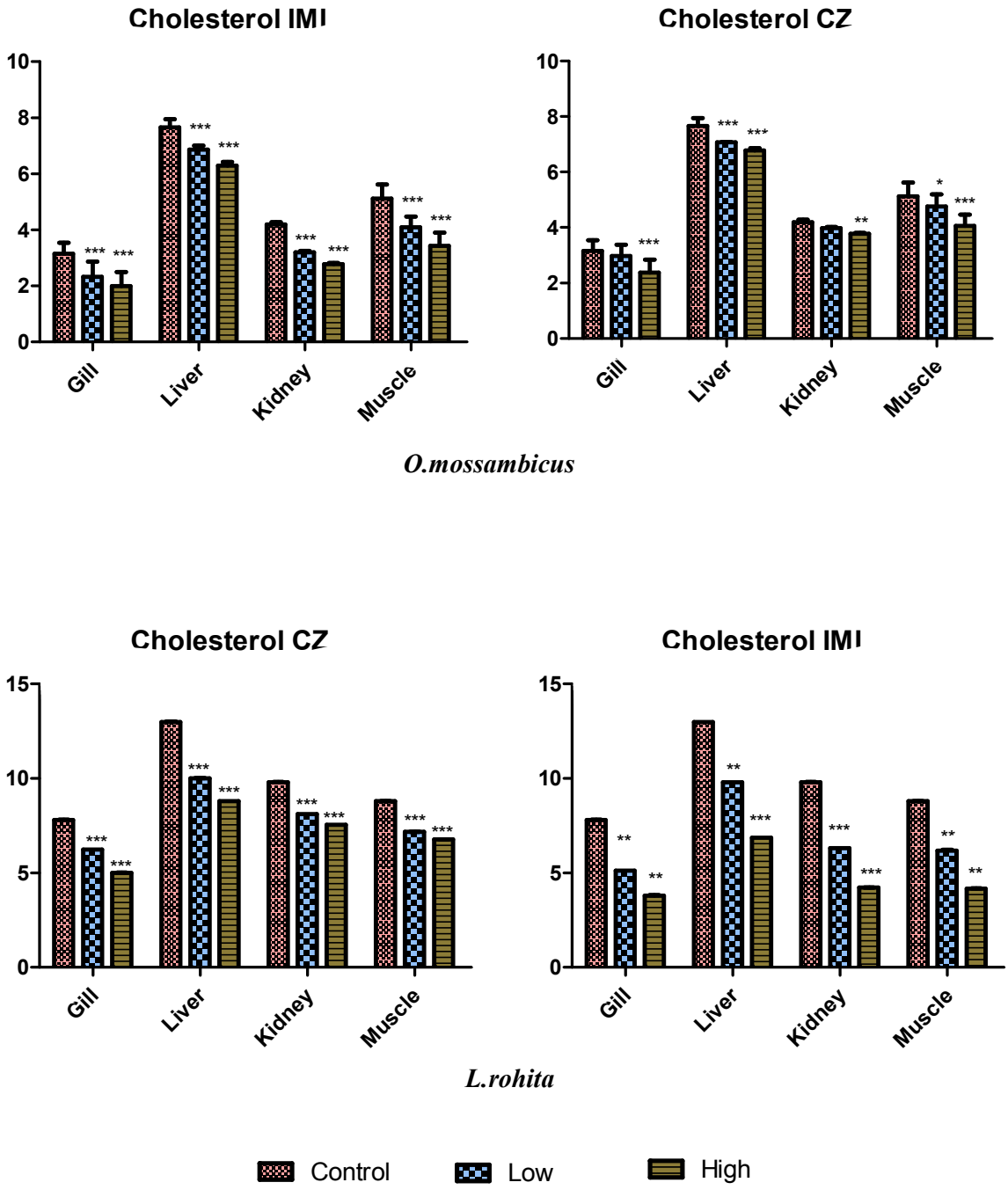
Two-factor ANOVA followed by Tukey's test showed that there was significant vatiation ($P<0.001$) in cholestrol level between treatments (Table 3.5 and Fig 3.5). Cholesterol level showed significant decrease with increase concentration of IMI and CZ in all the tissue of both the fishes.

Table: 3.5 Effect of IMI and CZ on level of Cholesterol (mean ± SEM) in *O.mossambicus* and *L.rohita.*

cholesterol							
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	3.150 ±0.399	2.330 ±0.541***	1.992 ±0.501***	3.150 ±0.399	2.980 ±0.399	2.380 ±0.468***
	Liver	7.654 ±0.288	6.864 ±0.140***	6.296 ±0.123***	7.654 ±0.288	7.070 ±0.009***	6.782 ±0.083***
	Kidney	4.198 ±0.078	3.208 ±0.050***	2.780 ±0.039***	4.198 ±0.078	3.982 ±0.040	3.770 ±0.032**
	Muscle	5.120 ±0.497	4.092 ±0.388***	3.428 ±0.479***	5.120 ±0.497	4.762 ±0.435*	4.060 ±0.399***
L.rohita	Gills	7.810 ±0.005	5.120 ±0.004**	3.790 ±0.025**	7.810 ±0.005	6.230 ±0.003***	5.010 ±0.003***
	Liver	12.980 ±0.040	9.810 ±0.002**	6.870 ±0.003***	12.98 ±0.040	10.01 ±0.005***	8.790 ±0.002***
	Kidney	9.810 ±0.003	6.310 ±0.001***	4.230 ±0.006***	9.810 ±0.003	8.120 ±0.004***	7.560 ±0.001***
	Muscle	8.790 ±0.005	6.180 ±0.044**	4.170 ±0.015**	8.790 ±0.005	7.180 ±0.008***	6.780 ±0.005***

- Values are expressed as mg / g wet wt of tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Figure: 3.5 Effect of IMI and CZ on level of Cholesterol (mean ± SEM) in *O.mossambicus* and *L.rohita.*



ALANINE AMINOTRANSFERASE:

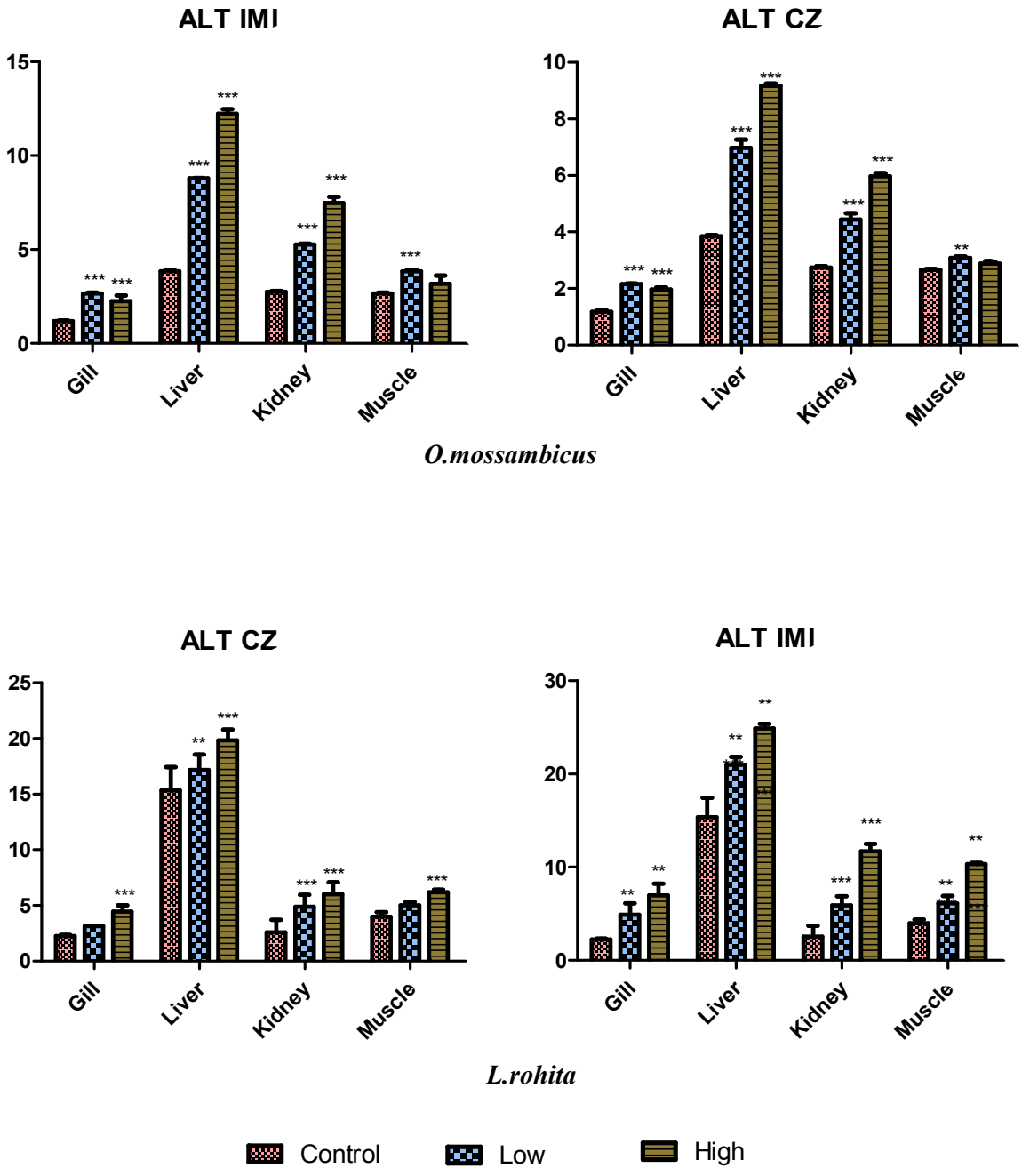
Two-factor ANOVA followed by Tukey's test showed that there was significant elevation in ALT activity in all the tissue of both the fishes on exposure of IMI and CZ compared to control (Table: 3.6 and Fig 3.6).

Table: 3.6 Effect of IMI and CZ on alanine aminotransferase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	ALT						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	1.180 ±0.042	2.660 ±0.040***	2.240 ±0.300***	1.180 ±0.042	2.150 ±0.031***	1.970 ±0.072***
	Liver	3.850 ±0.040	8.790 ±0.020***	12.240 ±0.242***	3.850 ±0.040	6.980 ±0.291***	9.170 ±0.076***
	Kidney	2.740 ±0.054	5.270 ±0.035***	7.480 ±0.322***	2.740 ±0.054	4.450 ±0.215***	5.970 ±0.112***
	Muscle	2.660 ±0.035	3.840 ±0.063***	3.170 ±0.438	2.660 ±0.035	3.090 ±0.040**	2.890 ±0.081
L.rohita	Gills	2.260 ±0.08	4.890 ±1.25**	6.97 ±1.25**	2.26 ±0.08	3.13 ±0.04	4.45 ±0.57***
	Liver	15.35 ±2.09	20.98 ±0.87**	24.89 ±0.48**	15.35 ±2.09	17.17 ±1.38**	19.83 ±0.97***
	Kidney	2.570 ±1.15	5.910 ±0.97***	11.71 ±0.79	2.57 ±1.15	4.89 ±1.07***	6.01 ±1.06***
	Muscle	3.980 ±0.42	6.130 ±0.79**	10.33 ±0.12**	3.98 ±0.42	5.01 ±0.30	6.17 ±0.23***

- Values are expressed as µmoles of pyruvate liberated / h/ mg protein.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.6 Effect of IMI and CZ on alanine aminotransferase activity (mean ± SEM) in *O.mossambicus* and *L.rohita.*



ASPARTATE AMINOTRANSFERASE:

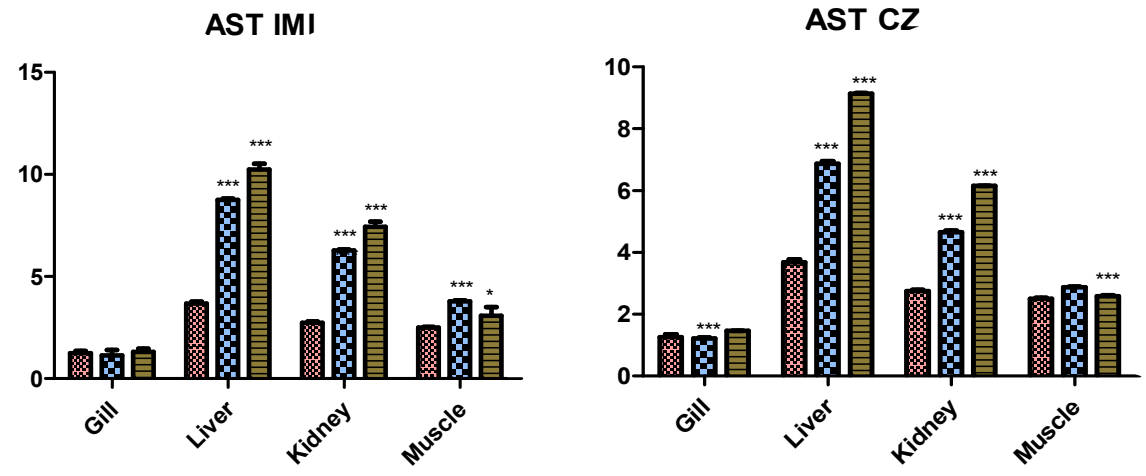
AST activity was found to be significantly elevated in tissues such as liver, kidney and muscle of both the treated groups compared to control in both the fishes. In the IMI and CZ treated group liver, muscle and kidney of *O.mossambicus* and *L.rohita* showed significantly elevated AST activity compared to control group. Gills of both the treated groups did not show any significant variation on IMI exposure as compared to control (Table 3.7 and Fig 3.7).

Table: 3.7 Effect of IMI and CZ on aspartate aminotransferase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

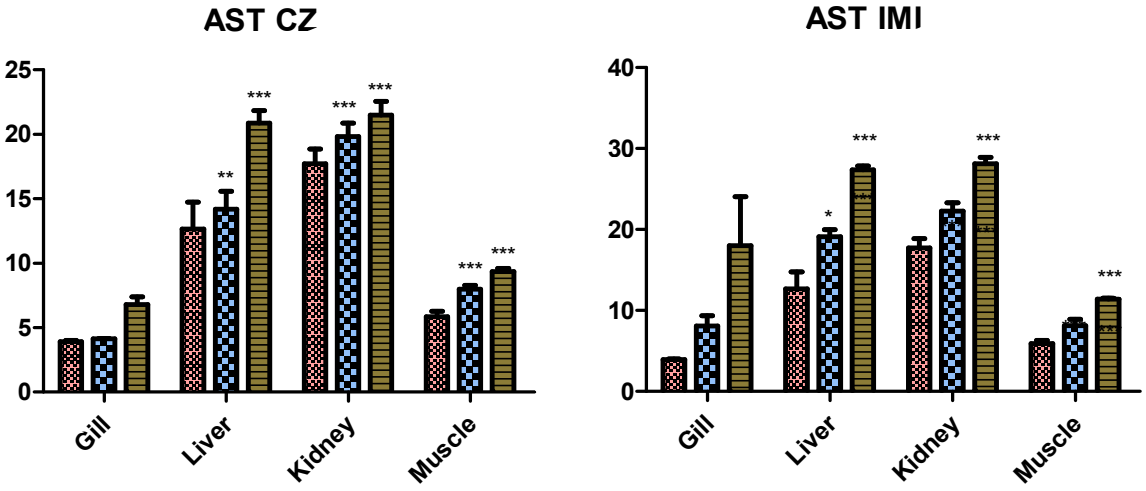
	AST						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	1.260 ± 0.094	1.140 ±0.264	1.310 ± 0.156	1.260 ±0.094	1.220 ± 0.025***	1.460 ±0.007
	Liver	3.680 ±0.089	8.760 ±0.058***	10.240 ± 0.282***	3.681 ±0.089	6.870 ±0.081***	9.140 ±0.015***
	Kidney	2.750 ±0.040	6.280 ±0.039***	7.440 ± 0.259***	2.749 ±0.040	4.650 ±0.054***	6.150 ±0.008***
	Muscle	2.500 ±0.027	3.800 ±0.030***	3.080 ±0.429*	2.500 ±0.027	2.870 ±0.028	2.590 ±0.029***
L.rohita	Gills	3.920 ±0.08	8.090 ±1.25	17.99 ±1.25	3.92 ±0.08	4.13 ±0.04	6.81 ±0.57
	Liver	12.65 ±2.09	19.10 ±0.87*	27.38 ±0.87***	12.65 ±2.09	14.21 ±1.38**	20.87 ±0.97***
	Kidney	17.72 ±1.15	22.30 ±0.97	28.13 ±0.97***	17.72 ±1.15	19.83 ±1.07***	21.48 ±1.06***
	Muscle	5.870 ±0.42	28.13 ±0.79	11.38 ±0.79***	5.87 ±0.42	7.98 ±0.30***	9.37 ±0.23***

- Values are expressed as µmoles of pyruvate liberated / h/ mg protein.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.7 Effect of IMI and CZ on aspartate aminotransferase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



O.mossambicus



L.rohita

Control Low High

ALKALINE PHOSPHATASE:

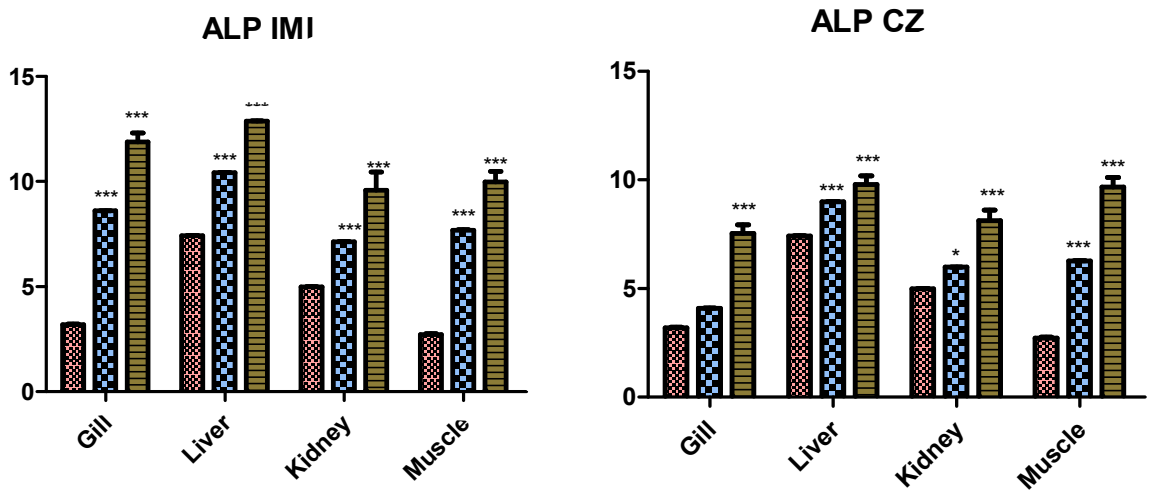
Statistical analysis showed significant (P<0.001) variation in ALP activity in liver, kidney, gill and muscle tissue in *O.mossambicus* exposed to IMI and CZ compared to control. Gill of CZ treated group showed a significant (P<0.001) elevation at high dose as compared to control whereas it did not showed any significant changes at low dose as compared to control. Whereas, there was a significant elevation in all the tissue of *L.rohita* exposed to both the agrochemicals as compared to control (Table: 3.8 and Fig 3.8).

Table: 3.8 Effect of IMI and CZ on alkaline phosphatase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

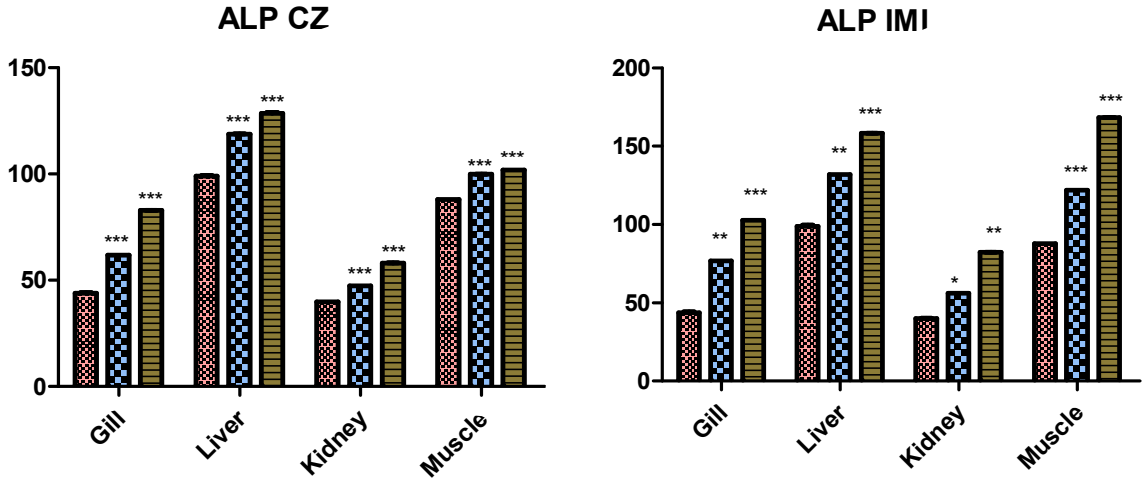
	ALP						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	3.190 ±0.026	8.600 ± 0.011***	11.880 ± 0.443***	3.190 ± 0.026	4.080 ± 0.020	7.530 ± 0.401***
	Liver	7.420 ± 0.007	10.420 ± 0.029***	12.878 ± 0.020***	7.420 ± 0.007	8.990 ± 0.020***	9.798 ±0.389***
	Kidney	4.980 ± 0.021	7.130 ± 0.010***	9.578 ± 0.872***	4.980 ± 0.021	5.980 ± 0.006*	8.120 ± 0.487***
	Muscle	2.720 ± 0.031	7.680 ± 0.027***	9.980 ± 0.496***	2.720 ± 0.031	6.270 ± 0.008***	9.670 ± 0.432***
L.rohita	Gills	43.78 ±0.451	76.71 ±0.003**	102.71 ±0.038***	43.78 ±0.451	61.81 ±0.020***	82.78 ±0.035***
	Liver	98.88 ±0.443	131.91 ±0.002**	158.31 ±0.025***	98.88 ±0.443	118.73 ±0.022***	128.48 ±0.463***
	Kidney	39.87 ±0.001	56.11 ±0.001*	82.17 ±0.015**	39.87 ±0.001	47.31 ±0.038***	58.12 ±0.008***
	Muscle	87.91 ±0.005	121.87 ±0.015***	108.31 ±0.044***	87.91 ±0.005	99.78 ±0.033***	101.81 ±0.005***

- Values are expressed as mg of phenol liberated / min / mg protein.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.8 Effect of IMI and CZ on alkaline phosphatase activity (mean ± SEM) in *O.mossambicus* and *L.rohita.*



O.mossambicus



L.rohita

Control Low High

PYRUVATE:

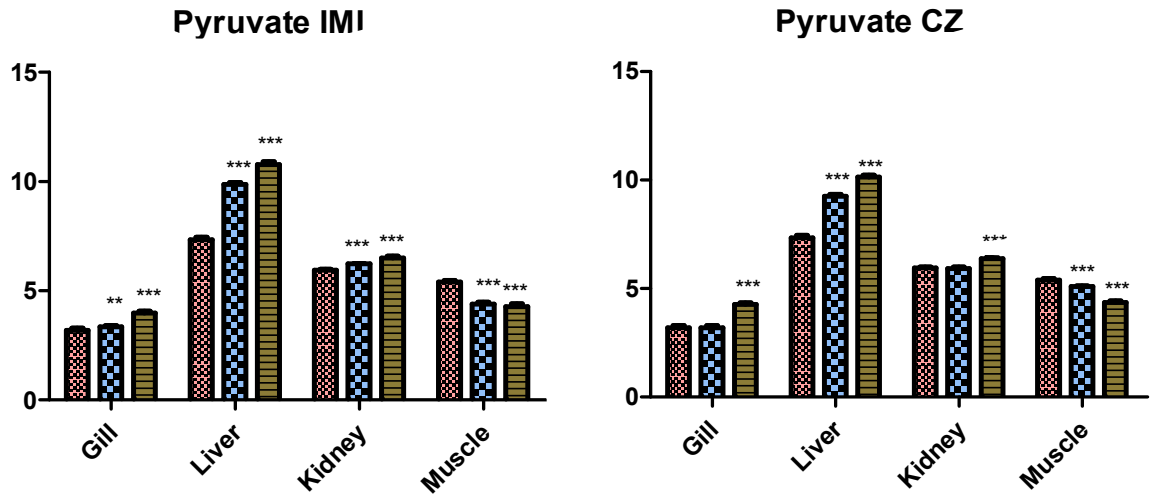
Two way ANOVA followed by Tukey's test showed that there was significant variation in pyruvate level between the IMI and CZ exposed fishes. On exposure of IMI and CZ, gills, liver and kidney of both the fish showed a significantly increased pyruvate level compared to control. Whereas, in *L.rohita*, muscle showed a significantly decreased pyruvate level on CZ exposure compared to control (Table 3.9 and Fig 3.9)

Table: 3.9 Effect of IMI and CZ on level of pyruvate (mean ± SEM) in *O.mossambicus* and *L.rohita.*

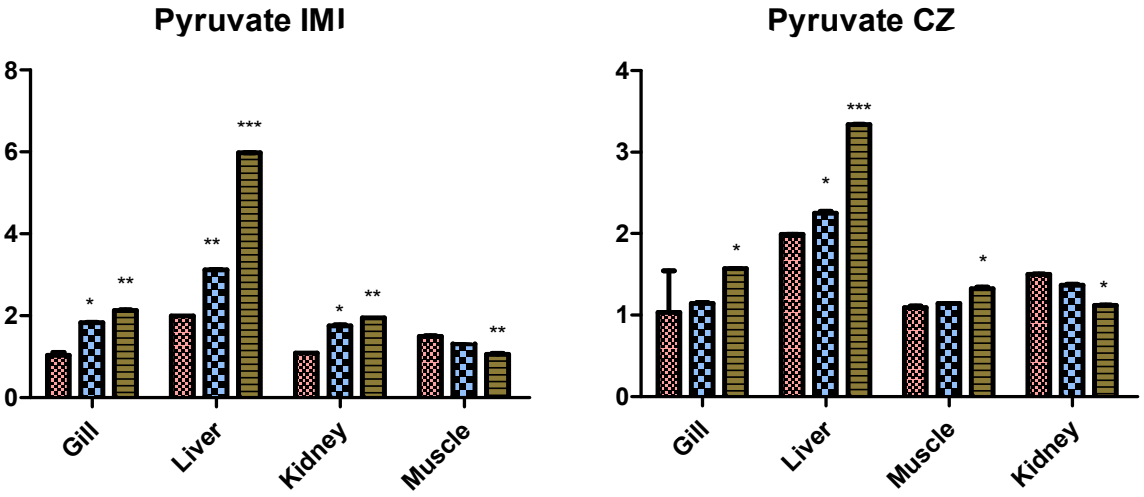
	Pyruvate						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	3.190 ±0.103	3.350 ±0.058**	3.980 ±0.090***	3.190 ±0.103	3.230 ±0.103	4.270 ±0.076***
	Liver	7.330 ±0.123	9.870 ±0.081***	10.780 ±0.123***	7.330 ±0.123	9.254 ±0.089***	10.130 ±0.094***
	Kidney	5.950 ±0.040	6.230 ±0.031***	6.490 ±0.100***	5.950 ±0.040	5.924 ±0.067	6.380 ±0.076***
	Muscle	5.390 ±0.085	4.390 ±0.089***	4.270 ±0.130***	5.390 ±0.085	5.098 ±0.015***	4.350 ±0.084***
L.rohita	Gills	1.030 ±0.516	1.830 ±0.004*	2.120 ±0.024**	1.030 ±0.516	1.140 ±0.015	1.570 ±0.003*
	Liver	1.990 ±0.002	3.120 ±0.001**	5.980 ±0.003***	1.990 ±0.002	2.250 ±0.025*	3.340 ±0.002***
	Kidney	1.090 ±0.020	1.750 ±0.024*	1.950 ±0.002**	1.090 ±0.020	1.140 ±0.001	1.320 ±0.024*
	Muscle	1.500 ±0.005	1.310 ±0.018	1.060 ±0.015**	1.500 ±0.005	1.370 ±0.007	1.120 ±0.005*

- Values are expressed as µmoles of pyruvate / g wet wt of tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.9 Effect of IMI and CZ on level of pyruvate (mean ± SEM) in *O.mossambicus* and *L.rohita.*



O.mossambicus



L.rohita

Control Low High

LACTATE DEHYDROGENASE:

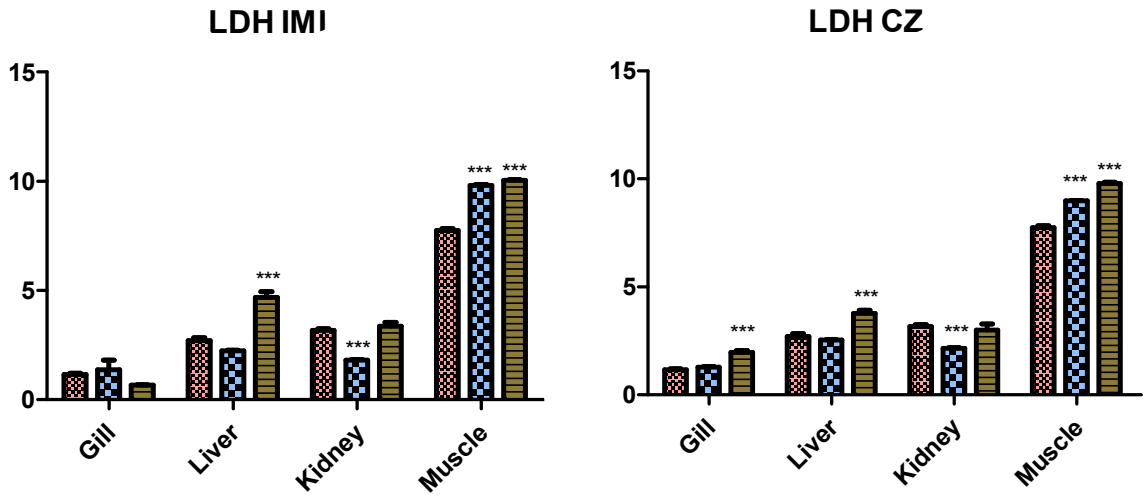
LDH activity in different tissues of *O.mossambicus* and *L.rohita* treated with different agro-chemicals showed significant variations compared to control (Table 3.10 and Fig 3.10). In the Curzate treated group, tissues such as liver, kidney and muscle showed significantly elevated activity as compared to control. Among the tissue of Imidacloprid treated group the gills and muscle showed a significantly elevated activity and the liver and kidney showed a significantly decreased activity compared to control.

Table: 3.10 Effect of IMI and CZ on Lactate dehydrogenase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

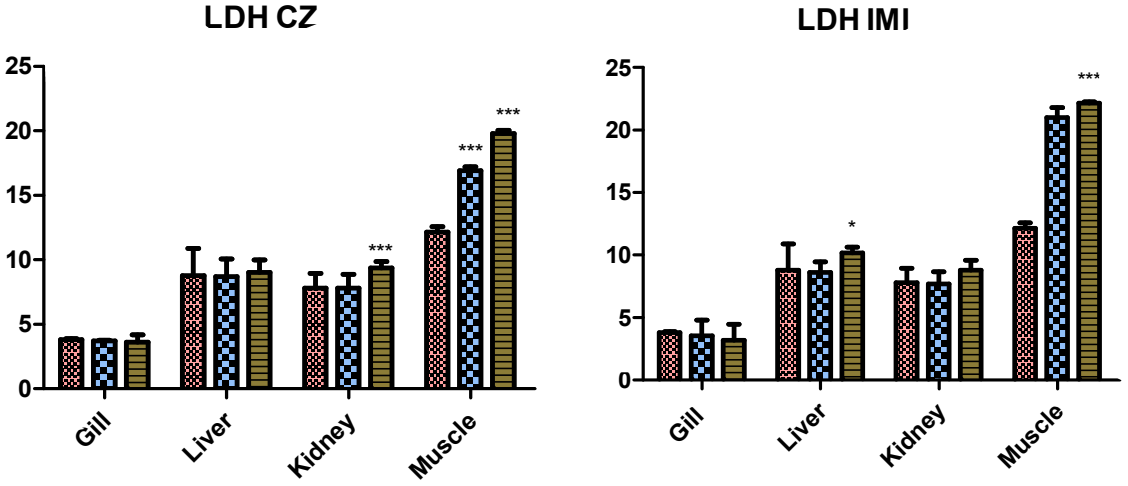
	LDH						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	1.160 ±0.040	1.370 ±0.441	0.670 ±0.013	1.160 ±0.040	1.280 ±0.021	1.970 ±0.080***
	Liver	2.710 ±0.123	2.230 ±0.040	4.680 ±0.258***	2.710 ±0.123	2.540 ±0.022	3.768 ±0.152***
	Kidney	3.170 ±0.075	1.810 ±0.035***	3.370 ±0.174***	3.170 ±0.075	2.160 ±0.020***	2.990 ±0.286
	Muscle	7.740 ±0.089	9.810 ±0.049***	10.040 ±0.024***	7.740 ±0.089	8.980 ±0.029***	9.790 ±0.058***
L.rohita	Gills	3.810 ±0.08	3.560 ±1.25	3.21 ±1.25	3.81 ±0.08	3.71 ±0.04	3.62 ±0.57
	Liver	8.790 ±2.09	8.610 ±0.87	10.17 ±0.48*	8.79 ±2.09	8.69 ±1.38	9.01 ±0.97
	Kidney	7.810 ±1.15	7.690 ±0.97	8.79 ±0.79	7.81 ±1.15	7.80 ±1.07	8.01 ±1.06***
	Muscle	12.16 ±0.42	20.98 ±0.79	22.13 ±0.12***	12.16 ±0.42	16.91 ±0.30***	19.81 ±0.23***

- Values are expressed as µmoles of pyruvate liberated / h/ mg protein.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.10 Effect of IMI and CZ on Lactate dehydrogenase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



O.mossambicus



L.rohita

Control Low High

GLUTAMATE DEHYDROGENASE:

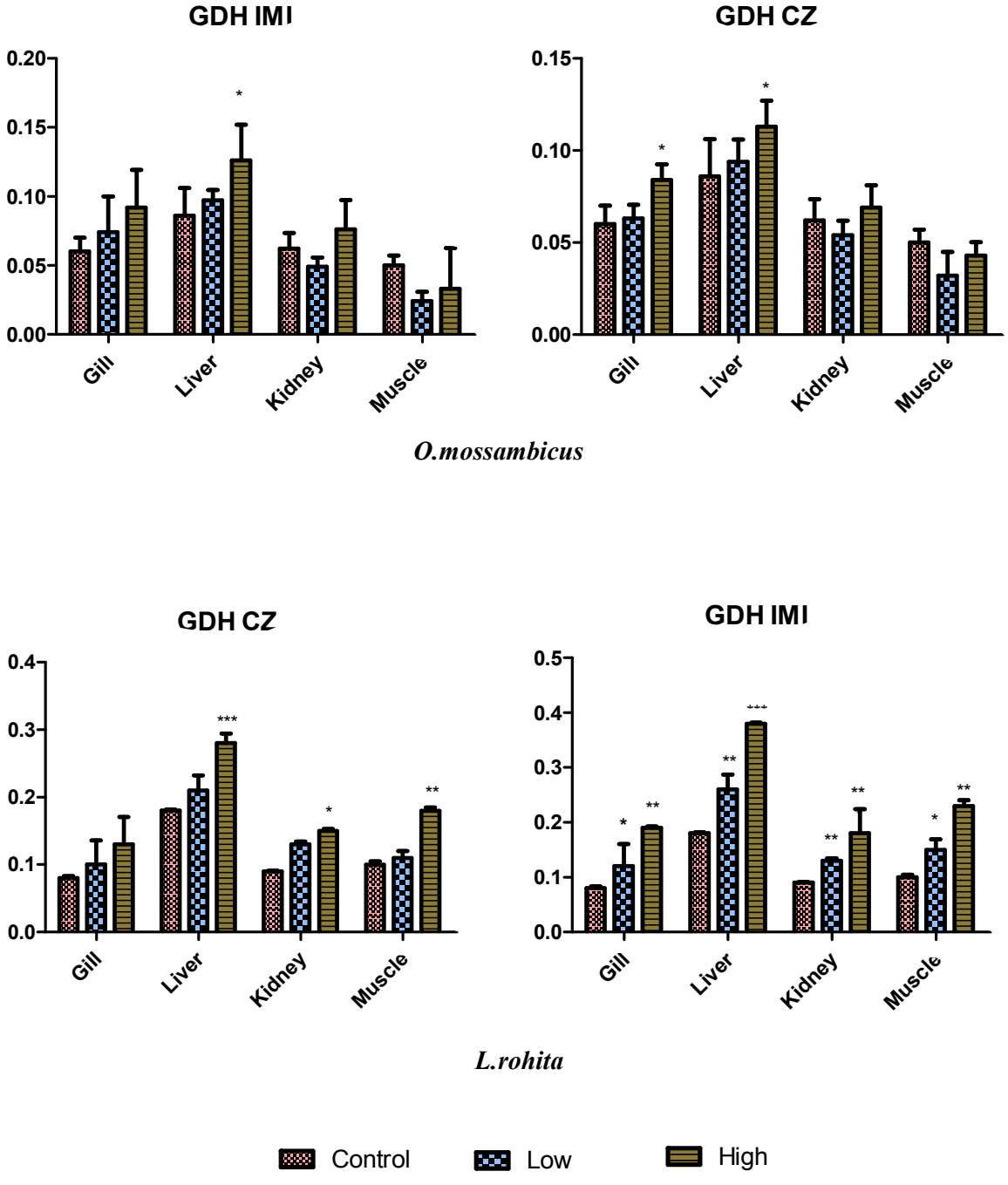
GDH activity showed statistically significant (P<0.05) increase in liver and gills tissue of *O.mossambicus* exposed to IMI and CZ. Whereas on exposure of IMI GDH activity showed a significant increase in all the tissue *L.rohita* and on CZ exposure only liver, kidney and muscle showed a significant increase at high dose compared to control (Table 3.11 and Fig 3.11).

Table: 3.11 Effect of IMI and CZ on Glutamate dehydrogenase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	GDH						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	0.060 ± 0.01	0.074 ± 0.026	0.092 ± 0.027	0.06 ± 0.01	0.063 ± 0.008	0.084 ± 0.008*
	Liver	0.086 ± 0.02	0.097 ± 0.008	0.126 ± 0.026*	0.086 ± 0.02	0.093 ± 0.012	0.112 ± 0.014*
	Kidney	0.062 ± 0.012	0.049 ± 0.007	0.076 ± 0.021	0.062 ± 0.01	0.054 ± 0.008	0.068 ± 0.012
	Muscle	0.05 ± 0.007	0.024 ± 0.007	0.033 ± 0.030	0.05 ± 0.01	0.032 ± 0.013	0.042 ± 0.013
L.rohita	Gills	0.080 ±0.003	0.120 ±0.040	0.190 ±0.003	0.080 ±0.003	0.100 ±0.036*	0.130 ±0.040**
	Liver	0.180 ±0.002	0.260 ±0.027	0.380 ±0.002***	0.180 ±0.002	0.210 ±0.022**	0.280 ±0.014***
	Kidney	0.090 ±0.001	0.130 ±0.004	0.180 ±0.044*	0.090 ±0.001	0.110 ±0.004**	0.150 ±0.003**
	Muscle	0.100 ±0.005	0.150 ±0.019	0.230 ±0.010**	0.100 ±0.005	0.110 ±0.010*	0.180 ±0.005**

- Values are expressed as mg of phenol liberated / min / mg protein.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.11 Effect of IMI and CZ on Glutamate dehydrogenase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



GLUCOSE-6-PHOSPHATASE:

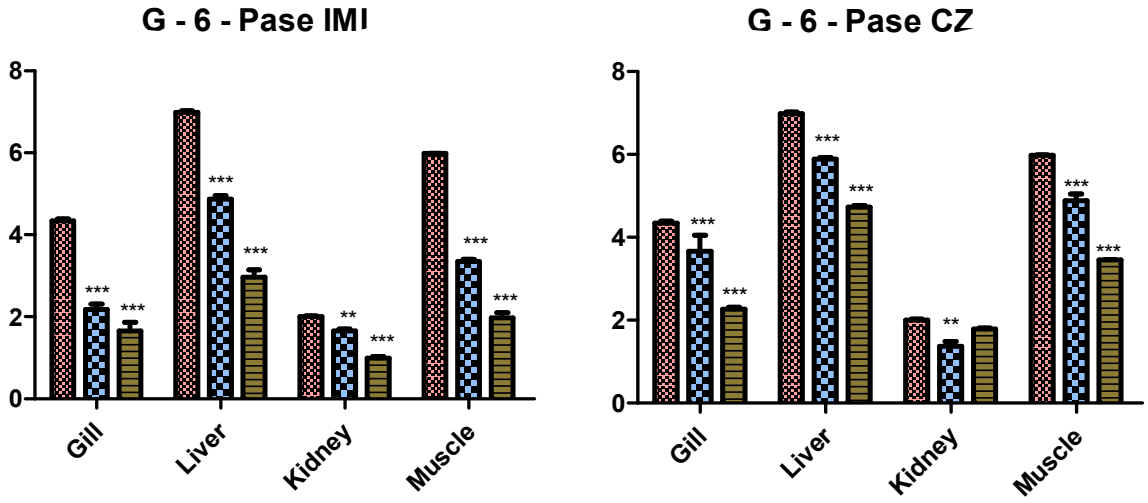
Two-way ANOVA followed by Tukey's test showed that there was significant decrease in glucose-6-phosphatase activity in both the fishes exposed to IMI and CZ compared to control (Table 3.12 and Fig 3.12).

Table: 3.12 Effect of IMI and CZ on glucose-6-phosphatase activity (mean ± SEM) in *O.mossambicus* and *L.rohita.*

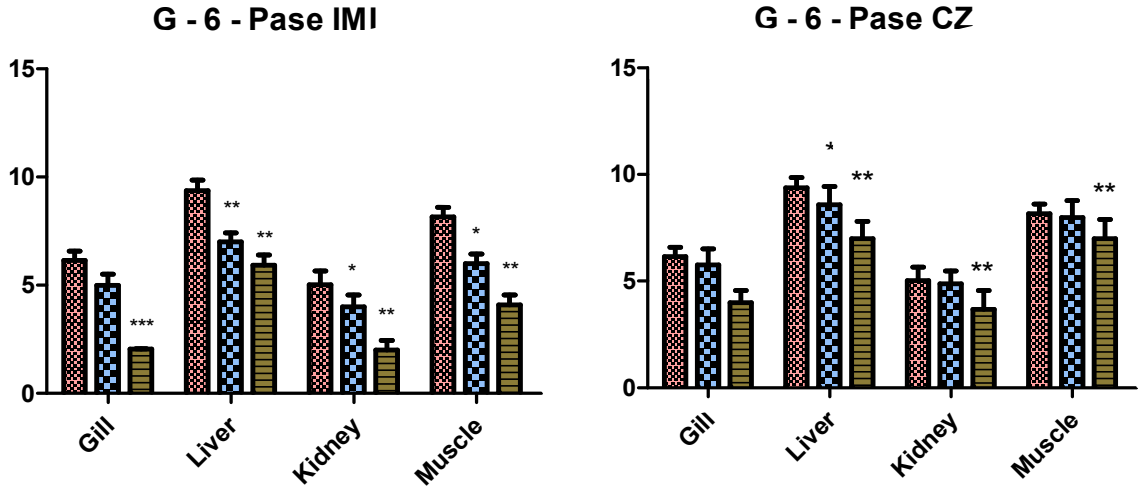
	Glucose – 6 – Po_4						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	4.342 ± 0.046	2.178 ± 0.130***	1.654 ± 0.209***	4.342 ± 0.046	3.664 ± 0.389***	2.262 ± 0.046***
	Liver	6.98 ± 0.043	4.87 ± 0.086***	2.97 ± 0.172***	6.98 ± 0.043	5.89 ± 0.043***	4.732 ± 0.038***
	Kidney	2.002 ± 0.021	1.658 ± 0.044**	0.988 ± 0.044	2.002 ± 0.021	1.37 ± 0.111**	1.792 ± 0.021
	Muscle	5.982 ± 0.010	3.352 ± 0.048***	1.978 ± 0.119***	5.982 ± 0.010	4.88 ± 0.172***	3.454 ± 0.010***
L.rohita	Gills	5.768 ±0.046	3.587 ±0.353	2.456 ±0.209***	5.676 ±0.413	0.752 ±0.305	3.156 ±0.363
	Liver	7.321 ±0.432	5.273 ±0.308**	3.167 ±0.378**	7.353 ±0.378	6.964 ±0.363*	5.467 ±0.038**
	Kidney	2.998 ±0.353	1.658 ±0.392*	1.687 ±0.418**	2.002 ±0.258	2.578 ±0.308	2.678 ±0.319**
	Muscle	6.356 ±0.258	3.988 ±0.048*	2.976 ±0.155**	6.555 ±0.010	5.389 ±0.172	3.454 ±0.353**

- Value are expressed as µg of inorganic phosphorus liberated/min/mg protein.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 3.12 Effect of IMI and CZ on glucose-6-phosphatase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



O.mossambicus



L.rohita

Control Low High

Discussion:

The changes in the biochemical constituents in the gills, muscle, kidney and liver of the fish (*O.mossambicus* and *L.rohita*) exposed to sub-lethal concentration of IMI and CZ at different dose were observed in the present study. The changes in the glycogen, protein and lipid profile exhibited a significant decrease in all the tissues in a dose dependent manner (Table 3.1, 3.2 & 3.4 and Fig 3.1, 3.2 & 3.4).

Proteins are indispensable constituents required by organisms in tissue building and play a important role on energy metabolism (Yeragi *et al.,* 2003; Remia *et al.,* 2008; Pang-Hung *et al.,* 2008). In the present investigation there was an overall decrease in the protein content in liver, muscle, kidney and gills (Table 3.1 and Fig 3.1). The physiological status of animal is usually indicated by the metabolic status of proteins (Nelson and Cox, 2005; Magar and Shaikh, 2012). The depletion in the protein may have been due to their degradation and possible utilization for metabolic purposes. Furthermore, decreased protein content was found to be maximum in liver followed by muscle, kidney and gill respectively, these variations in response of the pesticides in both the fishes suggests difference in metabolic calibers of individual tissue (Satyanarayana, 2005; Venkataramana *et al.,* 2006). Decrease in the protein content of all the tissues is also suggestive of impairment of protein synthesis or increase in the rate of its degradation to amino acids due to stress induced pesticide exposure, which can result in the production of free amino acids in the tricarboxylic acid cycle for energy production (Jenkins *et al.,* 2003; Radha *et al.,* 2005; Naveed *et al.,* 2010; Ganeshwade, 2011). The free amino acid pool can be used for ATP production by transamination reactions or by gluconeogenic pathway. Thus, the decrease in protein content under stress induced by pesticides may be attributed to the utilization to the amino acids in various cataboilc reactions. Behavioural responses of fishes, exposed to sub-lethal concentration of IMI and CZ, showed that they were under stress condition (Chapter 1). It has been reported earlier (Yadav *et al.,* 2007; Tripathi and Singh, 2003) that the animal exposed to chemicals obtain extra energy requirement from the tissue protein. The depletion of cellular proteins might be caused by one or more of the following factors: inhibition of amino acid incorporation, breakdown of protein into amino acids

and diffusion out of the cells. The decline in protein content may be related to tissue repair and the detoxification mechanism during stress (Neff, 1985; Remia *et al.,* 2008; Varadarajan, 2010). Our results are in concurrence with the earlier reported depletion in the protein content of *O.*mossambicus (Vijuen and Steyn 2003; Aniladevi, 2008; Varadarajan, 2010; Al-Kahtani, 2011) and *L.*rohita (Ramesh *et al.,* 1993; Das and Mukherjee 2003; Sivaperumal, 2008; Indirabai *et al.,* 2010; Rajput *et al.,* 2012).

Carbohydrates are the primary and immediate source of energy. As suggested by Arasta *et al.,* (1996), in stress condition carbohydrate reserves gets depleted to meet energy demand. Glycogen levels are found to be highest in liver as it is the chief organ of carbohydrate metabolism in animals, followed by muscle. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself (Bedii and Kenan, 2005; Sobha *et al.,* 2007). Depletion of glycogen in the present study in *O.mossambicus* and *L.rohita* was maximum in liver followed by muscle, gills and kidney, may be due to direct utilization for energy generation, a demand caused by pesticide stress induced hypoxia (Chapter II). During stress an organism needs sufficient energy which is supplied from reserved glycogen. Thus, in the present study probably the depletion in glycogen level (Table 3.2 and Fig 3.2) clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to pesticides (Kawade and Khillare, 2012). Besides, the decrease in the level of total protein, and glycogen and concentrations of pesticide caused an increase in the glucose level leading to lethargy (Chapter I). The significant increase in blood glucose which was dose dependent may be considered to be manifestation of stress induced by IMI and CZ exposure (Table 3.3 and Fig 3.3). Glucose increase is a general response of fish to acute and sub-lethal pollutant effects (Luskova *et al.,* 2002). As proposed by Wedemeyer and Mcleay, (1981) high level of blood glucose is caused by disturbances in carbohydrate metabolism due to physical and chemical stress. A variety of pesticides as a stressor have been known to stimulate adrenal tissue, resulting in increased level of glucocorticoids (Hontela *et al.,* 1996) and catecholamines. Both of these groups of hormones produced hyperglycemia. The dose dependent accumulation of glucose reported in this investigation revealed the *O.mossambicus* and *L.rohita* exposed to

sublethal concentration of both the pesticides became hyperglycaemic (Omoregie *et al.,* 1990; Bhavan and Geraldine, 1997; Haggag, 2004; Sweilum, 2006; Venkataramana *et al.,* 2006). Blood glucose is considered as indicator for stress response in fish (Abdel-Baky, 2001). The hyperglycaemic condition induced by IMI might be explained in part by inhibition of choline esterase (de Aguiar *et al.,* 2004) at neuro-effector sites in the head kidney, leading to hyper secretion of cortisol which stimulates the breakdown of glycogen to glucose (glycogenolysis) (Witold *et al.,* 2007; Francesco *et al.,* 2008; Logaswamy and Remia, 2009). Thus, hyperglycemia can be viewed as a physiological response of the fishes to meet the critical need for energy under toxic stress. Furthermore, the elevated glucose level observed in CZ exposed fished may be due to enhancement of the breakdown of liver glycogen. These findings are in agreement with those of Reddy and Leatherland, (1998); Bakhshwan *et al.,* (2009).

Lipids play very important role in the architectural dynamics of the cell and transport mechanism across cell membrane. Any stress is found to change the course of events associated with the lipid synthesis. Lipids also contribute to energy production as they are having high calorific values (Guyton, 2006) and play a vital role during the biochemical adaptations of the animals to stress conditions (Tayyaba *et al.,* 1981; Swami *et al.,* 1994). Extensive literature is available on the effects of different pesticides on tissue lipid fraction of various animals (Srinivas *et al.,* 1991; Chetty and Indira, 1994; Govindan *et al.,* 1994; Martin *et al.,* 2007). In the present study a significant decrease in the total lipid content of all the tissues (liver < muscle < kidney < gills) exposed to IMI and CZ in a dose dependent manner (Table 3.4 and Fig 3.4). Decreased lipid content suggests that an impairment of the lipid storage has taken place in the fishes and that the lipid might have been channelled for other metabolic functions in which it probably plays a vital role during stress condition. Since lipids form the rich energy reserves whose calorific value is reported to be twice than that of an equivalent weight of carbohydrates or proteins (Sobha *et al.,* 2007; Gijare *et al.,* 2011). Lipids serve as energy reserves to meet the metabolic demand for more energy to mitigate toxic stress. The decreased lipid content in the present investigation is parallel with the earlier reported altered lipid profile in *Orechromis mossambicus* (Amudha *et al.,* 2002; Leela Sivaparvathi *et al.,* 2002); in *Perca flavescens* (Levesque *et al.,* 2002); in

Cyprinus carpio (Swapna *et al.,* 2006); in *Gambusia affinis* (Revathi *et al.,* 2005); in *Anguilla Anguilla* (Pierron *et al,* 2008) and in *Channa punctatus* (Maruti and Rao, 2001), provide substantial support to the present findings.

Cholesterol is an important normal body constituent used in the structure of cell membranes, synthesis of bile and steroid hormones. The results presented in Table 3.5 and Fig 3.5 show a significant decrease on exposure of IMI and CZ compared to control in a dose dependent manner. Reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis particularly in the liver as it plays a major role in cholesterol homeostasis by regulating lipoprotein metabolism and lipid output in bile (Marzolo et al., 1990; Dietschy *et al.,* 1993). The liver is a key organ in the synthesis and excretion of cholesterol, hence any type of obstruction in the liver will cause alterations in cholesterol. Pesticide induced toxicity has probably resulted into destruction of liver cells hence, the cholesterol level eventually falls below normal due to decrease synthesis (Kamath, 1972). Reduction in cholesterol could also be due to reduce absorption of dietary cholesterol (Rao *et al.,* 1984; Kanaraj *et al.,* 1993; Shakoori *et al.,* 1996). However, Remia *et al.,*(2008) reported that the decline of cholesterol may be due to utilization of fatty deposits instead of glucose for energy purpose. Similar results were observed by Fahmy (2012) in *O.mossambicus* on exposure to Malathion, Sardamani and Selvarani, (2009) in *O.mossambicus* by exposure of Metribuzin; Ganeshwade, (2011) in *Punctius ticto* on exposure of Dimethoate and Singh et al., (2010) in *Channa punctatus* on exposure of Phorate.

Amino transferases are widely acknowledged for their significance in protein metabolism by virtue of their ability to regulate both synthesis and degradation of amino acids. Changes in their activities are often associated with changes in many other metabolic functions and thus represent widespread alterations in the organisms physiological state. Aminotransferases such and alanine amino transferase (ALT) and aspartae aminotransferase (AST) catalyse the reaction of transamination of alanine, glutamic and aspartic acids. They couple protein, carbohydrate and fat metabolism and tricarboxylic cycle under altered physiological, pathological and induced environmental stress conditions (Murugesan *et al.,* 1999). Changes in AST and ALT enzyme activity

in fish have been used frequently as indicators of toxicant and contamination of aquatic ecosystem (Kim *et al.,* 2008; Hedayati *et al.,* 2010). ALT is an enzymatic stress biomarker and its change identify damages in several tissues and organs of fish. ALT and AST are liver specific enzymes and are sensitive measures of hepato toxicity (Balint *et al.,* 1997). However, Oluah (1999) is of the view that alterations in the ALT and AST indicate tissue damage in liver, kidney, muscle and gills. Various scientists have reported the alterations in ALT and AST on exposure of pesticides (Jyothi and Narayan, 2000; Atamanalp *et al.,* 2002 a, b; Adhikari *et al.,* 2004; Begum, 2005; Gabriel *et al.,* 2012). Contrarily, Yildirim *et al.,* (2006) observed an increase in AST and ALT enzyme activities in gills, liver and kidney and have proposed that elevated enzyme activity is with the intension to increase the role of proteins in the energy production during stress. In the present study, compared to control, ALT and AST were found to be significantly elevated in all the tissues of fishes exposed to IMI and CZ in a dose dependent manner (Table 3.6 and 3.7 and Fig 3.6 and 3.7). The highest activity was observed in liver followed by kidney and muscle. As proposed by Vardharajan, (2010), the primary energy currency in fish is amino acids. Elevated activity of transferases is possibly a result of a response to stress induced by pesticides to generate keto acids like α-keto glutarate and Oxaloacetate for contributing to gluconeogenesis and/or energy production necessary to meet the access energy demand. According to Gabriel and George (2005), transamination is one principle pathway for synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuating energy demands of the organism under various adaptive conditions. A significant increase in the ALT activity in all the tissues can be assume as an attempt by these tissues to overcome pesticide toxicity. ALT is liver specific cytoplasmic transaminase. The increased ALT activity in tissues suggests either increased operation of transamination or increase synthesis of amino acids. This clearly indicates that stress brings about, the metabolic reorientation in the tissues by raising energy recourses through transaminases system. Similar studies have been reported by (Arshad *et al.,* 2007; Gabriel *et al.,* 2011; Rao, 2006; Velmurugan *et al.,* 2008) and they have inferred that the increased enzyme activity was due to increase utilization of amino acids for energy synthesis, as consequents of a fish suffering from toxic stress and energy crisis.

Furthermore, change in the AST has been correlated with mitochondrial damage (Diehl *et al.,* 1986; de Aguiar *et al.,* 2004). Thus, in the present study increase in AST activity can be considered to be the manifestation of the biochemical action of the pesticides leading to damage and affecting the mitochondrial membranes of the tissues. Our results are in agreement with the earlier scientists.

Alkaline phosphatase (ALP) is a brush border enzyme, which catalyses dephosphorylation of many molecules including nucleotides, proteins and alkalides at alkaline pH. It is well known that phosphatases are involve in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzyme, secretary activity and transport of phosphorylated intermediate across the cell membranes. Hydrolysis of phosphoester, phosphate transfer, phosphate transport activity, protein transport activity, phosphate transport, modulation of organic cation transport and involvement in cell proliferation have been suggested as possible function of ALP. ALP is also one of the important markers for liver and kidney. A dose dependent response was observed in liver, kidney, gills and muscle (Table 3.8 and Fig 3.8). A significant increase in enzyme activity in liver might be due to a stress induced over activity of hepatobiliary cells, which have involved in detoxification mechanism. Further increased ALP activity also may be due to pathological processes such as liver impairment and kidney disfunction (Barse *et al.,* 2006). Thus in the present study, increase in the levels of ALP and AST reflects liver damage, whereas an elevation in ALP activity may be indicative of renal and liver damage (Gill *et al.,* 1990; Bhattacharya *et al.,* 2005; Vardharajan, 2010; Stalin and Das, 2012).

On exposure of IMI and CZ gills, liver and kidney showed an elevated pyruvate levels in a dose dependent manner compared to control (Table 3.9 and Fig 3.9). This might be due to the higher glycolysis rate which is the only energy producing pathway for the animal when it is under stress condition. Furthermore, the end product of glycolytic pathway is pyruvate. Pyruvate occupies an important junction between various metabolic pathways it may be decarboxylated to acetyl CoA which can enter the TCA cycle or it may be utilized for fatty acid synthesis. Further pyruvate may be carboxylated to oxaloacetate which can be used for gluconeogenesis. Muscle of both

treated pesticides showed a decreased pyruvate level compared to control. Lactate dehydrogenase (LDH) is an enzyme recognized as a potential marker for assessing the toxicity of toxicant. LDH activity is marker for tissue damage in fish (Ramesh *et al.,* 1993), muscular damage (Balint *et al.,* 1997) and hypoxic conditions (Das *et al.,* 2004) and thus serves as a good diagnostic tool in toxicology. LDH interconverts lactate and pyruvate and has very important role in carbohydrate metabolism. LDH acts as a pivotal enzyme between glycolytic pathway and TCA cycle. It catalyses the conversion of pyruvate into lactate, under anaerobic conditions (Lehninger, 1993). A fish under stress preferentially meets its energy requirements through anaerobic oxidation (Luiz, 1998). The LDH in the tissues (liver, kidney, muscle and gills) of fishes treated with IMI and CZ showed an elevated activity in a dose dependent manner compare to control (Table 3.10 and Fig 3.10). Increased LDH activity suggests a significant increase in the conversion of pyruvate to lactic acid, thereby leading to the accumulation of lactic acid. Higher increase LDH in muscle on exposure to pesticides suggests that the final product of glycolysis-pyruvate was preferently used to produce lactate. Lactate form is an important gluconeogenic substrate which can be used to cope with the high and rapid demand of energy due to stress. several reports have revealed altered LDH activity in tissues under toxic conditions (Tripathi *et al.,* 1990; Mishra and Shukla, 2003; Rao, 2006) and have opined that LDH an important glycolytic enzyme is inducible by oxygen stress and therefore, the activity of regulatory enzymes gets altered to meet the required energy demands under toxic stress including the activity of LDH, which sustains the continued process of glycolysis under anaerobic conditions (Diamntino *et al.,* 2001; Agrahari and Gopal, 2009). Thus, the observed increased LDH can be interpreted as a shift in the respiratory metabolism from aerobic to anaerobic in order to meet the enhanced energy demand under the toxic stress (Singh and Shrivatava, 1982; Ansari and Kumar, 1988; Ferrando and Andreu-Moliner, 1991; Kamalaveni *et al.,* 2003; Gorbatiuk, 2010).

Glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalyses the oxidative deamination of glutamate, providing α-ketoglutarate to the kreb's cycle (Reddy and Vanugopal, 1990). This enzyme is having several metabolic functions with great physiological significance. It is closely associated with the detoxification mechanism of

tissues. GDH in extra hepatic tissues could be utilized for channelling of ammonia released during proteolysis for its detoxification into urea in the liver hence, activities of GDH along with AST and ALT are considered as sensitive indicators of stress (Gould *et al.,* 1976). An increase was observed in activity of GDH in all the tissues of the fish exposed to IMI and CZ (Table 3.11 and Fig 3.11). This suggests the active transdeamination of amino acids for the incorporation of keto acids in to the TCA cycle to release necessary energy required for the synthesis of new protein (Sreedevi *et al.,* 1992; Shivramkrishna and Radhakrishnaiah, 1998; Prashanth and Neenagund, 2008). GDH is also known to play a crucial role in ammonia metabolism and is known to be affected by a variety of factors (David, 1995). After exposure of toxicants, several metabolic functions with great physiological significance are known to be closely associated with detoxification mechanism of tissues. GDH in extra hepatic tissues could be utilize for its ultimate detoxification to urea in the liver. In the present study the significant elevation in activity of GDH indicate that association of oligomers in response to toxic stress, this shows that oxidative deamination is contributing higher ammonia production. The high levels of ammonia produce is not eliminated but is salvaged through GDH activity which is utilize for aminoacid synthesis through transaminases (Deva, 2000; Prashanth, 2000; Prashanth and Neenagund, 2008). According to Nelson and Cox (2005) and Sathyanarayana (2005), increased GDH activity may indicate an increased rapid utilization of amino acids and onset of detoxification mechanism (Prashanth, 2006; Ganesh *et al.,* 2006). GDH activity was found to be elevated in almost all tissues treated with pesticides compared to control. This increased activity may have helped in funnelling more α-ketoglutarate into TCA cycle for more energy generation. This indicates higher oxidation of amino acids to combat the toxic effect of pesticide and the higher activity of GDH may result in efficicent operation of oxidative deamination under toxic effect of IMI and CZ (Kumar *et al.,* 2010). The oxidation of glutamate in kreb's cycle leads to increased energy (Narasimha and Ramana, 1985; Naveed *et al.,* 2010).

Glucose-6-phosphatase (G-6-Pase) is an enzyme which catalyses the reaction causing the hydrolysis of glucose-6-phosphate formed either through glycolysis or gluconeogenesis, to glucose and phosphate in a characteristic manner. G-6-Pase activity

showed a dose dependent decrease in both the fishes on exposure of IMI and CZ as compared to control (Table 3.12 and Fig 3.12). Since this enzyme plays a role in the final stage of gluconeogenesis, its physiological functions or properties merit attention. G-6-Pase thus plays a critical role in blood glucose homeostasis. One of the important functions of the liver and, to a lesser extent, of the kidney cortex is to provide glucose during condition of starvation. Glucose is formed form gluconeogenic precursor in both the tissues and in the liver also from glycogen. The increase in blood glucose concentrations is known as a general secondary response to stress of fish to acute toxic effects and is considered as a reliable indicators of environmental stress (Sepici-Dincel, 2009). Increase in glucose level in fish under stress is reported by Cicik and Engin (2005); Rathnamma *et al.,* (2008); Firat *et al.,* (2011). Hyperglycaemic response illustrated in the preset study is an indication of disruption in carbohydrate metabolism possibly due to enhance glucose-6-phosphatase activity, elevated breakdown of liver glycogen, or the synthesis of glucose from extra hepatic tissue proteins and amino acids. Pesticide exposure have shown to increase the glucose content in blood because of intensive glycogenolysis and the synthesis of glucose from extrahepatic tissue proteins and amino acids (Almeida *et al.,* 2001). Increase in blood glucose by pesticide treatement may indicate disrupted carbohydrate metabolism due to enhance breakdown of liver glycogen, possibly mediated by increasing adrenocorticotrophic and glucagone hormones and/or reduced insulin activity. Our observations in the present study are in agreement with earlier reported work of pesticide induced hyperglycemia in fresh water fishes (Das and Mukherjee, 2003; Firat *et al.,* 2011).

Hence, from the present investigation on biochemical alterations of the fishes exposed to two different agrochemicals it can be concluded that decrease glycogen, lipid and protein suggest that in the pesticide exposed fishes, there was extensive mobilization of glycogen, lipid and protein. On exposure of the agrochemicals gills, liver and kidney showed an elevated pyruvate level compared to control possibly du rot high rate of glycolysis which is the only energy producing pathway for the animal when it is under stress condition. Further, elevated LDH suggests that the final product of glycolysis – pyruvate was preferentially used to produce lactate. Lactate is an important gluconeogenic sunstrate which helps to cope with the high and rapid energy demand

under toxic stress. Elevation in AST and ALT in different tissues of *O.mossambicus and L.rohita* can be considered as a response to the stress induced by agro-chemicals to generate keto acids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and/or energy production necessary to meet access energy demand. An elevation of ALP activity suggests an increase in lysosomal mobilization and tissue necrosis due to toxicity of agro-chemicals. This increase also suggests the supply of phosphate group for energy metabolism. Elevated GDH activity may have helped in funnelling more α-ketoglutarate in to TCA cycle for energy generation. Hyperglycaemic response illustrated in the preset study is an indication of disruption in carbohydrate metabolism possibly due to enhance glucose-6-phosphatase activity, elevated breakdown of liver glycogen, or the synthesis of glucose from extra hepatic tissue proteins and amino acids. Pesticide exposure have shown to increase the glucose content in blood because of intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids. Thus, it may be deduced that the pesticide exposure is stressful to the fishes.

Chapter IV

Effects of agro-chemicals on antioxidant enzymes and lipid peroxidation in *Oreochromis mossambicus* and *Labeo rohita*

Agrochemicals in the form of Insecticides, herbicides and fungicides are used extensively throughout the world. In general they are playing a pivotal role in meeting the food, cotton fibre and tobacco demand of escalating population and control of vector-borne diseases. Although they furnish some benefits for crop, they entail a number of risks and problems. Pesticide misuse in various sectors of the agriculture often has been associated with health problems and environmental contamination worldwide (Soares *et al.,* 2003; Mancini *et al.,* 2005; Remor *et al.,* 2009). Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides, is one of the major reasons for the high incidence of pesticide poisoning in developing countries (Konradsen *et al.,* 2003; Hurtig *et al.,* 2003; Atreya, 2008).

Environmental factors of both natural and anthropogenic origins have been known to induce alteration of different magnitudes in the physiological and biochemical status of animals (Shilov, 1981;Vosyliene and Kazlauskiene, 1999). Therefore, biomarker parameter assessment is a means of environmental monitoring, with the advantage of providing quantitative response as valuable information on ecological relevance as well as on the acute/chronic adverse effects caused by water pollution (De la Torre, 2005). Alteration in the chemical composition of a natural aquatic environment, due to contact with hazardous substances like heavy metals, pesticides, and effluents from industries usually affect the behaviours, biochemistry, and physiology of the fauna including fish (Radhaiah *et al.,* 1987). Water is one of the most precious natural resources on earth, and it creates a wide range of benefits to humans, including fisheries, wildlife, agriculture, urban, industrial and social development (Allan and Flecker, 1993). However, the unregulated release of agricultural chemicals especially pesticides into water bodies have caused environmental problems to all classes of organisms in the aquatic habitat. The aquatic ecosystem is faced with the threat of biodiversity loss due to indiscriminate use of pesticides (Rahman *et al.,* 2002).

The application of environmental toxicology studies on non mammalian vertebrates is rapidly expanding, and for aquatic system, fish have become an indication for the evaluation of the effects of noxious compounds (Ervnest, 2004). Pesticides occupy a unique position among many chemicals which are encountered daily by man. They are deliberately added to the environment for the purpose of killing, injuring, or at times enhancing the development of some forms of life. Water pollution by pesticides is a serious problem to all aquatic fauna and flora. In aquatic environment, pesticides may also cause several physiological and biochemical defects in fishes (Vasanbhi, *et al.,* 1989). Contamination of water with these recalcitrant chemicals often results in bioaccumulation in fish and other biota, sometimes to biologically active levels. These chemicals have been suspected to be cancer-causing agents in fish and other aquatic organism (GESAMP, 1991). Residues of these toxic chemicals found in water, sediments, fish, and other aquatic biota can pose risk to organisms, predators, and humans. Pesticides at high concentrations are known to reduce the survival, growth, and reproduction of fish and produce many visible effects on fish (Rahman *et al.,* 2002; Joseph and Raj, 2010). Water pollution also is recognized globally as a potential threat to both human and other animal population, which interact with the aquatic environment (Biney *et al.,* 1987; Svensson *et al.,* 1995).

Toxicity data for IMI, a new group of insecticides for aquatic invertebrate are far from enough (Tomizawa and Casida, 2003; Beketov and Liess 2008; Pestana *et al.,* 2009; Barbee and Stout, 2009; Stoughton, 2010; Lukancic, 2010; Azevedo-Pereira, 2011; Malev *et al.,* 2012). CZ is a mixture of cymoxanil and mencozeb. Cymoxanil is reported to be slightly toxic to fish and other estuarine and marine organisms on an acute basis. Mancozeb is very toxic to aquatic life; goldfish, rainbow trout, catfish and carp (Reddy and Bashamohideen, 1989; Grande *et al.,* 1994; Haya, 1989). The process of physiological stress response starts from the moment the body realizes the presence of the stressor, followed by the sending of signals to the brain, and to the specific sympathetic and hormonal responses to eliminate, reduce or cope with the stress. A stressor is a stimulus that acts on a biological system and a stress response is the animal's reaction to the stimulus (Pickering 1981; Barton, 2002). According to general adaptation syndrome, a stress response consists of three stages: alarm, resistance and exhaustion (Pickering, 1981). Acute or short-term stress can have salubrious or adaptive

effects. In contrast, chronic or long-term stress is generally harmful (Wedemeyer and McLeay 1981; Teles *et al.,* 2007; Dorval *et al.,* 2005; Thangavel *et al.,* 2005).

Pesticide exposure can lead to oxidative stress (OS) through unregulated generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical, peroxyl radicals and singlet oxygen. ROS are produced during normal process in the cell. Under normal conditions antioxidant systems of the cell minimize damage caused by ROS. When ROS generation increases to an extent that it overcomes the cellular antioxidant systems, the result is oxidative stress. It is known that pesticides can cause oxidative stress, resulting in the generation of free radicals (Banerjee *et al.,* 1999). It is suspected that pesticides induce alterations in antioxidants or free oxygen radical scavenging enzyme systems. In addition, it is generally believed that lipid peroxidation is one of the molecular mechanisms involved in pesticide induced toxicity (Akhgari *et al.,* 2003).

A pesticide produces stress condition in any organism, including fish (Ateeq *et al.,* 2002). In Pisces, three different pathways are exhibited followed by pesticide like stressor exposure. The basic pathway followed is the activation of HPI axis and altered levels of cortisol. However, Major complications arise when the stressor is very effective and the body starts expressing other two mechanisms of stress response. They include lipid peroxidation (LPO) and expression of various antioxidant mechanisms like GST, CAT, SOD and GPx and scavengers such as GSH and ascorbic acid. Expression of these two mechanisms is the clear indication of pesticide toxicity, as well as the counter mechanisms exhibited by the organism. Hence, in this study we have tried to investigate alterations in these two important mechanisms due to pesticide exposure and how the antioxidant responses and cellular defences are triggered due to pesticide exposure (Miller, 2006). Several studies demonstrated that changes in the levels of antioxidant enzyme activities can be used as possible biomarkers in different aquatic organisms (Orbea *et al.,* 2002; Gohil, 2012). These enzymes are biomarkers of tissue damage, thus their bioassay can serve as a diagnostic tool for assessing the functions of liver (Coppo *et al.,* 2002; Parthiban and Muniyan, 2011).

However, the exact mechanism(s) of their action in fresh water fishes has still not been completely understood particularly for IMI and CZ. ***Hence, in the present work, an***

endeavour has been made to explore the mechanisms on pesticides induced OS, cellular events influenced by OS, in various key organs of pesticide exposed fishes. After establishing the haematological alterations as well as the biochemical parameters, it is worth exploring the effect of these agrochemicals on lipid peroxidation as well as their antioxidant defence mechanisms as this aspect of the toxicity data for this new group of insecticides for aquatic invertebrate are far from enough.

Materials and methods:

Experimental designs:

Freshwater teleosts, O. mossambicus and L. rohita of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies

Based on the result of the 48 h LC50, 30 tilapia fish were divided in 3 groups, 10 fish for each group:

- Group 1 served as control without any treatment of Agro-chemicals.
- Group 2 were treated with low dose of IMI and CZ (LC 50 / 10).
- Group 3 were treated with high dose of IMI and CZ (LC 50 / 20) for a period of 21 days.

Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests.

Preparation of the tissue samples for the study.

At the end of the experiment (21 days) the fish were carefully netted to minimize stress, and weighed. Prior to sacrificing the fish, about 1 - 2ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes. The blood was stored in -4°C in deep freezer prior to analysis. Fishes were sacrificed by pithing (damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle). Tissues such as liver, kidney, gills and muscle were carefully removed, wiped

thoroughly, using blotting paper to remove blood and other body fluids. Then they were washed in chilled PBS and again blotted dry. After noting the total weight of the tissues, the desired amount of the tissues were weighed and used.

Enzyme estimation:

Estimation of ascorbic acid: (Roe and Oesterling 1944)

Principle:

Ascorbic acid is converted to dehydro ascorbic acid by shaking it with norit.It is then coupled with 2,4-DNPH in presence of thiourea as mild reducing agent then converted into a red coloured compound which is assayed colorimetrically.

Reagents:

a. Standard ascorbic acid

b. 2,4- DNPH solution

c. 85% H2SO4, 6% TCA.

Procedure:

Homogenize the weighed tissue in 6% TCA. Add norit to it. shake well allow it to stand for 15 minutes. Filter with whatmann paper 42. The mixture containing 4ml homogenate followed by 1 ml and after addition of 4 drops of 2,4 DNPH and put it into water bath for 15 minutes. Lastly 5 ml of 85% H2SO4 added. Wait for 30 min and then read the absorbance at 540 nm against a blank containing all the reagents. A series of standards were run along with blank treated in a similar manner to determine the ascorbic acid content. Values were expressed as mg/ g wet tissue.

Assay of Redused Glutathione (GSH):

Total redused glutathione was estimated by the method os Ellman (1958).

Principle:

The Glutathione assay is a modification of the method first described by Tietze. The general thiol reagent, 5-5'dithiolbis(2-nitrobenzoic acid) (DTNB or ellman's reagent) react with GSH to form the 5 thionitrobenzoic acid (TNB) and Gs-TNB.

Reagents:

a. DTNB (0.6 Mm) in 0.2 M phosphate buffer (pH – 8.0)

b. TCA 5%

c. standard glutathione.

Procedure:

Precipitated protein in the homogenates of gills, liver, kidney and muscle with 0.1 ml 5% TCA and 0.4 ml distilled waer. Mixed the contents well for complete precipitation of proteins and centrifuged. To 0.5 ml clear supernatant, added 2.5 ml of 0.2 M phosphate buffer and 50 µl of DTNB. Read the absorbance at 412 nm against a blank containing all the reagents. A series of standards were run along with blank treated in a similar manner to determine the glutathione content. Values were expressed as nmoles/100 g wet tissue.

Assay of Superoxide Dismutase (SOD) (EC 1.15.1.1)

Superoxide dismutase in different tissues was determined using the method of Kakkar et al., (1984).

Principle:

The assay of SOD is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm.

Reagents:

a. 0.1 M PBS, n-butanol

b. 0.052 M sodium pyrophosphate buffer (pH 8.3)

c. 0.0025 M Tris-HCl buffer(pH 7.4)

d. 186 µM phenazine methosulpahte (PMS)

e. 300 µM Nitro blue tetrazolium (NBT)

f. 780 µM NADH and Glacial acetic acid.

Procedure:

Weighed samples of tissues were homogrnised in 0.1 M PBS and subjected to differential centrifugation under cold condition. The supernatant was used as the enzyme source. Assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT,1.3 ml of distilled water and 0.1 ml of the enzyme source. The tubes were kept at 30°C for 90 seconds and the reaction was stopped by the addition of 1 ml of glacial acetic acid. Reaction mixture was shaken vigorously with 4.0ml of n-butanol. The mixture was allowed to stand for 10 minutes and centrifuged. The upper butanol layer was removed. Absorbance of the chromogen in butenol was measured at 560 nm against n-butanol blank. A system devoid of enzyme served as control, one unit

of enzyme activity is defined as the enzyme concentration required to inhibit chromogen production by 50% in one minute under the assay conditions and specific activity is expressed as unit/ mg protein.

Assay of Catalase (CAT) (EC 1.11.1.6)

Catalyse level in different tissues was determined using the method of Maehly and Chance (1955).

Principal :This method is based on the fact that dichromate in acetic acid is reduced to the chromatic acetate .when heated in presence of hydrogen peroxide with formation of perchromic acid as unstable intermediate.the chromium acetate is measured colourimetically at 610 nm. The catalase preparation is allowed to spilt hydrogen peroxide at regular time interval and the reaction is stopped by addition of dichromatic acid .mixture of hydrogen peroxide liberated is determined colourimetrically.

Reagents:

a. 0.01M phosphate buffer (pH 7.0)

b.30 mM H_2O_2.

Procedure:

The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The reaction mixture contained 0.01 M phosphate buffer. 30 mM hydrogen peroxide and the enzyme extract prepared by homogenizing the tissue in phosphate buffer and centrifuging at 5000 rpm. Specific activity was expressed as international Units / mg protein. 1 IU = change in absorbance / min / extinction coefficient (0.021).

Assay of Glutathione Peroxidase (GPx) (EC 1.11.1.9)

Glutathione peroxidase in different tissues was estimated by the method of Rotruck et al., 1973.

Principal:

Glutathione Peroxidase catalyzes the reduction of an organic peroxide (ROOH), oxidizing reduced glutathione (GSH) to form (GSSG). The oxidized glutathione is then reduced by glutathione reductase (GR) and b-nicotinamide adenine dinucleotide phosphate (NADPH) forming NADP+ (resulting in decreased absorbance at 340 nm)

and recycling the GSH. Because GPx is limiting, the decrease in absorbance at 340 nm is directly proportional to the GPx concentration.

Reagents:

a. 0.4 M Tris buffer (pH 7.0)

b. 10 mM sodium azide solution.

c.10% Tricholoro acetic acid (TCA)

d. 0.4 mM Ethylene diamine tetra acetic acid (EDTA)

e. 0.2 mM Hydrogen peroxide (H_2O_2)

f. 2 mM glutathione solution (GSH).

Procedure:

Weighed samples of different tissues were homogenized in a known volume of tris buffer. To 0.2 ml of Tris buffer, 0.2 ml EDTA, 0.1 ml sodium azide and 0.5 ml tissue homogenate were added and mixed well. To this mixture 0.2 ml of GSH followed by 0.1 ml H2O2 solution were added. The contents were mixed and incubated at 37 ºC for 10 minutes along with a control containing all reagents except tissue homogenate. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10% TCA. Tubes were certrifuged and the supernatant was assayed for GSH. The value are expressed as µg of /min / mg protein.

Assay of glutathione-s-transferase (GST) (EC 2.5.1.18)

Glutathione-S-Transferase in different tissue was determined using the method of Beutler et al., (1986).

Principle:

Glutathione transferase catalyse the conjugation of 2,4, dinitrobenzene (DCNB) or 3,4 dichloronitrobenzene (DCNB) with reduce glutathione (GSH) to produce a yellow product that has a n absorbance maxima at 340-360 nm and rate of product formation, that indicate enzyme activity, can be calculated by following increasing aborbance at 340 nm.

Reagent

a. 0.5 M phosphate buffer (pH 6.5)

b. 25 mM of 1-chloro-2, 4- dinitrobenzene (CDNB) in 95% ethanol

c. 20 mM glutathione (GSH)

Procedure:

All the tissues were homogenized in 0.5 M phosphate buffer. The reaction mixture contained 200 µl phosphate buffer, 20 µl CDNB and 680 µl distilled water. Then the tubes were incubated at 37°C for 1 minutes and added 50 µl of GSH. After mixing well, added 50 µl of tissue extract to the tube. Increase in absorbance was noted at 340 nm for 5 minutes in a UV-visible spectrophotometer. Values are expresses in µmoles of CDNB complexed / min / mg protein. The extinction coefficient between CDNB-GSH conjugate is 9.6 m/M/cm.

Assay of Lipid peroxidase (LPO)

LPO was estimated by the method of Niehaus and Samuelson, 1958

Principal: Lipid peroxide leads to formation of an endoperoxide that is malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) and gives thiobarbituric acid reactive substance (TBARS). TBARS gives a characteristic pink colour that can be measured caloriemetrically at 532nm.

Reagents:

TCA-TBA-HCl reagent: 15% (W/V) Trichloro acetic acid, 0.375% (W/V) Thiobarbituric acid (TBA) in 0.25 N HCl. 0.1 M Tris-HCl buffer (pH 7.5).

Procedure:

The tissue homogenate of different tissues were prepared in Tris-HCl buffer and was combined with thiobarbituric acid reagent and mixed thoroughly and heated for 15 minutes in a boiling water bath. It was then cooled and centrifuged for 10 minutes at 600 g. The absorbance of the sample was read spectrophotometrically at 535 nm against a reagent blank that contained no tissue extract. The extinction coefficient for malondialdehyde is 1.56×10^5/ M/ cm. The values are exptresses as millimoles / 100g wet wt of tissue.

Statistical Analysis:

The statistical analysis was carried out using the software Graph pad prism 5 package. For determining the significant difference between different treatments in biochemical parameters, Two-way ANOVA followed by Tukey's test for multiple comparisons between different concentration of IMI and CZ was done. Significance level (P value) was set at 0.05 in all tests

Result:

Ascorbic acid:

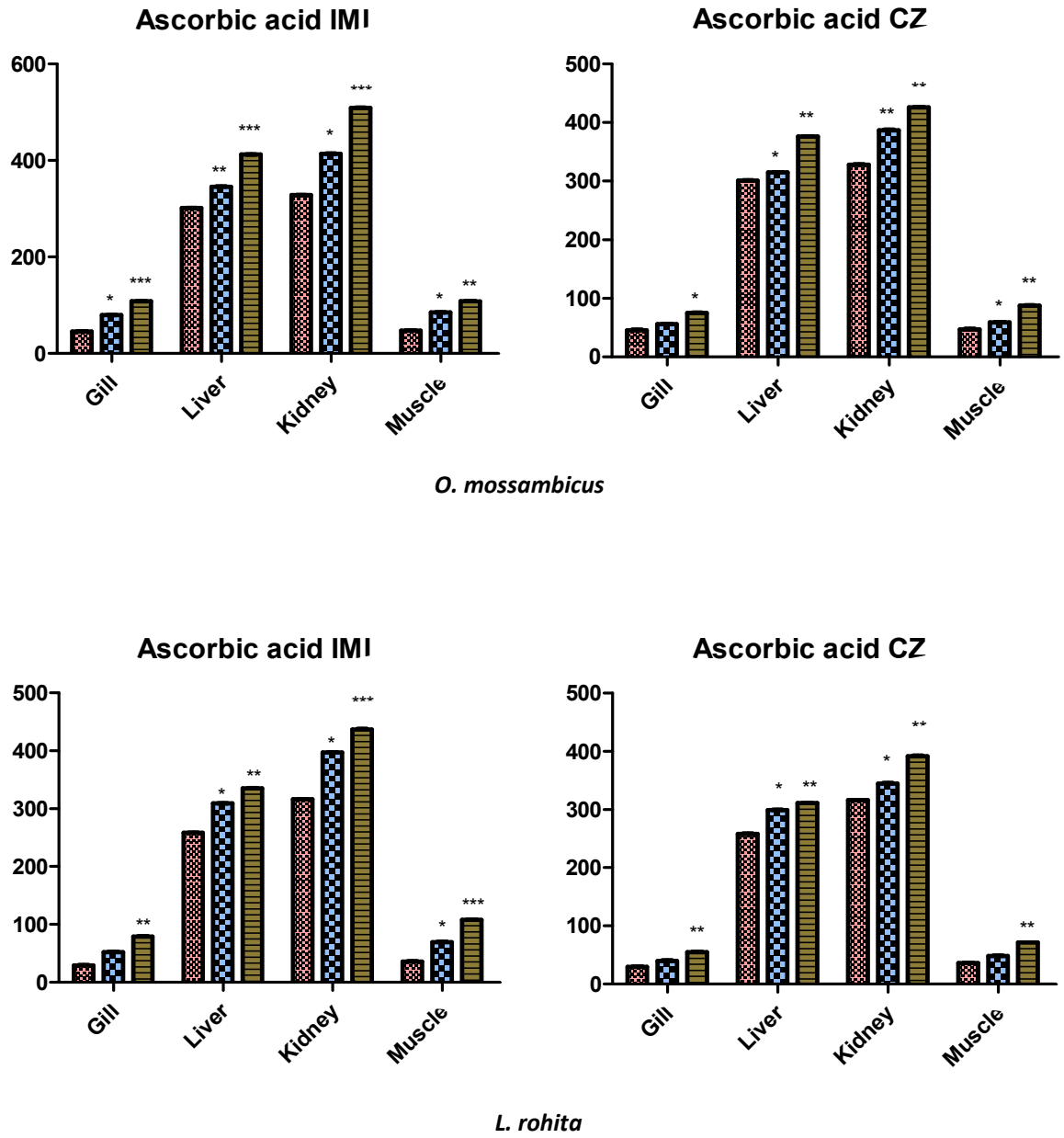
Two-factor ANOVA followed by Tukey's test showed that there was significant ($P<0.05$), variation in ascorbic acid content in both the fishes expose to IMI and CZ. There was statistically significant ($P<0.05$) different changes in the ascorbic acid level between the treated groups and the control. All the tissues, gills, liver, kidney and muscle showed significantly ($P<0.05$) elevated activity compared to control.

Table: 4.1 Effect of IMI and CZ on SOD activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	Ascorbic acid						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	29.23 ±0.401	52.000 ±0.537*	79.00 ±0.451***	29.23 ±0.401	39.89 ±0.550	54.780 ±0.577*
	Liver	258.00 ±0.491	309.000 ±0.389**	335.00 ±0.423***	258.00 ±0.491	299.00 ±0.306*	311.000 ±0.339**
	Kidney	316.00 ±0.462	397.000 ±0.397*	437.00 ±0.741***	316.00 ±0.462	345.00 ±0.787*	392.000 ±0.577**
	Muscle	36.00 ±0.446	69.500 ±0.451*	108.00 ±0.445**	36.00 ±0.446	48.00 ±0.889*	71.340 ±0.026**
L.rohita	Gills	45.23 ±0.89	79.000 ±0.537	108.00 ±0.451**	45.23 ±0.89	56.00 ±0.331	74.800 ±0.429**
	Liver	301.00 ±0.436	345.000 ±0.568*	412.00 ±0.885**	301.00 ±0.436	315.00 ±0.306*	376.000 ±0.427**
	Kidney	328.00 ±0.443	414.000 ±0.258*	509.00 ±0.030***	328.00 ±0.443	387.00 ±0.787*	426.000 ±0.532**
	Muscle	47.00 ±0.787	85.000 ±0.210*	108.00 ±0.613***	47.00 ±0.787	59.00 ±0.357	87.401 ±0.881**

- Values are expressed as mg/ g wet tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Figure: 4.1 Effect of IMI and CZ on SOD activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



Total reduced Glutathione (GSH)

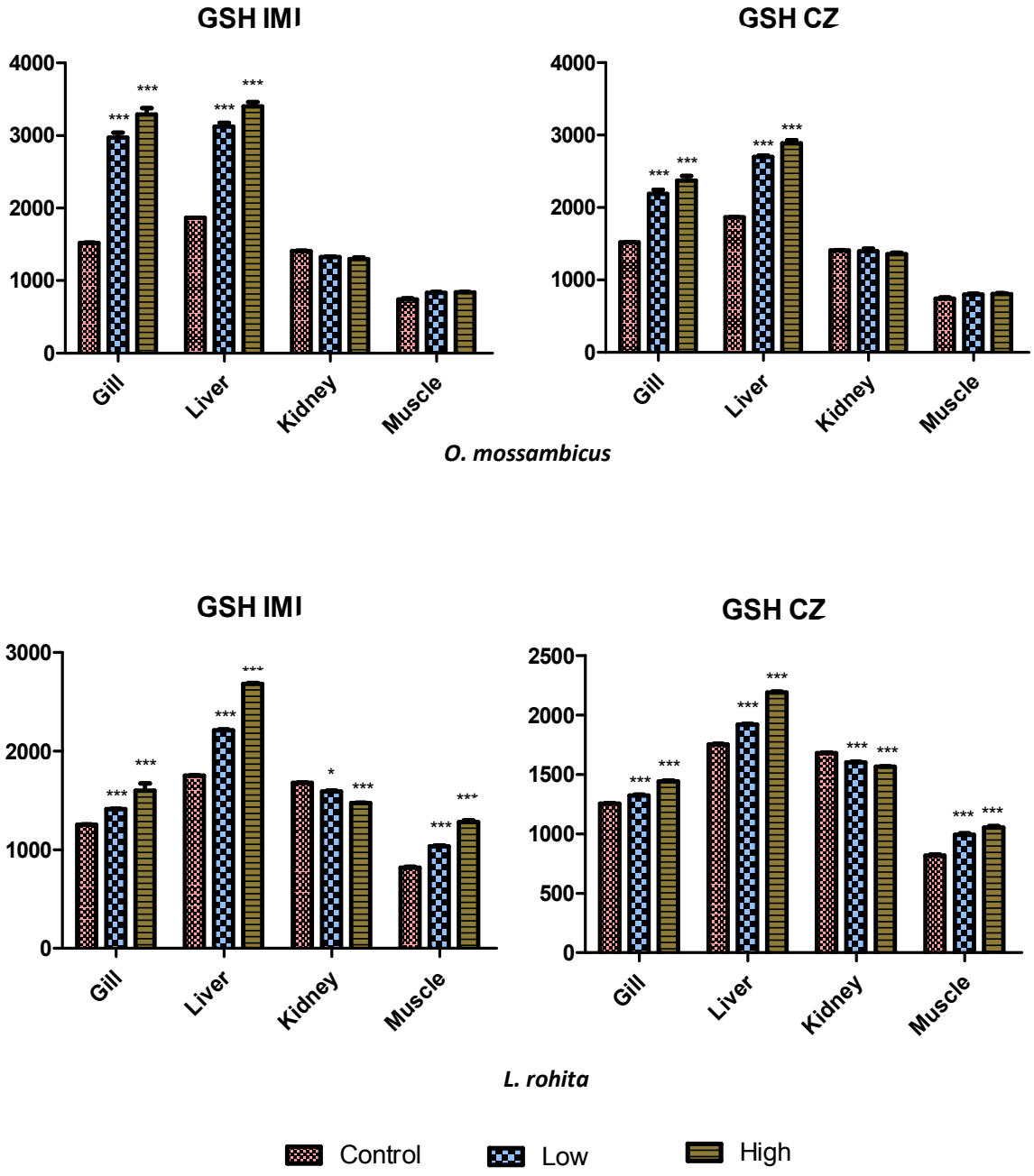
Two-factor ANOVA followed by Tukey's test showed that there was significant ($P<0.05$), variation in total reduced glutathione content between treated groups and between tissues treated with IMI and CZ. There was statistically significant ($P<0.05$) different changes in the GSH level among the treated groups compared to control. Among the tissues, gills, liver and muscle showed significantly ($P<0.05$) elevated activity compared to control but the kidney in both the treated groups showed statistically significant ($P<0.05$) reduced activity compared to control.

Table: 4.2 Effect of IMI and CZ on GSH activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	GSH						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	1532 ±0.985	2972 ±1.568***	3289 ±4.01***	1532 ±0.985	2189 ±1.88***	2376 ±2.01***
	Liver	1872 ±0.938	3121 ±2.48***	3401 ±3.87***	1872 ±0.938	2698 ±1.56***	2888 ±2.359***
	Kidney	1413 ±0.755	1324 ±3.872	1298 ±5.65	1413 ±0.755	1399 ±2.055	1356 ±2.95
	Muscle	742 ±0.564	832 ±0.788	857 ±1.992	742 ±0.564	798 ±2.44	806 ±3.54
L.rohita	Gills	1253 ±2.67	1410 ±2.69***	1598 ±7.21***	1253 ±2.67	1321 ±6.56***	1441 ±7.75***
	Liver	1751 ±3.89	2212 ±7.26***	2681 ±9.004***	1751 ±3.89	1919 ±5.02***	2189 ±6.43***
	Kidney	1678 ±4.26	1591 ±6.98*	1473 ±3.34***	1678 ±4.26	1601 ±3.98***	1563 ±3.78***
	Muscle	818 ±0.011	1037 ±5.71***	1281 ±18.29***	818 ±0.011	992 ±11.56**	1053 ±12.98***

- Value are expressed as mg / g wet wt of tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Figure: 4.2 Effect of IMI and CZ on GSH activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



Glutathione peroxidase (GPx):

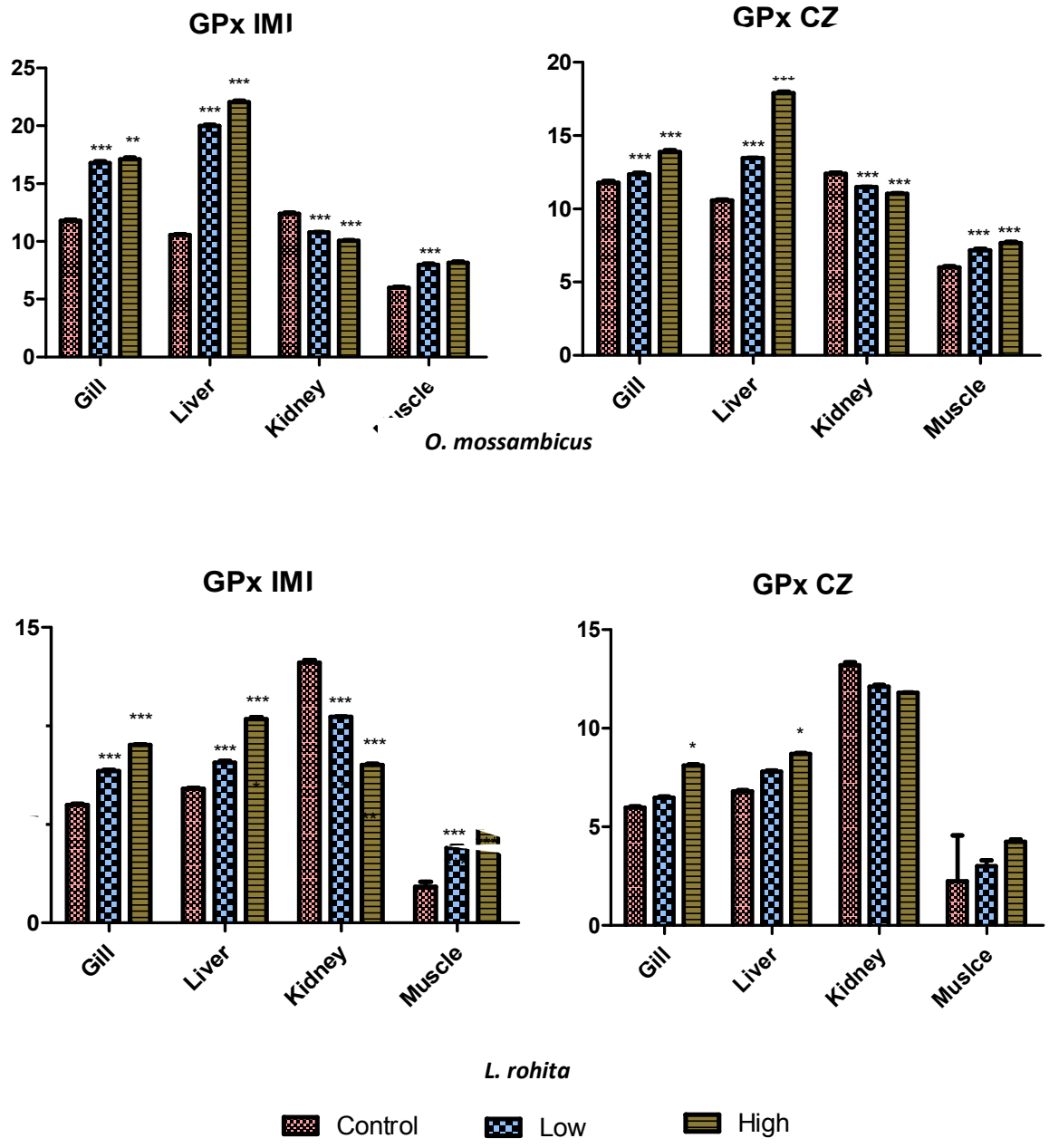
Glutathione peroxidase activity showed an overall significant change ($P<0.05$) in experimental groups of animal compared to control. Tukey's test showed significant difference between agro-chemical treated groups compared to control. Increased activity in liver and kidney of the treated groups compared to control. Whereas gills treated with IMI and CZ showed a decreased GPx activity compared to control. On treatment with both Curzate and Imidacloprid muscle showed a significantly ($P<0.05$) elevated activity compared to control.

Table: 4.3 Effect of IMI and CZ on GPx activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	GPx						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	11.78 ±0.103	16.76 ±0.129***	17.11 ±0.154***	11.78 ±0.103	12.36 ±0.102***	13.87 ±0.119***
	Liver	10.58 ±0.057	19.98 ±0.106***	22.06 ±0.124***	10.58 ±0.057	13.46 ±0.028***	17.91 ±0.084***
	Kidney	12.39 ±0.091	10.79 ±0.030***	10.07 ±0.054***	12.39 ±0.091	11.48 ±0.024***	11.02 ±0.048***
	Muscle	6.01 ±0.062	7.99 ±0.089***	8.16 ±0.100	6.01 ±0.062	7.18 ±0.072***	7.66 ±0.079***
L.rohita	Gills	5.97 ± 0.068	7.71 ±0.062***	9.03 ±0.033***	5.97 ± 0.068	6.47 ±0.054	8.12 ±0.053*
	Liver	6.80 ±0.046	8.13 ±0.084***	10.34 ±0.099***	6.80 ±0.046	7.80 ±0.052	8.69 ±0.048*
	Kidney	13.21 ±0.128	10.45 ±0.005***	8.01 ±0.051***	13.21 ±0.128	12.12 ±0.077	11.80 ±0.005
	Muscle	2.24 ±2.32	3.81 ±0.114***	5.72 ±0.008***	2.24 ±2.32	3.01 ±0.296	4.23 ±0.126

- Value are expressed as µg of GSH/min/mg protein.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Figure: 4.3 Effect of IMI and CZ on GPx activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



Glutathione-S-transferase (GST)

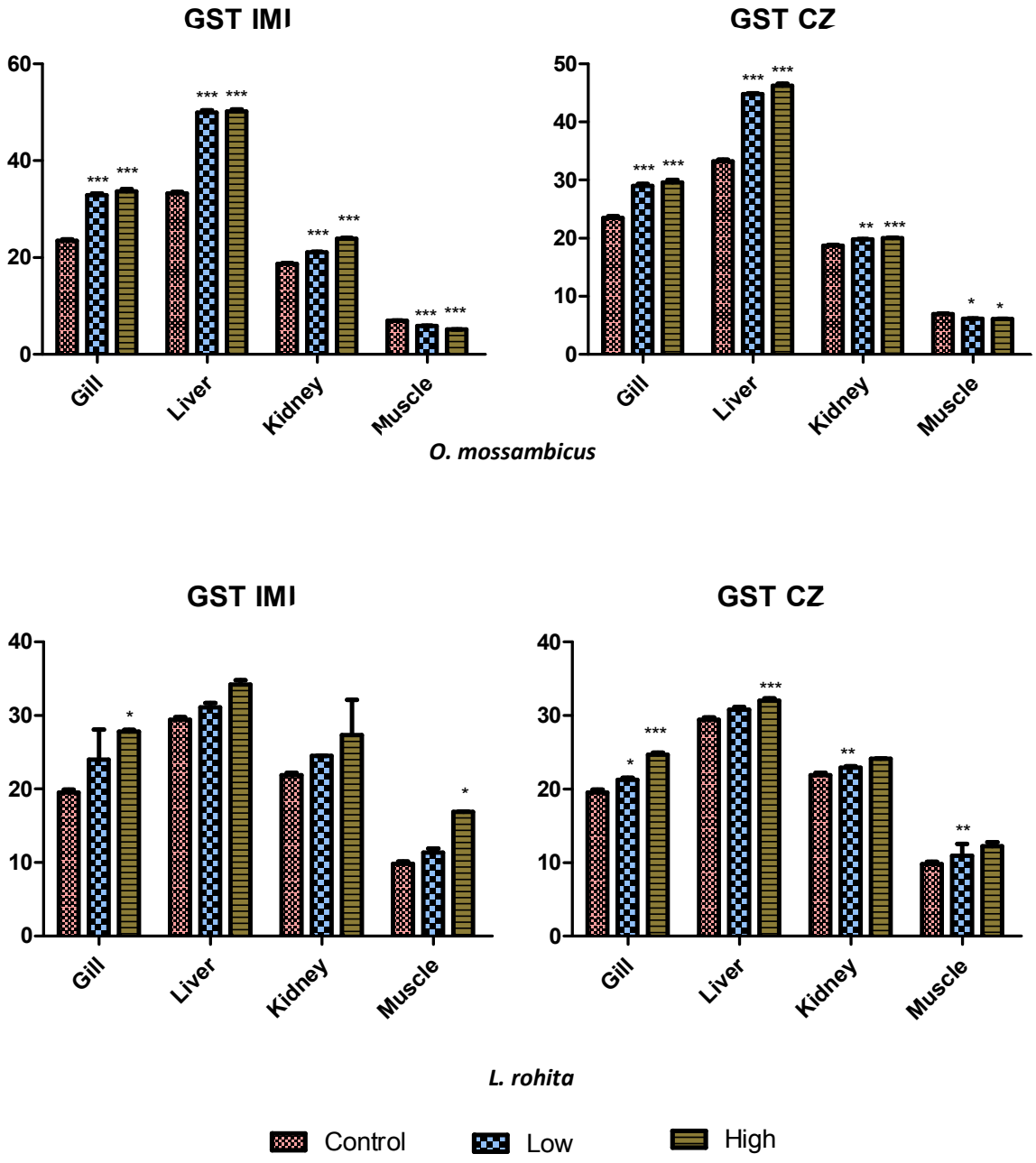
In the present study, glutathione-s-transferase activity in different tissues of *O.mossambicus* and *C.mrigala* treated with different pesticides showed significant variations (P<0.05), compared to control group. Tukey's test showed significant difference among the pesticides treated groups and also with the control. Among the tissues treated with different pesticides highest GST activity was seen in liver. Both kidney and muscle showed significantly (P<0.05) decreased GST activity compared to control. Significant differences were found in GST activity between the Curzate and Imidacloprid treated groups and also with the control.

Table: 4.4 Effect of IMI and CZ on GST activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	GST					
		IMI			CZ M8	
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	23.48 ±0.309	32.89 ±0.377***	33.68 ±0.453***	11.78 ±0.103	12.36 ±0.102***	13.87 ±0.119***
	Liver	33.23 ±0.304	49.98 ±0.426***	50.18 ±0.442***	33.23 ±0.304	44.81 ±0.162***	46.27 ±0.353***
	Kidney	18.69 ±0.184	21.09 ±0.088***	23.890 ±0.204***	18.69 ±0.184	19.810 ±0.061**	19.98 ±0.128***
	Muscle	6.93 ±0.081	5.89 ±0.042***	5.10 ± 0.025***	6.93 ±0.081	6.12 ±0.051*	6.08 ±0.046*
L.rohita	Gills	19.53 ±0.380	23.98 ±4.108	27. 83 ±0.171*	19.53 ±0.380	21.24 ±0.301*	24.69 ±0.270***
	Liver	29.41 ±0.349	31.12 ±0.561	34.21 ±0.558	29.41 ±0.349	30.78 ±0.360	32.01 ±0.307***
	Kidney	21.87 ±0.299	24.48 ±0.019	27.31 ±4.81	21.87 ±0.299	22.91 ±0.216**	24.12 ±0.016
	Muscle	9.89 ±0.306	11.34 ±0.547	16.89 ±0.040*	9.89 ±0.306	10.94 ±1.609**	12.21 ±0.578

- Values are expressed in µmoles of CDNB complexed /min/mg protein,
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 4.4 Effect of IMI and CZ on GST activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



Superoxide dismutase:

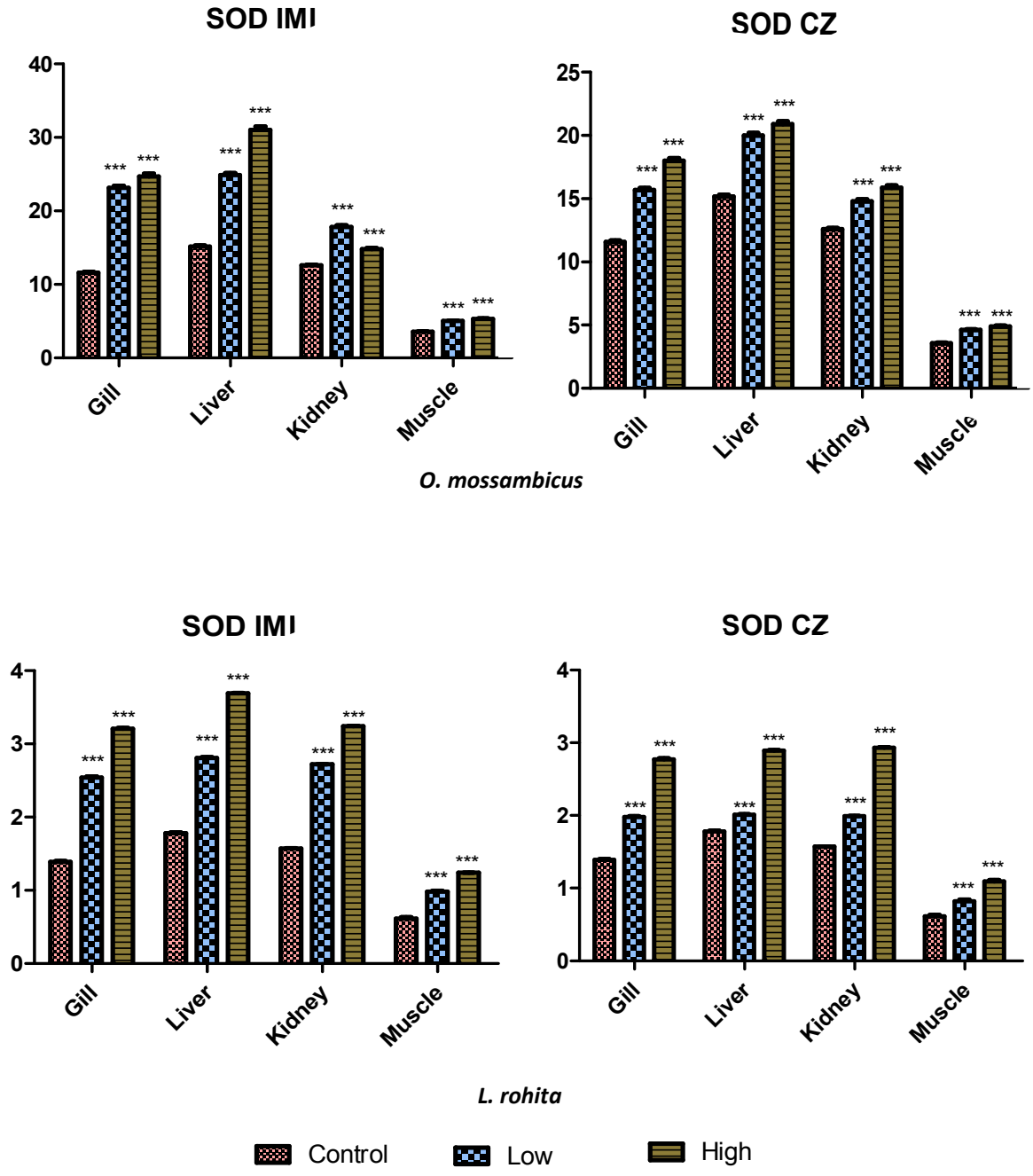
Two-factor ANOVA followed by Tukey's test showed that there was significant ($P<0.05$), SOD activity was found to be significantly ($P<0.05$) elevated in gills, liver and kidney of *O.mossambicus* treated with Curzate compared to control and among these tissues liver showed the maximum activity, whereas the fishes treated with Imidacloprid showed significantly elevated acivity in liver, kidney and muscle compared to control. A significantly ($P<0.05$) decreased activity compared to control was shown by gills treated with Imidacloprid and muscle treated with Curzate.

Table: 4.5 Effect of IMI and CZ on SOD activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	SOD						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	11.58 ±0.100	23.11 ±0.301***	24.68 ±0.369***	11.58 ±0.100	15.67 ±0.171***	17.99 ±0.212***
	Liver	15.16 ±0.132	24.84 ±0.302***	31.01 ±0.457***	15.16 ±0.132***	19.98 ±0.217***	20.89 ±0.233***
	Kidney	12.57 ±0.109	17.83 ±0.302***	14.79 ±0.148***	12.57 ±0.109***	14.79 ±0.148***	15.86 ±0.166***
	Muscle	3.560 ±0.028	5.010 ±0.052***	5.290 ±0.059***	3.560 ±0.028	4.62 ±0.045***	4.890 ±0.050***
L.rohita	Gills	1.390 ±0.010	2.54 ±0.014***	3.21 ±0.010***	1.39 ±0.010	1.98 ±0.013***	2.77 ±0.014***
	Liver	1.78 ±0.010	2.81 ±0.010***	3.69 ±0.010***	1.78 ±0.010	2.01 ±0.008***	2.89 ±0.010***
	Kidney	1.57 ±0.007	2.72 ±0.006***	3.24 ±0.005***	1.57 ±0.007	1.99 ±0.006***	2.93 ±0.007***
	Muscle	0.61 ±0.022	0.98 ±0.016***	1.24 ±0.003***	0.61 ±0.022	0.82 ±0.011***	1.09 ±0.023***

- Values are expressed as units/mg protein. One unit is defind as the amount of enzyme which gives 50% inhibition of formazon formation/minute.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Figure: 4.5 Effect of IMI and CZ on SOD activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



Catalase:

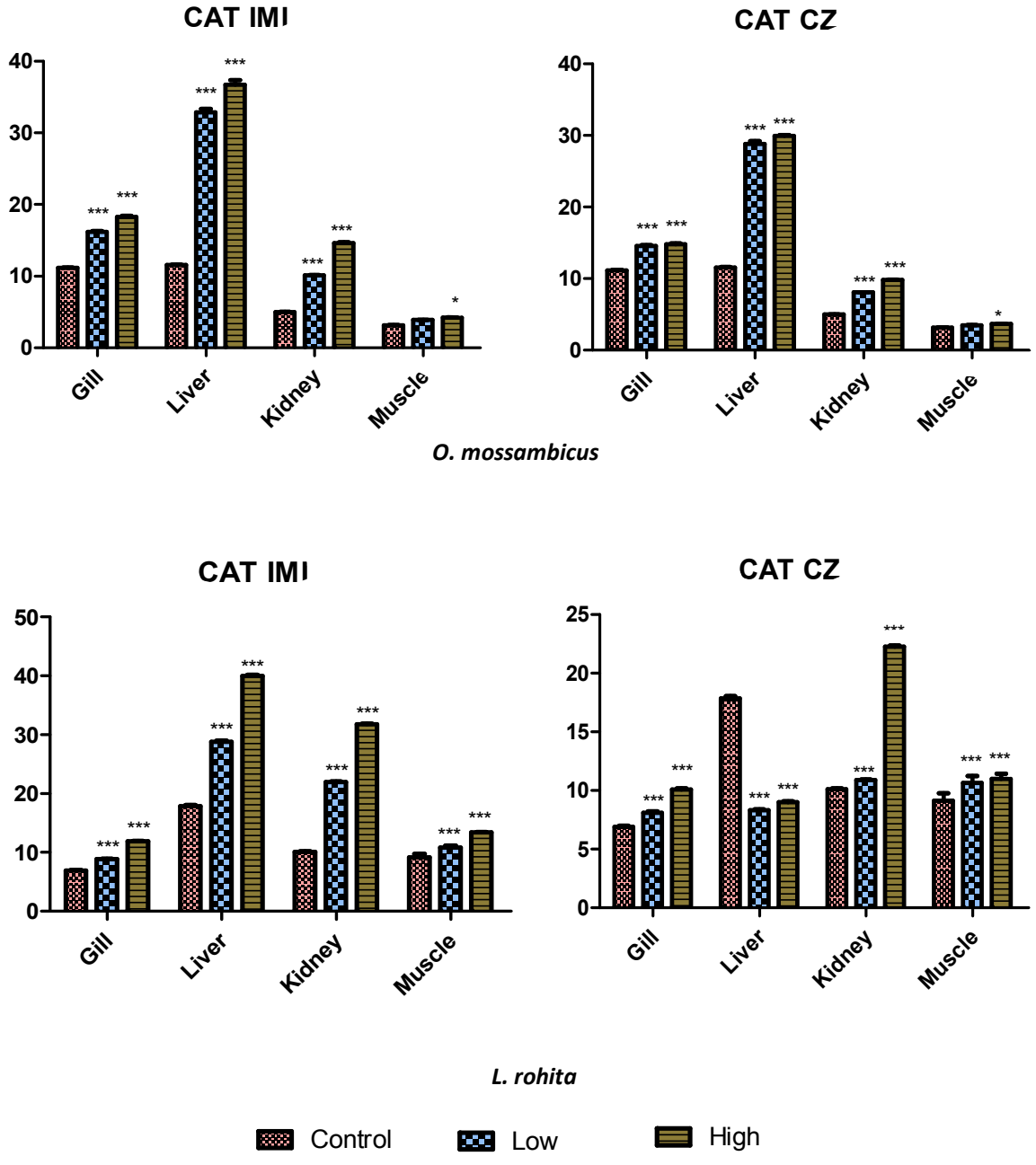
In the present study catalyse activity in different in different tissues of *O.mossambicus* treated with different pesticides showed significant variations (P<0.05), compared to control group. Tukey's test showed significant difference between pesticides treated groups and also with the control. On treatment with both Curzate and Imidacloprid gills, liver and kidney showed significantly elevated CAT activity compared to control. Comparison between groups treated with different pesticides revealed that there was significant increase (P<0.05) in CAT activity in all tissues compared to control except in muscle. Muscle showed a statistically significant decreased activity compared to control.

Table: 4.6 Effect of IMI and CZ on CAT activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

CAT							
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	11.14 ± 0.092	14.54 ± 0.146***	14.78 ± 0.136***	11.14± 0.092	14.54 ± 0.146***	14.78 ± 0.136***
	Liver	11.54 ± 0.069	32.84 ± 0.497***	36.71 ± 0.626***	11.54± 0.069	28.79 ± 0.409***	29.92 ± 0.435***
	Kidney	4.980 ± 0.023	10.12 ± 0.026***	14.61 ± 0.144***	4.98 ± 0.023	8.08 ± 0.013***	9.80 ±0.039***
	Muscle	3.160 ± 0.022	3.89 ± 0.028	4.210 ± 0.034*	3.16 ± 0.022	3.48 ± 0.023	3.670 ± 0.025*
L.rohita	Gills	6.910 ± 0.094	8.87 ± 0.084***	11.88 ± 0.064***	6.91 ± 0.094	8.12 ± 0.091***	10.09 ±0.087***
	Liver	17.87 ± 0.192	28. 79 ± 0.194***	39.98 ± 0.206***	17.87± 0.192	8.32 ± 0.060***	9.02 ±0.052***
	Kidney	10.12 ± 0.080	21.98 ± 0.098***	31.75 ± 0.102***	10.12± 0.080	10.89 ± 0.063***	22.28 ±0.095***
	Muscle	9.130 ±0.650	10.81 ±0.340***	13.44 ±0.053***	9.13 ±0.650	10.65 ±0.584***	10.99 ±0.453***

- Value are expressed as IU/change in absorbance at 230 nm/min, Extinction Coefficient = 0.021.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * (P<0.05); **(P<0.01);***(P<0.001)

Figure: 4.6 Effect of IMI and CZ on CAT activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



Lipid peroxidation (LPO):

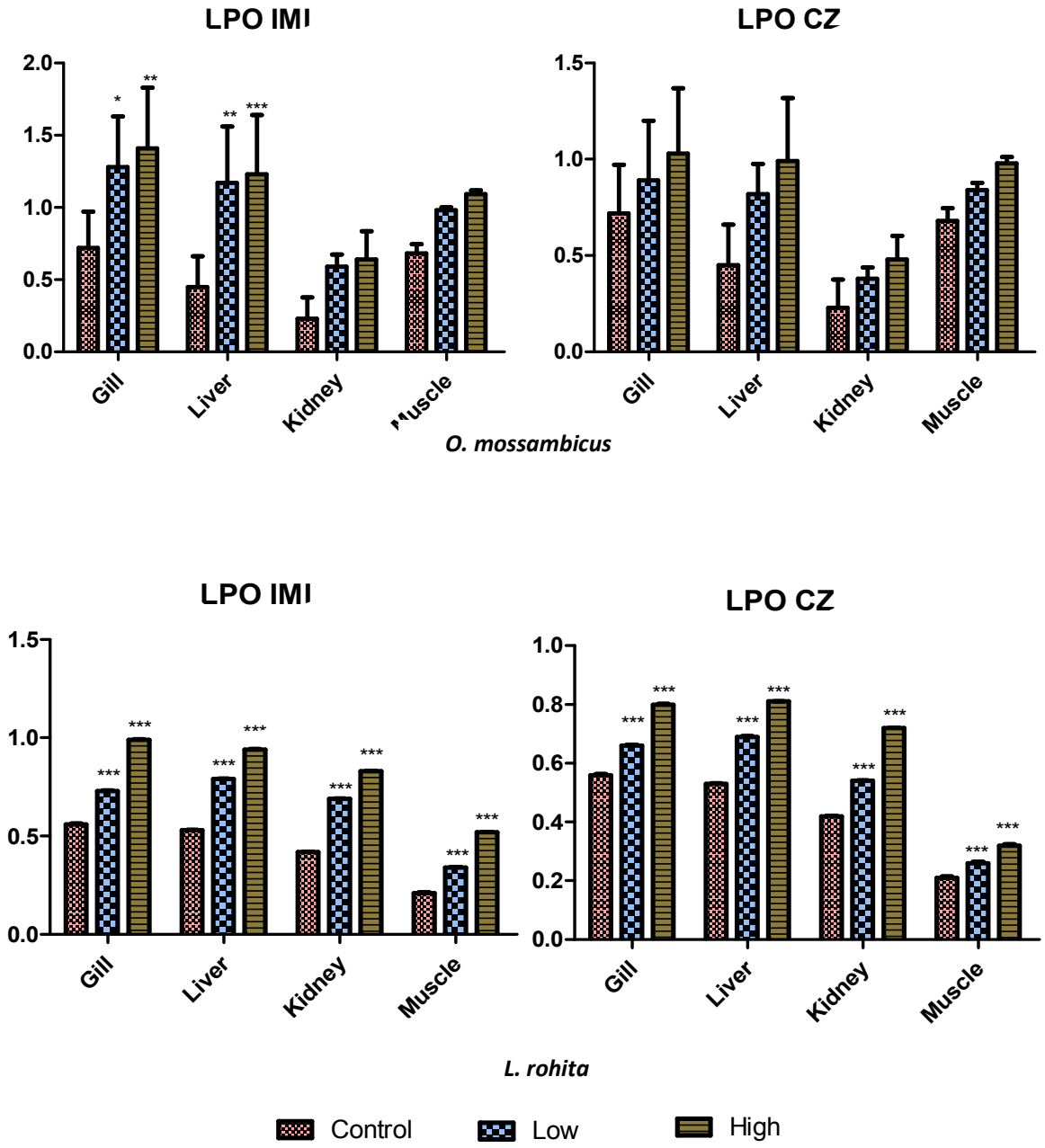
In the present study LPO level in different in different tissues of *O.mossambicus* treated with different pesticides showed significant variations ($P<0.05$), compared to control group. Tukey's test showed significant difference between pesticides treated groups and also with the control. Highest LPO level was found in the gill of fishes treated with Imidacloprid. On treatment with both Curzate and Imidacloprid liver, kidney and muscle showed significantly elevated LPO level compared to control (Table 4.5 and Fig 4.5)..

Table: 4.7 Effect of IMI and CZ on glucose-6-phosphatase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.

	LPO						
		IMI			CZ M8		
	Tissues	C	LD	HD	C	LD	HD
O.mossambicus	Gills	0.72 ±0.251	1.28 ±0.349*	1.41 ±0.338**	0.72 ±0.251	0.89 ±0.309	1.03 ±0.338
	Liver	0.45 ±0.0210	1.17 ±0.390**	1.23 ±0.409***	0.45 ±0.210	0.82 ±0.155	0.99 ±0.328
	Kidney	0.145 ±0.032	0.59 ±0.083	0.064 ±0.194	0.145 ±0.32	0.38 ±0.058	0.48 ±0.122
	Muscle	0.68 ±0.066	0.98 ±0.021	1.09 ±0.026	0.68 ±0.066	0.84 ±0.037	0.98 ±0.031
L.rohita	Gills	0.559 ±0.003	0.73 ±0.002***	0.99 ±0.002***	0.559 ±0.003	0.66 ±0.003***	0.799 ±0.002***
	Liver	0.529 ±0.002	0.79 ±0.006***	0.93 ±0.001***	0.529 ±0.005	0.69 ±0.002***	0.81 ±0.003***
	Kidney	0.419 ±0.001	0.69 ±0.001***	0.829 ±0.001***	0.419 ±0.001	0.54 ±0.001***	0.719 ±0.001***
	Muscle	0.209 ±0.004	0.339 ±0.003***	0.519 ±0.001***	0.209 ±0.004	0.26 ±0.002***	0.319 ±0.005***

- Values are expressed as mmoles MDA liberated/ 100g wet tissue.
- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Figure: 4.7 Effect of IMI and CZ on glucose-6-phosphatase activity (mean ± SEM) in *O.mossambicus* and *L.rohita*.



Discussion:

The effects of agro-chemicals in the form of insecticide (IMI) and fungicide (CZ) in the previous chapters have proved their toxicity on exposure of sub-lethal concentrations by way of showing alterations in their haematological and biochemical profile. Hence, in the present study an attempt is made to find out the modus operandi of detoxification. Detoxification path at tissue level can be detected by biochemical markers of OS (Van der Oost *et al.,* 2003).

The first line of defence to oxidative stress is the use of antioxidant scavengers, such as ascorbic acid (vitamin C), vitamin E, uric acid, carotenoid and glutathione. In the present study the response of the antioxidant scavengers (ascorbic acid and GSH) are presented in Table 4.1 and 4.2 and Fig 4.1 and 4.2. The second line of defence includes cellular mechanism which helps in removing excess ROS and avoids oxidative damage. It includes GPx, GST, CAT and SOD. The altered response of the enzymatic defense is presented in Table: 4.3 to 4.6 and Fig: 4.3 to 4.6.

In the present study agrochemical stress has significantly increase ascorbic acid content in liver, kidney and gills (Table 4.1 and Fig 4.1). Ascorbic acid content plays an important role in detoxification of the foreign bodies or toxicants in metabolic process. Ascorbic acid is an important water soluble antioxidant in biological fluid and essential micronutrient required for normal metabolic functioning. It acts as a biological reducing agent for hydrogen transport. It neutralizes reactive oxygen molecules and reduces oxidative DNA damage and genetic mutations (Frei, 1994). Furthermore, ascorbic acid also protects host cells against harmful oxidants released into the extracellular medium (Ray and Husain, 2002). The free metal ion-independent protein oxidation in cells is exclusively prevented by ascorbic acid. A recent review highlighted the bioregulatory rate of ascorbic acid to protect extracellular protein function through gene expression (Griffiths and Lunec, 2001). Ascorbic acid is necessary for the synthesis of collagen; growth and maintained of epithelial tissues. It can act as a hydrogen carrier. It may have an essential role in the metabolism of protein; fats and carbohydrates. The utilization of these components for production of energy, due to stress condition involves active

synthesis of new substances and a number of anabolic and catabolic reactions. These reactions may require ascorbic acid since it is known to act as a cofactor for many oxidations reduction reactions. Due to its anti-oxidant role and as a part of redox buffer system increased ascorbic acid is probably inhibiting the oxidative metabolism and preventing the production of electrophilic metabolites and is able to scavenge harmful free radical metabolites/ ROS (Sato *et al.,* 1990; Guha and Khuda-Bakhsh, 2002). Hence, the high level of ascorbic acid observed in the present study as agro-chemical induced stress condition is justifiable. The indication to detoxifying enzymes is reported to be accompanied by increase in ascorbic acid content of liver, kidney and gills, which stimulate detoxification of toxicant, suggestive of liver, kidney and gills to be the sites of detoxification. Our results are in agreement with earlier reported elevated ascorbic acid content in *Channa gachua* (Ali and Ilyas, 1981); in *Oreochromis mossambicus* (Guha and Khuda-Bukhsh, 2001); in *Clarias batrachus* (Kamble *et al.,* 2001; 2011) and *Puntius ticto* (Ganeshwade, 2011).

Glutathione is a tri peptide that is mainly present in cells in its reduced form (GSH), which basically acts as an intracellular reductant and neucleophile (Vardharajan, 2010). It functions in the synthesis of proteins and DNA, amino acid transport, maintenance of thiol – disulphide status, free radical scavenging, signal transduction, as an essential co-factor of several enzymes, as a non toxic storage form of cysteine, and as a defence against oxidizing molecules and potentially harmful pesticides (Di Mascio *et al.,* 1991; Kelly *et al.,* 1998; Pena-Llopis *et al.,* 2001; Dorval and Hontela, 2003; Elia *et al.,* 2003). In the present study there was a significant increase in the GSH activity in liver, kidney and gills on exposure of agrochemicals in a dose dependent manner (Table 4.2 and Figure 4.2). Among the tissues GSH level was found to be highest in the liver compared to other tissues which may be due to an adaptive mechanism to oxidative stress in its synthesis which can be provided for the increased GSH activity. However, a depletion of GSH was observed in kidney which illustrates that severe oxidative stress may have suppressed GSH levels due to loss of adaptive mechanisms and the oxidation of GSH to GSSG. During scavenging the ROS, GSH is oxidized and forms glutathione-protein mixed disulphides; hence, the ability of cell to reduce or synthesize GSH is the key to how effectively the cell can manage the oxidative stress. Total glutathione will be a protective biological index to indicate exposure to pesticides (Stein *et al.,* 1992). Due

to its function in resisting the reactive oxygen toxicity, the changing degree for total glutathione can serve as markers of exposure to pollutants which disturb the piscine oxyradicals (Vardharajan, 2010).

The second cellular mechanism to remove excess ROS and avoid oxidative damage is maintained through enzymatic defence strategy. These enzymatic defences include glutathione peroxidase (GPx), glutathione-s-transferase (GST), catalase (CAT) and superoxide dismutase (SOD). GPx activity showed an overall significant change in agrochemical exposed group of fishes (Table 4.3 and Fig 4.3) compared to control. GPx level was found to be increased in gills and liver which might be because of the induction, as in the case of any other defensive antioxidant enzyme. GPx is an enzyme with peroxidase activity and broad substrate spectrum (Lushchak, 2012). This enzyme is known to protect the fish from the damage caused by H_2O_2 and reduces it to lipid hydroperoxides (Flora *et al.,* 2008; Vinodhini and Narayana, 2009; Banaee, 2013). An increase in GPx activity in liver, kidney and gills is probably eliminating the access of H_2O_2 and lipid hydrogen peroxide produced in the fishes exposed to agro-chemicals. Similar results have been observed in the liver of *Cyprinus Carpio* (Li *et al.,* 2003; Vinodhini and Narayana, 2009); *Rainbow trout* (Orun *et al.,* 2003). Liver is a main detoxification organ of the fish for ingested of agro-chemical showing an adaptation to oxidative conditions and is seen reflecting the organotropism effect (Lenartova *et al.,* 1997; Mieiro *et al.,* 2011). Increase GPx activity have also been observed in kidney of *S.senegalensis* (Velma and Tchounwou, 2011; Oliva *et al.,* 2012), in *C.batracus* (Bhattacharya and Bhattacharya, 2007) and in *O.mossambicus* (Basha and Rani, 2003). As proposed by Tsangaris *et al.,* (2007), GPx is not only an important component of antioxidant defence system but its response is known to be accompanied by the action of other anti oxidants (GST) and Scavenger (GSH) molecules. Tissue specific increase of GPx in the present study indicates the adaptive approach by the fish to defend the OS, generated as a consequents of agro-chemical exposure and that the increased production of H_2O_2 due to OS is thus scavenged by the enzyme GPx. Elevated GPx activity also indicates that the regulation of ROS generated due to agro-chemicals is efficiently achieved by GSH pathway.

GST is one of the major phase II, GSH-dependent ROS- an electrophilic xenobiotic detoxifying enzyme (Meierjohann *et al.,* 2002; Comakli *et al.,* 2011) by making the xenobiotic chemicals more hydrophilic for transportation or excretion (Egaas *et al.,* 1993). This enzyme differs from the other enzymes and antioxidants in that it catalyses the direct conjugation of GSH with an offending toxicant (Ahmad, 1995). When severe oxidative damage prevents the primary antioxidants (mentioned above) from functioning GST can still remove the harmful substance, allowing the cell to regain homeostasis (Perl-Treves and Perl, 2002). In animals the toxicant conjugate is marked for excretion, GST is therefore considered a 'detoxification enzyme' rather than a traditional antioxidant (Ahmad, 1995). The elevated levels of GST in the present studies indicate the shift towards a detoxification mechanism under agro-chemical exposure (Table 4.4 and Fig 4.4). There is more GST activity in hepatic tissue compared to kidney and gills, which is due to effective role of liver in xenobiotic detoxification (Goering *et al.,* 1995).

Biotransformation reactions of toxic chemical in the organisms occur in three phases (transformation, conjugation and excretion). In the present study, fish organ-based GST assay reflected a dose dependent significant increase in liver, kidney and gills of both the fishes exposed to agro-chemicals. The increase in the activity of GST reported in the present study indicates the biotransformation pathway used a protective response in fish towards exposure to an oxidative stress inducing agro-chemicals. Similar kind of results have been reported earlier in liver, kidney and gills of freshwater murrel *C.punctatus* (Dabas *et al.,* 2012), obtained by in *O.niloticus* (Wengu *et al.,* 2009 and Gad, 2011) and in *O.mossambicus* (Anushia *et al.,* 2012).

Among the enzymes that compromise the defence system against toxicity also includes superoxide dismutase (SOD) and catalase (CAT) (Dorval and Hontela, 2003). Antioxidant enzymes are used by the organisms as natural endogeneous protection against the generation of ROS (Metwalli and Elmegd, 2002). SOD catalyses the destruction (dismutation) of superoxide free radicals produced during oxidation of pesticide (Otitoju and Onwurah, 2007). The action of SOD therefore results in the protection of the biological integrity of cells and tissues against the harmful effects of superoxide free radicals (Van der Oost *et al.,* 2003). To ameliorate the damage caused

by the hydroxyl radicals formed from superoxide radical and hydrogen peroxide, organisms have evolved mechanisms to regulate the concentrations of the two reactants. SOD is an important isoenzyme functioning as superoxide radicals' scavenger in the living organisms. It is an important enzyme family in living cells for maintaining normal physiological conditions and coping with stress (Otitoju, 2005). The antioxidant, CAT, is a hematin-containing enzyme based in the peroxisomes of cells and is an extremely important component of intracellular and antioxidant defences of aquatic organism (Jamil, 2002). It reduces the H_2O_2 into water and oxygen to prevent oxidative stress and in maintaining cell homeostasis. CAT is often induced concomitantly with the antioxidant SOD, as a result of OS. The formation of highly ROS is a normal consequence of essential biochemical reactions including mitochondrial and microsomal electron transport systems. The present study revealed that SOD and CAT activities in the liver, kidney and gills of *O.mossambicus* and *L.rohita* exposed to agrochemicals were increasing significantly (Table 4.5 and 4.6 and Fig 4.5 and 4.6). The induction of SOD and CAT may be a physiological adaptation for the elimination of ROS generation (Gad, 2011). As reported by Halliwall (1994), an increased in SOD is followed by a parallel increase in CAT, since both enzymes are linked functionally and occur in tandem. Also SOD is the enzyme metabolizing superoxide radical and its level is directly related to CAT activity. Therefore, the SOD-CAT system provides the first defense against oxygen toxicity induced by agro-chemicals. Similar results have been observed in the *Sparus aurata, Oreochromis mossambicus, Labeo rohita* and *Carassus auratus* (Sayeed *et al.,* 2003. Gull *et al.,* 2004; Zhang *et al.,* 2004; Nam *et al.,* 2005; Wilhelm-Filho *et al.,* 2005; Sun *et al.,* 2006; Correia *et al.,* 2007; Sivaperumal, 2008; Zaidi and Soltani, 2010). Considering the results for each tissue, it was found that the liver showed the highest SOD and CAT antioxidant activity compared to kidney and gills. Both enzymes appeared to have an important role in combating the generation of superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) from the intense metabolic activity characteristic of liver. Furthermore, significant increase in SOD and CAT activities in gills and kidney represents an adaptive response to protest the fish from free radical toxicity induced by agrochemicals.

The overall effect of pesticide radicals is the increased production of free radicals in the system and the concomitant decrease in the antioxidant activity due to the utilization of

the antioxidant enzymes to neutralize the free radicals generated. All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible to peroxidative damage (LPO) (Ray and Akhtar, 2002).

LPO has been identified as one of the basic deteriorative reactions in cellular mechanisms of the agro-chemical induced OS in fresh water fishes (Vardharajan, 2010). OS is a chain event which cascades into widespread chain reactions that produces many deleterious products in concentrations many magnitudes greater than the initiator (Ahmad, 1995). This is exemplified by the fact that thousands of polyunsaturated fatty acid molecules are destroyed by a LPO chain reaction initiated by a single initiator free radical. In order to prevent this vicious chain reaction the O_2 radical cascade to O_2^- and H_2O_2 must be attenuated, and the peroxides converted to innocuous metabolites. MDA is a major oxidation product of polyunsaturated fatty acids and increase MDA content is an indicator of LPO. MDA levels were measured as thiobarbituric acid reactive substances (TBARS) which served as an index of extent of lipid peroxidation. A dose dependent increase in the level of LPO, as expressed by MDA formed, was observed in liver, kidney and gills of the fishes exposed to IMI and CZ (Table 4.7 and Fig 4.7).

Elevated MDA level was observed in all the tissues on exposure to agrochemicals indicating that elevated antioxidant enzyme activities were not enough to prevent lipid peroxidation. Significant oxidative damage and lipid peroxidation should theoretically occur if antioxidant defences were overwhelmed by ROS production (Kappus, 1987; Halliwell and Gutteridge, 1989; Winston and Di Giulio, 1991; Vardharajan, 2010). Furthermore, significant increases in MDA ascribes to an excessive production of ROS, which could be related to antioxidant enzyme leakage (Yonar *et al.,* 2012). Our results are corroborated with previous studies reported by other investigators (Paulino *et al.,* 2005; Miller, 2006; Li *et al.,* 2007; Oruc, 2010; Kavitha and Rao, 2008; Sharbidre *et al.,* 2011; Lopez-Lopez, 2011).

Thus, from the present study it can be concluded that the response of antioxidant enzymes (SOD, CAT, GPx, and GST) and non-enzymatic antioxidant/scavengers (ascorbic acid and GSH) showed that the fishes are under severe oxidative stress and that the agro-chemicals are acting as potent free radicals generators. Lipid peroxidation (MDA) level proves that extensive lipid peroxidation has occurred on exposure of the agro-chemicals. And that both the antioxidants interact in a concerted manner to eliminate ROS and prevent damage to cellular components. This suggests that IMI and CZ at levels below median lethal concentration are capable of causing oxidative damage in *O.Mossambicus* and *L.rohita*.

CHAPTER V

Condition factor, organo-somatic indices and histoarchitecture of liver, spleen and gonads of freshwater teleosts on exposure to Imidacloprid and Curzate

Measurements of condition factor, which relates weight and length, and organo-somatic indices, which indicate the proportional sizes of target organs, are standard procedures in fish physiology studies and are used as indicators of the well-being of individual organisms (Di Giullo *et al.,* 2008). The condition factors are used as indicator of the well being of individual organism, because it integrates many levels of the organizational processes. For example, a decrease in condition factor is considered a reflection of depletion in energy reserves because these indices are positively related to muscle and livers energy content (Goede and Barton, 1990; Haque *et al.,* 1998; Jones *et al.,* 1999; Lizama *et al.,* 2002; Hasan and Secer, 2003). The condition factor is an organism-level response, with factors such as nutritional status, pathogen effects, and toxic chemical exposure causing greater-than-normal or less-than-normal weights (Andu and Kangur, 1996; Maxwell and Dutta, 2005; Azmat *et al.,* 2007). Organo-somatic indices reflect the status of organ systems, which may change in size due to environmental factors more rapidly than organism weights and lengths increase or decrease. Schmitt and Dethloff (2000) are of the opinion that: "The indices also integrate, at the organ system and organism level, the combined effects of multiple contaminants and the combined effects of contaminants and other stressors. Hence, they reflect adverse effects of chemical exposure that are not monitored routinely by water quality programs.

Commonly used organosomatic indices in various stress related studies include hepatosomatic index (HSI), viscerosomatic index (VSI), spleenosomatic index (SSI), gonadosomatic index (GSI) and Cardiosomatic index (CSI).

Singh and Canario (2004) observed that hepatosomatic index is one of the most investigated biomarker due to important role of liver in detoxification of pollutants, while Dogan and Can (2011), observed that organosomatic index is an appropriate bioindicator for endocrine disruption in fish consequent of chemical exposure. HSI is the weight of the liver expressed as a percentage of total body weight; it is also known as the liver somatic index. Gingerich (1982), in summarizing the extensive literature on the biology of the fish liver, reported that the liver constitutes, on average, about 2% of body weight in mature teleost fishes. Alterations in liver size may reflect changes in the metabolism and energy reserves of an individual fish (Busacker *et al.,* 1990). Because of the energy storage and metabolic functions of the liver, alterations in liver size due to environmental stressors are of interest. Evaluation of the HSI must consider the role of both endogenous and exogenous factors. The HSI varies with seasonal cycles (Saborowski and Buchholz, 1996; Delahunty and de Vlaming, 1980; Beamish *et al.,* 1996; Slooff *et al.,* 1983). Because of the liver's role in storage and metabolism, nutritional quality and regimes also affect relative liver size (Swallow and Fleming, 1969; Heidinger and Crawford, 1977; Fabacher and Baumann, 1985; Daniels and Robinson 1986; Förlin and Haux, 1990; Grady *et al.,* 1992; Scott and Pankhurst, 1992; Foster *et al.,* 1993).

Of the organo-somatic indices, the HSI is the one most often associated with contaminant exposure (Adams and McLean, 1985). Several investigators have suggested that relative liver enlargement in fish indicates exposure to environmental carcinogens or other toxic chemicals. Increased HSI has been reported in brown bullheads (*Ameiurus nebulosus*) from sites polluted with polycyclic aromatic hydrocarbons (PAHs) (Fabacher and Baumann, 1985; Gallagher and Di Giulio, 1989), in rainbow trout (*Oncorhynchus mykiss*), Atlantic cod, and winter flounder (*Pleuronectes americanus*) exposed to waters containing a mixture of PAHs and other pollutants (Poels *et al.,* 1980; Kiceniuk and Khan, 1987; Fletcher *et al.,* 1982) and in redbreast sunfish exposed to industrial discharge containing PAHs and polychlorinated biphenyls (PCBs) (Adams *et al.,* 1989).

In contrast with the studies described above, a number of laboratory studies found that liver size decreased following exposure to contaminants. Exposure of rainbow trout to sodium pentachlorophenate (Hickie and Dixon, 1987) caused a reduction in the HSI, as did

exposure of perch (*Perca fluviatilis*) to a mixture of metals (Larsson *et al.,* 1984); Atlantic salmon (*Salmo salar*) to cyanide (Ruby *et al.,* 1987); Asian redtail catfish (*Mystus nemurus*) to hydrogen sulfide (Haque *et al.,* 1998); and striped mullet (*Mugil cephalus*) to crude oil (Chambers, 1979). These decreases may have reflected glycogen loss in the liver as energy stores were utilized (Barton *et al.,* 1987). Exposure to carbofuran also decreased HSI in the green snakehead (*Channa punctatus*); the decrease was linked to histological changes in the liver, including hepatocyte damage and degeneration (Ram and Singh 1988; Adams *et al.,* 1992; McMaster *et al.,* 1991).

The spleno-somatic index (SSI) is the weight of the spleen expressed as a percentage of total body weight. Alterations in this index could indicate an abnormal condition in the spleen such as necrosis or swelling due to infection (Goede and Barton, 1990). Spleen size is considered a useful diagnostic factor because the spleen is a hematopoietic organ (Anderson, 1990) and dysfunction could have effects at the whole-organism level. The SSI has not been as thoroughly investigated as the HSI, but certain endogenous and exogenous factors are known to affect it. The range of spleen sizes varies among fishes (Anderson *et al.,* 1982) and among populations of the same species (Lipskaya and Salekhova, 1980). Relative spleen weight may also differ with gender, age, size, gonadal development, and growth rate (Krykhtin, 1976; Ruklov, 1979). Seasonal changes also affect the SSI (White and Fletcher, 1985). Finally, as with the HSI, factors that cause a disproportionate change in body weight will affect the SSI.

Nonspecific stressors (e.g., hypoxia) can result in altered spleen morphology. Studies on six species of teleost fish found that transient hypoxic conditions or severe exercise caused the spleen to contract fully and then decrease in size and hemoglobin content (Yamamoto and Itazawa, 1985; Yamamoto, 1988). Acute stressors including increased temperature, exhaustive exercise, hypoxia, and simulated transport led to spleen contraction and decreased spleen mass in young European sea bass (*Dicentrarchus labrax*), dab (*Limanda limanda*), and in *Trematomus bernachii*, a benthic Antarctic teleost (Hadj-Kacem *et al.,* 1987; Davison *et al.,* 1994; Pulsford *et al.,* 1994). Alterations in spleen morphology due to nonspecific stressors are paralleled by alterations at the cellular level: the release of erythrocytes into circulation, a decrease in the total numbers of white blood cells, an

increase in the proportion of atypical cells (erythrocytes and macrophage-like cells), and enhanced red blood cell degradation in the spleen (Yamamoto and Itazawa, 1983; Maule and Schreck, 1990).

Chemical contaminants can also alter the SSI. A trend of elevated SSI was seen with chronic exposure phenol of redbreast sunfish (Adams *et al.,* 1992). Decreased SSI occurred in cunners (*Tautogolabrus adspersus*) exposed to petroleum for six months, in Atlantic cod exposed to Venezuelan crude oil for 21 or more days, and in gobies (*Zosterisessor ophiocephalus*) residing at a polluted site (elevated PCBs, PAHs, metals) in the Venice Lagoon (Payne *et al.,* 1978; Kiceniuk and Khan, 1987; Pulsford *et al.,* 1995). Juvenile rainbow trout exposed for 24 h to a component of industrial effluent experienced significant decreases in the SSI and hemoglobin oncentration. A significant increase in the SSI and leukocrit, and significantly higher cumulative mortality after disease challenge were seen after a 25-d exposure (Johansen *et al.,* 1994). Histological data show cellular changes occurring in the spleen with exposure to contaminants, supporting the use of the SSI as a relevant indicator of spleen dysfunction. Chronic exposure of rainbow trout to bis(tri-n-butyltin) oxide resulted in a concentration-related splenic lymphocyte depletion. Reticuloendothelial cells proliferated in the spleen, suggesting an increased need for phagocytes to remove damaged blood cells, and increased erythrophagia was noted (Schwaiger *et al.,* 1992). Certain contaminants can affect organs such as the spleen directly or they can suppress immune system functions (Anderson *et al.,* 1989; Hutchinson and Manning, 1996), increasing disease prevalence and thus causing enlargement of the spleen. The histopathology of the spleen due to exposure to chemicals is not as well investigated as that of the gills and liver. The spleen being a haemopoietic and important immune organ is bound to be affected by chemical pollutants absorbed into the blood stream.

The gonado-somatic index, the weight of the gonads expressed as a percentage of total body weight. The GSI and gonadal histopathology fall into a category of indicators that provide structural, rather than functional, information about gonadal health and maturational stage (Ackermann, 2008). The GSI is one of several organosomatic indices, including the HSI and SSI, which establishes a ponderal relationship between the organ and the entire body. There is substantial evidence that most animal species undergo

reproductive cycling and, frequently, dramatic variation in gonadal size throughout the cycle (de Vlaming *et al.,* 1981). Consequently, calculating gonadal weight as a percentage of body weight has routinely been used to determine reproductive maturity, as well as assess gonadal changes in response to environmental dynamics or exogenous stresses.

Gonadal histopathology is often utilized alone, or in conjunction with the GSI, to confirm gonadal phenotype, determine the state of sexual development, and investigate reproductive impairment. Although gonadal histopathology is routinely used to detect higher level responses expressed as morphological abnormalities, observed alterations in cells and tissues are often reflective of previous biochemical and physiological modifications. The utilization of the GSI as a reproductive biomarker was first reported in 1927 in a study describing the yearly variations of female yellow perch (*Perca flavescens*) ovaries (Meien, 1927). Years later, Nikolsky (1963) endorsed this method on the premise that "…the effects of fish size on gonadal weight are eliminated by expressing gonadal weight as a percentage of body weight." There is significant evidence that exposure to various environmental pollutants can result in gonadal alterations such as a decreased GSI, morphological changes, or both. Pollutants may also cause alterations in these two indicators. A reduction in the GSI and impaired gonadal development (growth and structural pathologies) have been reported in response to environmentally relevant doses of dietary mercury in juvenile walleye (*Stizostedion vitreum*) (Friedmann *et al.,* 1996), organophosphate insecticides in female striped catfish (*Mystus vittatus*) (Choudhury *et al.,* 1993), and metacid-50 and carbaryl in climbing perch (*Anabas testudineus*) (Haider and Upadhyaya, 1985). Oocyte atresia, as defined by an involution or resorption of unfertilized eggs by the ovaries, is a normal physiological event in all fish, but it has become a pathological condition noted in fish after exposure to certain environmental contaminants (Johnson *et al.,* 1988; Cross and Hose, 1988; Kirubagran and Joy, 1988). The ability to detect increased degeneration or necrosis of developing oocytes by histological examination has inspired the use of oocyte atresia as a biomarker of reproductive impairment. Fish under stress will experience changes in tissue and organ function to maintain homeostasis.

The size or weight of the liver, spleen, and gonads relative to fish length or weight signifies overall health and reproductive status. The current investigation was undertaken to understand the toxicity of sublethal dose of IMI and CZ in O.mossambicus and L.rohita by determining its effects on condition factor and organosomatic indices (HSI, SSI and GSI). If a change in function exists, there will be a gross change in the structure of organs or tissues. Taking the aforementioned into account, along with condition factor morphological alterations were observed for liver, Gonads and Spleen.

Materials and Methods:

Experimental design:

Two freshwater teleosts, *O. mossambicus* and *L. rohita* of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies

Sub-lethal exposure:

Based on the result of the 48 h LC_{50}, 30 tilapia fish were divided in 3 groups, 10 fish for each group: Group 1 served as control without any treatment of Agro-chemicals. Group 2 were treated with low dose of IMI and CZ (LC_{50} / 10). Group 3 were treated with high dose of IMI and CZ (LC_{50} / 20) for a period of 21 days. Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests.

At the end of the experiment the fish were carefully netted to minimize stress, and the fish weighed. Fishes were sacrificed by pithing (damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle). Then, the liver, Gonads and spleen along with the other organs for histological observations was carefully removed and weighed.

Morphological observation:

Condition factor:

Sample of 10 fishes from each experimental setup was taken for measuring weight, length to determine K factor. The condition factor of fish was calculated according to the method of Anderson *et al.,* (1998) using the formula:

$K = W \times 100 / L^3$ (Where K= Condition factor; W= Weight of the fish; L=Length of the fish).

Organosomatic index

The organosomatic indices of the liver, spleen and Gonads were then calculated for the ten fish according to Dogan and Can (2011) to get the organ weight to the body weight ratios of the fish as follows: weight of the fish/ weight of the organ x 100

HSI: liver weight/ fish weight x 100

SSI: spleen weight/ fish weight x 100

GSI: Gonad weight/ fish weight x 100

Histological observation:

After measuring length and weight fresh tissues were fixed in 4% paraformaldehyde for 24 hours, dehydrated, embedded in paraffin wax and sectioned at 10-12µm then stained with heamatoxylin and eosin and examined microscopically and photographed using digital camera.

Statistical analysis:

Data were analyzed using two-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test to determine differences between treatments means as well as control means at significant rate of $P<0.05$. Data were represented in mean ± SEM. All statistics were carried out using Statistical Analysis program Graph pad prism 5.

Results:

The mean organosomatic index and condition factor of *O.mossambicus and L.rohita* exposed to different concentrations of IMI and CZ are presented in Table 5.1 and Fig: 5.1 and 5.2.

The condition factor (K) calculated for *O.mossambicus* varied from 1.72±0.041 to 1.64 ±0.019 (low dose) followed by 1.58 ±0.010 (high dose) on IMI exposure and from 1.72 ±0.046 to 1.67 ± 0.031 (low dose) followed by 1.60 ± 0.027 (high dose) on CZ exposure. While that for *L.rohita* varied from 1.08 ± 0.035 to 1.01 ± 0.009 (low dose) followed by 0.94 ± 0.011 (high dose) on IMI exposure and from 1.08 ±0.035 to 1.03 ± 0.034 (low dose) followed by 0.97 ± 0.019 (high dose) on CZ exposure.

SSI index of *O.mossmbicus* showed a significant decrease from 0.048 ± 0.0016 to 0.032 ± 0.002 ($P<0.01$) followed by 0.0182 ± 0.003 ($P<0.05$) on IMI exposure and from 0.0514 ± 0.001 to 0.039 ± 0.02 ($P<0.05$) followed by 0.027 ± 0.002 ($P<0.001$) on CZ exposure in a dose dependent manner. SSI index of *L.rohita* showed a significant decrease from 0.0506 ± 0.003 to 0.036 ± 0.003 ($P< 0.01$) followed by 0.024 ± 0.002 ($P< 0.001$) on IMI exposure. A non-significant decrease from 0.0516 ± 0.003 to 0.043 ± 0.003 ($P>0.05$) at low dose whereas a significant decrease up to 0.032 ± 0.003 ($P<0.001$) at high dose on CZ exposure.

Of the HSI the significant decrease was observed only on IMI exposure from 1.216 ± 0.052 to 1.050 ± 0.028 ($P< 0.05$) at high dose for *O.mossmabicus* and from 1.224 ± 0.043 to 1.154 ± 0.029 ($P< 0.01$) at high dose for *L.rohita.*

One of the most noticeable observations was at the end of the experiment for *L.rohita* showed that all the control as well as treated fishes turned out to be only females, hence male GSI was not reported. The female GSI exhibited a non-significant decrease on CZ exposure whereas, on IMI exposure there was a significant decrease from 1.098 ± 0.037 to 0.960 ± 0.019 ($P<0.05$) followed by 0.872 ± 0.013 ($P<0.001$). Male GSI of *O.mossambicus* revealed a significant decrease on IMI exposure ($P<0.05$) at low dose and ($P<0.01$) at high dose. And that for CZ was ($P<0.01$) at low dose and ($P<0.001$) at high dose. Female GSI of *O.mossambicus* also exhibited a significant decrease ($P<0.01$) at low dose and ($P<0.001$) at

high dose of IMI exposure. High dose exposure of CZ demonstrated a significant decreased in female GSI ($P<0.01$).

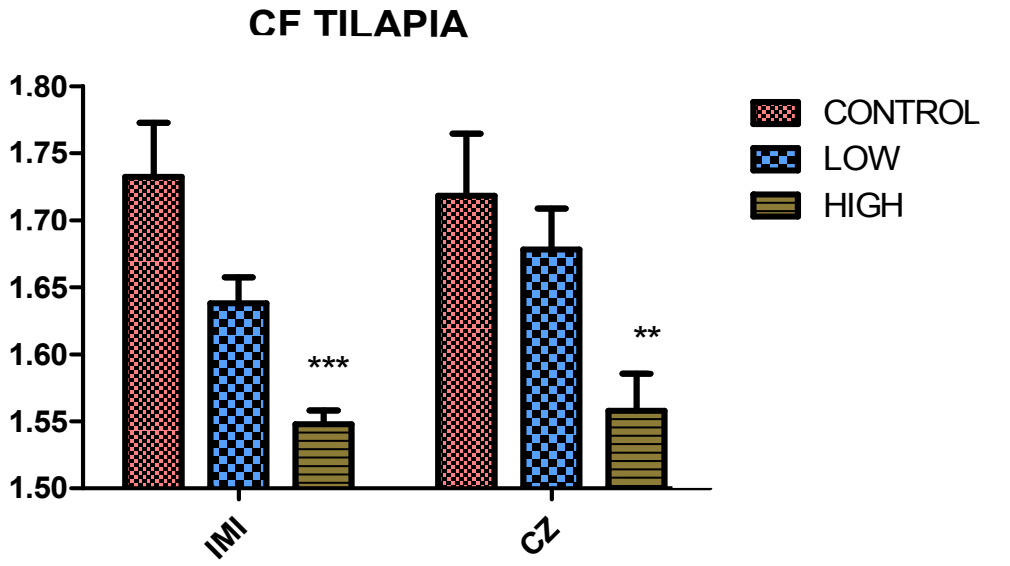
The histological alterations of the spleen, liver and gonad were observed and were found to exhibit dose dependent changes for both the agro-chemicals in both the fishes. However the alterations were more pronounced in IMI exposed tissues of the fishes. The histoarchitecture of the tissues sections are presented (Photomicrogarph 1 to 7). Spleen showed mild to severe depletion of white pulp which was replaced by empty space and activation of melanomarophage centers (MMCs) along with hemorrhage, fibrosis and intracellular edema. Liver histology too showed distinct altered features such as necrosis in hepatocytes, intracellular edema, lipid infiltration, hemorrhage and cytoplasmic vacuolation. The histological alterations of the testis on exposure of agro-chemicals consists of altered structure of seminiferous tubules associated with damage and presence of large number of inter and intra tubular vacuoles and severe necrosis. Female gonads showed oocyte atresia and vacuolation.

Table: 5.1 Condition factor, HSI, SSI and GSI of *O.mossambicus* and *L.rohita* subjected to sub-acute concentrations of IMI and CZ.

		IMI			CZ		
	INDICES	**C**	**LD**	**HD**	**C**	**LD**	**HD**
O.mosambicus	**HSI**	1.216 ±0.052	1.108 ±0.028	1.050 ±0.027*	1.224 ±0.043	1.192 ±0.300	1.154 ±0.029
	MALE GSI	0.240 ±0.012	0.212 ±0.012*	0.150 ±0.013**	0.246 ±0.014	0.232 ±0.08**	0.146 ±0.009***
	FEMALE GSI	0.582 ±0.011	0.508 ±0.016*	0.404 ±0.009***	0.518 ±0.012	0.450 ±0.07	0.404 ±0.012**
	SSI	0.048 ±0.007	0.032 ±0.002***	0.0182 ±0.003***	0.0514 ±0.001	0.039 ±0.02**	0.027 ±0.002***
	CF	1.72 ±0.041	1.64 ±0.019	1.58 ±0.010***	1.72 ±0.046	1.67 ±0.031	1.60 ±0.027**
L..rohita	**HSI**	1.200 ±0.032	1.090 ±0.023	1.006 ±0.028**	1.198 ±0.04	1.148 ±0.02	1.068 ±0.037*
	FEMALE GSI	1.098 ±0.037	0.960 ±0.019*	0.872 ±0.013***	0.992 ±0.044	0.91 ±0.022	0.880 ±0.012
	SSI	0.0506 ±0.003	0.036 ±0.003**	0.024 ±0.002***	0.0516 ±0.003	0.043 ±0.003	0.032 ±0.003***
	CF	1.08 ±0.035	1.01 ±0.009**	0.94 ±0.011***	1.08 ±0.035	1.03 ±0.034	0.97 ±0.019***

- Each value represents the mean ± SEM of six separate experiments.
- Signifiacant level indicated by * ($P<0.05$); **($P<0.01$);***($P<0.001$)

Fig: 5.1 Condition factor of *O.mossambicus* and *L.rohita* subjected to sub-acute concentrations of IMI and CZ.



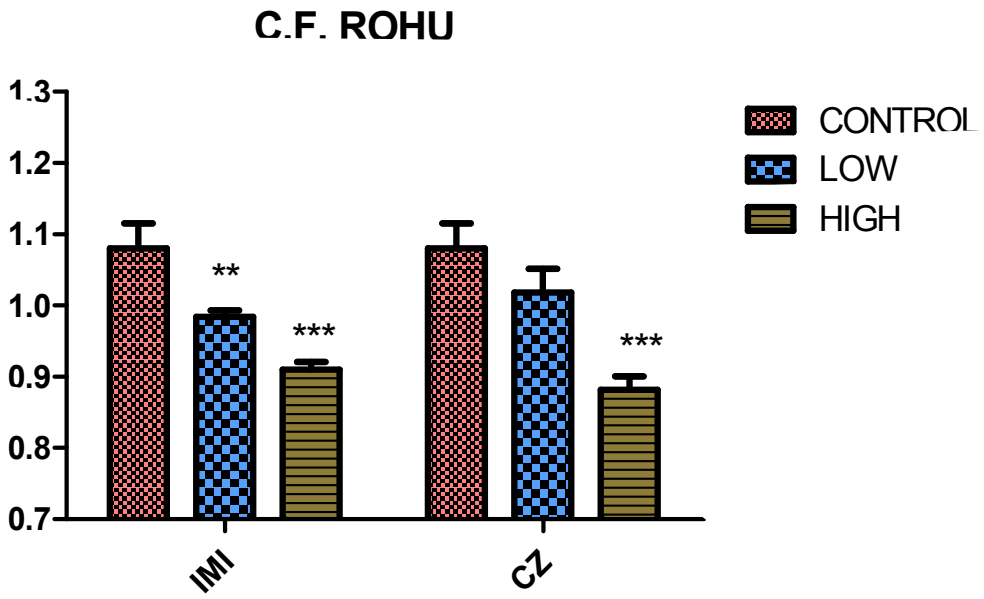


Fig: 5.2 HIS and SSI of *O.mossambicus* and *L.rohita* subjected to sub-acute concentrations of of IMI and CZ.

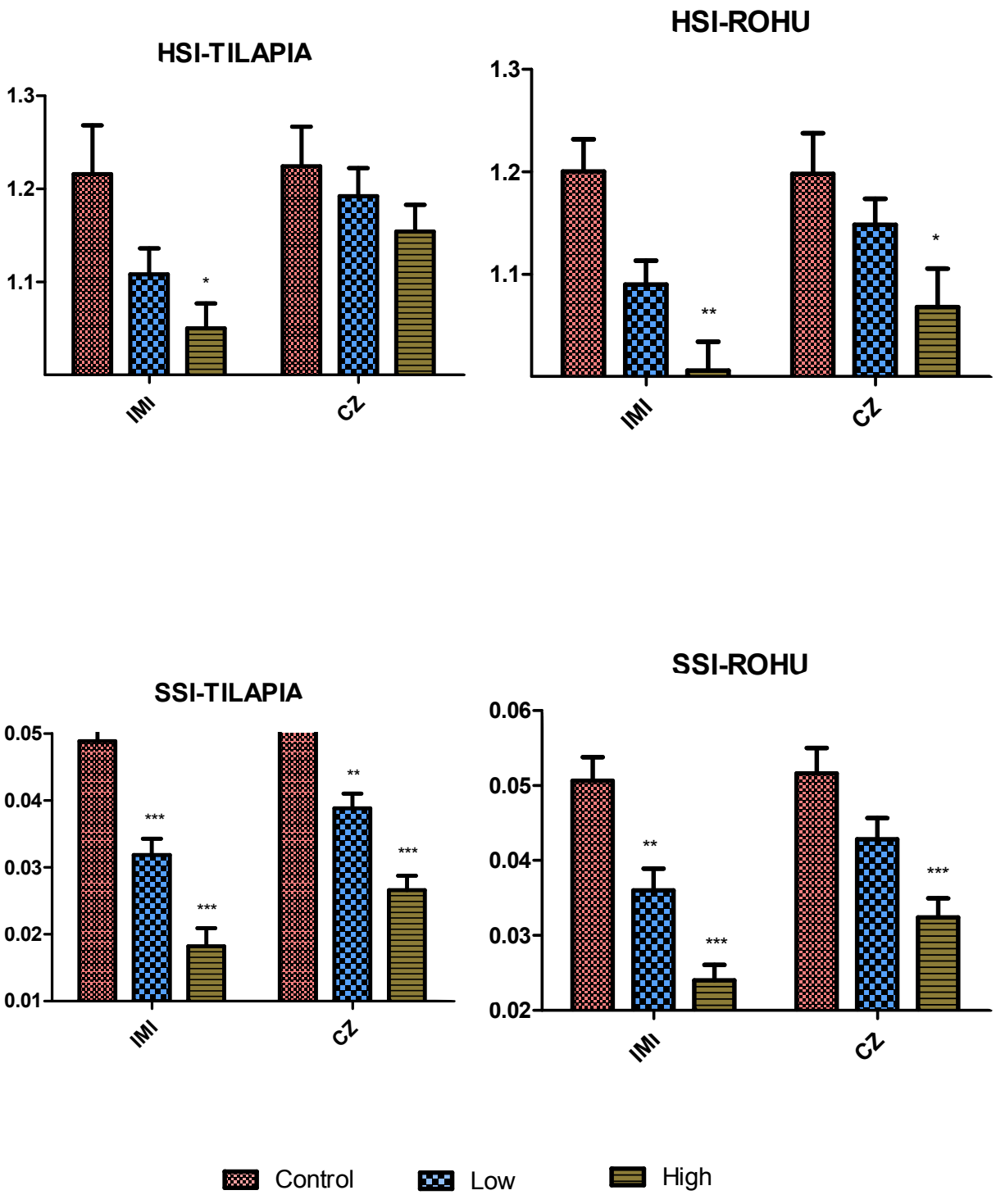
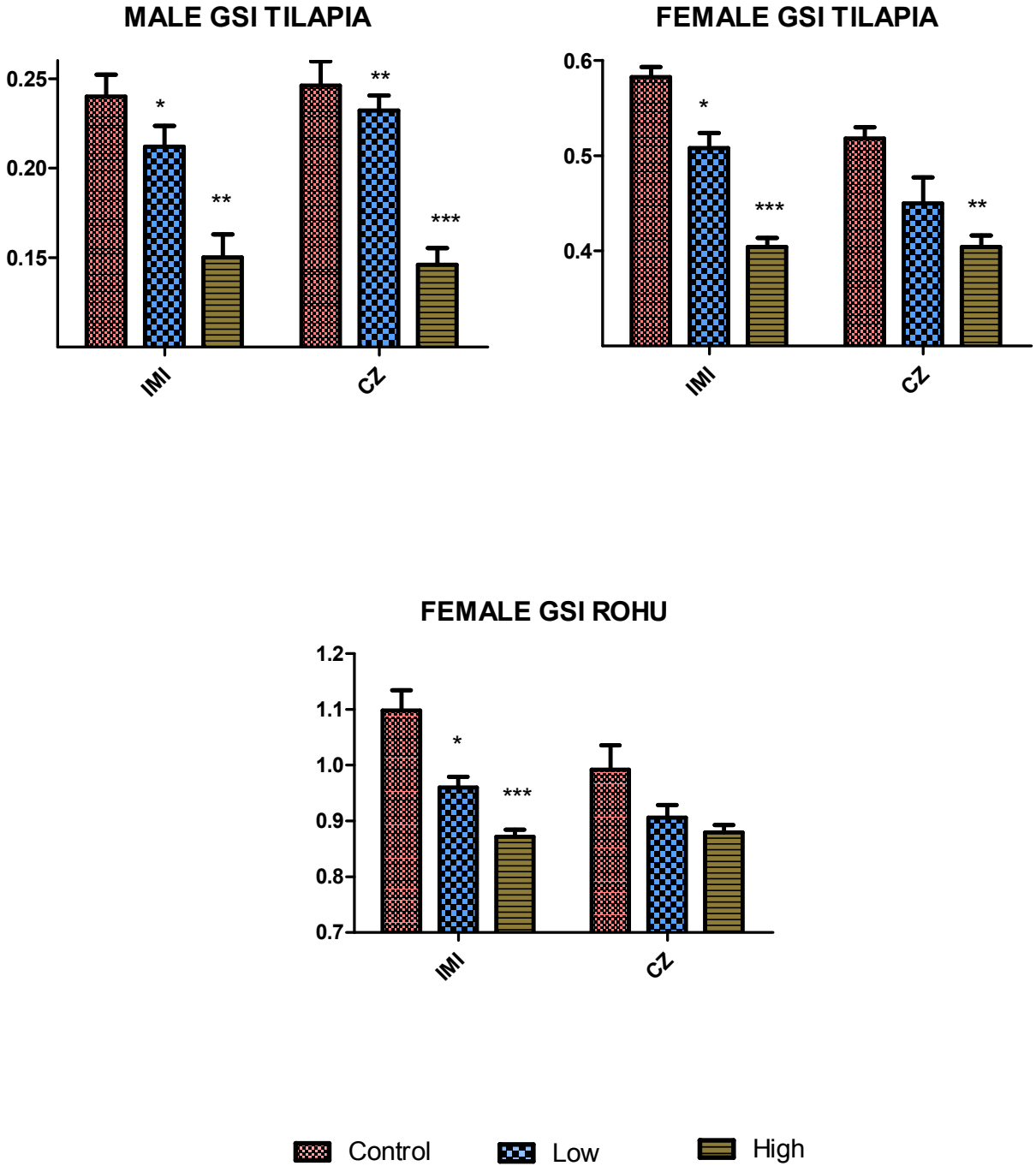


Fig: 5.3 GSI of *O.mossambicus* and *L.rohita* subjected sub-acute concentrations of IMI and CZ.



Discussions

The use of various organosomatic indices is based on the assumption that there is proportional relationship between fish size and the particular ratio in assessing fish stress by pollutant (Ronald and Bruce, 1990). In the present study, HSI revealed that there was a significant decrease in weight of the liver as the concentration of agro-chemicals increased. However, liver of both the fishes exhibited a greater diminution in HSI on IMI exposure compared to that of CZ. Decreased HSI value is indicative of that the fish were under stress and that IMI was more toxic compared to CZ. Altered liver function markers (AST and ALT) also indicate liver damage. The alterations in the enzymatic activities directly reflect the metabolic disturbances and cell damage in specific organs (Chapter III). Decrease in the weight of liver suggests a decrease in the production of endoplasmic reticulum for protein synthesis in liver tissue under toxicant exposure (Bennet and Wolke, 2004). Liver reduction could also be as a result of decreased lipid storage (Gabriel *et al.,* 2010; Areweriokuma *et al.,* 2011). A similar report has been made by Soufy et al, (2007) when the fish *Oreochromis niloticus* was exposed to carbofuran; in rainbow trout after long-term exposure to a mixture of heavy metals (Vosylienë and Sveceviėius, 1997); and of perch exposed to heavy-metal-containing effluent (Larsson *et al.,* 1984 and Figueiredo-Fernandes *et al.,* 2007).

Parallel to the decreased HSI, alterations in the histological structures were also observed. In the control fish, the liver was primarily composed of polyhedral hepatocytes typically with central nuclei and a prominent nucleolus (Photomicrograph 1A). Hepatocellular necrosis with parenchymal vacuolization, hypertrophy of hepatocytes, hemorrhages and widening of blood sinusoids were the distinct altered features in the agro-chemical exposed fish liver. At low dose only slight vacuolation and mild swelling of hepatocytes in which the nucleus retained a nearly normal shape was recorded (Photomicrograph 1B and 1D). At high dose the main alterations found in the liver were: irregular-shaped nuclei, nuclear hypertrophy, nuclear vacuolation and the presence of eosinophilic granules in the cytoplasm (Photomicrograph 1C and 1E). Cytoplasmic and nuclear degeneration was also

very common; melanomacrophages were identified as rounded aggregates of cells containing dark-yellowish granules of various sizes, normally close to the vessels (Photomicrograph 2B and 2D). Liver showed degeneration of the hepatocytes and intravascular haemolysis in blood vessels as shown in Photomicrograph 2C and 2E, congestion of central vein, hemorrhages, and nuclear pyknosis in the majority of hepatic cells. Liver showed fatty infiltration, these findings were apparent as the liver is considered the organ of detoxification and excretion. Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, or in other organs. Several authors recorded many histopathological changes in the liver of freshwater fish treated by insecticides diazinon, dimethoate, malathion and glyphosate, respectively (Neskovic *et al.,* 1996; Sakr *et al.,* 2001; Van Dyk, 2003; Stentiford *et al.,* 2003; Fanta *et al.,* 2003; Mela *et al.,* 2007 de Melo *et al.,* 2008; Kunjamma *et al.,* 2008; Mataqueiro *et al.,* 2009).

In the present study the SSI values were reported to be decreased in the agro-chemical exposed fishes compared to control. Alterations in relative spleen size could signal a dysfunction capable of affecting fish health. Decreased size has often been seen with acute, nonspecific stressors, but chronic exposure to a number of chemical contaminants also leads to this effect. The decrease seems to be due to necrosis and perturbations in cell processing, both of which could impact the overall condition of the individual fish. Reduced SSI may be the response of the fish to combat agro-chemical stress (Gabriel *et al.,* 2010). The SSI is of interest due to the spleen's hematopoietic function which also makes it an immune system organ.

It is also possible that environmental toxicants may increase the susceptibility of aquatic animals to various stressors by interfering with the normal functioning of their immune, reproductive and developmental processes (Couch and John, 1985). MMCs are focal accumulations of macrophages found in the spleen, head kidney, and sometimes liver of teleost fishes. in various MMCs parameters (e.g., number, size, percent area occupied) in relation to environmental contamination have been reported by several investigators (Wolke *et al.,*1985; Spazier *et al.,*1992; Wolke, 1992; Blazer *et al.,*1994; Couillard and

Hodson, 1996; Meinelt *et al.,* 1997; Facey *et al.,* 1999). Because MMCs are known to change in number, size, and pigment content in relation to fish health and environmental degradation, they qualify as anatomical and cytological biomarkers (Wolke 1992). The value of using MMCs as histological biomarkers lies in their ubiquity, availability, ease of measurement, and association with degraded environmental conditions. They are key cells for dealing with foreign material and cellular debris (Blazer *et al.,* 1994; Evans, 1998). The role of MMC and their pigments has been used as a biomarkers for environmental pollution by several authors, but the relationship between these structure and the endogenous factors is not completely explained (Rabitto *et al.,* 2005; Suresh, 2009). In the present study, Melanomacrophage centers (MMC) were scattered throughout spleen (Photomicrograph 3B, 3C, 3D and 3E). There are published data on alterations in the number of MMC caused by environmental variation (Fournie *et al.,* 2001; Schwaiger *et al.,* 1996; Garcia-Abiado *et al.,* 2004). Furthermore, the associated alterations observed in the spleen such as mild to severe depletion of white pulp which was replaced by empty space and activation of MMCs along with hemorrhage, fibrosis and intracellular edema is suggestive of an adaptive immune response to agrochemical stress. These findings are consistent with the earlier reports (Suresh and Veeraraghavan, 1998; Pulsford *et al.,* 1992; Blazer *et al.,* 1987; Brown and George, 1985; Falk *et al.,* 1995; Simko *et al.,* 2000; Decostere *et al.,* 2001 and Ekman and Norrgren, 2003). MMCs are physiological features in fish spleen and kidney (Agius & Roberts, 2003). Wolke *et al.,* (1985) first suggested MMC as potential monitors of fish health. MMC are a nonspecific response and numerous factors are involved in their formation and distribution. They are the deposition site for materials of exogenous (metals, biologically active particles) and endogenous (melanin, lipofuscin, ceroid, hemosiderin) origin (Vijayan and Leatherland, 1988).

GSI values decreased in dose dependent manner. GSI is said to be the state of gonadal development and maturity. It has been use to assess the gonadal changes in response to environmental dynamics or contaminant exposure (Schmitt and Dethloff, 2000). Temperature is one of the important factors known to regulate GSI. Kamanga *et al.,* (2002) in their studies have reported low GSI and are of the view that low temperature is one of the factor responsible for the reduce GSI. Hence, the room temperature in the present study

could be one of the possible causes in lowering the GSI. Furthermore, dose dependent decreased GSI observed has also been reported by Masud *et al.,* (2003) in *Cyprinus carpio and* in *Siganus rivulatus* by Olfat and El-Griesy, (2007), in *Rasbora danconius* by Anjali and Kulshrestra, (1990), in *Channa punctatus* by Kaur and Kaur, (2006) and *Clarius batrachus* by Begum & Vijayraghavan, (1995). Reduced GSI also indicates lowered reproductive activity (Bernard *et al.,* 2001 and Hassanin *et al.,* 2002). The reduced GSI in the present study may be due to lowered gonadal activity under agro-chemical stress. Deleterious effects of pesticides have been observed in earlier studies such as delayed maturity Dey & Bhattacharya (1989), abortion in Gambusia (Wani and latey, 1984), reduction reproductive efficiency (Mani and Saxena, 1985 and Kirubagran and Joy, 1992) and decrease in the percentage of different staged of oocytes along with reduction in GSI (Kulshretha and Arora, 1984; Pandey, 2000 and Mir *et al.,* 2011). Histomorphological alterations are also supporting the reduced GSI observed and probably may be associated with the impairment of the production of steroid hormones which might have arrested the formation of germ cells and cause degeneration or necrosis (Photomicrograph 5, 6 and 7). Vacuolated follicular epithelium and oocyte atresia were the prominent observations in the present studies in female gonads. The histological alterations of the testis on exposure of agro-chemicals consists of altered structure of seminiferous tubules associated with damage and presence of large number of inter and intra tubular vacuoles and severe necrosis. Our results are in agreement with earlier studies reported by, Pandey and Shukla (1982), Dey and Bhattacharya (1989), Lakhani and Pandey, (1985), Khillare, (1992), Ruby *et al.,* (1993) and Mir *et al.,* (2011).

As general indicators of the overall health and well-being of the fish, alterations in the indices indicate deleterious effect of the agro-chemicals. Thus, from the present study it is apparent that IMI and CZ have resulted in to considerable alteration in CF as well as HSI, SSI and GSI. It can be concluded that the histological changes induced by agro-chemicals varied in their expression and IMI was found to be more toxic than CZ.

Chapter VI

Histological changes in the tissues of *Oreochromis mossambicus* and *Labeo rohita* on exposure to IMI and CZ

Health of aquatic organisms cannot be measured directly. Instead, only indicator of health can be measured and in turn used to assess the "health" status. Histology and histopathology can be used as biomonitoring tools or indicators of health in toxicity studies as they provide early warning signs of disease (Meyers and Hendricks, 1985). Histopathological alterations are biomarkers of effect of exposure to environmental stressors, revealing prior alterations in physiological and/or biochemical function (Hinton *et al.,* 1992). Fish is a suitable indicator for monitoring environmental pollution because they concentrate pollutants in their tissues directly from water and also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web structural damages may occur in their target organs, histological structure may change (Fisk *et al.,* 2001; Boon *et al.,* 2002). Due to being exposed to pollutants, major and physiological stress may occur. This stress causes some changes in the metabolic functions. The changes in the functions are initiated with the changes in the tissue and cellular level. Although qualitative data are used in most cases to study the pathologies the environmental pollutants cause, quantitative data show better reactions of the organisms to pollutants (Jagoe, 1996).

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish for several reactions (Teh *et al.,* 1997; van der Oost *et al.,* 2003). The gill surface is more than half of the entire body surface area. In fish the internal environment is separated from the external environment by only a few microns of delicate gill epithelium and thus the branchial function is very sensitive to environmental contamination. Gills are the first organs which come in contact with environmental pollutants. Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver (Mallatt, 1985;

Evans, 1987). Additionally, absorption of toxic chemicals through gills is rapid and therefore toxic response in gills is also rapid. Gills have frequently been used in the assessment of impact of aquatic pollutants in marine as well as freshwater habitats (Haaparanta *et al.,* 1997; Athikesavan *et al.,* 2006; Craig *et al.,* 2007; Fernandes *et al.,* 2007; Jimenez-Tenorio *et al.,* 2007). Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic processes of fish. Gills are important not only for gaseous exchange but also for osmoregulation and excretion of toxic waste products (Robert, 2001), thus any harm in the gills leads to impairment of such vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes. The gills are important organs in fish to perform respiration, osmoregulation, acid base balance and nitrogenous waste excretion (Evans *et al.,* 2006). Fish gills are also vulnerable to pollutants in water because of their large surface area and external location. For this reason, fish gills are considered to be the most appropriate indicators of water pollution levels (Alazemi *et al.,* 1996). Various authors such as Karlsson-Norrgren *et al.* (1985), Mazon *et al.* (2002), Cerqueira and Fernandes (2002), Oliveira-Ribeiro *et al.* (2002) and Thophon *et al.* (2003) can be cited in regard to utilising the gills of fish as a tool for determining the toxicity of various pollutants in laboratory tests.

In fish, as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen-containing waste products from the metabolism such as ammonia, urea and creatinine. Following exposure of fish to toxic agents such as pesticides, tissue alterations have been found at the level of the tubular epithelium and glomerulus (Teh *et al.,* 1997). Hence, fish serve as excellent bioassay animal for toxicological impact studies and has been widely used for this purpose. Kidney of fishes receives much the largest proportion of postprandial blood and therefore renal lesion might be expected to be good indicators of environmental pollution (Hinton and Lauren, 1990; Ortiz *et al.,* 2003). There is evidence of glomerular and tubular lesions in fish kidney as a consequence of nephrotoxic effects of pesticides (Visoottiviseth *et al.,* 1999) as well as antibiotics used in aquaculture (Hicks and Geraci, 1984). Lesions in kidney interstitial tissue are mainly associated with viral and bacterial infections

(Roberts, 2001). Several renal pathological conditions, characterised by nephrolithiasis and granuloma, have been reported in cultured fish. Etiology is, at least partly, associated with nutrition (Paperna, 1987). Hence, in the present study an attempt is made to evaluate the effect of IMI and CZ on the histopathological alterations in gills and kidney of *Oreochromis mossambicus* and *Labeo rohita*.

Materials and Methods:

Experimental design:

Two freshwater teleosts, *O. mossambicus* and *L. rohita* of similar size in length and weight (12 ± 2 cm; 25 ± 1.9 g) and (25 ± 3 cm; 110 ± 5 g) respectively were brought from a local pond of Baroda district. Animals were transported to laboratory in large aerated plastic container and were acclimatized in glass aquaria containing 50 liter of well aerated dechlorinated tap water (with physic-chemical characteristics: pH 6.5- 7.5, temperature 25±3ºC and dissolved oxygen content of 7-8ppm) for ten days. During an acclimation period of 10 days, the fish were kept under natural photoperiod and fed two times a day (10:00 and 16:00h) with commercial pelleted diet. The acclimatized healthy fishes of both sexes were selected randomly for the studies

Sub-lethal exposure:

Based on the result of the 48 h LC50, 30 tilapia fish were divided in 3 groups, 10 fish for each group: Group 1 served as control without any treatment of Agro-chemicals. Group 2 were treated with low dose of IMI and CZ (LC 50 / 10). Group 3 were treated with high dose of IMI and CZ (LC 50 / 20) for a period of 21 days. Each concentration was replicated two times. Constant amount of the test chemical and test media were changed every 24 hours to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests.

At the end of the experiment the fish were carefully netted to minimize stress, and the fish weighed. After this, Fishes were sacrificed by pithing (damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle). Then, the liver, Gonads and spleen along with the other organs for histological observations was carefully removed and weighed.

Histological observation:

After measuring length and weight fresh tissues were fixed in 4% paraformaldehyde for 24 hrs, dehydrated, embedded in paraffin wax and sectioned at 10-12μm then stained with heamatoxylin and eosin and examined microscopically and photographed using digital camera.

Semiquantitative scoring:

Histopathological alterations were assessed using a score ranging from – to + + + depending on the degree and extent of the alteration: (-) none, (+) mild occurrence, (+ +) moderate occurrence, (+ + +) severe occurrence. A total of 10 slides were observed from each treatment.

Results:

Photomicrographs 1A and 2A shows normal histological structures of the gills of *O. mossambicus* and *L. rohita*. The common histopathological observations in the gills of *O. mossambicus* and *L. rohita* includes ploriferation of the epithelium of the gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, severe degenerative necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucus cells proliferations. Edematous changes, characterised by epithelial detachment were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Also, dialation and congetion in gill filaments were observed. Atrophy of secondary lamellae was seen. However, comparatively the degree of pathological changes observed on IMI exposure was more prominent compared to CZ for *O. mossambicus* as well as *L. rohita.* Distinct feature observed was hyperemia and hemorrhages in primary and secondary gill lamellaes at high dose of CZ (Fig 1B, 1C, 1D and 1E) exposure and at low dose of IMI (Fig 2B, 2C, 2D, and 2E) in *L. rohita.*

Photomicrograph 3A and 4A shows the normal histological structure of kidney. Histological alterations in the kidney of both the fishes consist of severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and haemorrahage, haemolysis. Vacuolar degenerations in the epithelium of renal tubules and dialation in the capillary tubes of renal tubules were observed. Also edema of Bowman's capsule with atrophy in the glomeruli and dialation in the renal blood vessels were observed. Kidney tissue from *O. mossambicus and L. rohita* showed mild necrosis and tubular degeneration on CZ (3B, 3C, 3D and 3E) exposure where as on IMI (4B, 4C, 4D and 4E) exposure it showed severe necrosis, vacuolation and tubular degeneration.

Table: 6.1 Summary of histological changes observed in the gills and kidney of *O.mossmbicus and L.rohita* subjected to IMI and CZ are presented

Tissues	*O.mossambicus*				*L.rohita*			
	IMI		CZ		IMI		CZ	
	HD	LD	HD	LD	HD	LD	HD	LD
Gills								
Depicting proliferation of the epithelium of the Primary lamellae	++	+++	+	+	+++	+	++	+
Curling of secondary lamellae	+	++	+	++	++	+	++	+
Enlargement of primary lamellae	+++	++	++	+	+++	++	++	+
Degeneration of primary lamellae	+	++	-	-	-	-	-	-
Cubbing of secondary lamellae	+++	++	+	+	-	-	-	-
Loss of secondary lamellae	+++	+	+	++	-	-	-	-
Distortion of epithelial lining of primary lamellae	++	+	+	+	+++	++	++	+
Proliferation of epithelial cells	+++	++	+	+	+++	++	++	+
Branchial filament with hyperplasia	+++	+	++	-	+++	+	+	-
Fusion of secondary lamellae	-	-	-	-	+++	++	+	
Hyperplasia at the tip of secondary lamellae	+++	++	+	-	+++	++	+	+
Branchial hemorrhage	-	-	-	-	+++	+	-	-
Uplifting epithelial lining of secondary lamellae	++	+	-	-	+++	++	+	-
Kidney								
Intracellular vacuolation	+++	++	+++	+	+++	++	++	+
Degeneration of tubular epithelial cells	++	+	+++	++	++	+	++	+
Cytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and lumen tubules diminished	+++	++	+++	++	++	+	++	+
Renal tubule degeneration	+++	++	++	+	+++	++	+	++
Haemorrhage in the epithelial cells of renal tubules	+++	++	+	++	++	+	++	+
Swelling in the epithelial cells of renal tubules	+++	++	++	+	+++	++	++	+
Shrinkage of glomeruli	+++	++	++	+	+++	+	++	
Expansion of space inside the bowman's capsule	+++	+	++	+	++	+	+++	++
Increase inter cellular space	++	+	+		++	+	++	
Increase intracellular space	+++	++	++	+	+++	++	++	+
Severe degenerative and necrotic changes in Renal tubules and glomeruli	+++	++	++	+	+++	++	+	+
Severe necrosis in the epithelium of renal tubules	+++	-	+	-	+++	-	+	-

(-) none, (+) mild occurrence, (+ +) moderate occurrence, (+ + +) severe occurrence.

Discussion:

Results of the present study revealed that *O. mossambicus* and *L. rohita* on exposure of IMI and CZ manifest histopathological changes in gills and kidney. It is possible that the pathological alterations in the tissues of both studied fish with both agro-chemicals could be a direct result of the pesticides induced stress. The gills, which participating in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water and thus are considered the primary target of the toxicant (Chandra and Banerjee, 2003; Moitra, 2012). The gills of both studied fish showed degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae, edema in gill filaments and sencondary lamellae along with congestion in blood vessels of gill filaments. These pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the entry of the toxicant through the gill surface. Moreover, alterations like proliferation of epithelial cells, partial and total fusion of secondary lamellae as well as lifting of epithelium are defense mechanisms as this would result in the increase of the distance between the external environment and the blood thereby serving as a barrier to the entrance of the agro-chemicals (Fernandes *et al.,* 2007; Mohamed, 2009). The cellular damage observed in the gills in terms of epithelial proliferation, separation of epithelial layer from supported tissue and necrosis can adversely affect the gas exchange and ionic regulation (Dobreva *et al.,* 2008; Arnaudoa *et al.,* 2008). The observed edematous changes in the gill filaments and secondary lamellae probably due to increased capillary permeability. Our results are parallel with earlier findings on the histopathological changes in the gills of different fish species exposed to pesticides (Sinhaseni and Tesprateep 1987; Gill *et al.,* 1988; Richmonds and Dutta, 1989; Alazemi *et al.,* 1996; Erkmen et al., 2000).

More prevalent and more pronounced changes in the gills of both the fish on IMI exposure were curling of secondary lamellae followed by disorganization, rupture in the secondary lamellae. Haemorrhage at primary lamellae and bulging at the tip of primary filament were also noticed. Our results are in agreement with earlier reported severe damage to gill architecture by Tkatcheva *et al.,* 2004; Velcheva *et al.,* 2010; Susithra *et al.,* (2007).

The functional unit of the kidney is the nephron. The kidney of fish from the control group, had normal histological structure. Morphologically, the nephron of the control fish consists of intact structures of glomerulus, tubules and collecting ducts. The glomeruli, a cluster of capillaries surrounded by the Bowman's capsule were very clearly seen. The structure of the proximal and distal convoluted tubules was undamaged. The teleostean kidney is one of the first organs to be affected by contaminats of the water (Thophon *et al.,* 2003). The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for selective reabsorption which helps in maintaining volume and pH of blood and body fluids as well as erythropoesis (Iqbal *et al.,* 2004). Kidney tissue from *O.mossambicus* on low dose exposure of CZ showed mild necrosis and shrunken glomeruli. However, at high doses the changes were more severe and the normal histoarchitecture of the kidney was lost. At low dose of IMI exposure led to complete degeneration of blood vessels in the glomeruli. The interstices of the tubules were seen to be enrich with haematopoetic tissue. At high dose there was complete degeneration of tubular epithelial cells and complete disorganized Bowman's capsules. Kidney tissue from *L. rohita* on low dose exposure of CZ showed mild swollen proximal tubular epithelial cells with dilated nuclei and at high dose it showed severe swelling of tubules with necrosis. At low dose of IMI exposure kidney showed expansion of space inside the Bowman's capsule and glomerular atrophy. At high dose of IMI exposure, severe degeneration of tubules, cloudy swelling and severe necrosis in nephritic tissue was observed.

The degenerative necrosis of the renal tubules affects the metabolic activities and may promote metabolic abnormalities in the fish (Camargo *et al.,* 2007). The present result are in agreement with those observed in *P. lineatus* exposed trichlorfon (Veiga, 2002), *L. calcarifer* exposed to cadmium (Thophon *et al.,* 2003); *C. mrigala* exposed to lambda-cyhalothrn and fenvalerate (Velmurugan *et al.,* 2007); *L. rohita* exposed to fluoride (Bhatnagar *et al.,* (2007), in *O.niloticus* exposed to alchlor (Peebua *et al.,* 2008) and in *O.mossambicus* exposed to Dimethoate (Parikh *et al.,* 2010).

It is believed that kidney tissues are a sensitive indicator of environmental pollution as they act as primary osmoregulatory organs and function in cellular immunity (de Bravo

et al., 2005). As an important organ of the immunity response (Zapata and Cooper, 1990) the observed mild to severe changes in the histoachitecture of the kidney may induce defense system changes harming the animal's homeostasis and health. Adaptive immune system of several teleost has been explored by immunotoxicological analysis by various scientists (Roales and Perlmutter, 1977; Ansari and Kumar, 1987; Arunachalam and Palanichami, 1982; Babu *et al.,* 1986; Zelikoff, 1994; Anderson and Zeeman, 1995; Hansen, 1997; Tsujii and Seno, 1990; Spazier *et al.,* 1992; Lin *et al.,* 2005; Lee and Anderson, 2005; Duffy and Zelikoff, 2006; Reynaud and Deschaux, 2006; Hansen *et al.,* 2007; Costa *et al.,* 2009; Bravo *et al.,* 2011). However, in the present study the main focus was to have an insight in to the behavioural, physiological, biochemical and histological aspects. Hence, at this juncture it is difficult to propose the immunotoxic effect of the agro-chemicals and these aspects demands more detailed analysis for understanding the immunotoxicological effects and mechanisms and the consequences as well as risks that may have on human consumers as consequence of the bioaccumulation.

As a conclusion, the findings of the present histological investigations demonstrate that the exposure of adult fresh water teleost fish, *O.mossambicus* and *L.rohita* caused moderate to severe damaging to gills and kidney.

General Consideration

Pesticides are used worldwide in agriculture (Sancho *et al.,* 2000, Oruç *et al.,* 2004 and de Menezes *et al.,* 2011). Considerable amounts enter runoff to become major pollutants in aquatic ecosystems causing disturbances of the delicate balance of aquatic ecosystems and affecting the health status of non-target aquatic organisms, such as fish (Oruç and Üner, 1999, Bretaudt *et al.,* 2000 and de Menezes *et al.,* 2011).Because there are thousands of different pesticides used around the world, data on aquatic contamination for any particular pesticide is usually quite limited. However, studies conducted in lentic and lotic systems have detected a variety of pesticides including the insecticides malathion, endosulfan and diazinon as well as the herbicides atrazine and glyphosate (LeNoir et al. 1999, Hayes et al. 2002; Kolpin et al. 2002, Thompson *et al.,* 2004). Interestingly, many pesticides found in aquatic systems are not intended or legally registered for application to aquatic systems, but they still appear. The concentrations found in surveys of natural habitats are often lower than the concentrations used in experimental tests, although these surveys are typically snapshots in time that are not always designed to detect peak concentrations . In most cases, we simply lack extensive data on natural pesticide concentrations to properly evaluate the validity of concentrations used in experiments. Given that pesticides find their way into aquatic systems, the relevant question is whether they affect the species in these systems.

Of the vast number of substances that have been introduced to aquatic systems around the world, a number posing serious environmental threats have primarily been identified either through single-species toxicity testing in the laboratory, or they have been brought to light as a result of observing biological effects in situ. Examples include dichlorodiphenyl-dichloroethylene (DDE) and eggshell thinning in birds (Hickey *et al.,* 1968), sex changes in freshwater fish associated with endocrine disrupting chemicals, EDCs (Vos *et al.,* 2000), and tumors in marine fish associated with polycyclic aromatic hydrocarbon (PAH) accumulation (Baumann and Harshbarger , 1995). These examples highlight the need for techniques that not only detect overt damage to organisms exposed to pollutants, but also the less obvious biochemical and physiological impairment that might ultimately result in ecological damage (Depledge and Galloway, 2005). With more than 67 million organic and inorganic substances known to date

(CAS, 2012), monitoring and assessing effects of chemical pollution necessarily faces great challenges. The concentrations found in surveys of natural habitats are often lower than the concentrations used in experimental tests, although these surveys are typically snapshots in time that are not always designed to detect peak concentrations. In most cases, there is lack of extensive data on natural pesticide concentrations to properly evaluate the validity of concentrations used in experiments. Given that pesticides find their way into aquatic systems, the relevant question is whether they affect the species in these systems.

The aquatic environment is particularly sensitive to the toxic effects of contaminants since a considerable amount of the chemicals used in industry, urbanization and in agriculture enter marine and other aquatic environment. The stressor in the environment exert their adverse effect at the organisms level leading to impairs on physiological functions in aquatic organisms. Xenobiotics are potentially harmful to fish by inducing tissue damage in gill, kidney and liver (Ahmad *et al.,* 2004), growth retardation (Gad and Sadd, 2008), genotoxicity (Aas *et al.,* 2000), reproductive disturbances (Maradonna *et al.,* 2004), tissue bioaccumulation (Rice *et al.,* 2000; Hellou and Leonard, 2004; Archana *et al.,*2011)

Fish are highly vulnerable to the presence of contaminants and are considered to be important indicators of environmental pollution (Prusty *et al.,* 2011). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.,* 2004; Clarkson, 1998; Dickman and Leung, 1998). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants built up in the food chain and are responsible for adverse effect and death in the aquatic systems (Farkas *et al.,*2002; Yousuf and El-Shahawi, 1999 and Okocha and Adedeji, 2011). Furthermore, the evaluation of hematological and biochemical characteristics in fish blood has become an important means of understanding possible mechanisms of toxicological impacts (*Borges et al.,* 2007 ; Sudova *et al.,* 2009 and Kavitha *et al.,* 2010).

Oreochromis mossambicus and *Labeo rohita* are the most popular fish species which are economically important for fisheries, aquaculture, game fishing and are also used extensively in biological, physiological and behavioural research (Skelton, 1993). Tilapia is a good biological

model for toxicological (Casas-Solis *et al.,* 2007; Giron-Perez *et al.,* 2007 and 2008) studies due to diverse characteristics, namely their high growth rates, efficiency in adapting to diverse diets, great resistance to diseases and handling practices, easy reproduction in capacity at prolific rate and finally, good tolerance to a wide range of environmental conditions (Fontainhas-Fernandes, 1998; Kumar *et al.,* 2011).

The neonicotinoids are a new insecticide class which includes the commercial products imidacloprid, acetamiprid, nitenpyram and thiamethoxam (Stark et al. 1995; Yamamoto & Casida, 1999 and Suchail *et al.,* 2000 and Isawa et al., 2003). Toxicity data for these new group of insecticides for aquatic invertebrate are far from enough , moreover, very less data exists for these chemicals on non-target organisms, especially those inhabiting fresh water aquatic systems are either insufficiently known or not reported yet. Imidacloprid (IMI) is a systemic chloronicotinyl insecticide. Toxicological studies on rats and mice and dogs have proved IMI to be moderately toxic (Tomizawa & Casida, 2003)**.** Response to IMI toxicity in birds has shown varied behavioural changes in birds. Exposure to IMI has led to histopathological changes as reported by Kammon *et al*., (2010). Risks of this novel pesticides has also been reported to be genotoxic on frogs and toads (Feng *et al*., 2004; Li-tao *et al*., 2006). IMI has been proved to be moderately toxic to fish. Toxic responses of IMI has been studied by Rajput et al., (2012) on fresh water fish, C*larias batrachus* and have reported the adverse effect of these toxicant on the protein profile of the fish. IMI has also been found to have profound influence in serum biochemical profile of fresh water fish *Channa punctatus* (Padma priya *et al*., 2012). A review of toxicity data of IMI toxicity for terrestrial non-target organisms such as Mammals, birds, and amphibians as well as aquatic organisms such as fish, amphibians and various invertebrates presented here thereby suggests that they too are very sensitive to broad-spectrum neurotoxic insecticide IMI. Unfortunately, in spite of all the technical knowledge gathered in this area of science in recent decades, little effort has been made to study the toxicity of IMI insecticides to the non-target taxa particularly fresh water teleosts. Thus, it is important to assess the concentration at which these chemicals are toxic to non-target aquatic organisms. It is rational thus to select imidacloprid for the present study.

Fungicides are either chemicals or biological agents that inhibit the growth of fungi or fungal spores; they also inhibit or kill fungi underlying diseases important to man. As reported by

Lorgue *et al*., (1996) pesticides are the most common cause of animal poisoning (45.5%), with fungicides accounting for 6.1% of all pesticides. The two most commonly involved species are dogs and cattle. The types of fungicides used in agriculture and food processing and storage range from those of relatively low toxicity to those, which can be lethal to animals (Oruc *et al*., 2009). Understanding mechanisms of fungicide action and toxicity is important because humans and domesticated animals encounter these pesticides through a wide variety of applications. Unfortunately, most toxicity data are from model laboratory animal's i.e rats, mice, and rabbits and offer little information on fresh water organisms.

Curzate M8 (CZ) fungicide is formulated as a 72% wettable powder: a mixture of 8% cymoxanil and 64% Mancozeb. Cymoxanil is toxic to aquatic organisms, such as fish and crutaceans and chronic ecotoxicity of this compound has been proved in *Daphnia magna* (Baer, 1993 a & b; and Kraemer, 1996). Mancozeb, another constituent of CZ is chemically identified as ethylenebisdithiocarbamate (EBDC). Mancozeb and Cymoxanil have been individually studied in various animal models and found to be mild to moderately toxic. However no studies have been recorded on CZ which is a mixture of Mancozeb and Cymoxanil particular with reference to fresh water teleost fish.

Acute toxicity is expressed as the median lethal concentration (LC_{50}) that is the concentration in water which kills 50% of a test batch of fish within a continuous period of exposure which must be stated (Amweg *et al.,* 2005).The application of the LC50 has gained acceptance among toxicologists and is generally the most highly rated test of assessing potential adverse effects of chemical contaminants to aquatic life (Brando *et al.,* 1992; Kumar, 2004; Fagr *et al.,* 2008; Gad and Saad, 2008; Khayatzadeh and Abbasi, 2010). The use of 96-h, LC_{50} has been widely recommended as a preliminary step in toxicological studies on fishes (Chapman, 2000; Ali and SreeKrishnan, 2001; ASTM, 2002; USEPA, 2005; APHA, 1998, 2005; Parrott *et al.,* 2006; Moreira *et al.,* 2008).

Mortality is obviously not the only end point to consider and there is growing interest in the development of behavioural markers to assess the lethal effects of toxicants. Abnormal behaviour is one of the most conspicuous endpoints produced by these toxicants, but until recently it has been underused by ecotoxicologists (Little and Brewer, 2001; Dell'Omo, 2002; Gerhardt, 2007; Hellou, 2010). Behavioral disturbances may be observed in aquatic biota at

concentrations of contaminants that can exist in the field, the sensitivity of these responses thus allows improving environmental risk assessment (Amiard-Triquet, 2009). Therefore, the use of behavioral biomarkers, associated to biochemical and physiological markers in carefully selected species will reveal a risk of cascading deleterious effects at the community and ecosystem levels. Series of studies has been conducted on fingerlings (Ugwemorudong and Sunday. (2010); and adult fish (Gabriel Edwards *et al.*, 1991; Kidd and James, 1991; Santhakumar *et al.,* 2000; Battaglin and Fairchild, 2002; Chindah *et al.,* 2004; Prasanth *et al.,* 2005; Okey, 2009; Ujagwung *et al*., 2010; Parikh *et al.,* 2010; Singh *et al.,* 2010; Srivastava *et al.,* 2010; Zhang *et al.,* 2010; Barbieri and Ferreira, 2011; Maniyar *et al.,* 2011) with a variety of pesticides. Perusal of literature reveals paucity of information on acute toxicity of IMI and CZ on freshwater fish, *Oreochromis mossambicus* and *Labeo rohita*. Hence, keeping in mind the importance of the acute toxicity as well as the behavioral responses, the present study has been focused to first evaluate the acute toxic effects on mortality and behaviour .

IMI and CZ exposed fish exhibited reduced activity compared to the control fish. The intensity of the behavioural activities of the fish decreased with increasing concentration and duration of exposure. The fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium due to exposure of IMI and CZ. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over their bodies, was more pronounced at higher concentrations, suggesting sensitivity to the agrochemicals (Wu and Chen, 2004; Shwetha and Hosetti, 2009).

The probit analysis revealed the fact that the LC_{50} value for *L. rohita* (0.8536 – IMI, 51.2689 – CZ) was much higher than *O. mossambicus* (0.7319 – IMI, 39.84 – CZ) for both the agrochemicals. It is evident from the result that CZ is less toxic than IMI. The toxicity of IMI and CZ LC_{50} for freshwater fishes when compared, revealed the fact that *O. mossambicus* was more sensitive to both the agrochemicals than *L. rohita*.Hence, from the present studies one can conclude that the acute response of the both the agrochemicals demonstrated variation perhaps due to their physiological status and this reflected the change in their behaviour.

Blood is most important and abundant body fluid. Its composition often reflects the total physiological condition (Venkatesan *et al.,* 2012). The blood parameters have been considered as diagnostic indices of pathological condition, findings are important for the assessment of

systemic functions and overall health of animals. Furthermore, the findings also helps in diagnosing the structural and functional status of animals exposed to the toxicant (Atamanalp and Yanik, 2003; Talas *et al.,* 2009 and Suvetha *et al.,* 2010).Consequences of pesticides on hematological factors of a number of fish species have been investigated in several studies: in *Cyprinus carpio* (Gluth and Hanke , 1985; Satyanarayan *et al*., 2004; Salvo *et al*., 2008 and Abdulmotalib *et al*., 2012) and *Clarias batrachus* (Benarji and Rajendranath, 1990; Patnaik and Patra, 2006; Kharat and Kothavade, 2012; Summarwar and Verma, 2012) in *Oreochromis mossambicus* (Sampath *et al*., 1993; Ali and Ran, 2009; Desai and Parikh, 2012), in *Heteropneustes fossilis* (Singh and Srivastava, 1994; Nath and Banerjee, 1996; Deka and Dutta, 2012), in *Cyprinion wabsoni* (Khattak and Hafeez, 1996;), and in *Piaractus mesopotamicus* (Tavares et al., 1999; Saxena *et al.* 2002; Carraschi *et al*., 2012).

In spite of the immense literature available by way of scientific information available on the pesticide toxicity on fishes, limited information is available on the effect of IMI and CZ, particularly with reference to the sub-lethal concentration on the haematological modulation. Hence, the assessment of the haematological alterations in fresh water teleost fishes at different concentration of IMI and CZ was undertaken. A Significant decrease was observed in RBCs, Hb and PCV values in *O.mossambicus* exposed to IMI and CZ. While, in *L.rohita*, a significant elevated RBCs, Hb and PCV values was observed in a dose dependent manner as compared to control. A significant decrease in Hb on IMI and CZ exposure to *O. mossambicus* suggest that the fish was under stress of anaemia due to toxic action of agrochemicals on the erythropoietic tissue. A decrease in RBC, Hb content and PCV has been observed earlier in fishes exposed to different pesticides (Svobodova *et al*., 1997; Park *et al*., 2004; Kori-Siakpere and Oghoghene, 2008; Palanisamy *et al*., 2011 and Saravanan *et al*., 2011). In *O.mossambicus* there was significant decreased in PCV associated with significant increase in MCV and MCH. Our results are parallel with the experiments performed by Nte *et al*., (2011) on fish hematology and have correlated the increase in MCV and MCH with decreased in PCV. The decrease in PCV indicates hypoxic condition of the fish due to anaemia on exposure of the pesticide. Furthermore, the observed low concentration of MCHC during the present work might have resulted from decrease in Hb synthesis consequent of effluent toxicity (Joshi *et al*., 2002; Shah, 2006; Parma et al., 2007; Adam and Agab, 2008; Rao, 2010; Ada *et al*., 2011; Desai and Parikh, 2012; Venkatesan *et al*., 2012). On exposure of IMI and CZ in *L.rohita* a significant increment of

MCV, MCH and MCHC associated with increment of PCV and Hb value were observed. In the present study the species specific differences in haematological indices were evident which are in agreement with the earlier reported work (Kakuta and Nakai 1992; Adedeji *et al*., 2000; Orun *et al*., 2003; Velisek *et al*., 2009a &b; Adedeji and Adegbile, 2011; Dikic *et al*., 2013).

As a result from the present study it can be concluded that the exposure of fish to IMI and CZ pesticides resulted in significant alterations in haematological parameters. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment. And furthermore, the haematological studies provide a rapid and sensitive method for predicting the effects of sub-lethal exposure on general health and well being of fish.

The utility of biochemical approaches in environmental pollution monitoring and characterization of exposure to stressor for the use in environmental risk assessment is based on the assumption that low concentrations of a toxicant will cause biochemical responses within individual organisms before these effects are observed at higher levels of biological organization (Sarkar *et al*., 2006). Such biochemical responses are considered to be rapidly responding endpoints (Adams, 2002), and thus most biochemical biomarkers in the laboratory studies are assessed after acute exposure to chemicals. Changes in the biochemical profile indicate alterations in metabolism of the organism resulting from the effect of the pesticide and they make it possible to study the mechanisms of the effects of these pesticides (Luskova *et al*., 2002). In the view of scantiness of information available on IMI and CZ toxicity, to move a step ahead after observation of the heamatolgical alterations the present work was under taken to have an insight regarding its biochemical alterations in the four tissues i.e. Gills, Liver, Kidney and Muscles.

The changes in the glycogen, protein and lipid profile exhibited a significant decrease in all the tissues in a dose dependent manner. Proteins are indispensable constituents required by organisms in tissure building and play a important role on energy metabolism (Yeragi *et al*., 2003; Remia *et al*., 2008; Pang-Hung *et al*., 2008). In the present investigation there was an overall decrease in the protein content in liver, muscle, kidney and gills. The physiological status of animal is usually indicated by the metabolic status of proteins (Nelson & Cox, 2005; Magar & Shaikh, 2012). The depletion in the protein may have been due to their degradation and possible utilization for metabolic purposes. The decrease in the protein content was found to be maximum in liver followed by muscle, kidney and gill respectively, these variations in response of the

pesticides in both the fishes suggests difference in metabolic calibers of individual tissue (Satyanarayana, 2005; Venkataramana *et al*., 2006). Furthermore, the decrease in the protein content of all the tissues is also suggestive of impairment of protein synthesis or increase in the rate of its degradation to amino acids due to stress induced pesticide exposure, which can result in the production of free amino acids in the tricarboxylic acid cycle for energy production (Jankins *et al*., 2003; Radha *et al*., 2005; Naveed *et al*., 2010; Ganeshwade, 2011). Our results are in agreement with the earlier reported depletion in the protein content of *O.mossambicus* (Vijuen and Steyn, 2003; Aniladevi, 2008; Varadarajan, 2010; Al-Kahtani, 2011) and *L.rohita* (Ramesh *et al*., 1993; Das and Mukherjee 2003; Sivaperumal, 2008; Sharma and Singh, 2009; Indirabai *et al*., 2010; Rajput *et al*., 2012).

Depletion of glycogen in the present study in *O.mossambicus* and *L.rohita* was maximum in liver followed by muscle, gills and kidney, may be due to direct utilization for energy generation, a demand caused by pesticide stress induced hypoxia. During stress an organism needs energy which is supplied from reserved glycogen. Thus, the depletion in glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to pesticides (Kawade and Khillare, 2012). Furthermore, the decrease in the level of total protein, and glycogen and concentrations of pesticide caused an increase in the glucose level leading to lethargy. Glucose increase is a general response of fish to acute and sub-lethal pollutant effects (Luskova *et al*., 2002). The dose dependent elevation in the glucose was reported for both the telesot fish. Thus, hyperglycemia can be viewed as a physiological response of the fishes to meet the critical need for energy under toxic stress. A significant decrease in the total lipid content of all the tissues (liver < muscle < kidney < gills) exposed to IMI and CZ in a dose dependent manner was observed. Decreased lipid content suggests that an impairment of the lipid storage has taken place in the fishes and that the lipid might have been channeled for other metabolic functions in which it probably plays a vital role during stress condition. Since lipids form the rich energy reserves whose calorific value is reported to be twice than that of an equivalent weight of carbohydrates or proteins (Sobha *et al*., 2007 and Gijare *et al*., 2011). Lipids serve as energy reserves to meet the metabolic demand for more energy to mitigate toxic stress. The decreased lipid content in the present investigation is parallel with the earlier reported altered lipid profile in *Orechromis mossambicus* (Amudha *et al*., 2002; Leela *et al*., 2002 and Shivaparvathi *et al.*, 2002); in *Perca flavescens* (Levesque *et al*., 2002); in *Cyprinus carpio* (Swapna *et al.,* 2006); in

Gambusia affinis (Revathi *et al.,* 2005); in *Anguilla Anguilla* (Febien pierron *et al,* 2007) and in *Channa punctatus* (Maruti and Rao, 2001), provide substantial support to the present findings. A significant decrease in cholesterol was reported, reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis particularly in the liver as it plays a major role in cholesterol homeostasis by regulating lipoprotein metabolism and lipid output in bile (Marzolo and Rigotti, 1990; Dietschy and Turley, 1993). The liver is a key organ in the synthesis and excretion of cholesterol, hence any type of obstruction in the liver will cause alterations in cholesterol. Pesticide induced toxicity has probably resulted into destruction of liver cells hence, the cholesterol level eventually falls below normal due to decrease synthesis (Kamath, 1972). Reduction in cholesterol could also be due to reduce absorption of dietary cholesterol (Jayntha Rao *et al*., 1984; Kanagaraj *et al*., 1993 and shakoori *et al.,* 1996). However, Remia *et al.*, (2008) reported that the decline of cholesterol may be due to utilization of fatty deposits instead of glucose for energy purpose. Similar results were observed by Fahmy (2011) in *O.mossambicus* on exposure to Malathion, by Shardamani and Shelvarani, (2009) in *O.mossambicus* by exposure of Metribuzin; by Ganeshwade, (2012) in *Punctius ticto* on exposure of Dimethoate and Singh *et al*., (2010) in *Channa punctatus* on exposure of Phorate.

In the present study, compared to control, ALT and AST were found to be significantly elevated in all the tissues of fishes exposed to IMI and CZ in a dose dependent manner. The highest activity was observed in liver followed by kidney and muscle. As proposed by Vardharajan, (2010), the primary energy currency in fish is amino acids. Elevated activity of transferases is possibly a result of a response to stress induced by pesticides to generate keto acids like α-keto glutarate and Oxaloacetate for contributing to gluconeogenesis and/or energy production necessary to meet the access energy demand. This clearly indicates that stress brings about, the metabolic reorientation in the tissues by raising energy resources through transaminases system. Similar studies have been reported by (Arshad *et al*., 2007; Gabriel *et al*., 2011; Rao, 2006 and Velmurugan *et al.,* 2008) and they have inferred that the increased enzyme activity was due to increase utilization of amino acids for energy synthesis, as consequents of a fish suffering from toxic stress and energy crisis.

ALP is also one of the important markers for liver and kidney. A significant increase in enzyme activity in liver might be due to a stress induced over activity of hepatobiliary cells, which have involved in detoxification mechanism. Further increased ALP activity also may be due to

pathological processes such as liver impairment and kidney dysfunction (Barse *et al.,* 2003). Thus in the present study, increase in the levels of ALP and AST reflects liver damage, whereas an elevation in ALP activity may be indicative of renal and liver damage (Gill *et al*., 1990; Bhattacharya *et al*., 2005; Vardharajan, 2010; Stalin and Das, 2012). On exposure of IMI and CZ gills, liver and kidney showed an elevated pyruvate levels in a dose dependent manner compared to control. This might be due to the higher glycolysis rate which is the only energy producing pathway for the animal when it is under stress condition. Furthermore, the end product of glycolytic pathway is pyruvate. Pyruvate occupies an important junction between various metabolic pathways it may be decarboxylated to acetyl CoA which can enter the TCA cycle or it may be utilized for fatty acid synthesis.

LDH acts as a pivotal enzyme between glycolytic pathway and TCA cycle. It catalyses the conversion of pyruvate into lactate, under anaerobic conditions (Lehninger, 1993). A fish under stress preferentially meets its energy requirements through anaerobic oxidation (Wallice - Luiz, 1998). The LDH in the tissues (liver, kidney, muscle and gills) of fishes treated with IMI and CZ showed an elevated activity in a dose dependent manner compare to control. Increased LDH activity suggests a significant increase in the conversion of pyruvate to lactic acid, thereby leading to the accumulation of lactic acid. Thus, the observed increased LDH can be interpreted as a shift in the respiratory metabolism from aerobic to anaerobic in order to meet the enhanced energy demand under the toxic stress (Singh and Shrivatava, 1982; Ansari and Kumar, 1988; Ferrando and Andreu-Moliner, 1991; Kamalaveni *et al*., 2003; Gorbatiuk, 2010).

An increase was observed in activity of GDH in all the tissues of the fish exposed to IMI and CZ. This suggests the active transdeamination of amino acids for the incorporation of keto acids in to the TCA cycle to release necessary energy required for the synthesis of new protein (Sreedevi *et al*., 1992; Shivramkrishna and Radhakrishnaiah, 1998; Prashanth and Neenagund, 2008). This increased activity may have helped in funneling more α-ketoglutarate into TCA cycle for more energy generation. This indicates higher oxidation of amino acids to combat the toxic effect of pesticide and the higher activity of GDH may result in efficient operation of oxidative deamination under toxic effect of IMI and CZ (Kumar et al., 2010). The oxidation of glutamate in kreb's cycle leads to increased energy (Narasimha and Rama, 1985 and Naveed *et al.,* 2010).

A pesticide produces stress condition in any organism, including fish (Ateeq *et al.,* 2002). In Pisces, three different pathways are exhibited followed by pesticide like stressor exposure. The

basic pathway followed is the activation of HPI axis and altered levels of cortisol. However, Major complications arise when the stressor is very effective and the body starts expressing other two mechanisms of stress response. They include lipid peroxidation and expression of various antioxidant mechanisms like GST, CAT, SOD and GPx and scavengers such as GSH and ascorbic acid. Expression of these two mechanisms is the clear indication of pesticide toxicity, as well as the counter mechanisms exhibited by the organism. An extensive survey of available literature indicates that for the last two decades pesticide-induced OS has been considered as a possible mechanism of toxicity and hence it has been a focus of toxicological research even today. The pesticides have been shown to induce production of ROS by altering the balance between the oxidants / prooxidants and antioxidants through promoting lipid peroxidation (LPO) and depleting the antioxidative cellular reserves (both the enzymatic and non enzymatic) leading to a condition of OS. The range of its impact spans from tissue injury, and aging through apoptosis, to onset of various known/unknown diseases. However, the exact mechanism(s) of their action in fresh water fishes has still not been completely understood particularly for IMI and CZ. Hence, in the present work, an endeavour has been made to explore the mechanisms on pesticides induced OS, cellular events influenced by OS, in various key organs of pesticide exposed fishes. After establishing the haematological alterations as well as the biochemical parameters, it is worth exploring the effect of these agrochemicals on lipid peroxidation as well as their antioxidant defence mechanisms as this aspect of the toxicity data for this new group of insecticides for aquatic invertebrate are far from enough.

Detoxification path at tissue level can be detected by biochemical markers of oxidative stress. The first line of defense to oxidative stress is the use of antioxidant scavengers, such as ascorbic acid (vitamin C), vitamin E, uric acid, carotenoid and glutathione. The second line of defense includes cellular mechanism which helps in removing excess ROS and avoids oxidative damage. It includes GPx, GST, CAT and SOD. The present study agrochemical stress has significantly increase ascorbic acid content in liver, kidney and gills. Ascorbic acid content plays an important role in detoxification of the foreign bodies or toxicants in metabolic process. It is identified that ascorbic acid is an antioxidant which might inhibit the oxidative metabolism of agro-chemicals and thus probably could prevent the production of electrophilic metabolites and as a part of redox buffer system it can scavenge harmful free radical metabolites/ reactive oxygen species (Sato *et al*., 1990; Guha and Khuda-Bakhsh, 2002). Hence, the high level of ascorbic acid

observed in the present study as agro-chemical induced stress condition is justifiable. The indication to detoxifying enzymes is reported to be accompanied by increase in ascorbic acid content of liver, kidney and gills, which stimulate detoxification of toxicant, suggestive of liver, kidney and gills to be the sites of detoxification. Our results are in agreement with earlier reported elevated ascorbic acid content in *Channa gachua* (Ali and Ilyas, 1981); in *Oreochromis mossambicus* (Guha and Khuda-Bukhsh, 2001); in *Clarias batrachus* (Kamble *et al.,* 2001 & 2010) and *Puntius ticto* (Ganeshwade, 2011).

In the present study there was a significant increase in the GSH activity in liver, muscle and gills on exposure of agrochemicals in a dose dependent manner. Due to its function in resisting the reactive oxygen toxicity, the changing degree for total glutathione can serve as markers of exposure to agrochemicals and the alterations to be an adaptive mechanism to oxidative stress.

GPx level was found to be increased in gills and liver which might be because of the induction, as in the case of any other defensive antioxidant enzyme. An increase in GPx activity in liver, kidney and gills is probably eliminating the access of H_2O_2 and lipid hydrogen peroxide produced in the fishes exposed to agro-chemicals. Similar results have been observed in the liver of *Cyprinus Carpio* (Li et al., 2003; Vinodhini and Narayanan, 2009); *Rainbow trout* (Orun *et al.*, 2005). Tissue specific increase of GPx in the present study indicates the adaptive approach by the fish to defend the oxidative stress, generated as a consequents of agro-chemical exposure and that the increased production of H_2O_2 due to OS is thus scavenged by the enzyme GPx. Elevated GPx activity also indicates that the regulation of ROS generated due to agro-chemicals is efficiently achieved by GSH pathway.The elevated levels of GST in the present studies indicate the shift towards a detoxification mechanism under agro-chemical exposure. There is more GST activity in hepatic tissue compared to kidney and gills, which is due to effective role of liver in xenobiotic detoxification (Goering *et al.,* 1995). Organ-based GST assay reflected a dose dependent significant increase in liver, kidney and gills of both the fishes exposed to agro-chemicals. The increase in the activity of GST reported in the present study indicates the biotransformation pathway used a protective response in fish towards exposure to an oxidative stress inducing agro-chemicals. Similar kind of results have been reported earlier in liver, kidney and gills of freshwater murrel *C.punctatus* (Dabas *et al.,* 2012), obtained by in *O.niloticus* (Wengu *et al.,* 2009 ; Gad, 2011) and in *O.mossambicus* (Anushia *et al*., 2012).

The present study revealed that SOD and CAT activities in the liver, kidney and gills of *O.mossambicus* and *L.rohita* exposed to agrochemicals were increased. Furthermore, a dose dependent increase in the level of LPO, as expressed by MDA formed, was observed in liver, kidney and gills of the fishes exposed to IMI and CZ . Elevated MDA level was observed in all the tissues on exposure to agrochemicals indicating that elevated antioxidant enzyme activities were not enough to prevent lipid peroxidation.

Thus, from the present study it can be concluded that the response of antioxidant enzymes (SOD, CAT, GPx, and GST) and non-enzymatic antioxidant/scavengers (ascorbic acid and GSH) showed that the fishes are under severe oxidative stress and that the agro-chemicals are acting as potent free radicals generators. Lipid peroxidaton (MDA) level proves that extensive lipid peroxidation has occurred on exposure of the agro-chemicals. And that both the antioxidants interact in a concerted manner to eliminate ROS and prevent damage to cellular components. This suggests that IMI and CZ at levels below median lethal concentration are capable of causing oxidative damage in *O.mossambicus* and *L.rohita*.

The size or weight of the liver, spleen, and gonads relative to fish length or weight signifies overall health and reproductive status. Hence, in the current investigation an attempt was also made to understand the toxicity of sublethal dose of IMI and CZ in *O.mossambicus* and *L.rohita* by determining its effects on condition factor and organosomatic indices (HSI, SSI and GSI). If a change in function exists, there will be a gross change in the structure of organs or tissues. Taking the aforementioned into account, along with condition factor morphological alterations were observed for liver, Gonads and Spleen. In the present study, HSI revealed that there was a significant decrease in weight of the liver as the concentration of agro-chemicals increased. However, liver of both the fishes exhibited a greater diminution in HSI on IMI exposure compared to that of CZ. Decreased HSI value is indicative of that the fish were under stress and that IMI was more toxic compared to CZ. Parallel to the decreased HSI, alterations in the histological structures were also observed.

Hepatocellular necrosis with parenchymal vacuolization, hypertrophy of hepatocytes, hemorrhages and widening of blood sinusoids were the distinct altered features in the agro-chemical exposed fish liver. At low dose only slight vacuolation and mild swelling of hepatocytes in which the nucleus retained a nearly normal shape was recorded. At high dose the

main alterations found in the liver were: irregular-shaped nuclei, nuclear hypertrophy, nuclear vacuolation and the presence of eosinophilic granules in the cytoplasm. Liver showed fatty infiltration, these findings were apparent as the liver is considered the organ of detoxification and excretion. Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, or in other organs. Several authors recorded histopathological changes in the liver of freshwater fish treated by insecticides diazinon, dimethoate, malathion and glyphosate, respectively (Neskovic et al. 1996; Sakr *et al* 2001; Van Dyk, 2003; Stentiford *et al*., 2003; Fanta *et al.,* 2003; Mela *et al.,* 2007 de Melo *et al.,* 2008; Kunjamma *et al.,* 2008; Mataqueiro *et al.,* 2009 ; Parikh *et al.,* 2010).

In the present study the SSI values were reported to be decreased in the agro-chemical exposed fishes compared to control. The decrease seems to be due to necrosis and perturbations in cell processing, both of which could impact the overall condition of the individual fish. Reduced SSI may be the response of the fish to combat agro-chemical stress (Gabriel *et al.,* 2010). Histological observations also revealed the presence of Melanomacrophage centers (MMC) scattered throughout spleen. Furthermore, the associated alterations observed in the spleen were mild to severe depletion of white pulp which was replaced by empty space and activation of MMCs along with hemorrhage, fibrosis and intracellular edema is suggestive of an adaptive immune response to agrochemical stress. These findings are consistent with the earlier reports (Pulsford *et al.,* 1992; Falk *et al.,* 1995; Suresh and Veeraraghavan, 1998; Simko *et al.,* 2000; Decostere *et al.,* 2001; kman and Norrgren, 2003).

GSI values decreased in dose dependent manner. Reduced GSI indicates lowered reproductive activity (Bernard *et al.,* 2001 and Hassanin *et al.,* 2002), under agro-chemical stress. Histomorphological alterations are also supporting the reduced GSI observed and probably may be associated with the impairment of the production of steroid hormones which might have arrested the formation of germ cells and cause degeneration or necrosis. Vacuolated follicular epithelium and oocyte atresia were the prominent observations in the present studies in female gonads. The histological alterations of the testis on exposure of agro-chemicals consists of altered structure of seminiferous tubules associated with damage and presence of large number of inter and intra tubular vacuoles and severe necrosis.

As general indicators of the overall health and well-being of the fish, an alteration in the indices indicates deleterious effect of the agro-chemicals. Hence, from the present study it is apparent that IMI and CZ have resulted in to considerable alteration in CF as well as HSI, SSI and GSI. It can be concluded that the histological changes induced by agro-chemicals varied in their expression and IMI was found to be more toxic than CZ. Histological alterations are biomarkers of effect of exposure to environmental stressors, revealing prior alterations in physiological and/or biochemical function (Hinton *et al.,* 1992). Hence, in the present study an attempt is made to evaluate the effect of IMI and CZ on the histological alterations in gills and kidney of *Oreochromis mossambicus* and *Labeo rohita*. The common histological observations in the gills of *O.mossambicus* and *L.rohita* includes proliferation of the epithelium of the gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, severe degenerative necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucus cells proliferations. Edematous changes, characterized by epithelial detachment were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Also, dilation and congestion in gill filaments were observed. Atrophy of secondary lamellae was seen. However, comparatively the degree of pathological changes observed on IMI exposure was more prominent compared to CZ for *O.mossambicus* as well as *L.rohita*. Distinct feature observed was hyperemia and hemorrhages in primary and secondary gill lamellae at high dose of CZ exposure and at low dose of IMI in *L.rohita.*

Histological alterations in the kidney of both the fishes included severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and hemorrhages, haemolysis. Vacuolar degenerations in the epithelium of renal tubules and dilation in the capillary tubes of renal tubules were observed. Also edema of Bowman's capsule with atrophy in the glomeruli and dilation in the renal blood vessels were observed. Kidney tissue from *O.mossambicus* and *L.rohita* showed mild necrosis and tubular degeneration on CZ exposure where as on IMI exposure it showed severe necrosis, vacuolation and tubular degeneration. Thus from the present studies it can be concluded that both the agrochemicals i.e. IMI and CZ are toxic to *O. mossambicus* and *L.rohita* and has resulted into deleterious changes in liver and kidney. The present study reports the acute and sublethal toxicity of IMI and CZ on biochemical profile, organo-somatic index, physiological stress response, behavioral alterations and histological

changes in gills, liver, muscle and liver as well as the kidney tissues of *O. mossambicus* and *L.rohita* fishes.

The important observations can be summarized.

- The probit analysis revealed the fact that the LC50 value for *L. rohita* (0.8536 – IMI, 51.2689 – CZ) was much higher than *O. mossambicus* (0.7319 – IMI, 39.84 – CZ) for both the agrochemicals. This indicated that IMI, even in microgram quantities can induce toxicity to fish. Of the two agro-chemicals CZ is less toxic than IMI.
- IMI and CZ exposed fish exhibited reduced activity compared to the control fish. The intensity of the behavioural activities of the fish decreased with increasing concentration and duration of exposure. The fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium due to exposure of IMI and CZ. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over their bodies, was more pronounced at higher concentrations, suggesting sensitivity to the agrochemicals.
- The exposure of fish to IMI and CZ pesticides resulted in significant alterations in haematological parameters. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment. And furthermore, the haematological studies provide a rapid and sensitive method for predicting the effects of sub-lethal exposure on general health and well being of fish.
- Depletion in protein, Lipids, Cholesterol and Glycogen content indicated the requirement of large amounts of these metabolites under a toxic stress to compensate the energy demand and the metabolic requirements. The remarkable changes in activities of enzymes like LDH, GDH and Pyruvate suggested the impaired oxidation of carbohydrates through TCA cycle. Variations in the liver and kidney-specific ALT, AST and ALP enzyme activities indicated the role of the tissue in detoxification processes under agro-chemicals toxicity. The toxicity resulted in impaired metabolism leading to disturbed homeostasis.
- The lipid peroxidation product namely malondialdehyde recorded an increase with increased concentration. A dose dependent increased level of antioxidant enzymes (CAT, SOD, GPx and GST) and scavengers (GSH and Ascorbic acid) was also observed. Thus, increase in their activity showed the efficiency of antioxidant system to defend the agro-chemical-induced stress.

- As general indicators of the overall health and well-being of the fish, an alteration in the indices indicates deleterious effect of the agro-chemicals. From the altered values of CF and indexes in the present study it is apparent that IMI and CZ have resulted in to considerable alteration in the overall health and well-being of the fish. Further, the histological changes induced by agro-chemicals varied in their expression and IMI was found to be more toxic than CZ.
- Histological observations envisaged the deleterious anatomical and morphological alterations induced in gill, liver, kidney, spleen and Gonads (Testis and Ovary) tissues by sub-lethal toxicity of the IMI and CZ agrochemicals. Each tissue showed specific sterical changes and revealed the incapability of these tissues to withstand the toxic effects induced by IMI and CZ.Histological damages in the tissues were found to intensified with increase in concentration and duration. Hepatocellular necrosis with parenchymal vacuolization, hypertrophy of hepatocytes, hemorrhages and widening of blood sinusoids were the distinct altered features in the agro-chemical exposed fish liver.The histopathological changes observed in the kidney were severe necrosis of tubular epithelial cells, thickening of the bowman's capsule and shrinkage of the glomeruli along with severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and haemorrahage, haemolysis. Vacuolar degenerations in the epithelium of renal tubules.

The present study revealed that the agrochemicals IMI and CZ are potent to cause toxic responses, even structural alterations, in non target aquatic organism like fish. Though it is degrading very readily, because of short half-life, the chances for acute toxicity are not avoidable. The hazards of environmental contamination are usually associated with unexpected side effects due to pesticides or pesticide-derived compounds. This report proves that IMI is highly toxic and that the CZ is moderately toxic. The toxic responses are reflected by the behavioral, biochemical and pathological changes. But concerted effort in reducing the use of pesticides and implementing natural remedies for pest-encroachment through organic farming can help resolving the problem of agrochemical pollution. Regulations limiting the use of agrochemicals, along with alternative solutions that are safer and non-toxic to the environment and humans should be encouraged. One such alternative is so called "natural pesticides" that are

not synthetically produced, but are derived from nature such as botanicals pesticides, microbial/biological agents and inorganic minerals. These solutions are generally assumed to be less toxic for human health than synthetic pesticides and could represent an interesting alternative. Further studies are needed on the occurrence, fate and impact of such pesticides on the ecosystem and public health. There is also need to undertake such research that will give early warning signal on the lethal limits of pesticides in fresh water fish species. It is therefore imperative that safe limits/standard for fish and other aquatic fauna in the fresh waters should be developed using data obtained from agrochemical induced ecotoxicological studies. Genotoxicity can be expected on exposure to agrochemicals. The reviews of related work have suspected the chances for significant changes at the molecular level. But this perspective can be considered for future studies.

BIBLIOGRAPHY

Aas, E., Baussant, T., Balk, L., Liewenborg, B. and Andersen, O.K. 2000. PAH metabolites in bile, cytochrome P4501A and DNA adducts as environmental risk parameters for chronic oil exposure: a laboratory experiment with Atlantic cod. *Aquat. Toxicol*. **51**: 241-258.

Abdel-Baky, T.E. 2001. Heavy metals concentrations in the catfish, *Clarias gariepinus* (Burchell, 1822) from River Nile, El - Salam canal and lake Manzala and their impacts on cortisol and thyroid hormones. *Egypt. J. Aquat. Biol. & Fish.*, **5**(1): 79 - 98.

Ackermann, C. 2008. A quantitative and qualitative histological assessment of selected organs of *Oreochromis mossambicus* after acute exposure to cadmium, chromium and nickel. M.Sc. Dissertation. University of Johannesburg, Johannesburg.

Adam, H.M. and Agab, H. 2008. Haematological and biochemical indices of *Clarias garipeinus* collected from River Nile, Sudan. *Sudan Academy of Sciences J*., **2**(1): 67-90.

Adams, S.M. 2002. Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland.

Adams, S.M. and McLean, R.B. 1985. Estimation of largemouth bass, *Micropterus salmoides* Lacepede, growth using the liver somatic index and physiological variables. *Journal of Fish Biology*, **26**: 111-126.

Adams, S.M., Crumby, W.D., Greeley, M.S.Jr., Shugart, L.R. and Saylor, C.F. 1992. Responses of fish populations and communities to pulp mill effluents: a holistic assessment. *Ecotoxicol. Environ. Saf.*, **243**: 347-60.

Adams, S.M., Shepard, K.L., Greeley, M.S.Jr., Jimenez, B.D., Ryon, M.G., Shugart, L.R. and McCarthy, J.F. 1989. The use of bioindicators for assessing the effects of pollutant stress on fish. *Mar. Environ. Res.,* **28**: 459-64.

Adedeji A., Weller T.M. and Gray J.W. 2007. MRSA in children presenting to hospitals in Birmingham, UK. *J. Hosp. Infect.*, **65**: 29-34

Adedeji, O. B., Adedeji, A.O., Adeyemo, A.K. and Agbede, S.A. 2008. Acute toxicity of diazinon to African catfish (*Clarias gariepinus*). Afr. J. Biotechnol., **7**: 651-654.

Adedeji, O.B. 2010. Acute effect of diazinon on blood plasma biochemistry in the African catfish (*Clarias gariepinus*). *Journal of Clinical Medicine and Research,* **2**(1): 001-006.

Adedeji, O.B. and Adegbile, A.F. 2011. Comparative Haematological Parameters of the Bagrid Catfish (*Chrysichthys Nigrodigitatus*) and the African Catfish (*Clarias Gariepinus*) from Asejire Dam in Southwestern Nigeria. *Journal of Applied Sciences Research*, **7**(7): 1042-1046.

Adedeji, O.B., Adeyemo, O.K. and Agbede, S.A. 2009. Effects of diazinon on blood parameters in the African catfish *(Clarias gariepinus)*. *African J. Biotechnology*, **8**(16): 3940-3946.

Adedeji, O.B., Taiwo, V.O. and Agbede, S. 2000. Comparative haematology of five Nigerian freshwater fish species. *Nig. Vet. J*., **21**: 75-84.

Adeyemo, O.K. 2008. Histological alterations observed in the gills and ovaries of *Clarias gariepinus* exposed to environmentally relevant lead concentrations. *J Environ Health,* **70**: 48-51

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C.T. and Ayyappan, S. 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost; *Labeo rohita* (Hamilton). *Ecotoxicol. Environ. Safety*, **58**: 220-226.

Agius, C. and Roberts, R.J. 2003. Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases,* **26**(9): 499-509.

Agrahari, S. and Gopal, K. 2009. Fluctuations of certain biochemical constituents and markers enzymes as a consequence of monocrotophos toxicity in the edible freshwater fish, *Channa punctatus. Pestic Biochem Phys.,* **94**(10): 5-9.

Agrahari, S., Pandey, K.C. and Gopal, K. 2007. Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch), *Pest.Biochem. Physiol*., **88**: 268-272.

Agrawal, A. and Sharma, B. 2010. Pesticides induced oxidative stress in mammalian systems: A review. *International Journal of Biological and Medical Research,* **1**(3), 90-104.

Ahel, M., McEvoy, J. and Giger, W. 1993. Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. *Environ. Pollut*., **79**: 243-248.

Ahmad, I., Hamid, T., Fatima, M., Chand, H.S., Jain, S.K., Athar, M. and Raisuddin, S. 2000. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. *Biochim. Biophys. Acta*., **1519**: 37-48

Ahmad, S. 1995. Antioxidant mechanisms of enzymes and proteins, in: Ahmad, S. (Ed.), Oxidative Stress and Antioxidant Defences in Biology, Chapman & Hall, New York, pp. 238-272.

Ahmed, S., Palermo, C., Wanand, S. and Walworth, N.C. 2004 Anovel proteinwith similaritiestoRb binding protein 2 compensatesfor loss of Chk1 function and affects histone modification in fission yeast. *Mol. Cell. Biol.,* **24**: 3660-3669.

Ainsworth, A.J. 1992. Fish granulocytes, morphology, distribution, and function. *Ann. Rev. Fish Dis.*, **2**: 123-148.

Akhgari, M., Abdollahi, M., Kebryaeezadeh, A., Hosseini, R. and Sabzevari, O. 2003. Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for Subchronic toxicity of malathion in blood and liver of rats. *Hum. Exp. Toxicol*., **22**: 205-211.

Alavanja, M.C.R., 2009. Pesticides Use and Exposure Extensive Worldwide Rev Environ Health. **24**(4): 303-309.

Alazemi, B.M., Lewis, J.W. and Andrews E.B. 1996. Gill damage in the freshwater fish *Gnathonemus ptersii* (Family: Mormyridae) exposed to selected pollutants: an ultra structural study. *Environ Technol.,* **17**: 225-238

Ali, H.A.J and Rani, V.J. 2009. Effect of phosalone on haematological indices in the tilapia, *Oreochromis mossambicus. Turk J Vet Anim Sci.,* **33**(5): 407-411.

Ali, M. and Sreekrishnan, T.R. 2001. Aquatic toxicity from pulp and paper mill effluents: A review. *Adv. Environ. Res.* **5**: 175-196.

Ali, R. and Ilyas, R. 1981. Change in Ascorbic acid concentration of air, breathing fish *Channa gachua* (Hamilton) after exposure to Dimecron. *J. Environ. Res.* II ; II

Al-Kahtani, M.A. 2011. Effect of an Insecticide Abamectin on some biochemical characteristics of Tilapia fish (*Oreochromis Niloticus*). American *Journal of Agricultural and Biological Sciences,* **6**(1): 62-68.

Allan, J.D. and Flecker, A.S. 1993. Biodiversity conservation in running waters. *Bioscience*, **43**: 32-43.

Allin, C.J. and Wilson, R.W. 2000. Effects of pre-acclimation to aluminum on the physiology and swimming behaviour of juvenile rainbow trout (*Oncorhynchus mykiss*) during a pulsed exposure. *Aquatic Toxicology*, **51**: 213-224.

Almeida, J.A., Novelli, E.L., Dal Pai Silva, M., and Junior, R.A. 2001. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environ Pollut.,* **114**(2): 169-175.

Alwan, S.F., Hadi, A.A., Shokr, A.E., 2009. Alterations in haematological parameter of fresh water fish. *Tilapia Zilli* exposed to Aluminium. *Journal of Science and Its Applications,* **3**(1): 12-19.

American Public Health Association (APHA). American Water Work Association (AWWA) and Water Pollution Control Federation (WPCF) (1998). Standard method for the examination of water and waste water. 20th ed. Washington, DC.

Amiard-Triquet, C. 2009. Behavioral Disturbances: The Missing Link between Sub-Organismal and Supra-Organismal Responses to Stress? Prospects Based on Aquatic Research. *Hum. Ecol. Risk. Assess.,* **15**(1), 87 - 110.

Amudha, P., Sangetha, G. and Mahalingam, S. 2002. Diary induced alterations in the protein, carbohydrate and lipid metabolism of a freshwater teleost fish *Oreochromis mossambicus. Poll. Res.,* **21**: 51-53.

Amweg, E.L. and Weston, D.P. 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. *Environ Toxicol Chem.,* **24**:1300-1301.

Andersen, O., Eijsink, V.G.H. and Thomassen, M. 2000. Multiple variants of the peroxisome proliferator-activated receptor (PPAR) y are expressed in the liver of the Atlantic salmon (*Salmo salar*). *Gene,* **255**: 411-418.

Anderson, B.G. 1960. The toxicity of organic insecticides to Daphn [a.Tf, ans. of the 1959 seminar on biological probtems in water pottution, R.A. Taft sanitar,y Engineer, Centre, Technicat Report of WHO-3.

Anderson, D.P. 1990. Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. *Am. Fish. Soc. Symp*., **8**: 38-51.

Anderson, D.P. and Zeeman, M.G. 1995. Chapter 12: Immunotoxicology in fish. In *Fundamentals of aquatic toxicology: Effects, environmental fate & risk assessment.* (ed. G. Rand), pp. 371-404. Taylor and Francis, Washington, DC.

Anderson, D.P. and Zeeman, M.G. 1995. Immunotoxicology in fish. In: G.M. Rand (Ed.) *Fundamentals of Aquatic Toxicology*., Taylor and Francis, Washington, 371-404.

Anderson, D.P., Dixon, O.W., Bodammer, J.E. and Lizzio, E.F. 1989. Suppression of antibody-producing cells in rainbow trout spleen sections exposed to copper in vitro. *J. Aquat. Anim. Health*, **1**: 57-61.

Anderson, D.P., Roberson, B.S. and Dixon, O.W. 1982. Immunosuppression induced by a corticosteroid or an alkylating agent in rainbow trout (*Salmo gairdneri*) administered a *Yersinia ruckeri* bacterin. *Dev. Comp. Immunol*., **6**(2): 197-204.

Andu, A.K. and Kangur, P. 1996. The Condition, length and age distribution of pikeperch. *Hydrobiologica,* **338**: 179-183.

Aniladevi, K P. 2012. Biochemical effects of the pesticide Chlorpyrifos on the fish *Oreochromis mossambicus* Peters Thesis submitted to Cochin University of Science and Technology, India.

Anita, T., Sobha K. and Tilak K.S., 2010. A study on acute toxicity, oxygen consumption and behavioral changes in three major carps, *Labeo rohita* (ham), *Catla catla* (ham) and *Cirrhinus mrigala* (ham) exposed to fenvalerate. *Bioresearch Bulletin,* **1**:33-40.

Anjali, R. and Kulshrestha, S.K. 1990. Effect of sublethal doses of three pesticides on the ovary of a carp minnow *Rasbora daniconius*. *Bulletin of environmental contamination and toxicology,* **45**(5): 742-747.

Ansari, B.A. and Kumar, K. 1988. Diazinon toxicity: effect on protein and nucleic acid metabolism in the liver of zebrafish, *Brachydanio rerio* (Cyprinidae). *Sci. Total Environ*., **76**(1): 63-68.

Anushia, C., Sampath kumar, P. and Selva Prabhu, A. 2012. A Pilot Study on Effect of Copper and Cadmium Toxicity in *Tilapia Mossambicus*. *Journal of Research in Animal Sciences,* **1**: 020-027.

APHA 1985. American Public Health Association. Standard Methods for the examination of water and waste water 15th edition, 476.

APHA American Public Health Association. 2005. Standard methods for examination of water including bottom sediments and sludges. Standard Methods, (19th ed.), 874.

APVMA Australian Pesticides & Veterinary Medicines Authority, 2003. The reconsideration of registrations of products containing diazinon and their labels. Part 1: product cancellations. California DPR, 2004. California Department of Pesticide Regulation.Bayer technical information, Confidor, 2000. Bayer, Germany.

Arasta, T., Bais V.B. and Thakur P. 1996. Effect of Nuvan on some biochemical parameters of Indian catfish *Mystus vittatus*. *Journal of Environmental Research,* **80**: 80-83.

Archana, A., Sharbidre, Metkari, M. and Patode, P. 2011. Effect of Diazinon on Acetylcholinesterase Activity and Lipid Peroxidation of *Poecilia reticulata*. *Research Journal of Environmental Toxicology*, **5**: 152-161.

Ardali, Y. 1990. Heavy Metal Exposure from IIndustrial Waters by Absorption. MS Thesis, Universitesi Fen Bilimleri Ens.Samsun (in Turkish).

Areweriokuma, S.V., Akinrotimi, O.A. and Gabriel, U.U. 2011. Effects of Cypermethrin on condition factor and organosomatic indices of *Clarias gariepinus*. *Journal of Agriculture and Social Research,* **11**(2): 67-72.

Armbrust, K.L. and Peeler, H.B. 2002. Effects of formulation on the run-off of imidacloprid from turf. *Pest Manag. Sci*., **58**: 702-706.

Arnaudova D., Tomova, E., Velcheva, I. and Arnaudov, A. 2008. A study on the lead, zink and cadmium in various organs in fishes from Cyprinidae and Percidae families in "Studen kladenes" and "Kardzali" dam lakes. (eds: I.A. Velcheva and Tsekov) Anniversary scientific conference of ecology. 1 November 2008, Plovdiv, 327-336.

Arockia, J.J. and Mitton, J.M.C. 2006.Effect of carbamate pesticide lannate (methomy1) on the biochemical components of the freshwater cichlid *Oreochromis mossambicus* (Peters). *Ind. J. Environ Ecoplan*., **12**: 263-268.

Arshad, N., Shabbir, G., Aleem, S. and Arshad M. 2007. GOT is one of the enzymes, which gives valuable diagnostic information for a number of disease conditions. *Asian J. Exp. Sci.,* **21**(2): 239-24.

Arunachalam, S. and Palanichamy, S. 1982. Toxic and sublethal effects of carbaryl on a freshwater catfish, *Mystus vittatus* (Bloch), *Arch. environ Contam Toxicol.,* **9**: 307-316.

ASTM (American Society for Testing and Materials) 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macro vertebrates and amphibians. E729/96. In Annual Book of ASTM standards, ASTM, Philadelphia, PA., 11.05: 179-200.

Atamanalp, M. and Yanik, T. 2003. Alterations in haematological parameters of rainbow trout *(Oncorhynchus mykiss)* exposed to mancozeb. Turk. *J. Vet. Anim. Sci*., **27**: 1213-1217.

Atamanalp, M., Keles, M.S., Halilogu, H.I. and Aras, M.S. 2002a. The effects of cypermethrin (a synthetic pyrethroid on some biochemical parameters: (Ca, P, Na and TP) of rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Veterinary Animal Science,* **26**: 1157-1160.

Atamanalp, M., Yanik, T., Haliloglu, H.I. and Aras, M.S. 2002b. Alterations in the hematological parameters of rainbow trout *(Oncorhynchus mykiss)* exposed to cypermethrin. *Israeli J. Aquacult. Bamidgeh*., **54**: 99-103.

Ateeq, B., Abul Farah, M., Niamat Ali, M. and Ahmad, W. 2002. Induction of micronuclei and erythrocyte alterations in the catfish *Clarias batrachus* by 2, 4- dichlorophenoxyacetic acid and butachlor. *Journal of Mutation Research,* **518**(2): 135-44.

Athikesavan, S., Vincent, S., Ambrose, T. and Velmurugan, B. 2006. Nickel induced histopathological changes in the different tissues of freshwater fish, *Hypophthalmichthys molitrix* (Valenciennes). *J. Environ. Biol.,* **27:** 391-395.

Atif, F., Parvez, S., Pandey, S., Ali, M., Kaur, M., Rehman, H., Khan, H.A. and Raisuddin, S. 2005. Modulatory effect of cadmium exposure on deltamethrin-induced oxidative stress in*Channa punctata* Bloch. *Arch. Environ. Contam. Toxicol*., **49**: 371-377

Atli, G., Alptekin, O., Tukel, S. and Canlin, M. 2006. Response of catalase activity to Ag^{+}, Cd^{+}, Cr^{6+} , Cu^{2+} and Zn^{2+} in five tissues of fresh water fish *Oreochromis niloticus*. *Comp. Biochem. Physiol.*, **143:** 218-224.

Atreya, K. 2008. Health costs from short-term exposure to pesticides in Nepal. Soc. *Sci. Med*., **67**: 511-519.

Aulakh, J.S., Malik, A.K. and Mahajan, R.K. 2005. Solid phase microextraction-high pressure liquid chromatographic determination of nabam, thiram and azamethiphos in water samples with UV detection. *Preliminary data, Talanta.,* **66**(1): 266-270.

Avci, A., Kacmaz, M. and Durak, I. 2005. Peroxidation in muscle and liver tissues from fish in a contaminated river due to petroleum refinery industry. *Ecotoxicol. Environ. Saf*., **6**: 101-105.

Azevedo-Pereira, H.M.V.S., Lemos, M.F.L. and Soares, A.M.V.M. 2011. Effects of imidacloprid exposure on *Chironomus riparius* Meigen larvae: Linking acetylcholinesterase activity to behaviour. *Ecotoxicol. Environ. Saf.,* **74**: 1210-1215.

Azmat, R., Talat, R. and Ahmed, K. 2007. The length - weight relationship, Condition factor and impact of fluoride concentration in *Johnius belangerii of* Arabian Sea. *Research Journal of Environmental Toxicology,* **1**(3): 138-143.

Baatrup, E. 2009. Measuring complex behavior patterns in fish — Effects of endocrine disruptors on the guppy reproductive behavior. *Human and Ecological Risk Assessment*, **15**(1): 53-62.

Babu S.B.R., Jayasundaramma B. and Ramamurthi R., 1986. Behavioural abnormalities of juveniles of the fish *Cyprinus carpio* exposed to methyl parathion. *Environ. Ecol*., **4**: 95-97.

Baer 1993a Baer, K.N., 15-Jan-93. Static, acute, 48-hour EC50 of DPX-T3217-113 (cymoxanil) to Daphnia magna. Report HLR 736-92. DuPont Haskell Laboratory. Unpublished.

Baer 1993b Baer K.N., 13-Oct-93. Chronic toxicity of DPX-T3217-113 (cymoxanil) to Daphnia magna: 24-hour renewal (revision 1). Report HLR 354-93 RV1. DuPont Haskell Laboratory. Unpublished

Bailey, G.S., Williams, D.E. and Hendricks, J.D. 1996. Fish models for environmental carcinogenesis: The rainbow trout. *Environ Health Perspec.,* **104**: 5-21

Bakhshwan, S.A., Marzouk, M.S., Hanna, M.I. and Hamed, H.S. 2009. Some investigations on

the clinical and biochemical alterations associated with dizinon toxicity in *Clarias gariepinus*. *Egypt J. Aquat. Biol. & Fish.,* **13(**2): 173-197.

Bakthavallhsalam, R. and Reddy, S.Y. 1982. Toxicity and behavioural response of Anabos lestudineus (Bloch) exposed to pesticides. *Indian J. Environ. Health,* **24**: 65.

Bakthavathsalam, R. 1991. Hematology of the fish Anabas testudineus exposed to lindane and carbofuran at submerged condition and on exposure on air. *Environ Ecol*., **9**(1): 124-127.

Balani, T., Agrawal, S., and Thaker, A.M. 2011. Hematological and biochemical changes due to short-term oral administration of imidacloprid. *Toxicol Int*., **18**: 2-4.

Baligar, P.N. and Kaliwal, B.B. 2001. Induction of gonadal toxicity to female rats after chronic exposure to mancozeb, *Ind. Health*, **39**: 235-243.

Balint, T., Ferenczy, J., Katai, F., Kiss, I., Kraczer, L. and Kufcsak, O. 1997. Similarities and differences between the massive eel (*Anguilla anguilla* L.) devastations that occurred in lake Ablation in 1991 and 1995. *Ecotoxicol. Environ. Saf.,* **37**: 17-23.

Banaee, M. 2013. Physiological dysfunction in fish after insecticides exposure. Chapter 4. "Agricultural and Biological Sciences : Insecticides Development of Safer and more effective technologies." Book edited by Stanislav Trdan.

Banaee, M., Mirvagefei, A.R., Rafei, G.R. and Majazi, B. A. 2008. Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. *International Journal Environmental Research***, 2**: 189-198

Banaee, M., Sureda, A., Mirvaghefi, A.R. and Ahmadi, K. 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss). Pestic Biochem Physiol* **99:**1-6.

Banerjee, B.D., Seth, V., Bhattacharya, A., Pasha, S.T. and Chakraborty, A.K. 1999. Biochemical effects of some pesticides on lipid peroxidation and freeradical scavengers. *Toxicol. Lett.*, **107**: 33-47.

Banerji, G. and Rajendranath, T. 1990. Haematological changes induced by an organophosphorus insecticide in a freshwater fish *Clarias batrachus (Linnaeus). Trop Freshwater Biol.,* **2**: 197-202.

Barata, C., Solayan, A. and Porte, C. 2004. Role of B-esterases in assessing toxicity of OP(chlorpyrifos, malathion) and carbamate (carbofuran) pesticides to *Daphnia magna*, *Aquatic Toxicology*, **66**: 125-139.

Barbee, G.C. and Stout, M.J. 2009**.** Comparative acute toxicity of neonicotinoid and pyrethroid insecticides to non-target crayfish *(Procambarus clarkii)* associated with rice-crayfish

crop rotations. *Pest. Manag. Sci.,* **65**:1250-1256.

Barbier, N. 2005. Bilan d'activité du Centre National d'Informations Toxicologiques Vétérinaires pour l'année 2003. *Thèse de doctorat vétérinaire,* 220, Lyon.

Barbieri, E. and Ferreira, L.A.A. 2011. Effects of the organophosphate pesticide Folidol 600® on the freshwater fish, Nile Tilapia (*Oreochromis niloticus*). *Pesticide Biochemistry and Physiology*, **99**(3): 209-214.

Barcellos, L.J.G., Kreutz, L.C., Rodrigues, L.B., Fioreze, I., Quevedo, R.M., Cericato, L., Conrad, J., Soso, A.B., Fagundes, M., Lacerda, L.A. and Terra, S. 2003. Haematological and biochemical characteristics of male jundia (Rhamdia Quelen, Quoy & GaimaRDT, Pimelodidae): changes after acute stress. *Aquacul. Res*., **34**: 1465-1469.

Barreto, M., Feitoza, J.M., Magalhaes, E.G., Da Silva, K., Manhaes-de- Castro, R.R., Manhães-de-Castro, F.M. and De-Castro, R. 2005. The expression of an intraspecific aggressive reaction in the face of a stressor agent alters the immune response in rats. *Braz. J. Biol*., **65**: 203-209.

Barse, A.V., Chakrabarti, T., Ghosh, T.K., Pal, A.K. and Jadhao, S.B. 2006. One tenth dose of LC_{50} of 4-tertbutylphenol causes endocrine disruption and metabolic changes in Cyprinus carpio. *Pesticide Biochem. Physiol*., **86**(3): 172-179.

Bartik, M. and Piskac, A. 1981. Fungicides, In: *Veterinary Toxicology*. (Eds.), 164-170, *Elsevier,*Praha.

Barton, B.A. 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integ Comp Biol.*, **42**:517-525.

Barton, B.A., Schreck, C.B. and Barton, L.D. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Dis. Aquat. Org.,* **2**: 173-85.

Barton, B.A.and Iwama, G.K.1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Ann. Rev. Fish Dis*., **1**: 3-26

Basha, P.S. and Rani, A.U. 2003. Cadmium induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicol. Environ. Saf.,* **56**: 218-221.

Baskaran, S., Kookana, R.S. and Naidu, R. 1999. Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal rates. *Pesticide Science,* **55**: 1222-1228.

Battaglin, W. A. and Fairchild, J. 2002. Potential Toxicity of Pesticides Measured in Midwestern Streams to Aquatic Organisms. *Journal of Water Science and Technology*. **45**(9):95-103.

Baumann, P.C. and Harshbarger, J.C. 1995. Decline in liver neoplasms in wild brown bullhead catfish after coking plant closes and environmental PAHs Plummet. Environmental Health Perspectives. *Journal of the National Institute of Environmental Health Sciences,* **103**(2):168-170.

Beamish, F.W.H. Jebbink, J.A., Rossiter, A. and Noakes, D.L.G. 1996. Growth strategy of juvenile lake sturgeon (*Acipenser fulvescens*) in a northern river. *Can. J. Fish. Aquat. Sci.,* **53**: 481-489.

Bebarji, G. and Rajendranth, T. 1990. Haematological changes induced by an organophosphorus insecticide in a fresh water fish *Clarias batrachus* (Linnaeus). *Trop. Fresh water. Biol*., **2**: 197-202.

Bedii, C. and Kenan, E. 2005. The effects of cadmium on levels of glucose in serum and glycogen reserves in theliver and muscle tissues of *Cyprinus carpio. Turk. J. Vet. Anim. Sci.,* **29**: 113-117.

Beena, S. and Viswarajan, S. 1987. Effect of cadmium and mercury on the haematological parameters of the fish, *Cyprinus carpio*. *Environ. Ecol*., **5**: 726-732.

Begum, G. 2005. In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure. *Pesticide Biochemistry and Physiology*, **82**: 185-196.

Begum, G. and Vijayaraghavan, S. 1995. In vivo toxicity of dimethoate on proteins and transaminases in the liver tissue of fresh water fish *Clarias batrachus* (Linn). *Bulletin of environmental contamination and toxicology,* **54**(3): 370-375.

Begum, G. and Vijayaraghavan, S. 1999. Effect of acute exposure of the organophosphate insecticide rogor on some biochemical aspects of *Clarias batrachus* (*Linnaeus*). *Environ. Res.,* **80**(1): 80-83

Beketov, M.A. and Liess, M. 2008. Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. *Environmental Toxicology and Chemistry*, **27:**461-470.

Bell, G.H., Davidson, J.N. and Emslie-Smith, D. 1972. "Textbook of physiology and biochemistry". Publisher : The English Language Book Society and Churchill Livingstone.

Bennet, R.O. and Wolke, R.C 2004. The effects of sublethal endrin exposure on rainbow trout *Salmo gaindineri. Journal of Fish Biology,* **31**: 375-385.

Bernard, S., Rodriguez, J.N., Cuisset, B., and Le Menn, F. 2001. Gonadosomatic index and seasonal variations of plasma sex steroids in skipjack tuna (Katsuwonus pelamis) and yellowfin tuna (Thunnus albacares) from the western Indian ocean. Aquatic Living Resources, 14(05): 313-318.

Bernet, D., Schmidt, H., Meier, W., Brkhardt-Holm, P. and Wahli, T. 1999. Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases*, **22**: 25-34.

Berny, P., Caloni, F., Croubels, S., Sachana, M., Vandenbroucke, V., Davanzo, F. and Guitart, R. 2009.Animal poisoning in Europe. Part 2: Companion animals. *The Veterinary Journal,* **183**(3): 255-259

Berrill, M., Coulson, D., McGillivray, L. and Pauli, B. 1998. Toxicity of endosulfan to aquatic stages of anuran amphibians. *Environ. Toxicol. Chem*., **17**: 1738-1744.

Beutler, E., 1986. Red cell Metabolism. Churchill Livingstone Pub, New York.70pp.

Bhatnagar, C., Bhatnagar, M. and Chand, B.R. 2007. Fluoride-Induced Histopathological Changes In Gill, Kidney, And Intestine Of Fresh Water Teleost, *Labeo Rohita. Research report Fluoride,* **40**(1): 55-61.

Bhattacharya, A. and Bhattacharya, S. 2007. Induction of oxidative stress by arsenic in *Clarias batrachus*: Involvment of peroxisomes. *Ecotoxicol. Environ. Safe.,* **66** (2): 178-187.

Bhattacharya, H., Lun, L. and Gomez, R.G.D. 2005. Biochemical effects to toxicity of CCl_4 on Rosy Barbs (*Puntius conchonius*). *Our Nature,* **3**(1): 20-25.

Bhavan, P.S. and Geraldine P. 1997. Alterations in concentrations of protein, carbohydrate, glycogen, free sugar and lipid in the prawn *Macrobrachium malcolmsonii* on exposure to sub lethal concentrations of endosulfan. *Pestic. Biochem. Physiol.*, **58**: 89-101.

Bielinska, I. 1987. Dielectric, haematological and biochemical studies of detergent toxicity in fish blood. *Phys. Med. Biol*., **32**: 623-635.

Bindali, B.B. and Kaliwal, B.B. 2002. Anti-implantation effect of a carbamate fungicide mancozeb in albino mice; *Ind. Health,* **40**(2): 191-197.

Biney, C., Calamari, D., Membe, T.W., Naeve, H., Nyakageni, B. and Saad, M.A.H. 1987. Scientific bases for pollution control in African inland waters. *FAO Fish. Rep.*, **369**: 9-23.

Bisson, M. and Hontela, A. 2002. Cytotoxic and Endocrine-Disrupting potential of Atrazine, Diazinon, Endosulfan and Mancozeb in Adrenocortical Steroidogenic cells of Rainbow trout exposed *in vitro*. *Toxicology and Applied Pharmacology*. **180**: 110-117.

Blaxhall, P.C. 1972. The hematological assessment of the health of freshwater fish. Review of selected literature. *J. Fish Biol.,* **4**: 593-604.

Blazer, V. S., D. E. Facey, J. W. Fournie, L. A. Courtney, and J. K. Summers. 1994. Macrophage aggregates as indicators of environmental stress. Pages 169-185 in J. S. Stolen and T. C. Fletcher, editors. Modulators of fish immune responses, volume 1. SOS Publications, Fair Haven, New Jersey.

Blazer, V.S., Wolke, R.E., Brown, J. and Powell, C.A. 1987. Piscine macrophage aggregate parameters as health monitors: effect of age, sex, relative weight, season and site quality in largemouth bass *(Micropterus salrnoldes*). *Aquat. Toxic.* **10**(4): 199-215

Boateng, J.O., Nunoo, F.K.E., Dankwa, H.R. and Ocran, M.H. 2006. Acute Toxic Effects of Deltamethrin on Tilapia, *Oreochromis niloticus* (Linnaeus, 1758). West Africa *Journal of Applied Ecology,*1-5**.**

Bobmanuel, N.O.K., Gabriel, U.U. and Ekweozor, I.K.E. 2006. Direct toxic assessment of treated fertilizer effluents to *Oreochromis niloticus* and catfish hybrid (*Heterobranchus bidorsalis Clarias gariepinus*). *Afr. J. Tech.,* **5**(8): 653-642.

Boeri, R.L., Kowalski, P.L. and Ward, T.J. 1996. Early life-stage toxicity of DPX-T3217-113 (cymoxanil) to the sheepshead minnow, *Cyprinodon variegatus* T. R. Wilbury Laboratories, Inc.

Boeri, R.L., Magazu, J.P., Ward, T.J. 1997. DPX-T3217-113 (cymoxanil): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss* T. R. Wilbury Laboratories, Inc.

BoeriI R.L., Magazu J.P. and Ward T.J., 06-Jun-97. DPX-T3217-113 (cymoxanil): Early life-stage toxicity to rainbow trout, Oncorhynchus mykiss (3 volumes). Report HLO 1013-96. T.R. Wilbury Laboratories, Inc. Unpublished

Bonmatin, J.M., Marchand, P.A., Charvet, R., Moineua, I., Bengsch, E.R. and Colin. M.E. 2005. Quantification of imidacloprid uptake in maize crops. *J. Agric. Food Chem*., **53**: 5336-5341.

Boon, J.P., Lewis, W.E., Choy, M.R., Allchin, C.R., Law R.J. and de Boer, J. 2002. Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. *Environ. Sci. Technol.,* **36**: 4025-4032, 2002.

Borges, A., Scotti, L., Siqueira, D., Zanini, R., do Amaral, F., Jurinitz, D. andWassermann, G. 2007. Changes in hematological and serum biochemical values in jundia´ Rhamdia quelen due to sub-lethal toxicity of cypermethrin. *Chemosphere*, **69**: 920-926

Bortolotto, T., Bertoldo, J.B., Zanette da Silveira, F., Defaveri, T.M., Silvano, J. and Tröger Pich, C., 2009. Evaluation of the toxic and genotoxic potential of landfill leachates using bioassays. *Environ. Toxicol. Pharmacol.*, 28, 288-293.

Bowman, J. and Bucksath, J. 1990. Acute toxicity of NTN 33893 to bluegill (Lepomis macrochirus). Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri (performing laboratory). Mobay Corporation, Kansas City, Missouri (submitting laboratory). 31 pp. Mobay Project ID: Report No. 100348.

Boyd, J. 1957. The use of *Daphnis magna* in the microbio-assay of insecticides. Ph.D. Thesis. Submitted to Pennsylvania State University, 194.

Brack, W., Schirmer, K., Kind, T., Schrader, S. and Schuurmann, G. 2002. Effect-directed fractionation and identification of cytochrome P450A-inducing halogenated aromatic hydrocarbons in contaminated sediment. *Environ. Toxicol. Chem*., **21**: 2654-2662.

Bradbury, S.P. and Coats, J.R. (1989). Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. Environmental Toxicology and Chemistry, Vol. 8, No. 5, (May 1989), 373-380.

Brando, C., Bohets, H.L., Vvyer, I.E. and Dierickx, P.J. 1992. Correlation between the in vivo cytotoxicity to cultured fathead minnow fish cells and fish lethality data for 50 chemicals. *Chemosphere*, **25**(2): 553-562.

Braunbeck, T. 1994. Detection of environmental relevant pesticide concentrations using cytological parameters: pesticide specificity in the reaction of rainbow trout liver. Oxford,15-29.

Bravo, C.F., Curtis, L.R., Myers, M.S., Meador, J.P., Johnson, L.L., Buzitis, J., Collier, T.K., Morrow, J.D., Laetz, C.A., Loge, F.J. and Arkoosh, M.R. 2011. Biomarker responses and disease susceptibility in juvenile rainbow trout *Oncorhynchus mykiss* fed a high molecular weight PAH mixture. *Environmental Toxicology & Chemistry*, **30**(3): 704-714.

Breitholtz M., Rudén C., Hansson S. O. and Bengtsson B-E. 2006. Ten challenges for improved environmental risk assessment. *Ecotoxicology and Environmental Safety* **63:** 324-335.

Breitholtza, M., Rudénb, C., Hanssonb, S. O. and Bengtssona, B.E. 2006. Ten challenges for improved ecotoxicological testing in environmental risk assessment. *Ecotoxicology and Environmental Safety,* **63**(2): 324-335

Brewer, S.K., Little E. E., DeLonay, A.J., Beauvais, S.B., and Jones, S. B. 1999. The use of automated monitoring to assess behavioral toxicology in fish. Linking behavior and physiology. In Environmental Toxicology and Risk Assessment 8th Volume. ASTM STP 1364. (eds: D.S. Henshel, M.C. Black, and M.C. Harrass). American Society for Testing and Materials, West Conshohocken, PA.

Brewer, S.K., Little, E.E., DeLonay, A.J., Beauvais, S.L., Jones, S.B. and Ellersieck, M.R. 2001. Behavioral dysfunctions correlate to altered physiology in rainbow trout (*Oncorynchusmykiss)* exposed to cholinesterase-inhibiting chemicals. *Arch. Environ. Contam. Toxicol*., **40**: 70-76

Brown, C.L. and George, C.J. 1985. Age-dependent accumulation of macrophage aggregation in the yellow perch, *Perca flavescens* (Mitchill). *Journal of Fish Diseases*, **8**: 135-138.

Buchholz, A. and Nauen. R. 2002. Translocation and translaminar bioavailability of two neonicotinoid insecticides after foliar application to cabbage and cotton. *Pest Manag. Sci.* **58**(1): 10-16.

Bunton, T.E. 1996. Experimental chemical carcinogenesis in fish. *Toxicol Pathol.,* **24**: 603-618

Busacker, G.P., Adelman, I.R., and Goolish, E.M. 1990. Methods for Fish Biology. (eds: C.B. Shreck and P.B. Moyle) American Fisheries Society, Bethesda, Maryland.

Cakmak, M.N. and Gorgon, A. 2003. Toxic effect of a synthetic pyrethroid insecticide (Cypermethrin) on blood cells of rainobow trout (*Oncorhynchus mykiss*, Walbaum). *J. Biological Sciences,* **3**: 694-698.

Caloni, F., Scarpa, P., Pompa, G. and Davanzo, F. 2004. Epidemiologia degli avvelenamenti degli animali domestici in Italia anni 2000-2002, Casistica del centro antiveleni diMilano. *Archivio Veterinario Italiano,* **55**: 1-6

Calviello, G., Piccioni, E., Boninsegna, A., Tedesco, B. Maggiano, N., Serini, S., Wolf, F.I. and Palozza, P. 2006. DNA damage and apoptosis induction by the pesticide Mancozeb in rat cells: involvement of the oxidative mechanism. *Toxicol Appl. Pharmacol.*, **211**: 87-96.

Camargo, M.M.P. and Martinez, C.B.R. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotrop. ichthyol*., **5**(3): 327-336.

Capowiez, Y., Rault, M., Mazzia, C. and Belzunces, L. 2003. Earthworm behaviour as a biomarker - a case study using imidacloprid. *Pedobiologia*, 47: 542-547.

Captan, *EPA-738-R-99-015*, Office of Pesticide Programs, U.S. Government Printing Office, Washington, DC.

Carragher, J. and Sumpter, J. 1990. The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. *Gen. Comp. Endocrinol*., **77**: 403-407.

Carraschi, S.P., *et al.* 2012. Histopathological biomarkers in pacu *(Piaractus mesopotamicus*) infected with *Aeromonas hydrophila* and treated with antibiotics. *Ecotoxicology and Environmental Safety,* **83**: 115-120.

Carriger, J.F., Hoang, T.C. and Rand, G.M. 2010. Survival analysis of mosquitofish (*Gambusia affinis*) and least killifish (*Heterandriaformosa*) exposed to endosulfan sulfate. *Arch Environ Contam Toxicol*., **58**: 1015-1022

CAS, 2012. CAS - A Division of the American Chemical Society. CAS Registry and CAS Registry Numbers. http://www.cas.org/expertise/cascontent/ registry/regsys.html, access: March 2012.

Casarez, E. 2001. Basic Prinicples of Toxicology BIOC 597c.

Casas-Solis, J., Santerre, A., Giron-Perez, M.I., R. Reynoso-Orozco. and Zaitseva, G. 2007.A comparative study of phagocytic activity and lymphoproliferative response infive varieties of tilapia *Oreochromis spp*, *J. Fish Biol.* **71**: 1541-1545

Cazenave, J., Wunderlin, D.A., Hued, A.C. and Bistoni, M.A. 2005. Hematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. *Hydrobiologia*, **537**(1-3): 25-33.

CCME (Canadian Council of Ministers of the Environment). 2007. Canadian water quality guidelines: Imidacloprid. Scientific Supporting Document. Winnipeg, Manitoba

Cengiz, E.I. and Unlu, E. 2002. Histopathological changes in the gills of mosquitofish, Gambusia affinis exposed to endosulfan. *B Environ Contam Tox.,* **68**: 290-296

Cengiz, E.I., Unlu, E. and Balci, K. 2001. The histopathological effects of Thiodan on the liver and gut of mosquitofish, *Gambusia affinis. J Environ Sci Heal B.,* **36**(1):75-85

Cerqueira, C.C.C. and Fernandes, M.N. 2002. Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. *Ecotoxicology and Environmental Safety,* **52**: 83-91.

Chambers, J.E. 1979. Induction of microsomal mixedfunction oxidase system components in striped mullet by short-term exposure to curde oil. *Toxicol. Lett.,* **4**: 227-230.

Chandrana, R., Sivakumara, A.A., Mohandassb, S. and Aruchamia, M. 2005. Effect of cadmium and zinc on antioxidant enzyme activity in the gastropod, *Achatina fulica*. C*omparative Biochemistry and Toxicology*, **140**(34): 422-426.

Chapman, P.M. 2000. Whole effluent toxicity test-usefulness, level of protection and risk assessment. *Environ. Toxicol. Chem.,* **19**:3-13.

Charmandari, E., Tsigos, C. and Chrousos, G. 2005. Endocrinology of the stress response. *Annu.Rev.Physiol*., **67**: 259-284.

Chauzat, M. P., Faucon, J. P., Martel, A. C., Lachaize, J., Cougoule, N. and Aubert, M. 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. *Journal of Economic Entomology*, **99**(2): 253-262.

Chellappa, N.T., Chellappa, S.L. and Chellappa, S. 2008. Harmful phytoplankton blooms and fish mortality in a eutrophicated reservoir of northeast Brazil. *Braz. Arch. Biol. Technol*., **51**: 833-841

Chen, L.Z., Cui, J.J., Ma, W.H., Niu, C.Y. and Lei, C.L. 2010. Pollen from Cry1Ac/ CpTI-transgenic cotton does not affect the pollinating beetle *Haptoncus luteolus*. *J Pest Sci.,* **84**(1): 9-14.

Chen, X., Yin, D., Hu, S. and Hou, K. 2004. Immunotoxicity of penta chlorophenol on macrophage immunity and IgM secretion of the crucian carp *(Carassius auratus)*. *J. Bull. Environ. Contam and Toxicol*., **73**: 153-160.

Chetty, A.N. and Indira, K. 1994. Alterations in the tissue lipid profiles of *Lamellidens marginalis* under ambient ammonia stress. *Bul. Environ. Contam. Toxicol.,* **53**: 693-698.

Chichester, C.O. 1965. Research in Pesticides. Academic press New York and London: 118-126

Chin Sue, H. 2002. Jamaica Country Report on Persistent Toxic Substances, Region XIRET/CSUCA, Regionally Based Assessment of Persistent Toxic Substances, GF/XG/XG/4030-00-20, FMAM/UNEP.

Chindah, A. C., Sikoki, F.D. and Vincent-Akpu, I. 2000. The effect of organochlorine pesticides on juveniles of a common wetland fish *Tilapia guineensis. J. Agric. Biotech. Environ.,* **1**(2): 75- 82.

Chindah, A.C., Braide S.A. and Nduaguibe, U.M. 2001. Tolerance of periwinkle (*Tympanotonus fuscatus* Linne) and shrimp (*Palemonetes africanus* Balss) to wastewater from Bonny light crude oil tank farm. *Polish J. Environmental Protection and Natural Resources,* **21/22**: 61-72.

Chindah, A.C., Sikoki, F.D. and Vincnet-Akpu, I. 2004. Toxicity of an Organnophosphate pesticide (Chloropyrifos) on a common Niger Delta wetland fish - *Tilapia guineensis* (Blecker 1862). *J. A ppl. Sci. Environ. M gt.,* **8**(2): 11-17.

Choudhury, C., Ray, A., Bhattacharya, S. and Bhattacharya, S. 1993. Non-lethal concentrations of pesticide impair ovarian function in the freshwater perch, Anabas testudineus. *Environ. Biol. Fishes,* **36**: 319-24.

Chrousos, G. P. 1998. Stressors, stress and neuro-endocrine integration of the adaptive response. *Ann. N. Y. Acad. Sci.* **851**: 311-335.

Chukwu, L.O. and Okpe, H.A. 2006. Differential responses of *Tilapaia guineensis* fingerlings inorganic fertilizer under various salinity regime Lockwoods. *J Environ Biol* **27**: 687-690.

Cicik, B. and Engin, K. 2005. The effect of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* L. (1758). *Turk. J. Vet. Anim. Sci*., **29**: 113-117.

Clark, G.M., Goolsby, D.A. and Battaglin, W.A. 1999.Seasonal and annual load of herbicides from the Mississippi River basin to the Gulf of Mexico. *Environ. Sci. Technol*., **33**(7):981-986.

Clarkson, T. W., 1998. Human toxicology of mercury. *J. Trace. Elem. Exp. Med*., **11**(2-3): 303-317

Clauss, T.M., Dove, A.D.M. and Arnold, J.E. 2008. Hematologic disorders of fish, Veterinary clinics of North *America. Ex. Anim. Pract.,* **11**: 445-462.

Clément, B., Persoone, G., Colin, J.R. and Le Dû-Delepierre, A., 1996. Estimation of the hazard of landfills through toxicity testing of leachates. 1: Determination of leachate toxicity with a battery of acute tests. *Chemosphere*, **33**: 2303-2320.

Cocco, P. 2002. On the rumors about the silent spring. Review of the scientific evidence linking occupational and environmental pesticide exposure to endocrine disruption health effects. *Cad. Saúde. Pública*., **18**(2): 379-402.

Cohen, S.Z., Creeger, S.M., Carsel, R.F. and Enfield, C.G. 1984. Potential pesticide contamination of groundwater from agricultural uses. (eds: R.F. Kruegar, and J.D. Seiber,) *Treatment and disposal of pesticide wastes*. *American Chemical Society Symposium, Series No. 259*. 297-325. American Chemical Society, Washington, DC.

Coldham, N.G., Sivapathasundaram, S., Dave, M., Ashfleld, L.A., Pottinger, T.G., Goodall, C. and Sauer, M.J. 1998. Biotransformation, tissue distribution, and persistence of 4-nonylphenol residues in juvenile rainbow trout (*Oncorhynchus mykiss*). *Drug Metabolism Dispososition*. b: 347-353

Comakli, V., Ciftci, M. and Kufrevioglu, I. 2012. Effects of some metal ions on rainbow trout erythrocytes glutathione S-transferase enzyme: an *in vitro* study. *Journal of Enzyme Inhibition and Medicinal Chemistry,* 1-6.

Connell, D.W. and Miller, A.J. 1984. Chemistry and Ecotoxicology of Pollution*,* Wiley, New York, 250.

Coppo, J.A., Mussart, N.B. and Fioranelli, S.A. 2002. Physiological variation of enzymatic activites in blood of bull frog, *Rana cetesbeiana* (Shaw, 1802). *Rev. Vet.,* **12/13**: 22-27.

Correia, A.D., Gonzalves, R., Scholze, M., Ferreira, M. and Henriques, M.A.R. 2007. Biochemical and behavioral responses in gilthead seabream (*Sparus aurata*) to phenanthrene. *Journal of Experimental Marine Biology and Ecology*, **347**: 109-122.

Costa, P.M., Caeiro, S., Diniz, M.S., Lobo, J., Martins, M., Ferreira, A.M., Caetano, M., Vale, C., DelValls, T,A. and Costa, M.H. 2009. Biochemical endpoints on juvenile Solea senegalensis exposed to estuarine sediments: the effect of contaminant mixtures on metallothionein and CYP1A induction. *Ecotoxicology,* **18**(8) 988-1000.

Couch, J.A. and John, C.H. 1985. Effects of carcinogenic agents on aquatic animals: an environmental and experimental overview. *Journal of Environmental Science & Health,* **3**(1): 63-105.

Couillard, C.M. and Hodson, P.V. 1996. Pigmented macrophage aggregates: A toxic response in fish exposed to bleached-kraft mill effluent? *Environmental Toxicology and Chemistry,* **15**(10): 1844-1854.

Craig, P.M., Wood, C.M., McClelland, G.B. 2007. Oxidative stress response and gene expression with acute copper exposure in zebrafish (*Danio rerio*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **293**: 1882-1892.

Crawford, N. 1958. An improved method for the determination of free and total cholesterol using ferric chloride reaction. *Lin. Chim. Acta.*, **3**:357-367.

Cross, J.N. and Hose, J.E. 1988. Evidence for impaired reproduction in white croaker (*Genyonemus lineatus*) from contaminated areas off southern California. *Mar. Environ. Res*., **24**: 185-188.

Cyriac, P.J., Antony, A. and Nambison, P.N.K. 1989. Haemoglobin and haematocrit values in the fish *Oreochromis mossambicus* (Reters) after shor-term exposure to copper and merary. *Bulletin of Environmental Contamination and Toxiocology,* **43**: 315-320.

Dabas, A., Nagpure, N. S., Kumar, R, Kushwaha, B., Kumar, P. and Lakra, W. S. 2012. Assessment of tissue-specific effect of cadmium on antioxidant defense system and lipid

peroxidation in freshwater murrel, *Channa punctatus. Fish Physiology and Biochemistry*, **38**: 469-482.

Daniels, W.H. and Robinson, E.H. 1986. Protein and energy requirements of juvenile red drum (*Sciaenops ocellatus*). Aquaculture, **53**: 243-52.

Das, A.C. and Mukherjee, D. 2000. Influence of insecticides on microbialtransformationof nitrogen and phosphorus in Typic Orchragualf soil. *J. Agric. Food Chem*., **48**(8): 3728-3732.

Das, B.K. and Mukherjee, S.C. 2000. Sublethal effect of quinalphos on selected blood parameters of *Labeo rohita* (Ham.) fingerlings. *Asian Fisheries Science*, **13**: 225-233.

Das, B.K. and Mukherjee, S.C. 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. *Comp Biochem Physiol*., **134C**: 109-121.

Das, P.C., Ayyappan, S., Jena, J.K. and Das, B.K. 2004. Acute toxicity of ammonia and its sublethal effects on selected haematological and enzymatic parameters of *Cirrhinus mrigala* (Hamilton). *Aquac. Res.,* **35**: 134-143.

Das, T., Pal, A.K., Chakraborty, S.K., Manush, S.M., Dalvi, R.S., Apte, S.K., Sahu, N.P. and Baruah, K. 2009. Biochemical and stress responses of *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) in relation to acclimation temperatures. *Journal of Fish Biology*, **74**(7): 1487-1498

Daughton, C.G. and Ternes, T.A. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ Health Perspect*., **6**: 907-38.

David, M. 1995. Effect of fenvlaterate on behavioural, physiological and biochemical aspects of freshwater fish, Labeo rohita. Ph.D. thesis, S. K. University, Anantapur India.

David, M., Mushigari, S.B. and Prashanth, M.S. 2002. Toxicity of fenvalerate to the freshwater fish, *Labeo rohita*. *Geobios*., **29**: 25-28.

David, M., Shivakumar, H.B., Shivakumar, R., Mushigeri, S.B. and Ganti, B.H. 2003. Toxicity evaluation of cypermethrin and its effect on oxygen consumption of the freshwater fish, *Tilapia mossambica. Indian J. Environ. Toxicol.,* **13**: 99-102.

Davis, J.C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *Journal of Fisheries Research Board Canada,* **32**(12): 2295-2332.

Davison, W., Franklin, C.E. and McKenzie, J.C. 1994. Haematological changes in an Antarctic teleost, *Trematomus bernacchii*, following stress. *Polar Biol*., **14**: 463-6.

de Aguiar, L.H., Moraes G., Avilez I.M., Altran A.E. and Correa C.F. 2004. Metabolical effects of Folidol 600 on the neotropical freshwater fish matrinxã, *Brycon cephalus*. *Environ. Res.,* **95**(2): 224-230.

De Bravo, M.I., Medina, L.,Marcano, S., Finol, H.J., Boada-Sucre, A. 2005**.** Effects of herbicide on the kidneys of two Venezuelan cultured fish: Caquetaia kraussii and Colossoma macropomum (Pisces: Ciclidae and Characeae). *Revista de Biologia Tropical,* **53**(1): 55-60.

De la Torre, F.R., Saliba, A. and Ferrair, L. 2005. Biomarkers of native fish species (*Cnesterodon decemaculatus*) application to the water toxicity assessment of a per-urban polluted river of Argentina. *Chemosphere,* **59**(4): 577-583.

De Melo, G., Donattib, L., Rudnikia, C. and Fanta, E. 2008. Hepatic alterations in the fish Rhamdia quelen contaminated with Folidol 600. *Ecotoxicol. Environ. Saf*., **71**: 821-829.

de Vlaming, V.L., Grossman, G. and Chapman, F. 1981. On the use of gonadosomatic index. *Comp. Biochem. Physiol*., **73**: 31-9.

Decostere, A., Haese, E. D., Lammens, M., Nelis, H. and Haesebrouck, F. 2001. In vivo study of phagocytosis, intracellular survival and multiplication of Flavobacterium psychrophilum in rainbow trout, *Oncorhynchus mikiss* (Walbaum), spleen phagocytes. *J. Fish Dis.,* **24**: 481-487.

Deka, C. and Dutta K. 2012. Effects of cypermethrin on some haematological parameters in *Heteropneustes fossilis* (bloch). *The Bioscan,* **7**(2): 221-223.

Delahunty, G. and de Vlaming, V.L. 1980. Seasonal relationships of ovary weight, liver weight, and fat stores with body weight in the goldfish, *Carassius auratus* (L). *J. Fish Biol.,* **16**: 5-13.

Dell'Omo, G. 2002. Behavioural Ecotoxicology. Published by Wiley and Sons, New York. 463 pp (hardcover). ISBN 0471968528.

Depledge, M.H. and Galloway, T.S. 2005. Healthy animals, healthy ecosystems. *Frontiers in Ecology and the Environment*, **3**: 251-258

Derbalah, A.S., Massoud, A.H. and Belal, E.B. 2008. Biodegrability of famoxadone by various microbial isolates in aquatic system. *Land Contamination Reclamation*, **16**: 13-23.

Desai, B. and Parikh, P. 2012. Impact of Curzate (fungicide) on Hematological Parameters of *Oreochromis mossambicus*. *International Journal of Scientific & Engineering Research*, **3**(7): 1-6.

Deva. Prakash Raju. 2000 Fenvalerate induced changes in the protein metabolism of freshwater fish, Tilipa mossambica (Peters). Ph. D. thesis, S. K. University, Anantapur India.

Devi, R. and Banerjee, T.K. 2007. Haematological disturbances due to ammonia toxicity in the air-breathing fish *Channa stiata*. *Biochem. Cell. Arch*., **7**: 217-223.

Dey, S. and Bhattacharya, S. 1989. Ovarian damage to *Channa punctatus* after chronic exposure to low concentrations of elsan, mercury, and ammonia. *Ecotoxicology and environmental safety*, **17**(2): 247-257.

Di Giulio, Richard T., and David E. Hinton, eds. The toxicology of fishes. *CRC,* 2008.

Di Mascio, P., Murphy, M. E. and Seis, H. 1991. Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. *American Journal of Clinical Nutrition*, **53**: 194-200.

Diamantino, T.C., Amadeu, E., Soaresa, M.V.M. and Guilherminoc, L. 2001. Lactate dehydrogenase activity as an effect criterion in toxicity tests with *Daphnia magna straus. Chemosphere,* **45**(4-5): 553-560.

Dickman, M.D. and Leung, K.M. 1998. Mercury and organochlorine exposure from fish consumption in Hong Kong. *Chemosphere*, **37**(5): 991-1015.

Diehl, P.A., Connat, J.L. and Dotson, E.M. 1986. Chemistry, function, and metabolism of tick ecdysteroids. In: *Morphology, Physiology and Behavioral Biology of Ticks* (Eds. J.R. Sauer and J.H. Hair), Chichester, Ellis Horwood: 165-193.

Dietschy, J.M., Turley, S.D., and Spady, D.K. 1993. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res*., **34**: 1637-1659.

Diez, S., Abalos, M. and Bayona, J.M. 2002. Organotin contamination in sediments from the Western Mediterranean enclosures following ten years of TBT regulation. *Water Res.,* **36**: 905-918.

Ding, Z., Yang, Y., Jin, H., Shan, Z., Yu, H., Feng, J., Zhang, X. and Zhou, J. 2004. Acute toxicity and bioconcentration factor of three pesticides on *Brachydanio rerio*. *Ying Yong Sheng Tai Zue Bao*, **15**: 888-890 (in Chinese).

Dobreva, V., Tsekov, A. and Velcheva, I. 2008. Study of the effect of zinc on gill functions of the crucian carp *Carassius gibelio* Bloch. *Bulgarian Journal of Agricultural Science,* **14**(2): 182-185.

Dobsikova, R., Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z. and Novotny, L. 2006. Effects of cypermethrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio L.). Neuroendocrinology Letter*, **27(**2): 101-105.

Dogan, D. and Can, C. 2011. Endocrine disruption and altered biochemical indices in male< i> *Oncorhynchus mykiss* in response to dimethoate. *Pesticide Biochemistry and Physiology,* **99**(2): 157-161.

Donaldson, D., Kiely, T. and Grube, A. 2002. Pesticides industry sales and usage: 1998 and 1999 market estimates. USEPA Report Number 733-R-02-001, U.S. Environmental Protection Agency, Washington, D.C., USA.

Donaldson, E.M. 1981. *The pituitary-interrenal axis as an indicator of stress in fish*. (eds: In: A.D. Pickering) Academic Press, New York: 11-47.

Dong, X.L., Zhu, L.S., Wang, J.H., Wang, J., Xie, H., Hou, X.X. and Jia, W.T. 2009. Effects of atrazine on cytochrome P450 enzymes of zebrafish (*Danio rerio*). *Chemosphere,* **77**: 404-412

Dorey, B. and Thatheyus, A.J. 2012. Acute Toxicity of Nickel and Cadmium to the Cichlid Fish, *Oreochromis mossambicus* (Peters). *Research in Zoology*, **2**(4): 19-22.

Dorucu, M. and Girgin, A. 2001. The effects of cypermethrin on some haematological parameters of *Cyprinus carpio*. *Aquaculture International,* **9**(2): 183-187.

Dorval, J. and Hontela, A. 2003. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Toxicol. Applied Pharmacol.,* **192**: 191-200.

Dorval, J., Leblond, V., Deblois, C. and Hontela, A. 2005. Oxidative Stress and Endocrine Endpoints in White Sucker (*Catostomus commersoni*) from a River Impacted by Agricultural Chemicals. *Environmental Toxicology and Chemistry*, **24**(5): 1273-1280.

Duffy, J.E. and Zelikoff, J.T. 2006. The relationship between noncoplanar PCB-induced immunotoxicity and hepatic CYP1A induction in a fish model. *Journal of immunotoxicology*, **3**(1): 39-47.

Dupoint de Nemours, E.l. 1983. Technical data sheet for Mancozeb, Biochemicals Department, Wilmington, DE, p 4-33 as reported in Ecotoxnet, Available. Accessed 2 February 2010. from:http://extoxnet.orst.edu/pips/mancozeb.htm).

Duthie, G.G. and Tort, L. 1985. Effect of dorsal aortic cannulation on respiration and haematology of the Mediterranean dogfish, *Scyliorhinus canicula. Comparative Biochem. Physiol*., **81**: 879-883.

Dutta, H.M. and Dalal, R. 2008.The Effect of Endosulfan on the Ovary of Bluegill Sunfish: A Histopathological Study (*Lepomis macrochirus*). *Int. J. Environ. Res*., **2**(3): 215-224.

Edwards, C. A. and Fusher, S.W. 1991. The use of chlorinesterases measurements in assessing the impacts of pesticides on terrestrial and aquatic invertebrates. In P. Mineau, ed. Chlorinesterases - inhibiting insecticides. Their impact on wild and the environment. Elsevier, Amsterdam, The Netherlands: 255 - 275.

Egaas, E., Skaare, J.U., Svendesen, N.O., Sandvik, M., Falls, J.G., Dauterman, W.C., Collier, T.K. and Netland, J. 1993. A comparative study of effect of atrazine on xenobiotic metabolizing enzymes in fish and insect, and on the in vitro phase 11 atrazine metabolisms in some fish, insect, mammals and one plant species. *Comp, Biochem*. *Physio.,* **106**: 141-149.

Ekinci, D. and Beydemir, S. 2010. Risk assessment of pesticides and fungicides for acid-base regulation and salt transport in rainbow trout tissues. *Pesticide Biochemistry and Physiology,* **97**: 66-70.

Ekman, E. and Norrgren, L. 2003. Pathology and immunohistochemistry in three species of salmonids after experimental infection with *Flavobacterium psychrophilum*. *Journal of fish diseases,* **26**(9): 529-538.

Ekweozor, I.K.E., Bobmanuel, N.O.K. and Gabriel, U.U. 2001. Sublethal effects of ammoniacal effluents on three commercial fish species from the Niger Delta, Nigeria. *J. Appl. Sci. Environ. M gt.,* **5**(2): 9-11.

Elahee, K.B. and Bhagwant, S. 2007. Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. *Ecotoxicol. Environ. Saf.*, **68**: 361-371.

Elbert, A., Nauen, R. and Leicht, W. 1998. Imidacloprid, a novel chloronicotinyl insecticide, biological activity and agricultural importance. (eds: In: I. Ishaaya and D. Degheele), Insecticides with Novel Modes of Action. Springer, Berlin, Germany, 50-73.

Elbert, A.B., Hartwig, B.J. and Erdelen, C. 1991. Imidacloprid -a new systemic insecticide. *Pflanzenschutz -Nachr Bayer,* **44**: 113-116.

Elbert, C., Erdelen, C., Kuehnho, ld.,J., Nauen, R., Schmidt, H.W. and Hattori,Y. 2000. Thiacloprid: a novel neonicotinoid insecticide for foliar application. Brighton Crop Protection Conference, Brighton, UK. Pests and Diseases, **2**A-1: 21-26.

El-Ezaby, M.M. 1994.Certain Studies on the Effect of Some Pollutants on Fresh water fish. Ph.D. Thesis Fac. Sci., Zagazig Univ. Benha Branch.

El-Hossary, G.G., Mansour, S.M. and Mohamed, A.S. 2009. Neurotoxic Effects of Chlorpyrifos and the Possible Protective Role of Antioxidant Supplements: an Experimental Study. *Journal of Applied Sciences Research*, **5**(9): 1218-1222.

Elia, A.C., Galarini, R., Taticchi, M.I., Dorr, A.J. and Mantilacci, L. 2003. Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure. *Ecotoxicol. Environ. Saf.,* **55**: 162-167.

Ellman, G.L. and Fiches, F.T. 1959. Quantitative determination of peptides by sulfhydryl groups. *Arch, Biochem Biophys.*, **82**: 70-72.

El-Shebly, A.A. and El-kady, M.A.H. 2008. Effects of Glyphosate Herbicide on Serum Growth Hormone (GH) levels and Muscle Protein Content in Nile Tilapia (*Oreochromis Niloticus L.*). *Res. Jour. Fish. Hydrobiol*., **3**(2): 84-88.

Environmental Protection Agency, Office of Prevention, Pesticides and Toxic substances 1998. Pesticide Fact Sheet, (Cymoxanil)

Erickson, T., Vanden Hoek, T.L., Kuritza, A. and Leiken, J.B. 1992. The emergency management of moray eel bites. *Ann. Emerg. Med.*, **21**: 212-216.

Erkmen B., Caliskan M. and Yerli S.V. 2000. Histopathological effects of cyphenothrin on the gills of the *Lepistes reticulates. Vet Hum Toxicol.,* **42**: 71-78

Erslev, A.J. 1977. Erthrocyte disorders Secondary polycythemia. Pages 295-299. (eds: E.B. Williams, A.J. Erslev and R.W. Rundles) Hematology. A Blakiston Publ., MaGraw-Hill.

Ervnest, H. 2004. A Textbook of modern toxicology (3rd ed), (John Wiley and Sons' Hoboken, New Jersey). 557.

Escher, B.I., Snozzi, M., Haberli, K. and Schwarzenbach, R.P. 1997. A new method for simultaneous quantification of the uncoupling and inhibitory activity of organic pollutants in energy transducing membranes. *Environ. Toxicol. Chem.,* **16**: 405-414

Evans, D. H., 1998. The physiology of fishes. Ed:CRC marine science series Press. Inc. 2000 Corporate Blvd., N. W., Boca Raton Florida.

Evans, D.H. 1987. The fish gill: site of action and model for toxic effects of environmental pollutants. Environ Health Perspect 71: 47-58.

Evans, D.H., Piermarini, P.M. and Choe, K.P. 2005. The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation, and Excretion of

Nitrogenous Waste. *Physiol Rev.,* 85: 97-177.

Ezzat, A.A., Abdel, A.S.H., El Nady, F.E. and Abdel-Barr, M. 1998. Impacts of sublethal concentrations of lead on haematology of *Siganus rivulatus*. Proceeding of the 8th inter. Confer. Environ. Protection is a Must.

Fabacher, D.L. and Baumann, P.C. 1985. Enlarged livers and hepatic microsomal mixed-function oxidase components in tumor-bearing brown bullheads from a chemically contaminated river. *Environ. Toxicol. Chem*., **4**: 703-10.

Facey, D.E., Leclerc, C., Dunbar, D., Arruda, D., Pyzocha, L. and Blazer, V. 1999. Physiological indicators of stress among fishes from contaminated areas of Lake Champlain. Pages 349-359 in Manley, T.O. and Manley, P.L. editors. Lake Champlain in transition: from research toward restoration. American Geophysical Union, Washington, D.C.

Fafioye, O.O., Adebisi, A.A. and Fagade, S.O. 2004. Toxicity of *Parkia biglossa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. *Afr J Biotech.,* **3**(11): 627-630.

Fagr, K.A., Saber, A.E., Farag, A.S. and Wagdy, K.B.K. 2008. Effect of water pollution on expression of immune response genes of *Solea aegyptiaca* in Lake Qarun. *African Journal of Biotechnology,* **7**(10):1418-1425.

Fahmy, G.H. 2012. Malathion toxicity: Effect on some metabolic activities in *Oreochromis niloticus*, the Tilapia fish. *International Journal of Bioscience, Biochemistry and Bioinformatics,* **2**(1): 52-55.

Falk, K., Press, C.McL., Landsverk, T. and Dannevig, B.H. 1995. Spleen and kidney of Atlantic salmon (*Salmo salar* L.) show histochemical changes early in the course of experimentally induced infectious salmon anaemia (ISA). *Vet. Immunol. Immunopathol*., **49**: 115-126.

Fanta, E., Rios, F., Romao, S., Vianna, A. and Freiberger, S. 2003. Histopathology of the fish Corydoras paleatus contaminated with sub-lethal levels of organophosphorus in water and food. *Ecotoxicol. Environ. Safe.,* **54**: 119-130.

FAO (Food and agriculture organization of the United Nations) - FAO specifications and evaluations for agricultural pesticides - Cymoxanil - (2005)

Farah, M.A., Ateeq, B., Ali, M.N., Sabir, R. and Ahmad, W. 2004. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. *Chemosphere*, **55**(2): 257-265

Farkas, A., Salanki, J. and Specziar, A. 2002. Relation between growth and the heavy metal concentration in organs of bream, *Abramis brama*L. populating lake Balaton. *Arch. Environ. Contam. Toxicol.,* **43**: 236-243.

Farombi, E.O., Mercy, C.U., Teclar, T.E., Oyeyemi, M.O. and Martins, E. 2008. Tetracycline-induced reproductive toxicity in male rats: effects of vitamin C and N-acetylcysteine. *Exp Toxicol Path.,* **60**: 77-85

Felsot, A., Racke, K.D. and Hamilton, D.J. 2003. Disposal and degradation of pesticide waste. *Reviews of Environmental Contamination and Toxicology,* **177**: 123-200.

Felsot, A.S. and Ruppert, J.R. 2002. Imidacloprid residues in Willapa Bay (Washington state) water and sediment following application for control of burrowing shrimp. *J Agric Food Chem.,* **50**: 4417-4423.

Felsot, A.S., Cone, W. and Ruppert, J.J.R. 1998. Distribution of imidacloprid in soil following subsurface drip chemigation. *Bull. Environ. Contam. Toxicol.,* **60**: 363-370.

Feng, S., Kong, Z., Wang, X., Zhao, L. and Peng, P. 2004. Acute toxicity and genotoxicity of two novel pesticides on amphibian, *Rana N.* Hallowell. *Chemosphere*, **56**(5): 457-463.

Fernandes, C., Fontainhas-Fernandes, A., Monteiro, S.M. and Salgado, M.A. 2007. Histopathological gill changes in wild leaping grey mullet (*Liza saliens*) from the Esmoriz-Paramos coastal lagoon. *Portugal. Environ.Toxicol*., **22**: 443-448.

Fernandez-Alba, A.R., Hernando, M.D., Pedra, L. and Chisti, Y. 2002. Toxicity evaluation of single and mixed antifouling biocides measured with acute toxicity bioassays*, Anal. Chim. Acta.*, **456**: 303-312.

Ferrando, M.D. and Andreu-Moliner, E. 1991. Effects of lindane on fish carbohydrate metabolism. *Ecotoxicol. Environ. Saf.,* **22**(1): 17-23.

Feussner, E.L., Christian, M.S. and Christian, G.D. 1982. Teratogenicity study of INT-3217 in New Zealand white rabbits (segment II evaluation) Argus Research Laboratories, Inc., Pennsylvania Report No. HL 467-82 GLP (Quality assurance unit final report statement available) not published.

Figueiredo-Fernandes, A.M., Fontaínhas-Fernandesm, A.A., Monteiro, R.A., Reis-Henrique, M.A. and Rocha, E. 2007. Spacial relationships of the intrahepatic vascular-biliary tracts and associated pancreatic acini of Nile tilapia, *Oreochromis niloticus* (Teleostei, Cichlidae): A serial section study by light microscopy. *Ann Anat.,* **189**: 17-30.

Finney, D.J. 1978. Statistical Method in Biological Assay, 3rd edition. London: Cambridge University Press: 508.

Firat, O., Cogun, H.Y., Yuzereroglu, T.A., Gok, G., Firat, O., Kargin, F. and Kotemen, K. 2011. A

comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. *Fish Physiol. Biochem*., **37**: 657-666.

Fishel, F.M. 2010. Pesticide Toxicity Profile: Neonicotinoid Pesticides.Institute of Food and Agricultural Sciences, University of Florida.

Fisk, A.T., Hobson, K.A. and Norstrom, R.J. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya food web. *Environ. Sci. Technol.,* **35**: 732-738.

Fiske, C.H., Subbarow, Y., 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.,* **66**: 375-400.

Fleeger, J.W. Carman, K.R. and Nisbet, R.M. 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci. Total Environ*., **317**: 207-233.

Fletcher, G.L., King, M.J., Kiceniuk, J.W. and Addison, R.F. 1982. Liver hypertrophy in winter flounder following exposure to experimentally oiled sediments. *Comp. Biochem. Physiol*., **73**: 457-462.

Flora, S.J., Mittal, M., Mehta, A. 2008. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J Med Res.,* **128**: 501-523.

Flores-Lopes, F. and Thomaz, A.T. 2011. A Histopathologic alterations observed in fish gills as a tool in environmental monitoring, *Braz. J. Biol.,* **71**(1): 179-188.

Folch, J., Lees, M. and Sloane-Stanley, G.H. 1957. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*: 497-509.

Fontaínhas-Fernandes, A., Luzio, A., Garcia-Santos, S., Carrola, J. and Monteiro, S. 2008. Gill Histopathological Alterations in Nile Tilapia, *Oreochromis niloticus* Exposed to Treated Sewage Water. *Brazilian Archives of Biology and Technology,* **51**(5): 1057-1063.

Fontaínhas-Fernandes, A.A 1998. Tilapia production, in: M.A. Reis-Henriques (Ed.), *Aquaculture* Handbook, 135-150.

Fontaınhas-Fernandes, M.P., Peixoto, A., Carrola, F.J. and Rocha, E. 2007. Biochemical and histological hepatic changes of Nile tilapia *Oreochromis niloticus* exposed to carbaryl. *Pesticide Biochemistry and Physiology*, **89**: 73-80.

Fontanetti, C.S.; Christofoletti, C.A.; Pinheiro, T.G.; Souza, T.S. & Pedro-Escher, J. (2010). Microscopy as a tool in toxicological evaluations, In: *Microscopy: Science, Technology, Applications and Education.* Méndez-Vilas, A. & Diaz, J, pp.(1001-1007), Badajoz: Formatex Research Center,

Foreman, W.T., Majewski, M.S., Goolsby, D.A., Wiebe, F.W. and Coupe, R .H. 2000. Pesticides in the atmosphere of the Mississippi River Valley, Part II - air. *Sci. Tot. Environ.,* **248**: 213-216.

Förlin, L. and Haux, C. 1990. Sex differences in hepatic cytochrome P-450 monooxygenase activities in rainbow trout during an annual reproductive cycle. *J. Endocrinol.,* **124**: 207-213.

Fossen, M. 2006. *Environmental fate of imidacloprid*. California Department of Pesticide Regulation.

Foster, A.R., Houlihan, D.F. and Hall, S.J. 1993. Effects of nutritional regime on correlates of growth rate in juvenile Atlantic cod (*Gadhus morhua*): Comparisons of morphological and biochemical measurements. *Can. J. Fish. Aquat. Sci*., **50**: 502-12.

Fournie, J.W., Summers, J.K., Courtney, L.A. and Engle, V.D. 2001. Utility of splenic macrophage aggregates as an indicator of fish exposure to degraded environments. *Journal of Aquatic Animal Health,* **13**: 105-116.

FRAC Code List 2012: Fungicides sorted by mode of action (including FRAC Code numbering): 1-70.

Francesco, P.I., Francesca, G.S., Umile, C., Giuseppe, C., Manuela, I. and Nevio, P. 2008. Polymer in Agriculture: a Review. *Am. J. Agri. Biol. Sci.,* **3**: 299-314.

Freeman, H.C. and Idler, D.R. 1973. Effect of corticosteroids on liver transaminases in two salmonids, the rainbow trout (*Salmo gairdnerii*) and the brook trout (*Salvelinus fontinalis*). *Gen. Comp. Endocrinol.,* **20**: 69-75.

Frei, B. 1994. Reactive oxygen species and antioxidant vitamins: mechanisms and action. *Am J Med.*, **97**: 5S-13S.

Friedemann. and Haugen, G.F. 1943. Collection of blood for the determination of pyruvic acid and lactic acid. *J. Biol. Chem.,* **144**: 67-77.

Friedmann, A.S., Watzin, M.C., Brinck-Johnsen, T. and Leiter, J.C. 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). *Aquat. Toxicol.,* **35**: 265-78.

Gabriel, A.A., Taga, I., Nguindex, D.R., Zaidi, M.A., Lehman, L.G., Altosaar, I. and Ngogang, J.Y. 2011. Effect of iodine supplementation on antioxidant status of normal alloxan monohydrate in toxicated rats. *International journal of Pharmacology,* **7**(6):726-731.

Gabriel, U.U. and Erondu, E.S. 2010. Toxicity of roundup (a glyphosate product) to fingerlings of *Clarias gariepinus*. *Animal Research International*. **7**(2): xxxx - xxxx.

Gabriel, U.U. and George, A.D. 2005. Plasma enzymes in *C. gariepinus* exposed to chronic levels of roundup (glyphosate). *Environ and Ecology,* **23**: 271-276.

Gabriel, U.U. and Okey, I.B. 2009. Effect of aqueous leaf extracts of *Lepidagathis alopecuroides* on the behaviours and mortality of hybrid cat fish (*Heterobranchus bidorsalis X Clarias gariepinus*) fingerlings. *Res. J. Appl. Sci. Eng. Tech*., **1**: 116-120.

Gabriel, U.U., Akinrotimi, O.A. and Ariweriokuma, V.S. 2012. Changes in Metabolic Enzymes Activities in Selected Organs and Tissue of *Clarias Gariepinus* Exposed to Cypermethrin. *Journal of environmental engineering and technology,* **1**(2): 13-19.

Gabriel, U.U., Ezeri, G.N.O. and Opabunmi, O.O. 2004. Influence of Salt excretion by the perfused head of trout adapted to sea water and its inhibition by adrenaline. *J. Comp Physiol*., 111: 77-91.

Gabriel, U.U., Macaulay, B.K. and Edori, O.S. 2010. Acute Toxicity and Behavioural Responses of African Catfish, *Clarias gariepinus* to amine Salt of 2,4-D. *Chin J Appl Environ Biol.* **16**(3): 347-352.

Gabriel, U.U., Mark, P. and Orlu, E.E. 2009. Toxicity and Behavioural Changes in *Heterobranchus bidorsalis* Fingerlings Treated with a Micronurient Fertilizer, Agrolyser *Research Journal of Environmental and Earth Sciences***, 1**(2): 34-38.

Gabriel, U.U., Obomanu, F.G and Edori, O.S 2010. Biochemical changes in hybrid catfish (*Heterobranchus biodorsalis x Clarias gariepinus*) treated with nuracron. *Chinese Journal of Applied Environmental Biology,* **16**(3): 1-5.

Gabriele, P. and Schwarzer, R. 1985. Changes in hemopoietic tissue of rainbow trout under influence of stress. *Diseases of Aquatic Organisms,* **1:** 1-10.

Gad, N.S. 2011. Oxidative stress and antioxidant enzymes in *Oreochromis niloticus* as biomarkers of exposure to crude oil pollution. *International Journal Of Environmental Science And Engineering (Ijese)*, **1**: 49-58.

Gad, N.S. and Saad, A.S. 2008. Effect of Environmental Pollution by Phenol on Some Physiological Parameters of *Oreochromis niloticus. Global Veterinaria,* **2**(6): 312-319.

Gallagher, E.P. and Di Giulio, R.T. 1989. Effects of complex waste mixtures on hepatic monooxygenase activities in brown bullheads (*Ictalurus nebulosus*). *Environ. Pollut*., **62**: 113-128.

Ganesh, K., Janaiah, C. and Venkateshwarlu, P. 2006. Specific activity levels of protease, aminases and GDH in fresh water fish *Clarias batrachus* on exposure to profenophos. *J. Aqua. Bio.,* **21**(2): 196-199.

Ganeshwade, R.M. 2011. Biochemical Changes Induced by Dimethoate in the Liver of Fresh Water Fish *Puntius ticto* (HAM). *Biological Forum-An International Journal*, **3**(2): 65-68.

Ganguly, S., Bhattacharya, S., Mandi, S. and Tarafdar, J. 2010. Biological detection and analysis of toxicity of organophosphateand azadirachtin-based insecticides in *Lathyrus sativus* L. *Ecotoxicology,* **19**: 85-95

Garcia-Abiado, M.A., Mbahinzireki, G., Rinchard, J., Lee, K.J. and Dabrowski, K. 2004. Effect of diets containing gossypol on blood parameters and spleen structure in tilapia, Oreochromis sp., reared in a recirculating system. *J. Fish Dis*., **27**: 359-368.

Garcia-Santos, S., Monteiro, S.M., Carrola, J. and Fontainhas-Fernandes, A. 2007. Alterac ¸o˜es histolo´ gicas em bra^nquias de tila´ pia nilotica *Oreochromis niloticus* causadas pelo ca´dmio. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia,* **59**(2): 376-381.

Gbore, F.A., Oginni, O., Adewole, A.M. and Aladetan, J.O. 2006. The effect of transportation and handling stress on hematology and plasma biochemistry in fingerlings of *Clarias gariepinus* and *Tilapia zillii*. *World Journal of Agriculrural Science,* **2**(2): 208-212.

George, SG. 1994. Enzymology and molecular biology of phase II xenobiotic-conjugating enzymes in fish in Aquatic Toxicology: Molecular, Biochemical and cellular Perspectives. In: Malins DC, Ostrander GK, editors. Boca Raton, FL: Lewis;. pp. 37-85.

Gerhardt, A. 2007. Importance of Exposure Route for Behavioural Responses in *Lumbriculus variegatus* Müller (Oligochaeta: Lumbriculida) in Short-Term Exposures to Pb. *Env Sci Pollut Res*. **14**(6) 430-434.

German Federal Environment Agency (2007) Endosulfan: draft dossier prepared in support of a proposal of endosulfan to be considered as a candidate for inclusion in the Annexes to the Stockholm Convention. Umweltbundesamt, Dessau

GESAMP (INO/FAO/UNESCO/WHO/IAEA/UN/UNDP), "Joint group of experts in the scientific aspects of marine pollution (1991): review of potential harmful substance carcinogen," Report study 40, GESAMP, 1991.

Ghosha, D., Bhattacharyab, S. and Mazumder, S. 2006. Perturbations in the catfish immune responses by arsenic: organ and cell specific effects. *Comp. Biochem. Physiol. C.,* **143**(4): 455-463.

Gibson, A.K. and Mathis, A. 2006. Opercular beat rate for rainbow darters *Etheostoma caeruleum* exposed to chemical stimuli from conspecific and heterospecific fishes. *Journal of Fish Biology,* **69**: 224-232.

Gijare, S.S., Raja, I.A., Tantarpale, V.T. and Kulkarni, K.M. 2011. Modulation in glycogen of

the freshwater fish *Ophiocephalus punctatus* exposed to cypermethrin. *The Bioscan*, **6**(1): 129-130.

Gil, F. and Pla, A. 2001. Biomarkers as biological indicators of xenobiotic exposure. *Journal of Applied Toxicology*, **21**(4), 245-255.

Gilham, I.D. and Baker, B.I. 1985. A black background facilitates the response to stress in teleosts. *J. Endocrinol*., **105**: 99-105.

Gill, T.S., Pant, J.C. and Pant ,J. 1988. Gill, liver and kidney lesions associated with experimental exposures to carbaryl and dimethoate in the fish (*Puntius conchonius* Ham). *Bull Environ Contain Toxicol.*, **41**:71-78.

Gill, T.S., Tewari H. and Pande J. 1990. Use of fish enzyme system in monitoring water quality: effects of mercury on tissue enzymes. *Comparative Biochemistry and Physiology,* **97C:** 287-292.

Gingerich, W.H. 1982. Hepatic toxicology of fi shes. In: Weber LJ, ed. Aquatic Toxicology. New York: Raven Press. 55-105.

Girard, C., Brodeur, J.C. and Hontela, A. 1998. Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test in vivo. *Can. J. Fish. Aquat. Sci.,* **55**: 438-450

Giron-Perez, M.I., Santerre, A., Gonzalez-Jaime, F., Casas-Solis, J., Hernandez-Coronado, M. and Peregrina-Sandoval, J. 2007. Immunotoxicity and hepatic functionevaluation in Nile tilapia (*Oreochromis niloticus*) exposed to diazinon. *FishShellfish Immunol*., **23**: 760-769.

Giron-Perez, M.I., Zaitseva, G., Casas-Solis, J. and Santerre, A. 2008. Effects of diazinon anddiazoxon on the lymphoproliferation rate of spleenocytes from Nile tilapia (*Oreochromis niloticus*): the immunosuppressive effect could involve anincrease in acetylcholine levels*, Fish Shellfish Immunol*., **25**: 517-521

Glusczak, L., Miron, D. D. S., Moraes, B. S., Simões, R. R., Schetinger, M. R. C., Morsch, V. M. and Loro, V. L. 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen).Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **146**(4), 519-524.

Glusczak, L., Miron, D.S., Crestani, M., Fonseca, M.B., Pedron, F.A., Duarte, M.F. and Vieira, V.L.P. 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens). Ecotoxicol. Environ. Saf*., **65**: 237-241.

Gluth, G. and Hanke, W. 1985. A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. *Ecotoxicol. Environ. Safety*, **9**: 179-188.

Goede, R.W. and Barton, B.A. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. *Am. Fish. Soc. S.,* **8**: 93-108.

Goering, P., Waalkes, M.P. and Klaassen, C.D. 1995. Toxicology of cadmium. In: Toxicology of Metals: Biochemical Aspects, Handbook of Experimental Pharmacology. (eds: Goyer R.A. and M.G.Cherian), Springer, New York, 189-213.

Gohil, D.R., Gadekar, S.B., and Sharbidre, A.A. 2012. Multibiomarker approach in *Oreochromis mossambicus* (Peters, 2983) fish to assess the impact of pollution in Mula River. *Indian Streams Research Journal*, **2**(4): 1-4.

Golovina, N.A. 1996. Morpho-functional characteristics of the blood of fish as objects of aquaculture. PhD. Thesis. Moscow: University of Moscow.

Gonzalez-Pradas, E., Fernandez- Perez, M., Villafranca-Sanchez, M. and Flores-Cespedes, F. 1999. Mobility of imidacloprid from alginatebentonite controlled-release formulations in greenhouse soils. *Pestic. Sci*., **55**: 1109-1115.

Gopi, R.A., Sathya, T.N., Goparaju, A. and Murthy, P.B. 2012. Endocrine Disrupting Effect of Fenvalerate 20% EC and Mancozeb 80 % WP in adult Zebra fish (*Danio rerio*) using Vitellogenin as a Biomarker Bull. *Environ. Pharmacol. Life Sci*., **1(**6): 66 -72.

Gorbatiuk, L.O. 2010. Physiological-Biochemical Response of Fish to the Pesticide Effect (A review). *Hydrobiological Journal*, **46**(4): 78-87.

Gormley, K.L. and Teather, K.L. 2003. Developmental, behavioral, and reproductive effects experienced by Japanese medaka (*Oryzias latipes*) in response to short-term exposure to endosulfan. *Ecotoxicol. Environ. Saf*., **54**(3): 330-338.

Gould, E., Collier, R.S., Karolous, J.J. and Givenus, S. 1976. Heart transaminase in the rock crab, *Cancer irroratus* exposed to cadmium salts. *Bull. Environ. Contam. Toxicol.,* **15**(6): 635-643.

Govindan, V.S., Jacob, L. and Devika, R. 1994. Toxicity and metabolic changes in *Gambusia affinis* exposed to phosphamidon. *Toxicol. Environ. Monit.,* **4**(1): 001-006.

Grady, A.W., McLaughlin, R.M., Caldwell, C.W., Schmitt, C.J. and Stalling, D.L. 1992. Flow cytometry, morphometry and histopathology as biomarkers of benzo(a)pyrene exposure in brown bullheads (*Ameiurus nebulosus*). *J App. Toxicol*., **12**: 165-77.

Graham, J.B. 1997. Air breathing fishes: Evolution, Diversity and Adaptation. *Academics Press, San Diego.*

Grande, M., Anderson, S. and Berg, D. 1994. Effect of pesticides on fish. *Norwegian J. Agric. Sci. Suppl.*, **13**: 195-209.

Grandjean, P., Budtz-Jorgensen, E., White, R.F., Jorgensen, P.J., Weihe, P., De-bes, F., Keiding, N. 1999. Methylmercury exposure biomarkers as indicators of neurotoxicity in 7-year-old children, *American Journal of Epidemiology*, **150**: 301-305.

Grau, R. 1988. The acute toxicity of NTN 33893 technical to rainbow trout (Salmo gairdneri) in a static test. Bayer AG, Leverkusen-Bayerwerk, Germany (performing laboratory). Mobay Corporation, Kansas City, Missouri (submitting company), Report No. 101303: 18.

Gravato, C. and Guilhermino, L., 2009. Effects of benzo[a]pyrene on sea bass(Dicentrarchus labrax L.): biomarkers, growth and behaviour. *Human Environ.Risk Assess*.,**15**: 121-137.

Greatti, M., Barbattini, R., Stravisi, A., Sabatini, A. and Rossi, S. 2006. Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho_ dressed seeds. *Bull Insectol* ., **59**: 99-103.

Greatti, M., Sabatini, A.G., Barbattini, R., Rossi, S. and Stravisi, A. 2003. Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. *Bulletin of Insectology*, **56**(1): 69-72.

Greaves, P. 2007. Histopathology of Pre-clinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation, 3rd edition, 102.

Gregus, Z. and Klaassen, C.D. 1996. Mechanisms of toxicity. In Casarett and Doull's Toxicology:the Basic study of poisons.Klaassen,c.d.(ed.): 35-74.Mcgraw Hill, New York.

Griffiths, H. R. and Lunec, J. 2001. Ascorbic acid in the 21st century - more than a simple antioxidant. *Environ. Toxicol. Pharmacol.,* **10**: 173-182.

Grigoras, G. 2008. Cercetari si experimentari privind optimizarea tehnologiei de iernare a ciprinidelor in varsta de o vara in vederea asigurarii durabile a productiei piscicole. Teza de doctorat. Universitatea "Dunarea de Jos" Galati.

Grinwisa, G.C.M., Boonstra, A., van den Brandhof, E.J., Dormans, J.A.M.A., Engelsma, M., Kuiper, R.V., van Loveren, H., Wester, P.W., Vaal, M.A., Vethaak, A.D. and Vosa, J.G. 1998. Short-term toxicity of bis (tri-n-butyltin) oxide in flounder *Platichthys flesus*: pathology and immune function. *Aquat. Toxicol.,* **42**: 15-36.

Grue CE, Gardner SC, Gilbert PL, 2002. On the significance of pollutant-induced alterations in behavior of fish and wildlife. In: Dell'Omo G (ed) Behavioural Ecotoxicology, John Wiley & Sons, LTD, West Sussex, UK, 1-90.

Guez, D., Suchail, S., Gauthier, M., Maleszka, R. and Belzunces, L.P. 2001. Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera). Neurobiol Learn Mem*., **76**: 183-191.

Guha, B. and Khuda-Bukhsh, A.R. 2002. Efficacy of vitamin-C (L-ascorbic acid) in reducing genotoxicity in fish (*Oreochromis mossambicus*) induced by ethyl methane sulphonate. *Chemosphere,* **47**: 49-56.

Guidaa, M., Inglesea, M. and Meriç, S.A. 2008. Multi-battery toxicity investigation on fungicides. *Desalination,* **226**: 262-270

Gul, S., Belge-Kurutas, E., Yildiz, E., Sahan, A. and Doran, F. 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey. *Environment International*, **30**: 605-609.

Gupta, N. and Yücel, Y.H. 2007. Glaucoma as a neurodegenerative disease. *Curr Opin Ophthalmol.,* **18**: 110-4.

Gupta, P.K. and Aggarwal, M. 2007.Toxicity of fungicides. In: *Veterinary Toxicology*, Gupta,R. C. (Ed.), 1st ed., Elsevier, New York, 587-601.

Gupta, S., Gajbhiye, V.T., Kalpana, and Agnihotri, N.P. 2002. Leaching behaviour of imidacloprid formulations in soil. *Bull. Environ. Contam. Toxicol.,* **68**: 502-508.

Guyton, C. 2006. Lipid metabolism. In: Text book of Medical physiology 9th edition, W. B. Saunders Co, Philadelphia, 849-859.

Haaparanta, A., Valtonen, E.T. and Hoffmann, R.W. 1997. Gill anomalies of perch and roach from four lakes differing in water quality. *Journal of Fish Biology,* **50**(3): 575-591.

Hadj-Kacem, N., Aldrin, J.F. and Romestand, B. 1987. Immediate effects of a rapid temperature increase on some blood parameters of European sea bass *Dicentrarchus labrax*. *Aquaculture,* **64**: 325-331.

Haggag, N.Z. 2004. Physiological studies on the changes induced by some non - traditional pesticides to the Nile catfish. M.V.Sc. thesis. Girls college, Ain shams Univ., Cairo.

Hahn, E.D. and Soyer, R. 2008. Probit and Logit Models: Differences in a Multivariate Realm. **28**: 1-14.

Haider, S. and Imbaray, R.M. 1986. Relative toxicity of technical material and commercial formulation of malathion and endosulfan to a fresh water fish, *Channa punctata*. *Ecotoxicol Environ Safe.,* **11**: 347-351.

Haider, S. and Upadhyaya, N. 1985. Effect of Commercial Formulation of Four Organophosphorus Insecticides on the Ovaries of a Freshwater Teleost, *Mystus vittatus* (Bloch)-A Histological and Histochemical Study. *Journal of Environmental Science & Health Part,* **20**(3): 321-340.

Halliwell, B. 1987. Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. *Chemistry and Physics of Lipids*, **44**(2-4): 327-334.

Halliwell, B. and Gutteridge, J.M.C. 1999. Free radicals in biology and medicine. Oxford University Press, Oxford.

Halliwell, B., 1994. Free radical antioxidant and human disease: Curiosity, cases or consequences? *Lancet*, **344**: 721-724.

Halliwell, B., Murcia, M.A. and Chirico, S. 1995. Free radicals and antioxidants in food and *in vivo:* What they do and what they work. *Critical Review in Food Science and Nutrition,* **35:** 7-20.

Haluzová, I., Modrá, H., Blahová, J., Havelková, M., Široká, Z. and Svobodová, Z. 2011. Biochemical markers of contamination in fish toxicity tests. *Interdiscip Toxicol.,* **4**(2): 85-89.

Hansen, B.H., Garmo, O.A. Olsvik, P.A. and Andersen, R.A. 2007. Gill metal binding and stress gene transcription in brown trout (*Salmo trutta*) exposed to metal environments: the effect of pre-exposure in natural populations. *Environmental Toxicology & Chemistry,* 26(5): 944-953.

Hansen, J.D. 1997. Characterization of rainbow trout terminal deoxynucleotidyl transferase structure and expression. TdT and RAG1 co-expression define the trout primary lymphoid tissues. *Immunogenetics*, **46**(5): 367-375.

Haque, S.M., Wahab, M.A., Wahid, M.I. and Haq, M.S. 1998. Impact of Thai silver barb (*Puntius gonionotus* Bleeker) inclusion of carp polyculture in pond culture system. *Bangladesh J. Fish. Res.,* **2**(1): 15-22.

Hart, L.J., Smith, S.A., Smith, B.J., Robertson, J. and Holladay, S.D. 1997. Exposure to tilapian fish to the pesticide lindane results in hypocellularity of the primary hematopoietic organ (pronephros) and the spleen without altering activity of phagocytic cells in these organs, *Toxicology*, **118**: 211-221.

Hartl, M.G.J., Hutchinson, S. and Hawkins, L. 2001. Organotin and Osmoregulation: quantifying the effects of environmental concentrations of sediment-associated TBT and TPhT on the freshwater adapted European flounder, *Platichthys flesus, L.J. Exp. Mar. Biol. Ecol.* **256**: 267-278.

Hartley, D. and Kidd, H. (Eds.) 1983. Royal Society of Chemistry, *The Agrochemicals Handbook,* Nottingham. England: A311.

Hasan, H.A. and Secer, S. 2003. Width length - weight relationship of the blue crab (Callinectes sapidus) population living in Beymelek Lagoon Lake. *Turkish Journal Veterinary and Animal Science,* **27**: 443 - 442.

Hassanin A., Kuwahara S., Nurhidayat, Tsukamoto Y., Ogawa K., Hiramatsu K. and Sasaki F. 2002. Gonadosomatic index and testis morphology of common carp (Cyprinus carpio) in rivers contaminated with estrogenic chemicals. *Journal of Veterinary Medical Science*, **64**: 921-926.

Haya, K.1989.Toxicity of pyrethroid insecticide to fish. Environ. *Toxicol. Chem*., **8**: 381-391

Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C. and Vonk, A. 2002. Herbicides: feminization of male frogs in the wild. *Nature*, **419**: 895-896

Hayes, W.J. and Laws, E.R. 1990. Classes of Pesticides. *Handbook of Pesticide Toxicology*, **3**, Academic Press, New York.

Heath, A.G. 1995. Water pollution and fish physiology. 2nd ed. Boca Raton, FL: Lewis Publishers.342

Hedayati, A., Safahieh, A., Savari, A. and Ghofleh, M.J. 2010. Assessment of aminotransferase enzymes in Yellowfin sea bream under experimental condition as biomarkers of mercury pollution. *World J. Fish Marine Sci.*, **2**(3): 186-192.

Hedayati, A., Tarkhani, R. and Shadi, A. 2012. Investigation of Acute Toxicity of Two Pesticides Diazinon and Deltamethrin, on Blue Gourami, *Trichogaster trichopterus* (Pallus). *Global Veterinaria,* **8**(5): 440-444.

Heidinger, R.C. and Crawford, S.D. 1977. Effect of temperature and feeding rate on the liver-somatic index of the largemouth bass, *Micropterus salmoides*. *J. Fish. Res. Board Can*., **34**: 633-638.

Hellou, J. 2011. Behavioural ecotoxicology, an “early warning” signal to assess environmental quality. *Environ Sci Pollut Res Int*. **18**(1): 1-11.

Hellou, J. and Leonard, J., 2004. Polycyclic aromatic hydrocarbons bioaccumulation and biotransformation products in trout exposed through food pellets. *Polycycl. Aromat. Compd*., **24**: 697-712.

Hellou, J., Beach, D.G., Erskine, S., Marklevitz, S.A., Saunders, L. 2010. Balanced fates of pyrene and 1-hydroxypyrene in snails, *Ilyanassa obsoleta*, exposed to spiked sediments. *Int J Polyc Arom Comp.,* **26**: 75-90.

Henderson, C., Pickering, Q. H. and Tarzweix, C. M. 1959. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. *Trans. Amer. Fish Soc.,* **88**:23-32.

Henderson, C., Pickering, Q.H. and Tarzwell, C.M. 1960. The toxicity of organic phosphate and chlorinated hydrocarbon insecticides of fishes. Pages 76-88 in Transactions of the Second Seminar on Biological Problems in Water Pollution. U.S. Public Health Service. R.A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

Henry, M.G. and Atchison, G.J. 1986. Behavioral changes in social groups of bluegills exposed to copper. *Trans. Am. Fish. Soc.,* **115**: 590-595.

Herskin, J. and Steffensen J.F. 1998. Energy savings in sea bass swimming in a school: measurements of tail beat frequency and oxygen consumption at different swimming speeds. *J Fish Biol.,* **53**:366-376.

Hesser, E.F. 1960. Methods for routine fish hematology. *Prog. Fish Cult*., **22**: 164-170.

Hickey, J.J. and Anderson, D.W. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. *Science,* **162**: 271-273.

Hickie, B.E. and Dixon, D.G. 1987. The influence of diet and pre-exposure on the tolerance of sodium pentachlorophenate by rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol*., **9**: 343-53.

Hicks, B.D. and Geraci, J.R. 1984. A histological assesment of damage in rainbow trout, *Salmo gairdneri* Richardson fed rations containing erythromycin. *J. Fish Dis.,* **7:** 457-66.

Hill, A.J., Teraoka, H., Heidemann, W. and Peterson, R.E. 2005. Zebrafish as model vertebrate for investigating chemical toxicity. *Toxicological Sciences*, **86**: 6-19

Hilmy, M., Lemke, A. and Jaicb, P. 1979. Haematological changes in Kuwait mullet, liza maeralepis (smith induces by heavy metals*). Ind. Mar. Sci*., **8**: 278-281.

Hine, P.M. 1992. The granulocytes of fish*. Fish Shell. Imm*., **2**: 79-98.

Hinton, D.E., and Lauren, D. J. 1990. Integrative histopathological approaches to detecting effects of environmental stressors on fishes. *In American Fisheries Society Symposium,* **8**: 51-66.

Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A. and Okihiro, M.S. 1992. Histopathological biomarkers, In: Huggett, R.J., Kimerle, R.A., Mehrle, P.M., Jr Bergman, H.L., (Eds.), Biomarkers: Biochemical,Physiological, and Histological Markers of Anthropogenic Stress, Lewis, USA, 155-196.

Hontela, A. 1997. Endocrine and physiological responses of fish to xenobiotics: Role of glucocorticosteroid hormones. *Rev. Toxicol*., **1**: 1-46.

Hontela, A. and Daniel, C. 1996. Effects of acute and sublethal exposure to Cadmium on the interregnal and thyroid function in rainbow trout *Onchorhyncus mykiss*. *Aquat. Toxicol*., **35**: 171-182.

Hontela, A., 1998. Interrenal dysfunction in fish from contaminated sites: in vivo and in vitro assessment. *Annual review. Environ. Toxicol. Chem*. **17:** 44-48.

Hontela, A., Daniel, C. and Ricard, A.C. 1996. Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. *Aquat Toxicol.,* **35**: 171-182

Hori, T. S. F., Avilez, I. M, Iwama, G.K., Jhonson, G. and Afonso L.O.B. 2008. Impairment of the stress response in matrinxa juveniles (*Brycon amazonicus*) exposed to low concentrations of phenol. *Comp. Biochem, Physiol. C. Toxicol. Pharmacol.,* **147**: 416-423.

Houston, A.H. 1997. Review: Are the classical hematological variables acceptable indicators of fish health? *Trans. Am. Fish. Soc*., **126**: 879-894

Howard, P.H. 1991 Handbook of Environmental fate and exposure data for organic chemicals: pesticides. Lewis Publishers, Chelsea.

Hrubec, T.C., Cardinale, J.L. and Smith, S.A. 2000. Hematology and Plasma Chemistry Reference Intervals for Cultured Tilapia (*Oreochromis* Hybrid) *Veterinary Clinical Pathology*, **29**(1): 7-12.

Hurtig, A.K., Sebastian, M.S., Soto, A., Shingre, A., Zambrano, D., Guerrero, W. 2003. Pesticide use among farmers in the Amazon Basin of Ecuador. *Arch. Environ. Health,* **14**(58): 223-228.

Hurtt, M.E. 1995. Reproductive and fertility effects with DPX-T3217-113 (cymoxanil) multigeneration reproduction study in rats (signed statement). Report HLR 568-93. DuPont Haskell Laboratory. Unpublished

Hutchinson, T.H. and Manning, M.J. 1996. Seasonal trends in serum lysozyme activity and total protein concentration in dab (*Limanda limanda* L.) sampled from Lyme Bay, UK. *Fish Shellfish Immunol.,* **6**: 473-482.

Hyliner, H., Andersson, T., Haux, C. and Olsson, P.E. 1989. Cortisol induction of metallothionein in primary culture of rainbow trout hepatocytes. *Journal of Cell Physiology,* **139**: 24-28.

Ibrahim, S.A., Tayel, S.I., Mahmoud, S.A. and El-Kasheif, M.A. 2009. Impact of the carbamate pesticide sevin on hematology and histology of teleost fish (*Oreochromis niloticus*). *Journal Global Veterinaria,* **3**(3): 196-203.

Ighwela, K.A., Ahmad, A.B. and Abol-Munafi, A.B. 2012. Haematological Changes in Nile Tilapia (*Oreochromis niloticus*) Fed with Varying Dietary Maltose Levels. *World Journal of Fish and Marine Sciences,* **4**(4): 376-381

Indirabai, W.P.S., Tharani G.G. and Seetha, P., 2010. Impact of sublethal concentration of endosulfan on biochemicals and histology of organ tissues of freshwater fish, *Labeo rohita* (hamilton, 1822). *The Bioscan*, **5**(2): 215-218.

Inodi, K.R., Okey, I.B. and Gabriel, U.U. 2010. Relative toxicity of aqueous leaf extracts of *Lepidagathis alopecuroides* (Vahl) R. Br. ex Griseb to the clariids, Clarias gariepinus and Heterobranchus bidorsalis fingerlings. *Agric. Biol. J. N. Am.,* **1**(5): 834-840

Iqbal, F., Qureshi, I.Z. and Ali, M., 2004. Histopathological changes on the Kidney of common Carp, *Cyprinus carpio* following nitrate exposure. *Journal of Research in Science*, **15**: 411-418.

Isidori, M., Lavorgna, M., Nardelli, A., and Parrella, A., 2003. Toxicity identification evaluation of leachates from municipal solid waste landfills: a multispecies approach. Chemosphere, 52, 85-94.

Isidori, M., Parrella, A., Piazza, C.M.L. and Strada, R. 2000. Toxicity screening of surface waters in southern Italy with Toxkit microbiotests. In: Persoone G., Janssen C.R. and De Coen W. (Eds.) New microbiotest for routine toxicity screening and biomonitoring Kluwer Academic / Plenum Publishers, New York, 289-293.

Iwama, G.K., Afonso, L.O.B. and Vijayan, M.M. 2004a. Stress in fish. AquaNet Workshop on Fish Welfare, Campbell River, BC, Canada.

Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, R. and Michael, M.R. 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera. Crop Protection*, **23**: 371-378.

Iwaya, K. and Kagabu, S. 1998, Biological properties of the chloronicotinyl insecticide imidacloprid: high selectivity and safer use in practice. *Reviews in Toxicology,* **2**: 121-132.

Jaffar, A. H. and Rani, V. J. 2009. Effect of phosalone on haematological indices in the tilapia, *Oreochromis mossambicus. Turk J Vet Anim Sci.,* **33**(5): 407-411.

Jagoe, C. H. 1996. Responses at the tissue level: quatitative methods in histopathology applied to ecotoxicology. In: Newman, M. C., Jagoe, C. H. (Ed.). Ecotoxicology: A hierarchial treatment. New York: Lewis Publishers; CRC Press.

Jagoe, C.H. and Haines, T.A. 1997. Changes in gills morphology of Atlantic Salmon (*Salmo salar*) Smolts due to addition of acid and aluminum to stream water. *Environmental Pollution*, **97**(12): 137-146.

Jain, S.K. and Punia, J.S. 2006. Hematological and biochemical changes in subacute imidacloprid toxicity. *Indian J Anim Sci.*, **76**: 233-235.

Jain, T.K., Morales, M.A., Sahoo, S.K., Leslie-Pelecky, D.L. and Labhasetwar, V. 2005. Iron oxide nanoparticles for sustained delivery of anticancer agents. *Mol. Pharmacol*., **2**: 194-205.

Jakoby, W.B. and Habig, W.H. 1980. In: Enzymatic Basis of detoxification: (Eds. W.B. Jakoby). Academic press, New York. pp. 63-94.

Jamil, K. 2002. Bioindicators and biomarkers of environmental pollution and risk assessment. *Science Publishers, Enfield,* **4**: 15-56.

Janssen, J. 1997. Comparison of response distance to prey via the lateral line in the ruffe and yellow perch. *Journal of Fish Biology*, **51**: 921-930

Javed, M. and Usmani, N. 2013. Haematological indices of Channa punctatus as an indicator of heavy metal pollution in waste water aquaculture pond, Panethi, India. *African Journal of Biotechnology*, **12**(5): 520-525.

Jee, L.H.; Masroor, F. and Kang, J.C. (2005). Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, Sebastes schlegeli (Hilgendorf). Aquaculture Research, Vol. 36, No. 9, (June 2005), pp. 898-905, ISSN 1365-2109

Jemec, A., Tisler, T., Drobne, D., Sepcic, K., Fournier, D. and Trebs, P. 2007. Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazinon to a non-target arthropod, the microcrustacean Daphnia magna. *Chemosphere,* **68**: 1408-1418.

Jenkins, F., Smith, V., Rajanna, B., Shameem, U., Umadevi, K., Sandhya, V. and Madhavi, R. 2003. Effect of sublethal concentrations of endosulfan on haematological and serum

biochemical parameters in the carp *Cyprinus carpio*. *Bulletin of Environmental Contamination and Toxicology*, **70**(5): 993-997.

Jensen, F.B., Nikinmaa, M. and Weber, R.E. 1993. Environmental pestubations of oxygen transport in teleost fishes. Causes, and consequences and compensations. In: Fish Ecophysiology. J.C. Rankin and F.B. Jensen, (Eds.). Chapman and Hall, London, pp: 161-179.

Jeschke, P. and Nauen, R. 2008. Neonicotinoids- from zero to hero in insecticide chemistry. **64**(11): 1084-1098.

Jhingran, A.G. 1984.some considerations of tilapia into Indian waters. *Burean of fish genetic resources,* C.I.F.R.I.,Allahabad: 10-12.

Jhosi, P.H.P and Bose, M. 2003. Effect of lindane and malathion exposures to certain blood parameters in a fresh water fish Clarias batrachus. *Pollut Res*., **21**: 55-57.

Jiang, J.Q, Lloyd, B. and Grigore, L.2001.Preparation and Evaluation of Potassium Ferrate as an Oxidant and Coagulant for Potable Water Treatment Environmental. *Engineering Science*, **18**(5): 323-328.

Jimenez-Tenorio, N., Morales-Caselles, C., Kalman, J., Salamanca, M.J., de Canales, M.L., Sarasquete, C., Del Valls, T.A., 2007. Determining sediment quality for regulatory proposes using fish chronic bioassays. *Environ. Int.,* **33**: 474-480.

Johansen, J.A., Kennedy, C.J., Sweeting, R.M., Farrell, A.P. and McKeown, B.A. 1994. Sublethal effects of tetrachloroguaiacol on juvenile rainbow trout, Oncorhynchus mykiss, following acute chronic exposure. Can. J. Fish. Aquat. Sci. 51:1967-74.

John, P., Saxena, R. and Prakash, A. 1990. Comparative acute toxicity of metasystox to *Heteropneustes fossilis* and *Ophiocephalus striatus. National Academy Science Letters*, **13**(11): 427-430.

John, P.J. 2007. Alteration of certain blood parameters of freshwater teleost, *Mystus vittatus* after chronic exposure to Metasystox and Sevin. *Fish Physiology and Biochemistry*, **33**: 15-20.

Johnson, L.L., Casillas, E., Collier, T.K., McCain, B.B. and Varanasi, U. 1988. Contaminant effects on ovarian development in English sole (*Parophyrs vetulus*) from Puget Sound, Washington. *Can. J. Fish. Aquat. Sci.,* **45**: 2133-46.

Johnson, W.W. and Finley, M.T. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. U.S. Department of the Interior, Fish and Wildlife Service Resource Publication 137.Washington, DC.

Jones, D.R., Langille, B.L., Randall, D.J. and Shelton, G. 1974. Blood flow in dorsal and ventral aortas of cod, *Gadus morhua*. *Am. J. Physiol*. **226**: 90.

Jones, R.E. Petrell, R.J. and Pauly, D. 1999. Using modified length-weight relationships to assess the condition of fish. *Aquacult. Eng.,* **20**: 261-276.

Joseph, B. and Raj, S.J. 2010. Effect of curacron toxicity on the total serum protein content of *Cyprinus carpio*. *Toxicol. Environ. Chem.,* **92**: 1889-1893.

Joshi, A. G. and Rege, M. S. 1980. Acute toxicity of some pesticides and a few inorganic salts to the masquito fish *Gambusia affinis* (Burid and Girard). *Indian J. Expt. Biol*., **8**: 435.

Joshi, P.K., Bose, M. and Harish, D. 2002. Haematological changes in the blood of *Clarias batrachus* exposed to mercuric chloride. *Ecotoxicological Environmental Monitoring*, **12**(2): 119-122.

Joshi, S.C., Gulati, N. and Gajraj, A. 2005. Evaluation of Toxic Impacts of Mancozeb on Testis in Rats. *Asian J. Exp. Sci.,* **19**(1): 73-83.

Junhong, B., Xiaoa, R., Cuia, B., Zhangb, K., Wangc, Q., Liua, X. and Gaoa, H. 2011. Assessment of heavy metal pollution in wetland soils from the young and old reclaimed regions in the Pearl River Estuary, South Chin, Laibin Huanga. *Environmental Pollution,* **159**(3): 817-824.

Jurd, R.D. 1985. Haematological and immunological 'metamorphosis' in neotenous urodeles. In Metamorphosis. (Eds. Balls, M. and N. Bownes). Clarendon Press, Oxford., 313-331.

Jyothi, B. and Narayan, G. 2000. Pesticide induced alteration of non protein nitrogenous constituents in the serum of a fresh water catfish, *Clarias batrachus Indian Journal of Experimental Biology.* **38**: 1058-1061.

Kackar, R., Shrivastava, M.K. and Raizada, R.B. 1999.Assessment of toxicological effects of mancozeb in male rats after chronic exposure. *Ind J Expt Biol*, **37**: 553-559.

Kackar, R., Srivastav, M.K. and Raizada, R.B. 1997. Studies on rat thyroid after oral administration of mancozeb: Morphological and Biochemical evaluations. *J Appl Toxicol,* **17**: 369-375.

Kagabu S. 2003. Molecular design of neonicotinoids: past, present and future. In *Chemistry of Crop Protection, Progress and Prospects in Science and Regulation*, ed. G Voss, G Ramos, pp. 193-212.

Kagabu, S. and Medej, S. 1995. Chloronicotinyl insecticides-stability comparison of imidacloprid and related compounds under simulated sunlight, hydrolysis conditions, and to oxygen. *Biosci Biotechnol Biochem,* **59**: 980-985,

Kakkar, P., Das, B., Viswanathan, D.N., 1984. A modified spectrometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys*. **21**:130.

Kakuta, I. and Nakai, T. 1992. Blood changes in Japanese *Anguilla*, *Anguilla japonica*, experimentally infected with typical or atypical *Aeromonas salmonicida*. *Comp. Biochem. Phys. A*., **103**: 151-155.

Kalavathy, K., Sivakumar, A.A. and Chandran, R. 2001. Toxic effect of the pesticide Dimethoate on the fish *Sarotherodon mossambicus*. *J Ecol Res Bioconserv.,* **2**(1-2): 27-32.

Kalender, Y., Yel, M. and Kalender, S. 2005. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin. *Toxicology*, **209**: 39-45.

Kamalaveni, K., Gopal, V., Sampson, U. and Aruna, D. 2003. Recycling and utilization of metabolic wastes for energy production is an index of biochemical adaptation of fish under environmental pollution stress. *Environ. Monit. Assess.,* **86**(3): 255-264.

Kamanga, L.J., Kaunda, E., Mtimuni, J.P., Maluwa, A.O. and Mfitilodze, W.M. 2004. Effect of temperature on oocyte development of Oreochromis karongae (Trewavas, 1941). Journal of Applied Ichthyology, 20(2): 139-145.

Kamath, S.H. 1972. Clinical Biochemistry for Medical Technologists. Churchill/Livingstone, London, 89.

Kamble S.M., Bhagwan H.K. and Chinte D.N. 2011. Acute toxicity of sevin concentration on mortality and behaviour of freshwater fish *Barilius barila, International Refered research journal*, **2**(23): 15-17.

Kamble S.M., Mohekar A.D. and Bhagwan H.K. 2001. Effect of sublethal concentrations of sevin (carbamate) on total ascorbic acid contents in ovaries of freshwater fish; Barilius bendelisis and B. burna. Proc. of National Seminar on Curr. Eng of develop.

Kammon, A.M., Brar, R.S., Banga, H.S., and Sodhi, S. 2010. Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. *Vet. Arhiv.,* **80**(5): 663-672.

Kanaraj, M.K., Ramesh, M., Sivakumari, K. and Manavalaramanujam, R. 1993. Impact of acid pollutaion on the serum haemolymph cholesterol of the crab, *Paratelphusa hydrodromous. J. Ecotoxicol. Eviron. Monit.,* **3**(2): 099-102.

Kane, A.S., Salierno, J.D. and Brewer, S.K. 2005. Fish models in behavioral toxicology: Automated techniques, updates and perspectives. In: Ostrander, GK, editor. Methods in Aquatic Toxicology (Chapter 32), Volume 2. Lewis Publishers, Boca Raton, FL. Pages 559-590

Kaoud, H.A., M.M., Zaki, A.R., El-Dahshan, S.S. and El-Zorba, H.Y. 2011. Amelioration of the toxic effects of cadmium-exposure in Nile Tilapia (*Oreochromis niloticus*) by using *Lemna gibba L. Life Sci. J.*, **8**: 185-195.

Kappus, H. 1987. Oxidative stress in chemical toxicity. *Arch. Toxicol.,* **60**: 144-149.

Karlsson-Norrgren, L., Runn, P., Haux, C. and Förlin, L. 1985. Cadmium-induced changes in gill morphology of Zebrafish, *Brachydanio rerio* (Hamilton-Buchanan), and Rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology*, **27**:81-95

Kathya, A., Modesto, C. and Martinez, B.R. 2010. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere*, **78**(3): 294-299.

Katira, R., Elhence, G.P., Mehrotra, M.L., Srivastava, S.S., Mitra, A., Agarwala, R. and Ram, A. 1990. A study of aluminium phosphide (Aluminium phosphide poisoning) poisoning with special reference to electrocardiographic changes. *J Assoc Physicians India*, **38**: 471-473.

Katja, S., George, B.O.S, Stephan, P. and Christian, E.W.S. 2005. Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behavior, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*). *Aquatic Toxicology,* **71**: 49-59

Kaur, A. and Kaur, K. 2006. Impact of nickel-chrome electroplating effluent on the protein and cholesterol contents of blood plasma of *Channa punctatus* (Bl.) during different phases of the reproductive cycle. *Journal of Environmental Biology,* **27**(2): 241-245.

Kavitha, C., Malarvizhi, A., Kumaran, S.S. and Ramesh, M. 2010**.** Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. *Food and Chemical Toxicology,* **48**: 2848-2854.

Kavitha, P. and Rao, J.V., 2008. Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, *Gambusia affinis*. *Environ. Toxicol. Pharmacol.,* **26**: 192-198.

Kawade, S.J. and Khillare, Y.K. 2012. Toxicity of copper on the protein content of certain tissues of freshwater fish, *Channa gachua* (Ham). *The Bioscan*, **7**(1): 53-56.

Kazianis, S. and Walter, R.B. 2002.Use of platyfishes and swordtails in biological research. *Lab Anim (NY),* **31**(3): 46-52.

Kelly, K.A., Harvilla, C.M., Brady, T.C., Abrano, K.H.and Leveir, E.D. 1998. Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. *Environ. Health Perspect.,* **106**: 375-384.

Keppel, G.E. 1969. Modification of the carbon disulfide evolution method for dithiocarbamate residues. *J. Ass. off. Analyt. Chem*., **52**(1):1 62-167.

Keppel, G.E. 1971. Collaborative study on the determination of dithiocarbamate residues of modified carbon disulphide evolution method. *J Assoc off Anal Chem.,* **54**: 528-532.

Khadre, S.E.M. and Shabana, M.B. 1991. Histopathological and physiological disorders of acute crude oil toxicity in *Mugil cephalus*. Inter.Conf.Marine fish Manag. and Develop. Alexandria Egypt.

Khan, A., Ahmad, L. and Khan, M. Z. 2012. Hemato-biochemical changes induced by pyrethroid insecticides in avian, fish and mammalian species. *Int. J. Agric. Biol*., **14**: 834-842.

Khangarot, B.S. and Tripathi, D.M. 1991. Changes in humoral and cell - mediated immune responses and in skin and respiratory surfaces of catfish, *Saccobranchus fossillis,* following copper exposure. *Ecot.Envir. Safety*, **22**(3): 291-308.

Kharat, S. and Kothavade, S. 2012. Hematological study of clarias batrachus with reference to trypanosomiasis. *Trends in Fisheries Research*, **1**(1): 6-9.

Khattak, I.U.D. and Hafeez, M.A. 1996. Effect of malathion on blood parameters of the fish, *Cyprinion watsoni*. *Pak. J. Zool*., **28**: 45-49.

Khayatzadeh, J. and Abbasi, E. 2010. The effects of heavy metals on aquatic animals. The 1st International applied geological congress, Department of Geology, Islamic Azad University-Mashad Branch, Iran: 688-694.

Khidr, M.B. and Mekkawy, I.A.A. 2008. Effect of separate and combined lead and selenium on the liver of the cichlid fish *Oreochromis niloticus*: ultrastructural study. *Egyptian Journal of Zoology*, **50**: 89-119.

Khillare, Y.K. 1992. Effect of ammonium sulphate on oocyte development of the fish, *Barbus stioma*. *Adv. Biosci.,* **11**: 75-78.

Kiceniuk, J.W. and Khan, R.A. 1987. Effect of petroleum hydrocarbons on Atlantic cod, Gadus morhua, following chronic exposure. *Canadian journal of zoology*, **65**(3): 490-494.

Kidd, H. and James, D.R., 1991. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK.

Kim, E., Mason, R.P. and Bergeron, C.M. 2008. A modeling study on methymercury bioaccumulation and its controlling factors. *Ecological Modelling*, **218**: 267-289.

Kimball, K.D. and Levin, S.A. 1985. Limitations to laboratory bioassays: the need for ecosystem-level testing. *Bioscience*, **35**: 165-171.

Kind, P.K., Grigg, G.C. and Booth, G.T. 2002 Physiological responses to prolonged aquatic hypoxia in the Queensland lungfish, *Neoceratodus forsteri. Resp Physiol Neurobiol* **132**: 179-190.

Kindemba, V. 2009. The impact of neonicotinoid insecticides on bumblebees, honey bees and other non-target invertebrates. *Buglife Invertebrate Conservation Trust, UK*.

King, J., 1965a. The dehydrogenases or oxidoreductases. Lactate dehydrogenase In: Practical Clinical Enzymology. London: Van Nostrand, D. Company Ltd. pp.83-93.

King, J., 1965b. The acid and alkaline phosphatases. In: D. Van (ed). Practical Clinical Enzymology. Nostrand Co., London, 191-208.

King, P.R. and King, E.J. 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol.,* **7**(4):322-326.

Kirubagran, R. and Joy, K.P. 1992. Toxic effects of mercury on testicular activity in freshwater teleost *Clarias batrachus*. *J.Fish. Biol.,* **41**: 305-315.

Kleerekoper, H., Westlake, J G.F., Matis, H. and P.J. Gensler. 1972. Orientation of goldfish (*Carassius auratus*) in response to a shallow gradient of a sublethal concentration of copper in an open field. *J Fish Res Bd Can.,* **29**: 45-54.

Knauer, K., Lampert, C. and Gonzalez-Valero, J. 2007. Comparison of in vitro and in vivo acute fish toxicity in relation to toxicant mode of action. *Chemosphere.* **68**: 1435-1441.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D. and Barber, L.B. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: a national reconnaissance. *Environ. Sci. Technol*., **36**: 1202- 1211.

Kolpin, D.W., Thurman, E.M. and Linhart S.M. 2000. Finding minimal herbicide concentrations in ground water??try looking for their degradates. *Science of the Total environment,* **248**:115-122.

Konradsen, F., Van der Hoek, W., Cole, D.C., Hutchinson, G., Daisley, H., Singh, S., Eddleston, M. 2003. Reducing acute poisoning in developing countries-options for restricting the availability of pesticides. *Toxicology*, **192**: 249-261.

Koprucu, S.S., Koprucu, K., Ural, M.S., Ispir, U. and Pala, M. 2006. Acute toxicity of organophosphorus pesticide diazinon and itseffects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). *Pesticide Biochem Physiol,* **86**: 99-105.

Kori-Siakpere, O. and Ubogu, E.O. 2008. Sublethal hematological effects of zinc on the freshwater fish, *Heteroclarias sp*. (Osteichthyes: Clariidae). *Afr. J. Biotechnol.,* **7**: 2068-2073.

Kori-Siakpere, O., Adamu, K.M. and Madukelum, I.T. 2007. Hematological effect of sublethal of paraquat on the African catfish, *Clarias gariepinus* (Osteichthyes: Clariidae). *Res. Jour. Environ. Sci*., **1**: 331-335.

Koundinya, P.R. and Raamurthi, R. 1979. Hematological studies in *Sarotherodon* (tilapia) *mossambicus* exposed to lethal ($LC_{50/48}$ hrs.) concentration of sumithion and sevin. *Curr. Sci*., **48**: 877-879.

Kraemer, G.L.C. 1996. DPX-T3217-113 (Cymoxanil): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss* DuPont Haskell Laboratory.

Kreutz, L.C., Barcellos, L.J.G., Silva, T.O., Anziliero, D., Martins, D. Lorenson, M., Marteninghe, A. and Silva, L.B. 2008. Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings. *Ciência Rural*., **38**(4):1050-1055.

Kreutzweiser, D.P., Good, K.P., Chartrand. D.T., Scarr, T.A. and Thompson, D.G. 2008. Toxicity of the systemic insecticide, imidacloprid, to forest stream insects and microbial communities. *Bull Environ Contam Toxicol*., **80**(3): 211-214.

Krishnappa, 1999a. Cymoxanil technical: 28-day dietary range finding study in Swiss Albino Mice,Reference:; Report No. 2141/96 ,Guideline: OECD 407 (1995)

Krishnappa, 1999b. Subchronic (90 day) oral toxicity study with cymoxanil technical in Swiss albino mice,Reference:; Report No. 2144/96 ,Guideline: OECD 408 (1981)

Krishnappa, 2002. Cancerogenicity study with cymoxanil technical in Swiss albino mice,Reference:; Report No. 2152/96 ,Guideline: OECD 451 (1981)

Kroes, R. 1988. Risk evaluation: the merging of science and art. pp 9 - 17. In: H.A.M. de Kruijf, D. de Zwart, P.N. Viswanathan and P.K. de Ray (eds.) Manual on aquatic ecotoxicology. Allied Publishers Private Ltd, New Delhi, India.

Krohn, J. 1989. Water solubility of NTN 33893. Miles report No. 99859. Bayer AG, Wuppertal-Elberfeld, Germany. 10 pp. (Reviewed in Mulye 1995).

Krohn, J. and Hellpointner, E. 2002. Environmental fate of imidacloprid. *Pflanzenschutz-Nachr.*, **55**: 1-25.

Krutzmann, H.L 1977. The effect of chloramphicol and Oxytetracyline on the haematopoiesis in the European ell *Anguilla anguilla*. *Aquaculture*, **10**: 323-334.

Krykhtin, M.L. 1976. Morphological and physiological indicators of the Kaluga sturgeon, Huso dauricus from the Amur Esturary. *J. Ichthyol.,* **16**: 259-70.

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

Kumar A., Prasad, M.R., Srivastava, K., Tripathi, S. and Srivastava, A.K. 2010. Branchial Histopathological Study of catfish *Heteropneustes fossilis* following exposure to purified Neem Extract, Azadirachtin. *World Journal of Zoology*, **5**(4): 239-243.

Kumar S. and Pant, S.C. 1984. Organal damage caused by aldicarb to a freshwater teleost *Barbus conchonius* (Hamilton). *Bull. Environ. Contam. Toxicol.,* **33**:50-55.

Kumar, A. 2004. Water pollution: New Delhi, A.P.H publishing corporation, p. 199.

Kumar, A., Prasad, M.R., Srivastava, K., Tripathi, S. and Srivastava, A.K. 2010. Branchial Histopathological Study of catfish *Heteropneustes fossilis* following exposure to purified Neem Extract, Azadirachtin. *World Journal of Zoology*, **5**(4): 239-243.

Kumar, H. and Gupta, A.B. 1997. Toxicity of organophosphorus, carbonate and synthetic pyrethroid pesticide to the Indian cat fish, *Hetropneustes fossilis. Journal for Nature Conservation*, **9**(1): 111-114.

Kumar, N., Jesu Prabhu, P. A., Pal, A. K., Remya, S., Aklakur, M.D., Rana, R.S., Gupta, S., Raman, R.P. and Jadhao, S.B. 2011. Anti-oxidative and immuno-hematological status of Tilapia *(Oreochromis mossambicus)* during acute toxicity test of endosulfan. *Pesticide biochemistry and physiology,* **99**: 45-52.

Kummerer, K. 2001. Drugs in the Environment: Emission of Drugs, Diagnostic Aids and Disinfectants into Wastewater by Hospitals in Relation to Other Sources-A Review. *Chemosphere*, **45**(6-7): 957-969

Kunjamma, A., Philip, B., Bhanu, S. and Jose, J. 2008. Histopathological effects on Oreochromis mossambicus (Tilapia) exposed to chlorpyrifos. *Journal of Environmental Research And Development*, **2**(4): 553-559.

Kurian, J. and Natarajan, K. 1997. Studies on Wastewater from Automobile Service Stations. *Indian Journal of Environmental Health*; **39**(1): 37-43.

Kwak, I.S., Chon, T.S., Kang, H.M, Chung, N.I., Kim, J.S., Koh, S.C., Lee, S.K. and Kim, Y.S. 2002. Pattern recognition of the movement tracks of medaka (*Oryzias latipes*) in response to sub-lethal treatments of an insecticide by using artificial neural networks. *Environmental Pollution*, **120**: 671-681.

Lages, N., Balcao, V.M. and Bruno, N.A. 2009. Risk assessment of human consumpation of potentially contaminated red swamp cryfish (*Procambarus clarkia*): a conceptual approach. *Rivesta da facudade de ciencias da saude.* **6**: 332-342.

Lakhani, L. and Pandey, A.K. 1985. Effect of dimecron stress on testis of *Sarotherodon mossambicus*. *Comparative Physiology and Ecology,* **10**(4): 171-175.

Lal, B., Singh, A., Kumari, A. and Singha, N. 1986. Biochemical and haematological changes following malathion treatment in the freshwater catfish *Heteropneustes fossilis* (Bloch). *Environ. Pollut*., **42**: 151-156.

Langiano, V.C. and Martinez, C.B.R. 2008. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* **147**(2): 222-223

Larsson, A., Haux, C., Sjobeck, M.L. and Lithner, G. 1984. Physiological effects of an additional stressor on fish exposed to a simulated heavy-metal-containing effluent from a sulfide ore smeltery. *Ecotox. Environ. Safe*., **8**: 118-128.

Larsson, A., Haux., C. and Sjobeck, M. 1985 . Fish physiology and metal pollution. Results and experiences from laboratory and field studies. *Ecotoxicol. Environ. Saf*., **9**: 275-281.

Larsson. 1975. Sublethal effects of toxic chemicals on aquatic animals. *Elsevier*, New York.

Latijnhouwers, M., de Wit, P.J. and Govers, F. 2000. Oomycetes and fungi: similar weaponry to attack plants. *Trends in Microbiology,* **11**: 462-469.

Law, J.M. 2001. Mechanistic considerations in small fish carcinogenicity testing. *ILAR J.,* **42**: 274-284.

Law, J.M. 2003. Issues Related to the Use of Fish Models in Toxicologic Pathology: Session Introduction. *Toxicologic Pathology*, **31**: 49-52

Lebedeva, N.E., Vosyliene, M.Z. and Golovkina, T.V. 1998. Changes in the biochemical composition of mucus of fish under the effect of environmental factors. *Reports of Russian Academy of Sciences,* **362**(5): 713-715.

Leblond, V.S., Bisson, M. and Hontela, A. 2001. Inhibition of cortisol secretion in dispersed head kidney cells of rainbow trout (*Oncorhynchus mykiss*) by endosulfan, an organochlorine pesticide. *Gen. Comp. Endocrinol*., **121**: 48-56.

Lee, R.F. and Anderson, J.W. 2005. Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. Marine Pollution Bulletin, **50**(7): 705-723.

Leela Siva Paravathi, M., Reddy, C.S. and Nadamunichettey 2002 . In vivo recovery and long term effect of phosalone on total lipid and triglycerides in fresh water fish *Tilapia mossambica*. (Peters) *Poll. Res*., **19**: 345-351.

Lehmann, K.P., Phillips, S., Sar, M., Foster, P.M. and Gaido, K.W. 2004. Dose-dependent alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di (n-butyl) phthalate. *Toxicol Sci.,* **81**(1): 60-68.

Lehninger, A.B., Nelson, D.L. and Cox, M.M. 1993. Principles of biochemistry, New York, NY: Worth Publishers.

Leight, A.E. and Van Dolah, R.F. 1999. Acute toxicity of the insecticide endosulfan, chlorophyrifos and malathion to the epibenthic estuarine amphipod *Gammarus palustris* (Bousfield). *Environ. Toxicol. Chem.,* **18**(50): 958-964.

Lenartova V., Holovska K., Pedrajas J.R., Martinez Lara E., Peinado J., Lopez Barea J., Rosival I. and Kosuth P. 1997. Antioxidant and detoxifying fish enzymes as biomarkers of river pollution. *Biomarkers*, **2:** 247-252.

LeNoir, J.S., Mcconnell, L.L., Fellers, G.M., Cahill, T.M. and Seiber, J.N. 1999. Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada Mountain Range, USA. *Environ. Toxicol. Chem*., **18:** 2715-2722.

Levesque, H., Moon, T.W., Campbell, P.G.C. and Hontela, A. 2002. Seasonal variation in carbohydrate and lipid metabolism of cortisolimpaired metalexposed yellow perch (*Perca flavescens*). *Aquat Toxicol*., **60**: 257-267

Lewis, A.E. 1970. Principles of hematology. Appleton Century Crofts, Meredith Corp. New York.

Lewis, S.K. and Lech, J.J. 1996. Uptake, disposition and persistence of nonylphenol from water in rainbow trout (*Oncorhynchus mykiss*). *Xenobiotica,* **26**: 813-819

Li, J.W., Fan, L.P., Ding, S.D. and Ding, S.L. 2007. Nutritional composition of five cultivars of Chinese jujube. *Food Chem.* **103**: 454-460.

Li, Q. and T. Kawada, 2006. The mechanism of organophosphorus pesticide-induced inhibition of cytolytic activity of killer cells. *Cell Mol. Immunol*., **3**: 171-178.

Li, X.Y., Liu, Y.D., Song, L.R., Liu, J.T. 2003 Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. *Toxicon,* **42**: 85-89.

Lin, J.H., Kao, W.C., Tsai, K.P. and Chen, C.Y., 2005 A novel algal toxicity testing technique

forassessing the toxicity of both metallic and organic toxicants. *Water Res.,* **39**(9):1869-1877.

Lin, Y.S., Gong, R.Z. and Zhu, Z.L. 2000. In: Pesticide and Ecoenvironmental Protection. Chemical & Technologies Publish House, Beijing, 14-15.

Lipskaya, N.Y. and Salekhova, L.P. 1980. Studies in nutrition and morphophysiological indicators of the genus Spicara fishes. *Ekol. Moria.,* **2**: 66-81.

Li-tao, Q., Shao-long, F. and Yang, Y. 2006. Toxicity of a Novel Pesticide,Imidacloprid,on Tadpoles and Frogs of *Rana Nigronaculata* Hallowell, *Journal of Nanhua University*, **34**(2): 181-184.

Little, E.E., and Brewer, S.K. 2001. Neurobehavioral toxicity in fish. In Target Organ Toxicity in Marine and Freshwater Teleosts New Perspectives: Toxicology and the Environment. Vol. 2. Systems. Schlenk, D. and W. H. Benson (eds). Taylor and Francis, London and New York, 139-174

Little, E.E., Fairchild, J.F., and DeLonay, A.J. 1993. Behavioral methods for assessing the impacts of contaminants on early life stage fishes. In American Fisheries Society Symposium 14 Water Quality and the Early Life Stages of Fishes. L. Fuiman (ed). American Fisheries Society, Bethesda.

Liu, W.P., Zheng, W., Ma, Y., and Liu, K.K. 2006. Sorption and degradation of imidacloprid in soil and water. *Journal of Environmental Science & Health - Part B (Pesticides)*, **41**(5): 623-634.

Livingstone, D.R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull*., **42**: 656-666.

Lizama, M., De Los, A.P. and Ambroso, A.M. 2002. Condition factor in nine species of fish of the characidae family in the upper Paraná river floodplain, *Brazil. Braz. J. Biol.,* **62**(1): 113-124.

Lloyd, R. 1992. Pollution and freshwater fish. Fishing New Books, Oxford, 176.

Lockhart, W.L., Metner, D.A. and Grift, N. 1973. Biochemical and residue studies on rainbow trout (*salmo gairdneri*) following field and laboratory exposure to fenitrothion. *Manitoba Entomologist,* **7**: 26-36.

Lockwood A. P. M. 1976. Effects of Pollutants on Aquatic Organisms. *Cambridge University Press,* 193.

Logaswamy, S. and Remia, K.M. 2009. Impact of Cypermethrin and Ekalux on respiratory and some biochemical activities of a fresh water fish, *Tilapia Mossambica*. *Current Biotica*, **3**(1): 65-73.

López-López, E.E., JE Sedeño-Díaz, J.E., Soto, C.C. and Favari, L.L. 2011. Responses of antioxidant enzymes, lipid peroxidation, and Na+/K+-ATPase in liver of the fish *Goodea atripinnis* exposed to Lake Yuriria water. *Fish Physiol Biochem.,* **37**(3): 511-522

Lorgue, G., Lechenet, J. and Rivière, A. 1996. *Clinical Veterinary Toxicology,* Blackwell, Oxford **5**: 194

Lovell, R.T. and Jantrarotai, W. 1991. Subchronic toxicity of dietary aflatoxin B1 to channel catfish. *Journal of Aquatic Animal Health*, **2**(4): 248-254.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randoll, R.J., 1951. Protein measurement with Folin Phenol reagent. *J. Biol. Chem*., **193**: 265-275.

Lucas, L.V., Cloern, J.E., Thompson, J.K. and Monsen, N.E.. 2002. Functional variability of habitats within the Sacramento-San Joaquin Delta: Restoration implications. *Ecol. Appl*., **12:** 1528-1547.

Luiz, W. 1998. Metabolic stress and cell damage in Colossoma macropomum and Hoplosternum littorale exposed to crude oil in the Amazon. Master Thesis. National Institute of Research in the Amazon.

Lukancic, S., Zibrat, U., Mezek, T., Jerebic, A., Simcic, T., Brancelj, A. 2010. Effects of Exposing Two Non-Target Crustacean Species, *Asellus aquaticus* L., and *Gammarus fossarum* Koch., to Atrazine and Imidacloprid. *Bull. Environ. Contam. Toxicol*., **84** :85-90.

Luo, Y.Z. and Zhang, M.H. 2010. Spatially distributed pesticide exposure assessment in the Central Valley, California, USA. *Environmental Pollution*, **158**(5): 1629-1637.

Lushchak, V.I. 2012. Glutathione homeostatis and functions: Potential targets for medical interventions. *Journal of amino acids:* 26.

Luskova V., Svoboda, M. and Koarova, J. 2002. The Effect of Diazinon on Blood Plasma Biochemistry in Carp *(Cyprinus carpio L.)*. *Acta Vet. Brno.,* **71**: 117-123.

Macek, K.J., and McAllister, W.A. 1970. Insecticede susceptibility of some common fish family representatives. *Trans. Am. Fish. Soc.,* **99**(1): 20.

Machado, M.R., Edith Fanta, Effects of the organophosphorous methyl parathion on the branchial epithelium of a freshwater fish *Metynnis roosevelti*, *Braz. arch. biol. technol.*, **46** (3): Curitiba June 2003.

Maehly, A.C., Chance, B. 1955. Assay of catalases and peroxidases. In: Methods in Enzymology, vol II, (ed. S. P. Colowick and N.O. Kaplan) Academic Press, New York, London, 764.

Magar, R.S. and Shaikh, A. 2012. Biochemical changes in proteins and amino acids in *Channa punctatus* in responses to sublethal treatment with the insecticide Malathion. *Trends in Life Sciences*, **1**(3): 19-23.

Magare, S.R. and Patil, H.T. 2000. Effect of pesticides on oxygen consumption. Red blood cell count and metabolites of fish, *Puntius ticto. Enviro and Eco.,* **18**(4): 891-894.

Maheshwari, R. 2005. Fish death in lakes. *Curr. Sci*., **88**: 1719-1721.

Majewski, M.S., Foreman, W.T. and Goolsby, D.A. 2000. Pesticides in the atmosphere of the Mississippi River Valley, Part I - rain. *Sci. Tot. Environ.,* **248**: 201-212.

Malato, S., Caceres, J., Aguera, A., Mezcua, M., Hernando, D., Vial, J. and Fernandez-Alba, A.R. 2001. Degradation of imidacloprid in Water by photo-fenton and TiO2 photocatalysis at a solar pilot plant*:* A comparative study. *Environmental science and technology,* **35**: 4359-4366.

Malev, O., Sauerborn Klobučar, R., Fabbretti, E. and Trebše, P. 2012. Comparative toxicity of imidacloprid and its transformation product 6-chloronicotinic acid to non-target aquatic organisms: Microalgae *Desmodesmus subspicatus* and amphipod *Gammarus fossarum*. *Pestic. Biochem. Physiol.,* **104**(3): 178-186.

Malik, R. and Hodgson, H. 2002. The relationship between the thyroid gland and the liver. Quarterly. *Journal of Medicine*, **95**: 559-569.

Mallatt, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**(4): 630-648.

Malleshappa, 2003. Combined chronic toxicity and carcinogenicity study with Cymoxanil technical in Wistar rats Reference:; Report No. 2153/96 ,Guideline: OECD 453 (1981)

Mancini, F., Van Bruggen, A.H.C., Jiggins, J.L.S., Ambatipudi, A.C. and Murphy, H. 2005. Acute pesticide poisoning among female and male cotton growers in India. *Int. J. Occup. Environ. Health,* **11**: 221-232.

Mani, K. and Saxena, P.K. 1985. Effect of safe concentrations of some pesticides on ovarian recrudescence in the freshwater murrel, *Channa punctatus* (Bl.): A quantitative study. *Ecotoxicology and environmental safety,* **9**(3): 241-249.

Maniyar, R.A., Nazeer Ahmed, R. and David M. 2011. Monocrotophos: toxicity evaluation and respiratory responses of *Cyprinus carpio* (Linnaeus). *Recent Research in Science and Technology.* **3**(1): 51-54.

Maradonna, F., Polzonetti, V., Bandiera, S.M., Migliarini, B., Carnevali, O., 2004. Modulation of the hepatic CYP1A1 system in the marine fish *Gobius niger*, exposed to xenobiotic compounds. *Environ. Sci. Technol*., **38**: 6277-6282.

Marchand, M.J., van Dyk, J.C., Pieterse, G.M., Barnhoorn, I.E.J. and Bornman, M.S., 2008. Histopathological Alterations in the Liver of the Sharptooth Catfish *Clarias gariepinus* from Polluted Aquatic Systems in South Africa. *Environmental Toxicology,* **24**(2):133-47

Martin,D.W., Mayes, P.A. and Rodwell, V.M. 2007. In Harper`s Review of Biochemisty 22nd edition, Large Medical Publication. Maruzen, Asia.

Maruthi, Y. A., and Rao, S. R. 2001. Effect of sugar mill effluent on organic reserves of fish. *Poll. Res.,* **20**(2): 167-171.

Marzolo, M.P., Rigotti, A. and Nervi, F. 1990. Secretion of biliary lipids from the hepatocyte. *Hepatol.*, **12**(3-2): 134-142.

Mason, C.F. 1981. Biology of Freshwater Pollution. Longman, London, 250 .

Masud, S., Singh, I.J. and Ram, R.N. 2003. First maturity and related changes in female *Cyprinus carpio* in response to long term exposure to a mercurial compound. *Journal of Ecophysiology and Occupatinal Health*, **3**: 14.

Mataqueiro, M., Nakaghi, S., de Souza, J., da Cruz, C., de Oliveira, G. and Urbinati, E. 2009. Histopathological changes in the gill, liver and kidney of pacu (Piaractus mesopotamicus, Holmberg, 1887) exposed to various concentrations of trichlorfon. *J. Appl. Ichthyol*., **25**: 124-127.

Matos, P., Fontaınhas-Fernandes, A., Peixoto, F., Carrola, J. and Rocha, E. 2007. Biochemical and histological hepatic changes of Nile tilapia *Oreochromis niloticus* exposed to carbaryl. *Pesticide Biochemistry and Physiology*, **89**: 73-80.

Matsuda, K., Buckingham, S.D., Kleier, D., Rauh, J.J., Grauso, M., and Sattelle, D.B. 2001. Neonicotinoids: Insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol. Sci*., **22**: 573 -580.

Matsumura, F., 1975. Toxicology of Insecticides. 2nd Edn. Plenum Press NY., 503.

Matta, J.L. 1995. Oxygen-derived free radicals as regulators of toxicity in the dinoflagellate *Ostreopsis lenticularis*. In Lassus,P., Arzul,G., Erand,E., Gentien,P. and Marcaillou,C. (eds), Harmful Marine Algal Blooms. Lavoisier, Paris, 469-474.

Maule, A.G. and Schreck, C.B. 1990. Changes in numbers of leukocytes in immune organs of juvenile coho salmon after acute stress or cortisol treatment. *Journal of aquatic animal health,* **2**(4): 298-304.

Maule, A.G., and Schreck, C.B. 1990. Changes in numbers of leukocytes in immune organs of juvenile coho salmon after acute stress or cortisol treatment. *Journal of aquatic animal health,* **2**(4): 298-304.

Maxwell, L.B. and Dutta, H.M. 2005. Diazinon-induced endocrine disruption in bluegill sunfish, *Lepomis macrochirus. Ecotoxicology and environmental safety,* **60**(1): 21-27.

Mayer, F.L.(Jr.), Versteeg, D.J., McKee, M.J., Folmar, L.C., Graney, R.L., McCume, D.C. and Rattner, B.A. 1992. Physiological and nonspecific biomarkers. In: Biomarkers: biochemical, physiological and histological markers of anthropogenic stress. (Eds. R.J. Huggett, R.A. Kimerle, P.M. Mehrle, and H.L. Bergman) Boca Raton (FL): Lewis Publishers.

Mazon, A.F., Monteiro, E.A.S., Pinheiro, G.H.D. and Fernandes, M.N. 2002. Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Braz. J. Biol.,* **62**(4): 621-631.

McCormick, M.I. 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology*, **79**: 1873-1883.

McKim, J.M., Christensen, G.M. and Hunt, E.P. 1970. Changes in the blood of the brook trout Salvelinus fontinalis after short-term and log-term exposure to copper. *J. Fish. Res. Board. Can*., **27**: 1883-1889.

McLeay, D.J. 1975. Sensitivity of blood cell counts in juvenile Roho, salmon (*Oncorhynchus kisutch)* to stressor including sublethal concentrations of pulp mill effluent and zinc. *J. Fish. Res. Bd. Can.,* **32**: 2357-2364.

McLeay, D.J. 1976. A rapid method for measuring the acute toxicity of pulpmill effluents and other toxicants to salmoid fish at ambient room temperature. *J. Fish. Res. Board Canada.* **33**: 1303-1311.

McMaster, M.E., Van der Kraak, G.J., Portt, C.B., Munkittrick, K.R., Sibley, P.K., Smith, I.R. and Dixon, D.G. 1991. Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. *Aquat. Toxicol.,* **21**: 199-218.

Mehrotra, N.K., Kumar, S. and Shukla, V. 1990. Enhancement of tumor-initiating activity of DMBA by the carbamate fungicide mancozeb. *Bull Environ Contam Toxicol*., **44**:39-45.

Meien, V.A. 1927. Observations on the yearly variation of the ovaries in perch. Perca fluviattius

RusskZool **7:** 4.

Meierjohann, S., Walter, R.D. and Muller, S. 2002. Regulation of intracellular glutathione levels in erythrocytes infected with chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. *Biochem J.,* **15**(368): 761-168.

Meinelt, T., R. Kruger, M. Pietrock, R. Osten, and C. Steinberg. 1997. Mercury pollution and macrophage centers in pike (*Esox lucius*) tissues. *Environmental Science and Pollution Research,* **4**(1): 32-36.

Mela, M., Randi, M.A.F., *et al*., 2007. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicology and Environmental Safety, **68**(3): 426-435.

Methomyl, ECH 178, 1996) International programme on chemical safety. Environmental health criteria 178, http://www.inchem.org/documents/ehc/ehc/ehc178.htm (accessed: February 2005).

Metwalli, A.M. and El-Magd, Y. 2002. Skin diseases and enzymatic antioxidant activity among workers exposed to pesticides. *Eastern Mediterranean Health J.*, **8**: 368 - 373.

Meyers, T.R. and Hendricks, J.D. 1985. Histopathology. In: (Eds., Loux, D.B., Dorfman, M.) Fundamentals of Aquatic Toxicology: Methods and Applications, Hemisphere USA, pp. 283-330.

Mgbenka, B.O., Oluah, N.S. and Umeike, I. 2003. Effect of gammalin 20 (Lindane) on the Differential white blood cell counts of the African catfish *Clarias albopunctatus*. *Bulletin of Environmental Contamination and Toxicology*, **71**(2): 248 - 254.

Michniewicz, M., Nalecz-Jawecki, G., Stufka-Olczyk, J. and Sawicki, J. 2000. Comparison of chemical composition and toxicity of wastewaters from pulp industry. In: Persoone G., Janssen C.R. and De Coen W. (Eds.) New microbiotest for routine toxicity screening and biomonitoring Kluwer Academic / Plenum Publishers, New York, 401-411.

Mieiro, C.L., Bervoets, L., Joosen, R., Blust, R., Duarte, A.C., Pereira, M.E. and Pacheco, M. 2011. Metallothioneins failed to reflect mercury external levels of exposure and bioaccumulation in marine fish - Considerations on tissue and species specific responses. *Chemosphere*, **85**: 114-121.

Miller, L.L. 2006. The effects of selenium on the physiological stress response in fish. Thesis

Mir, F.A., Shah, G.M., Jan, U., Mir, J.I. and Dubey, V.K. 2011. Studies on influences of sublethal concentrations of organophosphate pesticide- Dimethoate (Rogor), on Gonado Somatic

Index (GSI) of female common carp, *Cyprinus carpio communis. J. Ecophysiol. Occup. Hlth.,* **11**: 117-121.

Mirvagefei, B.M., Rafei. A.R.G.R. and Majazi Amiri, B. 2008. Effect of sub-lethal Diazinon Concentrations on Blood Plasma Biochemistry. *Int. J. Environ. Res*., **2**(2): 189-198

Mirvaghefei, B.M., Majazi Amiri, A.R., Rafei, B.G.R. and Nematdost, B. 2011. Hematological and Histopathological Study of Experimental Diazinon Poisoning in common carp fish (*Cyprinus carpio*). *Journal of Fisheries Iranian Journal of Natural Resources*, **64**(1): 1-14.

Mishra, R. and Shukla, S.P. 2003. Endosulfan effects on muscle malate dehydrogenase of the freshwater catfish *Clarias batrachus*. *Ecotoxicology and Environmental Safety,* **56**(3): 425-433.

Mishra, S. and Srivstava, A.K. 1980. The acute toxic effects of copper on the blood of a teleost. *Ecotoxicol. Environ. Safety*, **4**: 191-194.

Modesto, K.A. and Martinez, C.B.R., 2010. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere,* **78**: 294-299

Modra, H., Vrskova, D., Macova, S., Kohoutkova, J., Hajslova, J., Haluzova, I. and Svobodova, Z. 2011. Comparison of diazinon toxicity to embryos of *Xenopus laevis* and *Danio rerio* degradation of diazinon in water. *Bulletin of Environmental ,Contamination and Toxicology,* **86**: 601-604**.**

Mohamed, F.A.S. 2009. Histopathological Studies on Tilapia zillii and Solea vulgaris from Lake Qarun, Egypt. World Journal of Fish and Marine Sciences 1(1): 29-39.

Mohr, S., Berghahn, R., Schmiediche, R., Hübner, V., Loth, S., Feibicke, M., Mailahn, W.and Wogram, J.2012. Macro invertebrate community response to repeated short-term pulses of the insecticide imidacloprid, *Aquat. Toxicol*. 110-111 25-36

Mohun, A.F. and Cook, I.J.Y. 1957. Simple methods for measuring serum levels of glutamic-oxalo acetic and glutamic- pyruvic transaminase in routine laboratories. *J. Clin. Pathol.,* **10**: 394-399.

Monferran, M.V., Pesce, S.F., Cazenave, J., and Wunderlin, D.A. 2008. Detoxification and antioxidant responses in diverse organs of *Jenynsia multidentata* experimentally exposed to 1, 2-and 1, 4-dichlorobenzene. *Environmental toxicology,* **23**(2): 184-192.

Montagna, M.C. and Collins, P.A. 2008. Oxygen consumption and ammonia excretion of the fresh water crab Trichodactylus borellianus exposed to chlorpyrifos and endosulfan insecticides, *Pesticide Biochemistry & Physiology*, **92**: 150-155.

Montagna, M.C. and Collins, P.A., 2007. Survival and growth of Palaemonetes argentinus (Decapods; Caridae) exposed to insecticides with chlorpyrifos and endosulfan as active element. *Archives of Environmental Contamination Toxicology*, **53**: 371-378

Montero, D., Iyquierdo, M.S., Tort, L., Roaina, L. and Vergara, J.M. 1999.High stocking density produces crwoding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. *Fish Phisiol Biochem*., **20**: 53-60.

Moreira, S.M., Lima, I., Ribeiro, R. and Guilhermino, L. 2006. Effects of estuarine sediment contamination on feeding and on key physiological functions of polychaete *Hediste diversicolor*: Laboratory and in situ assays. *Aquatic Toxicology*, **78:**187-201.

Moreira-Santos, M., Donato, C., Lopes, I. and Ribeiro, R. 2008. Avoidance tests with small fish: determination of the median avoidance concentration and of the lowest-observed-effect gradient. *Environ. Toxicol. Chem.,* **27**: 1576-1582.

Morgan, J.D., Vigers, G.A., Farrell, A.P., Janz, D.M. and Maniville, J.F. 1991. Acute avoidance reactions and behavioral responses of juvenile rainbow trout (*Oncorhynchus mykiss*) to Garlon 4, G arlon 3A and vision herbicides. *Environ. Toxicol. Chem.,* **10**: 73-39.

Moriarty, F. 1988. Ecotoxicology: The study of pollutants in the ecosystems. 2nd edition. Academic Press Ltd., London. 289.

Mourente, G., Dıaz-Salvago, E., Bell, J.G. and Tocher, D.R. 2002. Increased activities of hepatic antioxidant defense enzymes in juvenile gilthead sea bream (*Sparus aurata* L.) fed dietary oxidized oil: attenuation by dietary vitamin E. *Aquaculture*, **214**: 343-361

Moza, P.N., K. Hustert, E. Feicht, and A. Kettrup. 1998. Photolysis of imidacloprid in aqueous solution. *Chemosphere,* **36**(3): 497-502.

Mubarik, A., Riazuddin, Z.P., Iqbal, S., Khuhro, M.I. and Bhutto, M.A. 2011. Monitoring of multiple pesticide residues in some fruits in Karachi, Pakistan. *Pak. J. Bot*., **43**(4): 1915-1918,

Mughal, A.L., Iqbal, M.J. and Shakoori, A.R.1993. Toxicity of short term exposure of sublethal doses of a systhetic pyrethroid, generate, on the Chinese grass carp, *Ctenopharyngodon idella*. *Proc. Sem. Aqua. Dev. Pak*., 49-74.

Mullins, J.W., 1993. Imidacloprid. A new nitroguanidine insecticide. *Am. Chem. Soc.*, 183-198.

Mulye, H.S. 1995. Environmental evaluation of imidacloprid insecticide and the end-use product

Admire 240F. Submission Numbers: 94-1706 and 94-1705. Pesticides Division, Commercial Chemicals Evaluation Branch, Environmental Protection Service, Environment Canada.

Murugesan, R., Palaniswamy, T.N. and Panneer, S. 1999. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities in different tissues of *Sarotherodon mossambicus* (Peters) exposed to a carbamate pesticides, carbaryl. *Pestic. Sci.,* **55**: 1217-1221.

Musa, S.O. and Omoregre, E. 1999. Haematological changes in the mud fish *Clarias gariepinus* exposed to malachite green. *Journal of Aquatic Sciences,* **14**: 37-47.

Mustafa, S. and Murad, A. 1984. Survival, behavioural and haematology profile of catfish. *Heteropneustes fossile* exposed to DDT. *Japan J.Ichthyol*., **31**: 55-65.

Nam, Y.K., Cho, Y.S., Choi, B.N., Kim, K.H., Kim, S.K., Kim, D.S. 2005. Alteration of antioxidant enzymes at the mRNA level during short-term starvation of rockbream *Oplegnathus fasciatus. Fisheries Science,* **71**: 1385-1387.

Narain, S. 2003. Stop the cycle of poison. *Down to Earth*., **2**(28): editor's page.

Narasimha, M.B. and Ramana, R.K.V. 1985. Alteration in the nitrogen metabolism in liver of fish *T. mossambica* under lindane toxicity. *J. Pids 6th Nat. Sym. Life Sci.,*: 17-19.

Narra, M.R., Regatte, R.R. and Kodimyala, R. 2012. Effects of chlorpyrifos on enzymes as biomarkers of toxicity in Fresh water field crab *Barytelphusa guerini*. *International Journal Of Environmental Sciences*, **2**(4): 2015-2023.

Nath, B.S., 2000. Changes in carbohydrate metabolism in haemolymph and fat body of the silkworm, *Bombyx mori* L., exposed to organophosphorus insecticides. *Pestic. Biochem. Physiol.* **68**: 127-137.

Nath, R. and Banerjee, V. 1995. Effects of various concentrations of lead nitrate on haematological parameters of an air breathing fish, *Clarias batrachus*. *J. Freshwater Biol*., **7**: 267-268.

Nath, R. and Banerjee, V. 1996. Effect of pesticides methylparathion and cypermethrin on the air-breathing fish *Heteropneustes fossilis*. *Environ. Ecol.,* **14**: 163-165.

National Research Council, 1989. Biological markers in reproductive toxicology. National Academic Press. Washington. D.C. 359

Naveed, A., Janaiah, C. and Venkateshwarlu, P. 2010a. The effects of lihocin toxicity on protein metabolism of the fresh water edible fish, *Channa punctatus* (Bloch). *Journal of*

Toxicology and Environmental Health Sciences, **3**(1): 018-023.

Naveed, A., Venkateshwarlu, P. and Janaiah, C. 2010b. Impact of sublethal concentration of triazophos on regulation of protein metabolism in the fish *Channa punctatus* (Bloch). *African Journal of Biotechnology,* **9**(45): 7753-7758.

Nebeker, A.V., Mckinney, P. and Cairns, M.A. 1983. Acute and chronic effects of diflubenzuron (Dimilin) on freshwater fish and invertebrates. *Environmental Toxicology and Chemistry*, **2**: 329-336.

Neff, J.M. 1985. Use of biochemical measurement to detect pollutant-mediated damage to fish. *ASTM Spec Tech Pub*., **1(**854): 155-183.

Nelson, D.L. and Cox, M.M., 2005. *Lehninger: Principios de Bioquímica*. 4th ed. Barcelona: Ediciones Omega S.A.

Nemeth-Konda, L., Fuleky, Gy., Morovjan, Gy. And Csokan, P. 2002. Sorption behaviour of acetochlor, atrazine, carbendazim, diazinon, imidacloprid and isoproturon on Hungarian agricultural soil. *Chemosphere,* 48: 545-552.

Neskovic, N.K. *et.al.* 1996. Biochemical and histopathological effects of glyphosate on carp, Cyprinus carpio. *Bulletin of Environmental Contamination and Toxicology,* **56**: 295-302.

Newman, D.J. 2008. Natural Products as Leads to Potential Drugs: An Old Process or the New Hope for Drug Discovery? *J. Med. Chem*. **51**: 2589-2599.

Newman, M.C. 1998. Fundamentals of Ecotoxicology. Ann Arbor Press, Chelsea, MI.

Nickerson, J.G., Dugan, S.G. and Drouin, G. 2001. A putative β2-adrenoceptor from the rainbow trout (*Oncorhynuchus mykiss*). *European J Biochemistry,* **268**: 6465-6472

Niehaus, W.G. Jr., Samuelson, B., 1968. Formation of malonaldialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem*., **6**: 126.

NIH (National Institutes of Health). 2002. http://www.nih.gov/science/models/zebrafish

Nikam, S.M. Shejule, K.B. and Patil, R.B. 2011. Study of acute toxicity of Metasystox on the freshwater fish, *Nemacheilus botia*, from Kedrai dam in Maharashtra, India. *Biology and Medicine,* **3**(4): 13-17.

Nikolsky, G.V. 1963. *The Ecology of Fish.* New York, London: Academic Press.

Nilza, L.R., Ligia, M.M., Dense, O.S., Rassia, B.P., Celso, V.N., Tania, U.N., Benicio, A.F. and Benedito, P.F. 2003. Haematological and biochemical values for Nile tilapia Orechromis niloticus) cultured in semi-intensive system. Acta Scientiarum Biological Sciences. Maringa. 25(2): 385-389

Nounou, A.H., Soliman, M.M., Rizkalla, E.H., Assad, M.N. 1997. Haematological and biochemical changes in *Clarias lazera* as a result of long term exposure to trial combination of lead, mercury and arsenic .Egypt. *J. Agric. Res*., **75**(1): 247-270.

Nte, M.E., Hart, A.E., Edun, O.M. and Akinrotimi, O.A. 2011. Effects Of Industrial Effluents On Haematological Parameters Of Black Jaw Tilapia *Sarothedon Melanotheron* (Rupell, 1852). *Continental J. Environmental Sciences,* **5**(2): 29-37.

Nuno, L., Victor, B. and Bruno, N. 2009. A risk assessment of human consumpation of potentially contaminated red swamp cryfish (*Procambarus clarkia*): A Conceptual approach. *Rivesta da facudade de ciencias da saude*. **6**: 332-342.

Nussey, G., Vuren, V.J. and Du Preez, H.H. 1995. Effect of copper on haematology and osmoregulation of the Mozambique tilapia, *Orechromis mossambicus* (Cichidae). *Comp. Biochem. Physiology*., **111**: 369-380.

Nwania, C.D., Lakrab, W.S., Nagpureb, N.S., Kumarb, R., Kushwahab, B. and Srivastavab, S.K. 2010. Mutagenic and genotoxic effects of carbosulfan in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Food and Chemical Toxicology*, **48**(1): 202-220

Oakes, K.D. and Van der Kraak, G.J. 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquat. Toxicol.,* **63**: 447-463

Oakes, K.D., McMaster, M.E. and van der Kraak, G.J. 2004.Oxidative stress responses in longnose sucker (*Catostomus catostomus)* exposed to pulp and paper mill and municipal sewage effluents. *Aquat. Toxicol.*, **67**: 255-271

Obomanu, F.G., Ogbalu, O.K., Gabriel, U.U., Fekarurhobo, G.K. and Abadi, S.U. 2007. Piscicidal effects of *Lepidagathis alopecuriodes* on mudskipper, *Periophthalmus papillio* form the Niger Delta. *Res. J. Appl. Sci.*, **2**(4): 282-387.

Ogbulie, J.N. and Okpokwasili, G.C. 1999. Haematological and Histological responses of *Clarias gariepinus* and *Heterobranchus bidon salis* to some bacterial disease in Rivers State, Nigeria. *Journal of National Science Foundation of Sri Lanka,* **27**(1): 1-16.

Okechukwu, E.O.A.J. and Balogun, J.K. 2007. Effects of acute nominal doses of chlorpyrifos-ethyl on some haematological indices of African catfish *Clarias gariepinus. Journal of Fisheries International,* **2**(2): 190-194.

Okocha ,R.O. and Adedeji, O.B. 2012. Overview Of Copper Toxicity To Aquatic Life. *Report and Opinion* , **4**:(8)

Olaifa, F. G., Olaifa, A. K. and Onwude, T. E. 2004. Lethal and sublethal effects of copper to the African Cat fish (*Clarias gariepnus*). *Afr. J. Biomed. Res*., **7**: 65-70

Olfat, M.N. and El-Greisy, Z.A. 2007. Comparative impact of different waste sources on the reproductive parameters and histology of gonads, liver and pituitary gland of *Siganus rivulatus. J. Applied Sci. Res.,* **3**: 236-244.

Oliva, M., Vicentem, J. J., Gravato, C., Guilhermino, L. and Galindo-Riano, M.D. 2012. Oxidative stress biomarkers in Senegal sole, Solea senegalensis, to assess the impact of heavy metal pollution in a Huelva estuary (SW Spain): Seasonal and spatial variation. *Ecotoxicol. Environ. Saf*., **75**: 151-162.

Oliveria-Ribeiro, C.A., Belger, L., Pelletier, É. and Rouleau, C. 2002. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). *Environmental Research,* **90**(2): 17-225.

Oluah, N.S. 1999. Plasma aspartate amino transferase activity in the catfish *Clarias albopunctatus* exposure to sublethal zinc and mercury. *Bulletin of Environmental Contamination Toxicology,* **63**: 343-349.

Oluah, N.S. and Mgbenka, B.O. 2004. Effect of actellic 25 EC on the differential leucocyte counts of the catfish *Clarias albopunctatus* (Nichole & Lamonte, 1953) *Animal Research International*, **1**(1): 52 -56.

Oluah, N.S. and Nwosu, V.O. 2003. The effect of Brewery waste water in the differential white Blood cell count of the catfish *Clarias albopunctatus*. *Journal of Biological Research and Biotechnology,* **1**: 111 - 118.

Olufayo, M.O. and Alade, O.H. 2012. Acute toxicity and histological changes in gills, liver and kidney of catfish, *Heterobranchus bidorsalis* exposed to cypermethrin concentration." *African Journal of Agricultural Research,* **7**(31): 4453-4459.

Omiama, S.E. 2004. Protective Effect of Vitamin C and Glutathione against the Histopathological Changes Induced by Imidacloprid in the Liver and Testis of Japanese Quail. *The Egyptian Journal of Hospital Medicine,* **16**: 39-54.

Omitoyin, B.O. 2007. Introduction to fish farming in Nigeria. University of Ibadan Press. 90.

Omitoyin, B.O. Ajani, E.K., Adesina, B.T. and Okuagu, C.N.F. 2006. Toxicity of lindane (gamma hexachlorocyclohexane) to *Clarias gariepinus* (Burchell 1822). *W. J. Zool*., **1**: 57-63.

Omitoyin, B.O., Ajani, E.K., Adeshina, B.T. and Okuagu, C.N.F. 2006. Toxicity of Lindane (Gamma Hexachloro - CycloHexane) to *Clarias gariepinus* (Burchell 1822). *World Journal of Zoology*. **1**(1): 57- 63.

Omitoyin, B.O., Ogunsanmi, A.O. and Adesina, B.T. 1999. Studies on acute toxicity of piscicidal plant extracts (*Tetrapleura tetraptera*) on tilapia (*Sarotherodon galilacus*) fingerlings. *Trop. J. Anim. Sci.,* **2**: 191-197.

Omoregie, E., Ufodike, E.B.C. and Keke, I.R. 1990. Tissue chemistry of *O. niloticus* exposed to sublethal concentrations of Gammalin 20 and Actellic 25EC. *J. of Aquatic Science*, **5**: 33-36.

Orbea, A., Ortiz-Zarragoitia, M., Solé, C. Porte, M. and Cajaraville, M.P. 2002. Antioxidant enzymes and peroxisome proliferation in relation to contaminant body bordens of PAHs and PCBs in bivalve molluscs, crabs and Wsh from the Urdaibai and Plentzia estuaries (Bay of Biscay). *Aquat. Toxicol.,* **58**:75-98.

Ortiz, J.B., González de Canales, M.L., and Sarasquete, C. 2003. Histopathological changes induced by lindane (?-HCH) in various organs of fishes. *Scientia Marina,* **67**(1): 53-61.

Ortolani, E.L., Antonelli, A.C. and Sarkis, J.E.S. 2004. Acute sheep poisoning from a copper sulphate footbath. *Veterinary and Human Toxicology,* **46**(6): 315-318

Oruç, E.Ö. 2010. Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. *Pestic. Biochem. Physiol.,* **96**: 160-166.

Oruç, E.Ö. and Üner N. 1999. Effects of 2,4-Diamin on some parameters of protein and carbohydrate metabolisms in the serum, muscle and liver of Cyprinus carpio. *Environ. Pollut.,* **105**(23): 267-272.

Oruc, E.O., Y. Sevgiler, Y. and Uner, N. 2004. Tissue-specific oxidative stress responses in fish exposed to 2,4-D and azinphosmethyl. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **137**(1): 43-51

Oruc, H.H., Cengiz, M. and Beskaya, H. 2009. Chronic copper toxicosis in sheep following the use of copper sulfate as a fungicide on fruit trees. *Journal of Veterinary Diagnostic Investigation,* **21**(4): 540-543.

Orun, I., Dorucu, M. and Yazlak, H. 2003. Haematological parameters of three cyprinid fish species from Karakaya Dam Lake, Turkey. *Online J. Biol. Sci*., **3**: 320-328.

Osweiler, G.D., Carlson, T.L., Buck, W.B. and Von Gelder, G.A. (Eds) 1985. Organic Synthetic Fungicides. In: *Clinical and Diagnostic Veterinary Toxicology*, 3rd ed.:231-242.

Oti, E.E. 2002. Acute toxicity of cassava mill effluent to the African catfish fingerlings. *J. Environ, Biology*. **20**(3): 112-117.

Otitoju, O. 2005. Sub-chronic effect of Rambo insect powder contaminated diet on superoxide

dismutase (SOD) activity in Wistar albino rats. *Biochemistri.*, **17**(2): 157-163.

Otitoju, O. and Onwurah, I.N.E. 2007. Glutathione S-transferase (GST) activity as a biomarker in ecological risk assessment of pesticide contaminated environment. *African J. Biotech.*, **6**(12): 1455-1459.

Pablos, M.V., Fernández, C., Babín, M.M., Navas, J.M., Carbonell, G., Martini, F., García-Hortigüela, P. and Tarazona, J.V., 2009. Use of a novel battery of bioassays for the biological characterisation of hazardous wastes. *Ecotoxicol. Environ. Saf.*, 72: 1594-1600

Pablos, M.V., Martini, F., Fernández, C., Babína, M.M., Herraez, I., Miranda, J., Martínez, J., Carbonell, G., San-Segundoa, L., García-Hortigüelaa, P. and Tarazonaa, J.V. (2011). Correlation between physicochemical and ecotoxicological approaches to estimate landfill leachates toxicity. *Waste Management*, 31: 1841-1847.

Packer, L. 1991. Protective role of vitamin E in biological systems. *American Journal of Clinical Nutrition,* 53: 1050-1055.

Padma Priya, B., Rachel, V. and Maruthi, Y.A. 2012. Acute toxicity effect of imidacloprid insecticide on serum biochemical parameters of fresh water teleost *Channa punctatus. Int. J. Int sci. Inn. Tech. Sec. A*., **1**(2): 18-22.

Pagano, G., Iaccarino, M., Biase, A. De, Meric, S., Warnau, M., Oral, R. and Trieff, N.M. 2001. Factors affecting R6 fungicide toxicity on sea urchin fertilization and early development: roles of exposure routes and mixture components. *Human Exp. Toxicol.*, **20**(8): 404-411.

Palace, V.P., Spallholz, J.E., Holm, J., Wautier, K., Evans, R.E. and Baron, C.L. 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. *Ecotox. Environ. Safe.,* **58**: 17-21

Palace, V.P.N., Khaper, Q.Q. and Singal, P.K. 1999. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radical Biology & Medicine*, **26**: 746-761

Palanisamy, P., Sasikala, G., Mallikarak, D., Bhuvaneshwai, N. and Natarajan, G.M. 2011. Haematological changes of fresh water food fish, *Channa Striata* on exposure to *Cleistanthus collinus* suicidal plant extract. *Res J Pharm, Bio & Chemi sci.*, **2**(2): 814-816.

Palmer, A.K., James, P., Cox, R. and Clark, R. 1981. Effect of H 12712 on pregnancy of the New Zealand white rabbit Haskell Laboratory, E.I. Du Pont de Nemours & Co., Delaware Report No. HLO-805-81 GLP not published

Palozza, P. and Krinsky, N.I. 1992. Antioxidant effects of carotenoids *in vivo* and *in vitro*: An overview. *Meth. Enzymol*., **213**: 403-420.

Pamila, D., Subbaiyan, P.A. and Ramaswamy, M. 1991. Toxic effect of chromium and cobalt on Sartherodon mossambicus (peters). Ind. *J. Environ. Hlth*., **33**: 218-224.

PAN pesticides database, 2006.

Pandey A.C. 2000. Aquatic pollution and fish reproduction : a bibliographical review *Indian J. Fish*., **47**(3) : 231-236.

Pandey B.N., Chanchal, A.K. and Singh, M.P. 1976. Effects of Malathion on the oxygen consumption and blood of *Channa punctatus* (Bloch). *Indian J. Zoatony,* **27**: 95-110

Pandey, A. K. and Shukla, L. 1982 Effec t of an organophosphorus insecticide Malathion on the testicular histology in *Sarotherodon mossambica*. *Nat. Acad. Sci. Lett.*, **5**, 141-143.

Panepinto A.S. 1992. Acute inhalation toxicity study with DPX-T3217-115 (cymoxanil) in rats (Revision 1). Report HLR 83-92 RV1. DuPont Haskell Laboratory. Unpublished.

Pang-Hung, Y., Jian S., Amartalingam R. and Choon-Fah J.B. 2008. Boric Acid Levels in Fresh Noodles and Fish Ball. *Am. J. Agril. Biol. Sci.*, **3**: 476-481.

Panigrahi, A. K. and Mishra, B.N. 1978. Effect of mercury on the morphology of erythrocytes in *Anabas scandens*. *Bull. Environ. Contam. Toxicol*., **23**: 784-787.

Paperna, I. 1987. Systemic granuloma of spariid fish in culture. *Aquaculture*, **67**(1), 53-58.

Parashar, R.S. and Banerjee, T.K. 2002. Toxic impact of lethal concentration of lead nitrate on the gills of air-breathing catfish *Heteropneustes fossilis* (Bloch). *Veterinarski Arhiv.,* **72**(3): 167-183

Parikh, P.H., Rangrez, A., Adhikari-Bagchi, R. and Desai, B.N. 2010. Effect of dimethoate on some histoarchitecture of freshwater fish *Oreocromis mossambicus* (Peters, 1852). *The Bioscan*, **5**(1): 55-58.

Park, J.H., Cho, K.S., Lee, W.H., Cho, Y.J. and Yeon, I.J. 2004. Change of actue toxicity and hematological parameters of *Pawsobagrus fulvidraco to lindane. Envrion Eng Res.,* **9**(4): 150-159.

Parma, M.J., Loteste, A., Campana M. and Bacchetta, C 2007. Changes of haematological parameters in *Prochilodus lineatus* (pisces, Prochilodontidae) exposed to sublethal concentration of cypermethrin. *Journal of environmental Science,* **141**: 216-228.

Parrott, J.L., McMaster, M.E. and Hewitt, L.M. 2006. A decades of research on the environmental impacts of pulp and papermill effluents in Canada: Development and application of fish bioassays. *J. Toxicol. Environ. Health*, **9**: 297-317.

Parthasarathy, R. and Joseph, J. 2011.Study on the changes in the levels of membrane-bound ATPases activity and some mineral status in cyhalothrin-induced hepatotoxicity in fresh water tilapia (*Oreochromis Mossambicus*). *African Journal of Environmental Science and Technology*, **5**(2): 98-103.

Parthiban, P. and Muniyan, M. 2011. Effect of Heavy Metal Nickel On The Lipid Peroxidation And Antioxidant Parameters In The Liver Tissue Of *Cirrhinus Mrigala* (Ham.). *International Journal of Development Research*, **1(**1): 001-004.

Parvez, S. and Raisuddin, S. 2005. Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). *Environmental Toxicology and Pharmacology*, **20**(1): 112-117.

Paterson, B.D. and Spanoghe, P.T. 1997. Stress indicators in marine decapod crustaceans, with particular reference to the grading of western rock lobsters (*Panulirus cygnus*) during commercial handling. *Marine and Freshwater Research*, **48**: 829-834.

Pathan, T.S., Thete, P.B., Shinde, S.E., Sonawane D.L. and Khillare Y.K. 2010. Histopahtological changes in the gill of freshwater fish, *Rasbora daniconius* exposed to paper mill effluent. *Iranica journal of energy and environment,* **1**(3): 170-175.

Patil, C., Paul, R. and Malkanna, K. 2008, Neuroendocrine reglulation and pesticidal impact on freshwater crab, *Barytelphusa guerini*. *Journal of Environmental Biology*, **29**: 887-892.

Patil, V.K. and David, M. 2008. Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). *Turk. J. Fish. Aquat. Sci.,s* **8**:233-237.

Patnaik, L. 2010. Biochemical Alterations Induced by Sevin in *Clarias batrachus*. *Asian j. Exp. Biol. Sci*., **1**(1): 124-127.

Patnaik, L. and Patra, A.K. 2006. Haematopoietic alterations induced by carbaryl in *Clarias batrachus* (Linn.) *J. Appl. SCI. Environ. Manage.*, **10**: 5-7.

Paul, A.J., Hutchens, D.E., Firkins, L.D. and Borgstrom, M. 2004. Dermal safety study with imidacloprid/moxidectin topical solution in the ivermectin-sensitive collie. *Veterinary Parasitology*, **121**: 285-291.

Paulino, M., Iribarne, F., Dubin, M., Aguilera-Morales, S., Tapia, O. and Stoppani, A.O. 2005. The chemotherapy of chagas' disease: an overview. *Mini-Reviews in Medicinal Chemistry*, **5**(5): 499-519.

Payne, J.F., Kiceniuk, J.W., Squires, W.R. and Fletcher, G.L. 1978. Pathological changes in marine fish after a 6-month exposure to petroleum. *J. Fish. Res. Board Can*., **35**: 665-7.

Peakall, D. 1994. Biomarkers-the way forward in environmental assessment. *Toxicol. Ecotoxicol.*, **1** :55-60.

Peebua, P., Kruatrachue, M., Pokethitiyook, P. and Singhakaew, S. 2008. Histopathological alterations of Nile tilapia, *Oreochromis niloticus* in acute and subchronic alachlor exposure. *Journal of Environmental Biology,* **29**(3): 325.

Peña-Llopis, S., Dolores Ferrando, M. and Peña, J.B. 2003. Increased recovery of brain acetylcholinesterase activity in dichlorvos-intoxicated European eels *Anguilla anguilla* by bath treatment with N-acetylcysteine. *Diseases of Aquatic Organisms*, **55**: 237-245.

Perez, M.H. and Wallace, W.G. 2004. Differences in prey capture in grass shrim, *Palaemonetes pugio*, collected along an environmental impact gradient, *Arch. Environ. Contam. Toxicol.*, **46**: 81-86.

Perkins, E.J.J. and Schlenk, D. 2005. In vivo acetylcholinesterase inhibition, metabolism and toxicokinetics of aldicarb in channel catfish: Role of biotransformation in acute toxicity. *Toxicol Sci*. **53**(2): 308-315.

Perl-Treves, R. and Perl, A. 2002. Oxidative stress: An introduction. In: Inzé D, Van Montagu M (eds), Oxidative Stress in Plants, pp.1-32. Taylor & Francis, London.

Perry, S.F., Gilmour, K.M., Swenson, E.R., Vulesevic, B., Chew, S.F. and Ip, Y.K. 2005. An investigation of the role of carbonic anhydrase in aquatic and aerial gas transfer in the African lungfish Protopterus dolloi. J. Exp. Biol. 208: 3805-3815.

Pestana, J.L., Loureiro, S., Baird, D.J. and Soares, A.M. 2010. Pesticide exposure and inducible antipredator responses in the zooplankton grazer, Daphnia magna Straus. *Chemosphere*, **78**(3): 241-248.

Pestana, J.L.T., Alexander, A.C., Culp, J.M., Baird, D.J., Cessna, A.J. and Soares, A.M.V.M., 2009. Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. *Environ. Pollut*., **157**: 2328-2334.

Pestana, M., Gouveia, M. and Abrentes, I. 2009. Effects of *Solanum sisymbriifolium* and *S. nigrum* on lesion nematode *Pratylenchus goodeyi*, on parasites of banana. *Review of Agricultural Sciences*, **32**:173-181.

Pickering AD. 1981. Stress and Fish. London: Academic Press. p 367.

Pierron, F., Baudrimont, M., Bossy, A., Bourdineaud, J.P., Brèthes, D., Elie, P. and Massabuau, J.C. 2007. Impairment of lipid storage by cadmium in the European eel (*Anguilla anguilla*). *Aquatic Toxicology*, **81**(3): 304-311.

Pierron, F., Baudrimont, M., Lucia, M., Durrieu, G., Massabuau, J.C. and Elie, P. 2008. Cadmium uptake by the European eel: Trophic transfer in field and experimental investigations. *Ecotox. Environ. Safe.,* **70**(1): 10-19.

Pierron, F., Bourret, V., St-Cyr, J., Campbell, P.G.C., Bernatchez, L.,Couture, P. 2009. Transcriptional responses to environmental metal exposure in wild yellow perch (*Perca flavescens*) collected in lakes with differing environmental metal concentrations (Cd, Cu, Ni). *Ecotoxicology*, **18**: 620-631

Pimentel, D. 1971. Ecological effects of pesticides on nontarget species. Executive Office of the President's Office of Science and Technology. U.S. Government Printing Office, Washington, DC.

Pimentel, D. and Greiner, A. 1997. Environmental and socioeconomic costs of pesticide use. In: Pimentel, D. (Ed.), Techniques for Reducing Pesticide Use: Economic and Environmental Benefits. John Wiley and Sons, *Chichester*, :51-78.

Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. and D'Amore, M., 1992. Environmental and human costs of pesticide use. *Bioscience,* **42**: 750-760.

Plummer. 1987. An introduction to Practical Biochemistry, Third ed., McGraw Hill Publishing Co. Ltd., New Delhi. pp. 268-270.

PMRA (Pest Management Regulatory Agency). 2001. Imidacloprid. Regulatory Note. REG2001-11. Ottawa: Health Canada, Pest Management Regulatory Agency. Available at http://www.pmraarla. gc.ca/english/pdf/reg/reg2001-11-e.pdf.

Poels, C.L.M., van der Gaag, M.A. and van de Kerkhoff, J.F.J. 1980. An investigation into the long-term effects of Rhine water on rainbow trout. *Water Res*., **14**: 1029-35.

Ponnana, D. 1999 Teratogenicity in rabbits with cymoxanil technical , Rallis Research Centre, India Report No. 2151/96 GLP ,not published

Powers, D.A. 1989. Fish as model systems. *Science,* **246**: 352-358.

Prasanth, M.S., David, M. and Mathed, S.G. 2005. Behavioural changes in freshwater fish *Ctenopharyngodon idellus* (Hamilton) exposed to cypermethrin. *Journal of Ecotoxicology and Environmental Monitoring.* **26**(1): 141-144.

Prashanth, M.S. 2003. Cypermethrin induced physiological and biochemical and histopathological changes in freshwater fish, Cirrhinus mrigala. Ph.D thesis, Karnataka University, Dharwad.

Prashanth, M.S. 2006. Impact of cypermethrin on protein metabolism in fresh water fish

Cirrihinus mrigala. Nature. Environ. Pollution. Tech., **5**(2): 321-32.

Prashanth, M.S. and Neelagund, S.E. 2008. Behavioural surveillance of Indian major carp *Cirrhinus mrigala* (Hamilton) exposed to freecyanide. *International Journal of Applied Environmental Sciences,* **3**(1): 31.

Prashanth, M.S., and Neelagund, S.E. 2008. Impact of Cypermethrin on enzyme activities in the freshwater fish *Cirrhinus mrigala* (Hamilton). *Caspian J. Env. Sci.* **6**(2): 91-95.

Prashanth, M.S., David, M. and Mathed, S.G. 2005. Behavioral changes in freshwater fish, *Cirrhinus mrigala* (Hamilton) exposed to cypermethrin. J. *Environ. Biol*., **26**: 141-144.

Press, C. McL, and Evensen, O. 1999. The morphology of the immune system in teleost fishes. *Fish & Shellfish Immunology,* **9**(4): 309-318.

Prusty, A.K., Kohli, M.P.S., Sahu, N.P., Pal, A.K., Saharan, N., Mohapatra, S. and Gupta, S.K. 2011. Effect of short term exposure of fenvalerate on biochemical and haematological responses in *Labeo rohita* (Hamilton) fingerlings. *Pestic. Biochem. Physiol*., **100**: 124-129.

Puangkaew, J., Kiron, V., Ishizaka, K., Satoh, S. and Watanabe, T. 2004. Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with three lipid sources. *Aquaculture*, **234**(1-4): 361-379.

Pulsford, A.L., K.P. Ryan and J.A Nott, 1992. Metals and mealnomacrophages in flounder, Platichthys flesus, spleen and kidney. J. Mar. Biol. Ass. U.K., 72, 483-498.

Pulsford, A.L., Lemaire-Gony, S., Tomlinson, M., Collingwoood, N. and Glynn, P.J. 1994. Effects of acute stress on the immune system of the dab, *Limanda limanda*. *Comp. Biochem. Physiol*., **109**: 129-39.

Pulsford, A.L., Thomas, M.E., Lemaire-Gony, S., Coles, J., Fossato, V.U. and Pipe, R.K. 1995. Studies on the immune system of the goby, *Zosterisessor ophiocephalus*, from the Venice Lagoon. *Mar. Pollut. Bull.,* **30**: 586-91.

Rabitto, I.S., Alves Costa, J.R.M., Silva de Assis, H.C., Pelletier, E., Akaishi, F.M., Anjos, A., Randi, M.A.F. and Ribeiro, O. 2005. Effects of dietary Pb(II) and tributylin an neotroptical fish Hoplias malabarius: Histopathological and biochemical findings. *Ecotoxicol. Environ. Saf.,* **60**: 147-156.

Radha, G., Logaswamy, S. and Logankumar, K. 2005. Sublethal toxicity of dimethoate on protein, glucose and cholesterol contents in the fish, *Cyprinus carpio. Nature Environment and Pollution Technology*, **4**(2): 307-310.

Radhaiah, V., Girija, M., Rao, K.J. 1987. Changes in selected biochemical parameters in the

kidney and blood of the fish, *Tilapia mossambica* (Peters), exposed to heptachlor. *Bull. Environ. Contam. Toxicol.,* **39**: 1006-1011.

Rahman M. Z; Hossain Z; Mollah M. F. A; Ahmad G. U. (2002). Effect of diazinum 60EC on *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* 'Naga'. *The ICLARM Quarterly,* **25**: 8-12.

Rahman, A., Gupta, M. V., Sollows, J. D., Mazid, M. A., Hussain, M. G., and Dey, M. M. 2002. Economics and adoption patterns of integrated rice-fish farming in Bangladesh. *Rural aquaculture*, 41-54.

Rajput, V., Singh, S.K., Arpita, Kirti and Abhishek. 2012. Comparative toxicity of Butachlor, Imidacloprid and Sodium fluoride on protein profile of the walking cat fish *Clarias batrachus*. *Journal of Applied Pharmaceutical Science*, **2**(06): 121-124.

Ram, R.N. and Singh, S.K. 1988. Carbofuran-induced histopathological and biochemical changes in liver of the teleost fish, *Channa punctatus* (bloch). *Ecotoxicology and environmental safety*, **16**(3): 194-201.

Ramachandra, M. 2000. Malathion induced changes in the ovary of fresh water fish *Glassobius quaris, Pollu. Res.,* **19**(1): 73-75

Ramasamy, P.K., Jeyaraaj, R., David, A.J. and Ramaswamy, M. 2007. Toxicity of an organophosphorus pesticide, Quinalphos to the catfish, *Mystus vittatus*. *J. Eeotoxicol. Environ. Monit.* **17**(4): 391-396

Ramaswamy, V., Boucher, O., Haigh, J., Hauglustaine, D., Haywood, J., Myhre, G., Nakajima, T., Shi, G.Y. and Solomon, S. (Eds.), Radiative Forcing of Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, New York, USA (2007)

Ramesh, M. and Sarvanan, M. 2008. Haematological and biochemical responses in a fresh water fish *Cyprinus carpio* exposed to chlorpyrifos. *International Journal of Integrative Biology*, **3**: 80-83.

Ramesh, M., Sivakumari, K., Kanagaraj, M.K. and Manavalaramanujam, K. 1993. Toxicity of dye effluent in lactate dehydrogenase activity in *Labeo rohita*. *J. Environ. Prot*., **13**: 124-127.

Rao, J., Azhar Baig, K. and Ramamurthy, K. 1984. Effects of a systemic pesticide phosphonidon some aspects of freshwater fish *Tilapia mossambica. Ind. J. Environ. Hlth.*, **26**: 60-64.

Rao, J.V. 2006. Biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sub-lethal concentrations of an organophosphorus insecticide, monocrotophos. *Chemosphere,* **65**(10): 1814-1820.

Rao, J.V., Kavitha, P., Jakka, N.M., Sridhar, V. and Usman, P. 2005. Toxicity of organophosphates on morphology and locomotor behavior in brine shrimp, *Artemia salina*. *Arch. Environ. Contam. Toxicol*., **539**: 227-232.

Ravikrishnan, R., Murugan, S.S., Pillai, K.S. and Murthy, P.B.K. 1997. Effect of a sublethal concentration of combination of two pyrethroids on acetylcholinesterase in brain of a freshwater fish, *Tilapia mossambicus. Journal of Aquatic Sciences*, **12**(122): 39-41.

Ray, G. and Husain, S.A. 2002. Oxidants, antioxidants and carcinogenesis. *Ind J Exp Biol.*, **40**: 1213-32.

Reavill, D. and Roberts, H. 2007.Diagnostic cytology of fish. *Vet. Clin. Exot. Anim.*, **10**: 207-234.

Reddy, M.P. and Bashamohideen, M. 1989. Fenvalerate and cypermethrin induced changes in the haematological parameters of Cyprinus carpio. *Acta Hydrochim. Hydrobiol.*, **17**: 101-107.

Reddy, N.M., Ghousia, B., Rajender, K. and Rao, J.V. 2011a. Toxic impact of two Organophosphate insecticides on biochemical parameters of a food fish and assessment of recovery response. *Toxicology & Industrial Health,* 1-10.

Reddy, N.M., Ghousia, B., Rajender, K. and Rao, J.V. 2011b. Sub-lethal effect of chlorpyrifos on protein metabolism of the food fish *Clarias batrachus* and monitoring of recovery. *Toxicology & Environmental Chemistry*, **93**(8): 1650-165

Reddy, P.K. and Leatherland, J.F. 1998. Stress Physiology, In: Fish disease and disorders, Vol.2 Non - infections Disorders. (Eds. Leatherland, J.F.and Woo, P.T.K.), Cabi Pub. 1998: pp.279 - 301.

Reddy, S.L.N. and Venugopal, N.B.R.K. 1990. Fuoride-induced changes in protein metabolism in the tissue of freshwater crab, *Barytelphusa guerini*. *Environ. Pollut.,* **67**(2): 97-108.

Regoli, F., Winston, G.W., Gorbi, S., Frenzilli, G., Nigro, M., Corsi, I. and Focardi, S. 2003. Integrating enzymatic responses to organic chemical exposure with total oxyradical absorbing capacity and DNA damage in the European eel *Anguilla Anguilla*. *Environ. Toxicol. Chem*., **22**: 2120-2129

Rehulka, J. 2002a. Aeromenas causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): Clinial Pathology, haematology and biochemistry. *Acta Vet Brno*. 71: 351-360.

Rehulka, J. 2002b. Effect of polychlorinated biphenyls Delor 103 on some haematological and biochemical indices of the blood plasma of the rainbow trout. *Oncorhynchus mykiss* (Walbaom). In: The second PCB workshop. Recent Advances in the Environmental

Toxicology and Health Effects of PCBs. Brno, Czech Republic. May 07-11, 2003. Book of Abstracts, p. 36.

Reish, D.L. and Oshida, P. S. 1987. Manual of methods in aquatic environment research. Part 10. Shortterm static bioassay. *FAO Fisheries Technical paper.* **247**:1-62.

Rekanović, E., Potočnik, I., Milijašević-Marčić, S., *et al.,* 2012. Toxicity of metalaxyl, azoxystrobin, dimethomorph, cymoxanil, zoxamide and mancozeb to *Phytophthora infestans* isolates from Serbia. *J Environ Sci Health B.,* **47**(5): 403-409.

Relyea, R.A. and Hoverman, J.T. 2006.Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. *Ecol. Lett.,* **9**:1157-1171.

Remia, K.M., Logaswamy, S., Logankumar, K. and Rajmohan, D. 2008. Effect of an insecticide (Monocrotophos) on some biochemical constituents of the fish *Tilapia mossambica. Poll. Res.,* **27**(3): 523-526.

Remor, A.P., Totti, C.C., Moreira, D.A., Dutra, G.P., Heuser, V.D. and Boeira, J.M. 2009. Occupational exposure of farm workers to pesticides: Biochemical parameters and evaluation of genotoxicity. *Environ. Int*, **35**: 273-278.

Revathi, K., Gulati, S. and Sharief, D. 2005. Tannary effluent induced biochemical charges in the larvivorous fish, *Gambusia affinis. Poll. Res.,* **24**(4): 815-818.

Reynaud, S. & Deschaux, P. (2006). The effects of polycyclic aromatic hydrocarbons on the immune system of fish: a review. Aquatic Toxicology, Vol.77, No.2, pp. 229-238, ISSN 0166-445X.

Reynaud, S., and Deschaux, P. 2006. The effects of polycyclic aromatic hydrocarbons on the immune system of fish: A review. *Aquatic Toxicology*, **77**(2): 229-238.

Rice, C.A., Myers, M.S., Willis, M.L., French, B.L., Casillas, E., 2000. From sediment bioassay to fish biomarker connecting the dots using simple trophic relationships. *Mar. Environ. Res*., **50**: 527-533.

Richmonds C. and Dutta H.M. 1989: Histopathological changes induced by malathion in the gills of bluegill *Lepomis macrochirus. Bull Environ Contam Toxicol.,* **43**: 123-130

Richter ED. 2002. Acute human poisonings. In: Encyclopedia of Pest Management (Ed D Pimentel). Dekker, New York, 3-6.

Riddell, D.J., Culp, J.M. and Baird, D.J. 2005. Sublethal effects of cadmium on prey choice and capture efficiency in juvenile brook trout (*Salvelinus fontinalis). Environmental Toxicology and Chemistry,* **24**(7): 1751-1758.

Roales, R.R. & Perlmutter, A. (1977) The effects of sub-lethal doses of mercu ry, and copper, apllied singly and jointly, on the immune response of the blue gourami (Trichogaster trichopterus) to viral and bacterial antigens. Arch. environ. Contam. Toxicol., 6: 325-331.

Roberts, A.P. and Oris, J.T. 2004. Multiple biomarker response in rainbow trout during exposure to hexavalent chromium, *Comp. Biochem. Physiol.*, **138**: 221-228

Roberts, R.J. 2001 Fish Pathology, 3rd edn. W.B. Saunders, Philadelphia, PA.

Roche, H. and Boge, G. 1996. Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. *Mar. Environ. Res*., **41**: 27-43.

Roe, J.H. and Oesterling, M.J. 1944. The determination of Dehydroascorbic acid and Ascorbic acid in plant tissues by the 2,4-dinitrophenylhydrazine method. *J Biol Chem.,* **152**: 511-17.

Ronald, W.G. and Bruce, A.B. 1990. Organosomic indices and an autopsy based assessment as indicators of health condition of fish. *American Fisheries Society,* **8**: 93-108.

Rortais, A., Arnold, G., Halm, M.P. and Touffet-Briens, F. 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees.*Apidologie*, **36** (1): 71-83.

Rosas, L.G. and Eskenazi, B. 2008. Pesticides and child neurodevelopment. *Current Opinion in Pediatrics*, **20**: 191-197.

Rotruck, J.J., Pope, A.L. Ganthe, H.E.., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G., 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science*, **179**: 588-590.

Rouchaud, J., Gustin, F. and Wauters, A. 1994. Soil biodegradation and leaf transfer of insecticide imidacloprid applied in seed dressing in sugar beet crops Miles Inc., Imidacloprid: Pesticide leaching potential model. Report No. 105008. 1993.

Rouchaud, J., Gustin, F. and Wauters, A. 1994. Soil biodegradation and leaf transfer of insecticide imidacloprid applied in seed dressing in sugar beet crops. *Bull. Environ. Contam. Toxicol.,* **53**(3): 344-350.

Roy, S., Alam, S. and Chowdhury, A. 2010. Leaching behaviour of imidacloprid formulations in soil. Dissipation of Curzate (Cymoxanil 8% + Mancozeb 64%) in Potato Tuber and Cropped Soil. *Pesticide Research Journal*, **22**(1): 5-9.

Ruby, S.M., Idler, D.R. and So, Y.P. 1987. Changes in plasma, liver, and ovary vitellogenin in landlocked antic salmon following exposure to sublethal cyanide. *Arch. Environ. Contam. Toxicol.,* **16**: 507-510.

Ruby, S.M., Idler, D.R. and So, Y.R. 1993. Plasma vitellogenin, 7-0 estradiol, T3 and T4 levels in sexually maturing rainbow trout *Oncorhynchus mykiss* following sublethal HCN exposure. *Aquatic Toxicology,* **26**: 91-102.

Ruklov, F.N. 1979. A description of some morphophysiological characters of salmon of the genus *Oncorhynchus. J. Ichthyol*., **19**: 23-40.

Saborowski, R. and Buchholz, R. 1996. Annual changes in the nutritive state of North Sea dab. *J. Fish Biol.,* **49**: 173-94.

Safahieh, A., Jaddi, Y., Yavari, V. and Zadeh, R.S. 2012. Sub-Lethal Effects of Herbicide Paraquat on Hematological Parameters of Benny Fish esopotamichthys Sharpeyi. *IPCBEE* 42(27): 141 - 145.

Saha, S. and Kaviraj, A. 2009. Effects of cypermethrin on some biochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish *Heteropneustes fossilis*. *Chemosphere*, **74**: 1254-1259

Sakr, S.A., Hanafy, S.M. and El-Desouky, N.E. 2001. Histopathological, histochemical and physiological studies on the effect of the insecticide, hostathion, on the liver of the catfish *Clarias gariepinus*. *Egypt. J. Aquatic Biol. Fish*., **6**(2): 103-124.

Salvo, L.G., Sinhorini, I.L., Malucelli, B.E., Klemz, C., Sanchez, D.C.O., Nicaretta, L., Malucelli, M.I.C., Bacila, M. and Assis, H.C.S. 2008. Effects of endosulfan sublethal concentrations on carp (*Cyprinus carpio*, Linnaeus, 1758): morphometric, histologic, ultrastructural analyses and cholinesterase activity evaluation. *Brazilian Journal of Veterinary Research and Animal Science*, **45**: 87-94.

Sampath, K., Velammal, S., Kennedy, I.J. and James, R. 1993. Haematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sublethal levels of Ekalux. *Acta Hydrobiol*., **35**: 73-83.

Sanchez, W., Palluel, O., Meunier, L., Coquery, M., Porcher, J.M. and Aït-Aïssa, S. 2005. Copper-induced oxidative stress in three-spine stickleback: relationship with hepatic metal levels. *Environ. Toxicol. Phar.,* **19**: 177-183.

Sanchez, W., Piccini, B. and Porcher, J. 2008. Effect of prochloraz fungicide on biotransformation enzymes and oxidative stress parameters in three-spined stickleback (*Gasterosteus aculeatus* L.). *J Environ Sci Heal B*., **43**: 65-70.

Sancho, E., Ceron, J.J. and Ferrando, M.D. 2000. Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in *Anguilla anguilla*. *Ecotoxicol. Environ. Saf.,* **46**: 81-86.

Sanders, H.O. and Cope, O.B. 1966. Toxicities of several pesticides to two species of cladocerans. *Trans. Am. Fish. Soc.,* **96**: 165.

Santhakumar, M., Balaji, M. and Ramadu, K. 2000. Effect of monocrotophos on plasma phosphatase activity of a freshwater fish *Anabus testudineus* (Bloch). *Pollution Research*, **19**(2): 257-259.

Santhakumar, M., Balaji, M.S. and Ramudu, K. 1999. Effect of sublethal concentrations of monocrotophos on erythropoietic activity and certain hematological parameters of the fish Anabas testudineus (Bloch). *Bulletin of Environmental Contamination Toxicology***, 63**: 379 - 384.

Saradhamani, N. and Selvarani, B.J. 2009. A study on the effect of herbicide metribuzin on the biochemical constituents of the freshwater fish, *Tilapia mossambica* Peters (Pisces: Cichlidae). *Curr. Biotica,* **3**: 220-231.

Saravanan, M., Ramesh, M., Malarvizh, A. and Petkam, R. 2011. Toxicity of Neem Leaf Extracts (Azadirachta indica A. Juss) on Some Haematological, Ionoregulatory, Biochemical and Enzymological Parameters of Indian Major Carp, *Cirrhinus mrigala*. *Journal of Tropical Forestry and Environment,* **1**(1): 14-26.

Sarkar, A.R., Shrivastava, A.N. and Sarker, S. 2006. Molecular Biomarkers: Their significance and application in marine pollution monitoring. *Ecotoxicology*, **15**(4): 333-340.

Sarkar, B., Chatterjee, A., Adhikari, S. and Ayyappan, S. 2005. Carbofuran and cypermethrin induced histopathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery. *J. Appl. Ichthyol.,* **21**: 131-135.

Sarver, J.W., 1992. Primary dermal irritation study with DPX-T3217-113 (cymoxanil) in rabbits (Revision 1). Report HLR 787-91 RV1. DuPont Haskell Laboratory. Unpublished.Sasaki, T. and Matsuvs, S.A. 1972. Effect of acetic acid concentration of the colour reaction in the O-toluidine boric acid for blood glucose determination. *Rinsho Kagaku*. **1**: 346-343

Sastry, K.V. and Sachdeva, S.S. 1994. Effect of water - borne cadmium and copper on the blood of the fish *Channa punctatus*. *Environ. Ecol*., **12**(2): 291-297.

Sathyanarayna, D. 2005. Biochemistry book and allied (P) std 8/1 Chintamani Das Lane Kolkata 70009. India 349.

Sato, K., Niki, E. and Shimasaki, H. 1990. Free radical-mediated chain oxidation of low density

lipoprotein and its synergistic inhibition by Vitamin E and Vitamin C. *Arch. Biochem. Biophy.,* **279**: 402-405.

Satyanarayan, S., Bejankiwar, R.S., Chaudhari, P.R., Kotangale, J.P. and Satyanarayan, A. 2004. Impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntius ticto*. *J Environ Sci*., **16**(4): 631-634.

Satyanarayana, U. 2005. Biochemistry books and allied (P) Ltd., 8/1 Chintamani Das Lane, Kolkata, 700009, India.

Saxena, K.K. and Seth, N. 2002. Toxic effects of cypermethrin on certain hematological aspects of fresh water fish *Channa punctatus*. *Bull. Environ. Contam. Toxicol*., **69**: 364-369.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haquee, R. and Raisuddin, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol. Environ. Saf*., **56**: 295-301.

Schmitt, C.J. and Dethloff, G.M. (Editors). 2000. Biomonitoring of Environmental Status and Trends (BEST) Program: selected methods for monitoring chemical contaminants and their effects in aquatic ecosystems. Information and Technology Report USGS/BRD-2000--0005. Columbia (MO): U.S. Geological Survey, Biological Resources Division.

Schmuck, R. 2004. Effects of a chronic dietary exposure of the Honeybee Apis mellifera (Hymenoptera: Apidae) to Imidacloprid. *Archives of Environmental Contamination and Toxicology,* **47**(4): 471-478.

Schwaiger, J., Bucher, F., Ferling, H. and Kalbfus, W.N.R.D. 1992. A prolonged toxicity study on the effects of sublethal concentrations of bis (tri- n-butyltin) oxide (TBTO); histopathological and histochemical findings in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol*., **23**: 31-48.

Schwaiger, J., Fent, K., Stecher, H., Ferling, H., and Negele, R.D. 1996. Effects of sublethal concentrations of triphenyltinacetate on raibow trout (*Oncorhynchus mykis*s). *Arch Environ Contam Toxicol*., **30**: 327-34.

Schwaiger, J., Spieser, O.H., Nardy, E., Kalbfus, W., Braunbeck, Th. and Negele, R.D. 1999. Toxic effects versus endocrine disruption Does the xenoestrogen nonylphenol influence physiological functions in fish? SETAC: 9th Annual Meeting, 25-29 May 1999, Leipzig, Germany.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W. and Triebskorn, R. 1997. The use of histopathological indicators to evaluate contaminant-related stress in fish. *J. Aquat. Ecosyst. Stress Recov.,* **6**: 75-86.

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson,C.A., von Gunten, U., and Wehrli, B. 2006.The challenge of micropollunts in aquatic system. *Science,* **313**: 1072-1077.

Scientific and Technical Committee 2004. A multifactorial study on the disturbance of bees: Imidacloprid used in coating seed (Gaucho) and the disturbance to bees. Final Report.

Scott, A.C. and Rogers, N.A. 1981. Haematological effects of prolonged sublethal hypoxia on channel catfish *Ictalurus punctatus* (Rafinesque). *Journal of Fish Biology*, **18**: 591 - 601.

Scott, G.R. and Sloman, K.A. 2004. The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. *Aquat. Toxicol*., **68**: 369-392.

Scott, S.G. and Pankhurst, N.W. 1992. Interannual variation in the reproductive cycle of the New Zealand snapper *Pagrus auratus* (Bloch and Schneider) (Sparidae). *J. Fish Biol*., **41**: 685-696.

Seifter, S., Dayton, S., Novic, B. and Muntwyler, E. 1950. *Arch. Biochem*., **25**: 191.

Seitz, A. and Ratte, H.T. 1991. Aquatic ecotoxicology: On the problems of extrapolation from laboratory experiments with individuals and populations to community effects in the field. *Comp. Biochem. Physiol. C.,* **100**: 301-304.

Selck, H., Riemann, B., Christoffersen, K., Forbes, V. E., Gustavson, K., Hansen, B. W. and Petersen, S. 2002. Comparing sensitivity of ecotoxicological effect endpoints between laboratory and field. *Ecotoxicology and environmental safety*, **52**(2), 97-112.

Sepici-dincel, A., Benli, A.C.K., Selvi, M., Sarikaya, R., Sahin, D., Ozkul, I.A. and Erkoc, F. 2009. Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio L.*) fingerlings: Biochemical, hematological, histopathological alterations. *Ecotoxicology and Environmental Safety*, **72**(5): 1433-1439.

Sepici-Dinçel, A., Benli, A.Ç.K., Selvi, M., Sarıkaya, R., Şahin, D., Özkul, I.A. and Erkoç, F. 2009. Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: biochemical, hematological, histopathological alterations. *Ecotoxicol Environ Saf*.,**72**:1433-1439.

Seth, N. and Saxena, K. 2003. Haematological references in fresh water fish *Channa punctatus* due to fenvalerate. *Bull. Environ. Contam Toxicol*., **71**: 1192 -1199.

Sevgiler, Y., Üner, N. and Oruç, E. 2005. Oxidative stress-related and ATPase effects of etoxazole in different tissues of *Oreochromis niloticus. Environmental Toxicology and Pharmacology*, **20**(1): 99-106.

Shah, S.L., 2002. Behavioural abnormalities and *Cyprinion watsoni* on exposure to copper and zinc. *Turk. J. Zool.,* **26**: 137-140.

Shahi, J. and Singh, A. 2011. Effect of bioactive compounds extracted from euphorbious plants on hematological and biochemical parameters of *Channa punctatus*. *Rev. Inst. Med. trop. S. Paulo*., **53**(5): 259-263.

Shakoori, A.R., Mughal, A.L. and Iqbal, M.J. 1996. Effects of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver, and muscles of a freshwater fish, *Ctenopharyngodon idella*. *Bull Environ Contam Toxicol*., **57**(3): 487-94.

Sharaf, S., Khan, A., Khan, M. Z., Aslam, F., Saleemi, M. K. and Mahmood, F. 2010. Clinico-hematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. *Exp. Toxicol. Pathol.,* **62**: 333-341.

Sharbidre, A.A., Metkari, V., Patode, P. 2011. Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, *Poecilia reticulata. Pestic. Biochem. Physiol.,* **101**: 132-141.

Sharma, R.K., Shandilya, S.A. and Sharma, S. 1983. Observations on the effect of Malathion on the mortality of fish *Clarius batrachus* (Linn.). *Comp.Physiol. Ecol*., **8**: 155-156.

Sharma, S. and Krishnamurthy, G. 1998. Bioefficacy of some insecticides against insect pests of tomato. *Pestology,* **22**(12): 34-36.

Shen, H.M. and Liu, Z.G. 2006. JNK signaling pathway is a key modification in cell death mediated by reactive oxygen and nitrogen species. *Free Radic. Biol. Med.,* **40**: 928-939.

Shilov, I.A. 1981. Stress as an ecological phenomenon. *J. Zool.,* **58**(6): 805-812.

Shrimpton, J.M. and McCormick, S.D. 1999. Responsiveness of gill Na^+/K^+-ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. *J. Exp. Biol*., **202**: 987-995.

Shrimpton, J.M. and Randall, D.J. 1994. Downregulation of corticosteroid receptors in gills of coho salmon due to stress and cortisol treatment. *Am. J. Physiol*., **267**: 432-438.

Shwetha, A., Dube, A. and Hosetti, B.B. 2012. Effect Of Exposure To Sublethal Concentrations Of Zinc Cyanide On Tissue Atpase Activity In The Fresh Water Fish, *Cirrhinus mrigala* (Ham). *Arch. Biol. Sci., Belgrade,* **64**(1), 257-263.

Siddiqui, A., Choudhary, M., Goriya, H.V., Bhavsar, S.K. and Thaker, A.M. 2007. Evaluation of immunotoxic effect of short-term administration of quinalphos and imidacloprid in white leghorn cockerels. *Toxicol Int*., **14**: 15-19.

Sies, H., and Stahl, W. 1995. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *The American journal of clinical nutrition,* **62**(6), 1315S-1321S.

Simko, E., Brown, L.L., MacKinnon, A.M., Byrne, P.J., Ostland, V.E. and Ferguson, H.W. 2000. Experimental infection of Atlantic salmon, Salmo salar L., with infectious salmon anaemia virus: a histopathological study. *J. Fish Dis*., **23**: 27-32.

Singh, A.P., Singh, S., Bhartiya, P. and Yadav, K. 2010. Toxic effect of phorate on the serum biochemical parameters of Snake headed fish *Channa punctatus* (Bloch). *Advances in Bioresearch*, **1**(1): 177-181.

Singh, H.H. and Srivastava, A.K. 1982. Effect of formothion on carbohydrate metabolism in Indian catfish (*Heteropneustes fossilis*). *Environ. Res.,* **28**(2): 335-339.

Singh, H.S. and Reddy, T. 1990. Effect of copper sulphate on hematological, blood chemistry and hepatosomatic index of an Indian catfish *Heteropneustes fossillis* and its recovery. *Ecotoxicol. Environ. Safety.*, **20**: 30.

Singh, N.N. and Srivastava, A.K. 1994. Formothion induced haematological changes in the freshwater Indian catfish *Heteropneustes fossilis*. *J. Ecotox. Environ. Monit*., **4**: 137-140.

Singh, P.B. and Canario, A.V.M. 2004. Reproductive endocrine disruption in the freshwater catfish, Heteropneustes fossilis in response to pesticide γ-hexachlorocyclohexane exposure. *Ecotoxicol. Environ. Saf.,* **58**: 77-83.

Sinhaseni P. and Tesprateep T. 1987. Histopathological effects of paraquat and gill function of *Puntius gonionatus,* Bleeker. *Bull Environ Contam Toxicol.,* **37**:308-312.

Sivaperumal, P. 2008. The influence of organophosphorus pesticide-methylparathion on protein, lipid metabolism and detoifing enzymes in rohu (Labeo rohita). Thesis.

Sivaramakrishna, B. and Radhakrishnaiah, K. 1998. Impact of sub lethal concentration of mercury on nitrogen metabolism of the freshwater fish, *Cyprinus carpio* (Linn). *J. Environ. Biol.,* **19**(2): 111-117.

Skelton, 2001. A complete Guide to the freshwater fishes of Southern Africa. Struik Publishsers.

Skelton, P.H., 1993, A complete guide to the freshwater fishes of southern Africa, Southern Book Publishers, Halfway House.

Slooff, W., Van Kreijl, C.F. and Baars, A.J. 1983. Relative liver weights and xenophobic-metabolizing enzymes of fish from polluted surface waters in the Netherlands. *Aquat. Toxicol.,* **4**: 1-14.

Smith, G. and Weis, J. S. 1997. Predator/prey interactions in *Fundulus heteroclitus*: effects of living in a polluted environment. *Journal of Experimental Marine Biology and Ecology.* **209:**75-87.

Soares, A.M.V.M. and Calow, P. 1993. Seeking standardization in ecotoxicology. In: Soares, A.M.V.M., Calow, P. (Eds.), Progress in standardization of aquatic toxicity tests. Chapter 1. Lewis, London.

Soares, W., Almeida, R.M.V.R. and Moro, S. 2003. Rural work and risk factors associated with pesticide use in Minas Gerais, Brazil. *Cad. Saúde Pública.,* **19:** 1117-1127.

Sobha, K., Pornima, A., Harini, P. and Veeraiah, K. 2007. A study on biochemical changes in the fresh water fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant cadmium chloride. *Khatmandu Univ. J. Sci. Eng. Tech*., **1**(I4): 1-11.

Soivio, A., Westman, K. and Nyholm, K. 1974. The influence of changes in oxygen tension on the hematocrit value of blood samples from asphyxic rainbow trout (*Salmo gairdneri*). *Aquaculture,* **3**: 395-401.

Spazier, E., V. Storch, and T. Braunbeck. 1992. Cytopathology of spleen in eelAnguilla anguilla exposed to a chemical spill in the Rhine River. *Diseases of Aquatic Organisms,* **14**: 1-22.

Spitsbergen, J.M. and Kent, M.L. 2003. The state of the art of the zebrafish model for toxicology and toxicologic pathology research-Advantages and current limitations. *Toxicologic Pathology*, **31**: 62-87.

Spragne, J.B. 1975. Measurement of pollution toxicity to fish: In Bioassay methods for acute toxicity. *Water Resour.,* **3**: 346-349.

Sreedevi, P.B., Sivaramakrishna, A., and Radhakrishaiah, S.K. 1992. Effect of nickel on some aspects of protein metabolism in the gill and kidney of the freshwater fish, *Cyprinus carpio (L). Environ. Pollut.,* **77**(1): 355-361.

Sreenivasan, R.S., Krishna Moorthy, P. and Deecaraman, M. 2011. Cypermethrin Induced Toxicity to phosphatases and dehydrogenases in gills and hemolymph of fresh water crab, Spiralothelphusa hydrodroma (Herbst). *International Journal of Biology & Medical Research*, **2**(3): 784-788.

Srinivas, T., Prasad, T.A., Raffi, G.M. and Reddy, D.C. 1991. Effect of atrazine on some aspects of lipid metabolism in fresh water fish. *Biochemistry International*, **23**(3): 603-609.

Srivastava, A.K., Mishra, D., Shrivastava, S., Srivastav, S.K. and Srivastav, A.K. 2010. Acute Toxicity And Behavioural Responses Of Heteropneustes Fossilis To An Organophosphate Insecticide, Dimethoate. *International Journal of Pharma and Bio Sciences.* **1**(4): B359 - B363.

Srivastava, S., Prabhakar, P. and Srivastava, B.C. 2007. Toxicity and behaviour of the fish *Labeo rohita* and *Channa punctatus* exposed to pulp paper mill effluent. *J. Ecotoxicol. Environ. Monit.* **17**(3): 241-244.

Stalin, I.S. and Das, S.S.M. 2012. Biochemical changes in certain tissues of *Cirrhina mrigala* (Hamilton) (Cyprinidae: Cyprniformes) exposed to fenthion. *International Journal Of Environmental Sciences*, **2**(3): 1268- 1277.

Stark, J.D., Jepson, P.C. and Mayer, D.F. 1995. Limitation to use of topical toxicity data for prediction of pesticide side effect in the field. *J. Econ. Entomol.,* **88**: 1081-1088.

Stein, J.E., Collier, T.K., Reichert, W.L., Casillas, E., Hom, T. and Varanasi, U. 1992. Bioindicators of contaminant exposure and sublethal effects: studies with benthic fish in Puget Sound. Washington. *Environ. Toxicol. Chem.,* **11**: 701-714.

Steinhausen, M.F., Steffensen, J.F. and Andersen, N.G. 2005. Tail beat frequency as a predictor of swimming speed and oxygen consumption of saithe (*Pollachius virens*) and whiting (*Merlangius merlangus*) during forced swimming. *Marine Biology,* **148**(1):1 97-204.

Stentiford, G.D., Longshaw, M., Lyons, B.P., Jones, G., Gren, M. and Feist, S.W. 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. *Mar. Environ. Res*., **55**(2): 137-159.

Stocco, D. 2000. Intramitochondrial cholesterol transfer. *Biochemica et Biophysica Acta,* **1486**: 184-187.

Stoughton, S.J., Liber, K., Culp, J. and Cessna, A. 2008. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse-exposure conditions, *Arch. Environ. Contam. Toxicol*., **54**: 662-673.

Stoyanova, S., Georgieva, E., Velcheva, I., Yancheva, Y. and Atanasova, P. 2012. Effects of the insecticide "Actara 25 WG" on the glyconeogenesis in the liver of common carp (*Cyprinus carpio* L.*). J. BioSci. Biotech*., **1**(3): 249-254.

Subburaju S, Selvarajan V R, 1988: Effect of acute toxic concentration of chlorpyrifos on the acetylcholinesterase activity in different regions of the brain of edible fish *Tilapia mossambica (Peters). Indian J. Comp. Anim. Physiol. 6,86-89 In: Mace G Baron and Kent B Woodburnt, 1995: Rev. Environ. Contam. Toxicol.,* **144**: 2-76.

Suchail, S., Guez, D. and Belzunces, L.P. 2000. Characteristics of imidacloprid toxicity in two Apis mellifera subspecies. *Environ. Toxicol. Chem*., **19**: 1901-1905.

Summarwar, S. and Verma, S. 2012. Study of biomarkers of physiological defense against reactive oxygen species during environmental stress. *Int. J. LifeSc. Bt & Pharm. Res*., **1**(3): 198-205.

Sun, Y., Yu, H., Zhang, J., Yin, Y., Shi, H. and Wang, X. 2006. Bioaccumulation depuration and

oxidative stress in fish *Carassius auratus* under under phenanthrene exposure. *Chemosphere,* **63**(8): 1319-1327.

Sunderam, R.I.M., Cheng, D.M.H. and Thompson, G.B. 1992. Toxicity of endosulfan to native and introduced fish in Australia. *Environ Toxicol Chem*., **11**: 1469-1476.

Suresh, N. 2009. Effect of cadmium chloride on liver, spleen and kidney melano macrophage centres in *Tilapia mossambica*. *Journal of Environmental Biology,* **30**(4): 505-508.

Suresh, N. and Veeraraghavan, K. 1998. Effects of sublethal concentration of distillery effluent on the occurrence of kidney melano macrophage centres in *Mystus vittatus* (Bloch). *J. Natcon*., **10**: 203-207.

Susan, T. A., Sobha, K., Veeraiah, K. and Tilak, K.S. 2010. Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade. *Journal of Toxicology and Environmental Health Sciences,* 2(5): 53-62,

Susithra, N., Jothivel, N., Jayakumar, P., Paul, V. I., Sen, M., Dastidar, M. G. and Ranade, P. 2007. Toxicopathological impact of cadmium chloride on the accessory respiratory organ of the air-breathing catfish *Heteropneustes fossilis*. *Iranian Journal of Environmental Health Science & Engineering,* **4**(1): 1-8.

Suvetha, L., Ramesh, M. and Saravanan, M. 2010. Influence of cypermethrin toxicity on ionic regulation and gill Na+/K+-ATPase activity of a freshwater teleost fish *Cyprinus carpio. Environ. Toxicol. Pharmacol.,* **29**: 44-49.

Suzuki, Y. and Iida, T. 1992. Fish granulocytes in the process of inflammation. *Ann. Rev. Fish Dis.*, **2**: 149-160.

Svensson, B., Nilsson, A., Jonsson, E., Schutz, A., Akesson B. and L. Hagmar, 1995. Fish consumption and exposure to persistent organochlorine compounds, mercury, selenium and methylamines among Swedish fishermen. *Scandinavian J. Work Environ. Health,* **21**: 96-105.

Svoboda, M., Luskova, V. and Drastichova, Z.U. 2001. The effect of diazinon on haematological indices of common carp *(Cyprinus carpio L*). *Acta vet. Brno*., **70**: 457-465.

Svobodová, Z., Kolářová, J. and Vykusová, B. 1997. Stress at fish generated by conventional breeding technologies. In Animal Protection and Welfare, *VFU Brno.*, 60-64.

Svobodová, Z., Lusková, V., Drastichová, J., Svoboda, M. and Źlábek, V. 2003. Effect of deltamethrin on haematological indices of common carp (*Cyprinus carpio L.). Acta Vet. Brno*., **72**: 79-85.

Svobodova, Z., Vykusova, B. and Machova, J. 1994. The effects of pollutants on selected haematological and biochemical parameters in fish. In: Sublethal and chronic effects of pollutants on freshwater fish (Eds.: R. Müller and R. Lloyd). Fishing New Books, London.

Swallow, R.L. and Fleming, W.R. 1969. The effect of starvation, feeding, glucose, and ACTH on the liver glycogen levels of *Tilapia mossambica*. *Comp. Biochem. Physiol.,* **28**: 95-106.

Swami, K.S., Jagannadhan Rao, K.S., Sathyavelu Reddy,K., Srinivasmurthy, K., Lingamurthy, G., Chetty, C.S. and Indira, K. 1983. The possible metabolic diversion adapted by the fresh water muscle to counter the toxic metabolic effect of selected pesticides. Ind. *J. Anim. Physiol.*, **1**: 95-106.

Swapna, I., Rajasekhar, M., Supriya, A., Raghuveer, K., Sreenivasulu, G., Rasheeda, M.K., Majumdar, K.C., Kagawa, H., Tanaka, H., Dutta-Gupta, A. and Senthilkumaran, B. 2006. Thiourea-induced thyroid hormone depletion impairs testicular recrudescence in the air-breathing catfish, *Clarias gariepinus*. *Comp. Biochem. Physiol. AMol. Integr. Physiol*., **144**(1): 1-10.

Sweilum, M.A. 2006. Effect of sublethal toxicity of some pesticides on growth parameters, haematological properties and total production of Nile tilapia, *Oreochromis niloticus L*. and water quality of ponds. *Aquacul. Res*., **37**(11): 1079 - 1089.

Szent Gyorgyi, A. 1958. Bioenergetics. Academic Press, New York.

Talas, Z.S. and Gulhan, M.F. 2009. Effects of various propolis concentrationson biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss). Ecotoxicol. Environ. Safe.,* **72**: 1994-1998.

Talebi, K. 1998. Diazinon Residues in the Basins of Anzali Lagoon, Iran. *Bulletin Environmental Contamination Toxicology*, **61**: 477-483.

Tan, X., Hou, S. and Wang, M. 2007. Enantioselective and diastereoselective separation of synthetic pyrethroid insecticides on a novel chiral stationary phase by highperformance liquid chromatography. *Chirality*, **19**: 574-580.

Tayyaba, K., Hasan, M., Islam, F. and Khan, N.H. 1981. Oranophosphate pesticides metasystax induced regional alterations in brain nucleic acid metabolism. *Ind.J.Exp.Biol*., **19**(7): 688-690.

TDC Environmental, 2003. Insecticide market trends and potential water quality implications.

Teh, S.J., Adams S.M. and Hinton, D.E. 1997. Histopathological biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. *Aquatic Toxicology,* **37**: 51-70.

Teles, M., Pacheco, M., and Santos, M.A., 2007. Endocrine and metabolic responses of *Anguilla anguilla* L. caged in a freshwater-wetland (Pateira de Fermentelos--Portugal). *The Science of the total environment,* **372**(2-3): 562-570.

Teraoka, H., Dong, W., Tsujimoto, Y., Iwasa, H., Endoh, D., Ueno, N., Stegeman, J.J., Peterson, R.E. and Hiraga, T. 2003. Induction of cytochrome P450 1A is required for circulation failure and edema by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in zebrafish. *Biochem Biophys Res Commun.,* **304:** 223-228.

Teunissen, 2003. 52 weeks oral dietary toxicity study with cymoxanil technical in male and female Beagle dogs Reference:; Report No. NOTOX Project 338355, Guideline: OECD 452 (1981)

Thangavel, P., Sumathiral, K., Karthikeyan, S., Ramaswamy, M. 2005. Endocrine response of the freshwater teleost, *Sarotherodon mossambicus* (Peters) to dimecron exposure. *Chemosphere*, **61**(8): 1083-1092.

Thirugnanam, M. and Forgash, A.T. 1977. Environmental impact of mosquito pesticide toxicity and anticholinesterase activity of choryopyrifos to fish in a salt marsh habitat. *Archives Environmental Contamination and Toxcology*, **5**: 415 - 425.

Thompson and Schuster 1968. Effects of certain metallic salts upon fishes. Tras. Am. Fish. Soc., 44: 20-124.

Thompson, D.G., Wojtaszek, B.F., Staznik, B., Chartrand, D.T. and Stephenson G.R. 2004. Chemical and biomonitoring to assess potential acute effects of Vision herbicide on native amphibian larvae in forest wetlands. *Environ. Toxicol. Chem*., **23**: 843-849.

Thophon, S., Kruatrachue, M., Upatham, E.S., Pokethitiyook, P., Sahaphong, S., and Jaritkhuan, S. 2003. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. *Environmental Pollution,* **121**(3): 307-320.

Tilak, K.S., K. Veeraiah and Yacobu, K. 2001. Studies on histopathological changes in the gill, liver and kidney of *Ctenopharyngodon idellus* (Valenciennes) exposed to technical fenvalerate and EC 20%. *Pollut. Res.,* **20**: 387-393

Tilak, K.S., Rangarao, D.M., Pnyamyuda, Devi, A.A. and Murthy, A.S. 1980. Toxicity of carbaryl and 1-naphthal to the fresh water fish *Labeo rohita. Indian J. Expt. Biol*., **188**: 75-76.

Tilman, D. 2001. Diversity and productivity in a long-term grassland experiment. *Science*, **294**: 843-845.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S. 2002. Agricultural sustainability and intensive production practices. *Nature*, **418**: 671-677.

Tiwari, S. and Singh, A. 2004. Effect of Oleandrin on a freshwater air breathing murrel, *Channa punctatus (Bloch.). Indian J Exp Biol*., **42**(4):413-8.

Tkatcheva V., H. Hyvärinen, J. Kukkonen, L. Ryzhkov and I. Holopainen. 2004. Toxic effects of mining effluents on fish gills in a subarctic lake system in NW Russia. - Ecotoxicology and Environmental Safety, 57(3): 278-289.

Tokieda, M., Ozawa, M., Kobayashi, S. and Gomyo, T. 1997. Method to determination of total residues of the insecticide acetamiprid and its metabolites in crops by gas chromatography. *J Pestic Sci.,* **22**: 77-83.

Tomizawa M. and Casida J.E. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu. Rev. Pharmacol. Toxicol*., **45**: 247-268.

Tomizawa, M. and Casida, J.E. 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology*, **48**: 339-364.

Tomizawa, M., Ostuka, H., Miyamoto, T., Eldefrawi, M.E. and Yamamoto, I. 1995. Permacological characteristics of insect nicotinic ion channel and the comparison of the effect of nicotinoids and neonicotinoids. *J. Pestic. Sci.,* **20**: 57-64.

Tomlin C.D.S. 2002. *The e-Pesticide manual*. 12th ed. British Crop Protection Council. version 2.1.

Tomlin, C.D.S. 1997. The Pesticide Manual. The British Crop Protection Council, UK.

Tomlin, C.D.S. 2000. A World Compendium, 12th ed., British Crop Protection Council, *The Pesticide Manual,*

Tomlin, E.D.S. [ed.] 2003. The pesticide manual. British Crop Protection Council, Hampshire, United Kingdom.

Tompkins E.C., 1994. Chronic toxicity study with DPX-T3217-113 (cymoxanil) one year feeding study in dogs. Report HLO 65-94. WIL Research Laboratories, Inc. (USA). Unpublished.

Toor, H.S. and Kaur. 1974. Toxicity of pesticides to the fish *Cyprino carrpio communis*. *Ind. J. Exp. Biol*. **12**: 334-336.

Tort, L. 2002. Cortisol and hematological response in sea bream and trout subjected to the anesthetic clove oil and 2-phenoxyethanol. *Aquac. Res*., New York, **33**: 907- 910.

Travares, D.M., Martins, M.L. and Nascimento, K.S. 1999. Evaluation of haematological parameters in praractus mesopotamicos. Holmberg (*Osteichthyes, characidae*) with Argulus sp. (*Crustacea, branchiura*) in festation and treatment with organophosphate. *Rev. Bras.,* **16**: 553-555.

Tripathi, P.K. and Singh A. 2003. Toxic effects of dimethoate and carbaryl pesticides on reproduction and related enzymes of the fresh water Snail Lymnaea acuminate. *J. Bull. Environ. Contam. Toxicol.*, **71**: 0535-0542.

Tripathi, S.N., Chandola, H.M., Singh, B.C. and Chadha, A.N. 1990. Study on hypoglycemic fraction of *C. tamala* and *T. belerica*. *Ayurveda.* **11**: 1-12.

Tsangaris, C., Papathanasiou, E., and Cotou, E. 2007. Assessment of the impact of heavy metal pollution from a ferro-nickel smelting plant using biomarkers. *Ecotoxicology and Environmental safety,* **2**(66): 232-243.

Tsujii, T. and Seno, S. 1990. Melano-macrophage centres in the aglomerular kidney of the sea horse (teleosts): morphologic studies on its formation and possible function. *Anatomical Record*, **206**: 460-470.

Turkar, K. S., Gupta, R. S., Banerjee, K. and Wanjari, R. R. 1998. Relative efficacy of new chemicals for the control of *Helicoverpa armigera* (Hub.) on chickpea. *Pestology* **22**(9):36-38.

Ujagwung, G.U., Koffi, M.B. and Simeon, E.O. 2010. Acute Toxicity and Behavioural Responses of African Catfish, Clarias gariepinus to Amine Salt of 2,4-D. Chin J Appl Environ Biol., **16** (3): 347-352.

Ulland, B.M., Weisburger, J.H., Weisburger, E.K., Rice, J.M. and Cypher, R. 1972. Thyroid cancer in rats from ethylene thiourea intake. *J. Nat Cancer Inst*., **49**: 583-584.

Üner, N. Oruç, E.Ö. Sevgiler, Y. Şahin, N. Durmaz, H. and Usta, D. 2006. Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. *Environmental Toxicology and Pharmacology,* **21**: 241-245.

United States Environmental Protection Agency (2002) Reregistration eligibility decision for endosulfan. EPA 738-R-02-013, United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances, Washington, DC.

United States Environmental Protection Agency (2007) Appendix 1 to 2007 addendum: environmental fate and ecological risk assessment of endosulfan. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, DC

United States Environmental Protection Agency (USEPA) (2005). Aquatic Toxicity Information Retrieve AQUIRE aquatic toxicology database.

Urbiati, E.C., de Abreu, J.S., da Silva, C.A.C. and Landinez P.M.A. 2004. Loading and transport stress of juvenile matrinxa (brycon cephalus, characidae) at various densities. *Aquaculture*, **229**: 389-400.

US EPA, 2004. Office of prevention, pesticides and toxic substances. EPA 738-R-04-006, Diazinon, interim reregistration eligibility decision.

Val, A.L., Silva, M.N.P. and Almeida-Val, V.M.F. 1998. Hypoxia adaptation in fish of the Amazon: a never ending task. *S Afri J Zool*., **33**(2): 107-114.

Valicre, E.J. and Stickney, C.J. 1972. Hypoxia, Univ of Chicago Press, Chicago, London In: Narendara N Singh and Anil K Srivastava, 1994: Formothion induced haematological changes in the freshwater Indian catfish Heteropneustes fossilis, J. Ecotoxicol. Environ. Monit., 4(2): 137-140.

Van der Oost, R., Beyer, J. and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment review. *Environ. Toxicol. Phar*., **13**: 57-149

Van Dyk, J.C. 2003. Fish histopathology as a monitoring tool for aquatic health: a preliminary investigation. M.Sc. thesis, Rand Afrikaans University, Johannesburg.

Varadarajan, R., 2010. Biochemical effects of different Phenolic compounds on Oreochromis Mossambicus Peters. Thesis submitted to the Cochin University of Science and Technology, India.

Vasait, J.D. and Patil, V.T. 2005. The toxic evaluation of organophosphorus insecticide monocrotophos on the edible fish species *Nemacheilus botia. Ecology Environment and Conservation,* **8**(1): 95-98.

Vasanbhi, R., Baskaran, P., Palanchyiny, S. and Aruna Chalam, S. 1989. Impact of carbofuran on feeding evergetice in some fresh water fishes. *Environmental Econology*, **8**: 40-45.

Veiga, M.L.D., Rodrigues, E.D.L., Pacheco, F.J. and Ranzani-Paiva, M.J.T. 2002. Histopathologic changes in the kidney tissue of *Prochilodus lineatus* Valenciennes, 1836 (Characiformes, Prochilodontidae) induced by sublethal concentration of trichlorfon exposure. *Brazilian Archives of Biology and Technology,* **45**(2): 171-175.

Velcheva, I G., Arnaudov, A.D. and Georgieva, E.S. 2010. Influence of Zinc on Gill Morphology of Gibelio Carp (Carassius gibelio). *Ecologia balkanica,*2: 19-23.

Velisek, J., Dobsikova, R., Svobodova, Z., Modra, H. and Luskova, V. 2006b. Effect of deltamethrin on the biochemical profile of common carp (*Cyprinus carpio* L.). *Bulletin of Environmental Contamination and Toxicology*, **76**(6): 992-998.

Velisek, J., Jurcikova, J., Dobsikova, R., *et al.* 2007. Effects of deltamethrin on rainbow trout (*Oncorhynchus mykiss*. *Environ. Toxicol. Pharmacol.,* **23**: 297-301

Velisek, J., Stastna, K., Sudova, E., Turek, J. and Svobodova, Z. 2009a. Effects of subchronic simazine exposure on some biometric, biochemical, hematological and histopatological parameters of the common carp (*Cyprinus carpio* L.). *Neuroendocrinology Letters*, **30**(1): 236-241.

Velisek, J., Svobodova, Z. and Piackova, V. 2009b. Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). *Veterinarni Medicina*, **54**(3): 131-137

Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z., Dobsikova, R., Novotny, L. and Dudzik, M. 2006a. Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). *Veterinarni Medicina*, **51**(10): 469-476.

Velma, V. and Tchounwou, P.B. 2011. Hexavalent chromium-induced multiple biomarker responses in liver and kidney of goldfish, *Carassius auratus*. *Environmental Toxicology,* **26**(6): 649-656.

Velmurugan, B., Selvanayagam, M., Cengiz, E. and Uysal, E. 2008. Levels of transaminases, alkaline phosphatase, and protein in tissues of *Clarias gariepienus* fingerlings exposed to sub-lethal concentrations of cadmium chloride. *Environ. Toxicol*., **23**: 672-678.

Velmurugan, B., Selvanayagam, M., Cengiz, E.I., and Unlu, E. 2007. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. *Journal of Environmental Science and Health Part B,* **42**(2), 157-163.

Venkata Rathnamma, V., Vijaya Kumar, M. and Philip, G.H. 2007**.** Effect of Deltamethrin on glycogen phosphorylase and glucose-6-phosphatase activity in freshwater fish *Labeo rohita*. *Bulletin of Pure & Applied Sciences-Zoology,* **26A**(2): 59-66.

Venkata Rathnamma,V., Vijayakumar, M. and Philip, G.H. 2008. Acute toxicity and behavioral changes in freshwater fish *Labeo rohita* exposed to Deltamethrin*. J.Aqua Biol*., **23**(2): 165-170.

Venkataramana, G.V., Sandhya Rani, P.N. and Murthy, P.S. 2006. Impact of Malathion on the biochemical parameters of gobiid fish, *Glossogobius giuris* (Ham). *J. Environ. Biol*., **27**: 119-122.

Venkatesan, S., Pugazhendy, K., Meenambal, M., Sangeetha, D., Vasantharaja, C., Jayachandren, K. and Prabakaran, S. 2012. Protective Role of Spirulina on the Variation of Haematological Parameter Induced by Herbicide Atrazine in the Fresh water Fish

Cyprinus carpio (Linn). *International Journal of Pharmaceutical & Biological Archives*, **3**(1): 249-254.

Venugopala, 1999. Subchronic (90 day) oral toxicity study with cymoxanil technical in Beagle dogs Reference: Report No. 2145/96 Guideline: OECD 409 (1981)

Verma, S.R., Bansal, S.K., Gupta, A.K., Pal, N., Tyagi, A.K., Bhatnagar, M.C., *et al.,* 1982. Bioassay trials with twenty three pesticides to a fresh water teleost, *Saccobranchus fossilis. Water Research,* **16**(5): 525-529.

Verma, S.R., Rani, S., Bansal, S.K. and Delala, R.C. 1980. Effect of pesticide thiotox, dichlorovous and chlorofurun on the fish *Mystus vittatus*. *Water, Air and Soil Pollution*, **13**(2): 229-234.

Vijayan, M.M., and Leatherland, J.F. 1988. Effect of stocking density on the growth and stress-response in brook charr, *Salvelinus fontinalis*. *Aquaculture*, **75**(1): 159-170.

Vijayan, M.M., Pereira, C., Grau, E.G. and Iwama, G.K. 1997. Metabolic responses associated with confi nement stress in tilapia: the role of cortisol. *Comp Biochem Physiol Pharmacol Toxicol Endocrinol*., **116**: 89-95.

Vijuen, A. and Steyn, G.J., 2003. Zinc effects on the embryos and larvae of the sharp tooth catfish, *Clarias gaiepinus. J. Burchell,* **10**: 22.

Vinodhini, R. and Narayanan, M. 2009. Biochemical changes of antioxidant enzymes in common carp (*Cyprinus carpio* L.) after heavy metal exposure. *Turk. J. Vet. Anim. Sci.,* **33**: 1-6.

Viran, R., Erkoc, F.U., Polat, H. and Kocak, O. 2003. Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata). Ecotoxicology and Environmental Safety*. **55**: 82-85.

Visoottiviseth P, Thamamaruitkun T, Sahaphogan S, Riengrojpitak S, Kruatrachue M. 1999. Histopathological effects of triphenyltin hydroxide on liver, kidney and gill of Nile tilapia *(Oreochromis niloticus). Appl Organomet Chem.,* **13**:749-763.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambre, C., Tarazona, J.V., Brandt, I. and Vethaaak, D.A. 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Critical Reviews in Toxicology*, **30**: 71-133.

Vosyliene, M.Z. and Kazlauskiene, N. 1999. Alterations in fish health status parameters after exposure to different stressors. *Acta Zool. Lithuanica Hydrobiol.,* **9**(2): 82-95.

Vosylienë, M.Z. and Sveceviėius, G. 1997. Sublethal effects on rainbow trout of chronic exposures to mixtures of heavy metals. *Fish physiology, toxicology and water quality*, 141-150.

Wahbi, O.M. 1992. Effect of Abu Qir Fertilizer Industrial effluent on the physiological characteristics of different stages of *Sparus auratus*. M.Sc. Thesis, Faculty of Science, Alexandria University.

Walker, C.H. and Livingstone, D.R. (Editors) (1992). Persistent Pollutants in Marine Ecosystems. Pergamon Press, Oxford.: 272

Walsh, L., McCormick, C., Martin, C. and Stocco, D. 2000. Roundup inhibits steroidogenesis by disrupting steroidogenic actue regulatory (StAR) protein expression. *Environmental and Health Perspectives* **108**: 769-776.

Wamhoff, H. and Schneider, V. 1999. Photodegradation of imidacloprid. *J. Agric. Food Chem*., **47**(4): 1730-1734.

Wan, M.T., Kuo, J., Buday, C., Schroeder, G., Van Aggelen, G. and Pasternak, J. 2005. Toxicity of α-, β-, (α+β)- endosulfan and their formulated and degradation products to *Daphnia magna, Hyalella azteca, Oncorhynchus mykiss, Oncorhynchuc kisutch*, and biological implications in streams. *Environ Toxicol Chem*., **24**: 1146-1154.

Wani, G.P. and Latey, A.N. 1984. Effect of thiourea treatment on cholesterol contents of the liver and the gonads of a freshwater teleost, *Gara mulya. Geobios,* **11**: 45-47.

Warren, C. E. 1971. Biology and water pollution control. W. B.Saunders Co., Philadelphia.

Weber, R.E. 1982. Interspecific adaptation of haemoglobin function in fish to oxygen availability. In: Exogenous and Endogenous Influences on Metabolic and Neural Control. Eds. A.D.F. Addink and N. Spronk, Pergamon Press, Oxford, UK. : 87 - 101.

Wedemeyer, G, and Mcleay, A. 1984. Assessing the Tolerance of Fish and Fish population to environmental stress. The problems and methods of monitoring in: Contaminate Effects on Fisheries on Fisheries Cairnus WV, Hodson PV and Nriagu JO (Eds). John Wiley and Son Inc New York, 164-195.

Wedemeyer, G. A. and McLeay, D. J. 1981. Methods for determining the tolerance of fishes to environmental stressors. In A. D. Pickering (ed.), Stress and fish, pp. 247-275. Academic Press, New York.

Wendelaar-Bonga, S.E. 1997. The stress response in fish. *Physiol. Rev.,* **77**: 591-625.

Wengu, X., Yuangou, I., Qingyang, W., Shuqi, W., Huaiping, Z. and Wenhua, L. 2009. Effect of phenanthrene on hepatic enzymatic activities in tilapia *Oreochromis niloticus*. *J. Environ. Science,* **21**: 854-857.

White, A., and Fletcher, T.C. 1985. Seasonal changes in serum glucose and condition of the plaice, *Pleuronectes platessa* L. *Journal of fish biology,* **26**(6): 755-764.

Whittle, D.M. and Flood, K.W. 1977. Assessment of the acute toxicity, growth impairment, and flesh taining potential of a bleached Kraft mill effluent on rainbow trout *Salvo gairdneri. J. Fish Res. Board Canada,* **34**: 869-878.

Wilhelm-Filho, D., Torres, M.A., Tribbes, T.B., Pedrosa, R.C. and Soares, C.H.L. 2001. Influence of season and pollution on the antioxidant defenses of the cichlid fish acará (*Geophagus brasiliensis*). *Braz. J. Med. Biol. Res.,* **34**: 719-72.

Wilhelm-Filho, D., Torres, M.A., Zaniboni-Filho, E. and Pedrosa, R.C. 2005. Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, *Leporinus elongates* Valenciennes, 1847. *Aquaculture,* **244:**349-357.

Winn, R.N. 2001.Transgenic fish as models in environmental toxicology. *ILAR J.,* **42**: 322-329.

Winston, G.W. and Di Giulio, R.T., 1991. Proxidant and antioxidant mechanisms in aquatic organisms. *Aquat. Toxicol.,* **19**: 137-161.

Wintrobe, M.M. 1978. Clinical Haematology. H. Kimpton, London, UK.

Witold, J., Marek, S., Robert, K., Józefa, C., Kinal, S., Korczynski, M. and Bartkowiak, A. 2007. Effect of Fat-Mineral Preparation From Fish Oil on Fatty Acid Content on Cow Milk. *Am. J. Agril. Biol. Sci.*, **2**(4): 276-283.

Wolke, R.E. 1992. Piscine macrophage aggregates: a review. *Annual Review of Fish Diseases,* **2**: 91-108.

Wolke, R.E., Murchelano, R.A., Dickstein, C.D. and George, C.J. 1985. Preliminary evaluation of the use of macrophage aggregates (MA) as fish health monitors. *Bulletin of Environmental Contamination and toxicology,* **35**(1): 222-227.

Wood, C.M., Hogstrand, C., Galvez, F. and Munger, R.S. 1996. The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss)*1. The effects of ionic Ag. *Aquatic Toxicology*, **35**(2), 93-109.

Wu, J.P. and Chen, H.C. 2004. Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation of white shrimp (*Litopenaeus vannamei*). *Chemosphere.* **5:**1591-1598.

Yadav, A., Gopesh, A., Pandey, R.S., Rai, D.K. and Sharma, B. 2007. Fertilizer industry effluent induced biochemical changes in fresh water teleost, *Channa striatus* (Bloch) *Bull. Environ. Contam. Toxicol.,* **79**: 588-595.

Yadav, A., Neraliya, S. and Gopesh, A. 2007. Acute toxicity levels and ethological responses of *Channa striatus* to fertilizer industrial waste water. *J. Environ Biol.* **28**(2):159-62. (ch. 1)

Yamamoto, I. and Casida, J.E. (Eds.). 1999. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. *Springer*, Tokyo.

Yamamoto, I., Casida, J.E. (Eds.), 1999. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. Springer,Tokyo. Suchail,S., Guez,D.,Belzunces, L.P.,2000. Characteristics of imidacloprid toxicity in two Apis mellifera subspecies. Environ. Toxicol. Chem. 19:1901-1905.

Yamamoto, K. 1988. Contraction of spleen in exercised freshwater teleost. *Comp. Biochem. Physiol.*, **89**: 65-6.

Yamamoto, K. and Itazawa, Y.K.H. 1983. Erythrocyte supply from the spleen and hemoconcentrationin hypoxic yellowtail. *Mar. Biol*., **73**: 221-226.

Yamamoto, K. and Itazawa, Y.K.H. 1985. Direct observation of fish spleen by an abdominal window method and its application to exercised and hypoxic yellowtail. *JPN. J. Ichthyol.,* **31**: 427-433.

Yanaguchi. F. and Brenner, S. 1997.Molecular cloning of 5-hydroxytryptamine (5-HT) type 1 receptor genes from the Japanese puffer fish, Fugu rubripes. *Gene* **191**: 219-223.

Yang J.L., Chen H.-C., 2003 Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere **53**:877-882.

Yang, C.F., Lui, J., Shen, H.M. and Ong, C.N. 2000. Dual role of glutathione in selenite-induced oxidative stress and apoptosis in human hepatoma cells, Free Radic. Biol. Med., **28**: 1115-1124.

Yeragi, S.G., Rana, A.M. and Koli, V.A. 2003. Effect of pesticides on protein metabolism of mud skipper *Boleophthalmus dussumieri*. *J. Ecotoxicol. Environ. Monit.,* **13**(3): 211-214.

Yildirim, M.Z., Benli, K.C., Selvi, M., Ozkul, A., Erko, F. and Kocak, O. 2006. Acute toxicity behavioural changes and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile Tilapia (*Oreochromis niloticus*) fingerlings. *Environmental Toxicology,* **21**: 614-620.

Yonar, E., Yonar, M., Ural, S.M., Silici, M.Ş. and Düşükcan, S.M. 2012. Protective role of propolis in chlorpyrifos-induced changes in the haematological parameters and the oxidative/antioxidative status of *Cyprinus carpio carpio*. *Food and Chemical Toxicology*, **50**(8): 2703-2708.

You, J., Schuler, L.J. and Lydy, M.J. 2004. Acute toxicity of sediment-sorbedendrin, methoxychlo and endosulfan to Hyalella azteca and Chironomus tentans. *Bull Environ Contam Toxicol*., **73**: 457-464

Yousuf, M.H.A. and El-shahawi, A. 1999.Trace metals in *Lethrinuslentjan* fish from Arabian Gulf: Metal accumulation inkidney and heart tissues. *Bull. Environ. Contam. Toxicol.,***62**: 293-300.

Yuill, T. and Miller, M. 2008. Dose-response relationship" in Encyclopedia of Earth. (eds: C.J. Cleve- land,) Environmental Information Coalition, National Council for Science and the Environment: Washington, D.C.

Zaidi, N. and Soltani, N. 2010. Chronic toxicity of flucycloxuron in the mosquitofish, *Gambusia affinis*: acetylcholinesterase and catalase activities and pattern of recovery. *Annals of Biological Research,* **1**(4) : 210-217.

Zaki, M.S., Fawzi, O.M., Mostafa, S.O. and Taha, N. 2010. Pathological and Biochemical Studies in Tilapia Zilli Infected with *Saprolegnia parasitica* and Treated with Potassium Permanganate. *J. American Science*, **6**(9): 395-398.

Zapata, A.G. and Cooper, E.L. 1990. Cells of the immune system. (eds: A.G. Zapata and Cooper, G.L.) The immune system: comparative histophysiology, Wiley, New York, 15-30

Zhang, J., Shen, H., Wang, X., Wu, J. and Xue, Y. 2004. Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish Carassius auratus. *Chemosphere,* **55**: 167-174

Zhang, W., Jiang, F. and Ou, J. 2011. Global pesticide consumption and pollution: with China as a focus. *Proceedings of the International Academy of Ecology and Environmental Sciences,* **1**(2): 125-144.

Zhang, Z.Y., Yu, X.Y., Wang, D.L., Yan, H.J. and Liu, X.J. 2010. Acute toxicity to zebrafish of two organophosphates and four pyrethroids and their binary mixtures. *Pest Management Science,* **66**(1): 84-89.

Zheng, W. and Liu, W. 1999. Kinetics and mechanism of the hydrolysis of imidacloprid. *Pesticide Science,* 55(4): 482-485.

ISSN 2229-5518

Impact of Curzate (fungicide) on Hematological Parameters of *Oreochromis mossambicus*

Bhavika Desai and Pragna Parikh

Abstract: Curzate, a fungicide, is currently registered for commercial use in over 50 countries on more than 15 crops, creates serious threat to the environment as well as target and non-target organisms like aquatic and land dwelling animals. The present investigation was carried out to study the impact of the fungicide on the hematological parameters of fresh water fish Oreochromis mossambicus. Adult fish of nearly similar weight (25 ± 1.9 g) and length (15.5 ± 1.2cm) were exposed to two sub lethal concentration i.e. 4.9 mg/l and 2.45 mg/l of Curzate for a period of 21 days. The hematological analysis showed significant reduction in red blood cells (RBCs) count, hemoglobin (Hb) value, packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC), while total white blood cells (WBCs) count, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly increased in the treated groups as compared the control group. The present study shows that Curzate causes alterations in hematological parameters leading to physiological dysfunctions thus validating the toxic effect of the fungicide on the fish.

Key words: fungicides, haematology, Blood indices and *Oreochromis mossambicus*

---

1 INTRODUCTION

Agricultural pesticides are indispensable in contemporary agriculture. They are beneficial by providing reliable, persistent and relatively complete control against harmful pests with less cost and effort [1]. Due to injudicious and indiscriminate use of these agrochemicals such as fertilizers, pesticides, insecticides and fungicides to boost crop production with the sole aim of getting more yield, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted. Normally these pesticides reach the aquatic environment through surface run off, sediment transport from treated soil and direct application as spray to water bodies to control the inhabiting pests [2]. [1]

These chemicals may be directly toxic, deteriorate the water quality by changing its physico-chemical nature and cause ecological imbalance leading to health hazards to different types of aquatic organisms in general and fishes in particular [3]. In extreme cases there are records of catastrophic mortality of the entire aquatic biota [2].

The use of agrochemicals in the field has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystem. Ayoola (2008) has reported that water pollution by pesticides is a serious problem to all aquatic fauna and flora and to a considerable extent even man. These agrochemicals adversely affect the non-target organisms, especially fish which are one of the most widely distributed organisms in an aquatic environment and being susceptible to environmental contamination may reflect the extent of the biological effects of environmental pollution in waters [5].

Blood analysis is crucial in many fields of ichthyological research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation [6]. Haematological indices are very important parameters for the evaluation of fish physiological status. The changes depend on fish species, age, the cycle of the sexual maturity of spawners, and diseases [7; 8 and 9]. In warm-blooded animals, changes in the blood parameters, which occur because of injuries or infections of some tissues or organs, can be used to determine and confirm the dysfunction or injuries of the latter i.e. organs or tissues. However in fish, these parameters are more related to the response of the whole organism, i.e. to the effect on fish survival, reproduction and growth.

A vast amount of scientific information is available on the pesticide toxicity on fishes but limited information is available on the effect of these pesticides, in minute concentration, on the physiology of haemopoietic system,

[1] *Author, Bhavika Desai, a research scholar, Department of Zoology, The M.S.University of Baroda, Vadodara-390002. Ph: 09979366015*
E-mail: bhavu1611@yahoo.co.in

2 Co- Author, Pragna Parikh, Associate professor, Department of Zoology, The M.S.University of Baroda, Vadodara-390002. Ph: 09825329148
E-mail: php59@yahoo.co.in

thought to be most sensitive indicator towards environmental pollutants. Therefore, the present study was undertaken to assess and contribute to knowledge on the haematological changes in fresh water fish, Oreochromis mossambicus at different concentration of Curzate.

2 MATERIALS AND METHODS

2.1 Experimental design:

Fresh water fish Oreochromis mosssambicus (15 ± 2.6 cm and 24 ± 3 g) were obtained from a local pond of Baroda district and were acclimatized under laboratory condition. They were kept in glass aquaria containing 50 L of dechlorinated tap water.

30 tilapia fish were divided in 3 groups, 10 fish for each group:
Group 1 served as control without any treatment of fungicide.
Group 2 were treated with fungicide Curzate i.e. 4.9 mg/l (LC 50 / 10).
Group 3 were treated with fungicide Curzate i.e. 2.45 mg/l (LC 50 / 20).

Constant amount of the test chemical and test media were changed every 24 hours and the experiment lasted for 21 days. The fishes were fed once in a day throughout the duration of the sub-lethal toxicity tests.

2.2 Haematological estimation of fish:

Test organism was removed, from each tank for blood analysis. About 4 - 5ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant; properly mixed and stored at -20°C for haematological analysis. The blood was stored in -4°C in deep freezer prior to analysis.

2.3 Blood Cell Count:

The red blood corpuscles (RBC) and White blood corpuscles (WBC) were counted using haemocytometer crystalline chamber using "Hayem's" and "Turch's" diluting fluid, respectively.

Haemoglobin Estimation (HB) and Pack Cell Volume (PCV):

They were analyzed in NIHON KOHDEN Automated Hematology Analyzer (Celtics α, Japan).

Mean Cell Haemoglobin Concentration (MCHC):

This refers to the percentage of haemoglobin in 100 ml of red blood cell. This was calculated by dividing the haemoglobin content in g/dL by the PCV % of red blood according to the formulae:

MCHC = HB/PCV*1000 g/dL

Mean Corpuscular Volume (MCV):

The value of the corpuscular volume was calculated from the haematocrit value (PCV %) and the erythrocyte count (106/ µL) using the formula

MCV =PCV*1000/ RBCs fL

Mean Corpuscular Haemoglobin (MCH):

Mean corpuscular Haemoglobin concentration expresses the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from the haemogobin value (HB) and from the erythrocyte count according to the following formulae

MCH = HB/RBCs pg

Leucocyte differential count:

Leucocyte differential count was done using Giemsa stain.

2.4 Statistical analysis:

Statistical analysis was performed using Graph pad prism 5 software. The data was analyzed using two-way ANOVA test. Results were presented as mean ± SE. The significance was set as $P<0.05$, $P<0.01$ and $P<0.001$.

3 RESULTS AND DISCUSSION

The changes of haematological parameters like, RBC, WBC, Hb, PCV, MCV, MCH and MCHC in the fish Oreochromis mossambicus both in control as well as sublethal concentrations of Curzate exposed after 21 days are shown in Table 1 and Fig: 1. The haematological analysis revealed a highly significant reduction in Red Blood Cell (RBCs) count from 1.807±0.006 106/µl in the control fish to 1.523±0.013 106/µl and 0.938±0.014 106/µl in the Low dose and High dose respectively. Also a significant decrease was recorded in hemoglobin (Hb) from 7.475±0.030 g/dl in control to 5.922±0.111 g/dl and 4.457±0.287 g/dl in low dose and high dose respectively. Haematocrit or PCV is essential in clinical haematology to determine alterations in blood.

Red blood cell mass as measured by packed cell volume (PCV) and Hemoglobin content (Hb) of exposed fish groups showed a progressive decrease parallel to the increasing concentration of the fungicide. Wahbi et al., (2004) and Zaki et al., (2008) attributed the decrease in the

RBC to heamolytic crisis that results in severe anemia in fish exposed to heavy metals and herbicide respectively. Furthermore, the reduction of RBC also leads to development of hypoxic condition which in turn leads to increase in destruction of RBC or decrease in rate of formation of RBC due to non availability of Hb content in cellular medium (Chen, et al., 2004). The damage of toxicant on erythrocyte may be secondary, resulting from a primary action of toxicant on erythropoietic tissues on which there exist a failure in red cell production and or due to increase in the erythrocyte destruction. These results are in affirmative agreement with that investigated by Wahbi, et al., (2004).

The values of MCV in the experimental groups showed significant increase ($p<0.01$), MCH values showed significant increase at high dose ($p<0.001$) and at low dose ($P<0.01$) respectively. MCHC values showed insignificant decrease at low dose and a significant decrease at high dose ($p<0.01$). The MCV, MCH and MCHC values are completely dependent upon the factors of PCV, RBC count and haemoglobin concentration. In the present study, the PCV, RBC and hemoglobin concentration is completely altered. So indirectly the values of MCV, MCH and MCHC were affected. In the present study the decreased PCV values with increased MCV and MCH associated with decreased MCHC values could probably due to stress induced by the fungicide and confirms the occurrence of haemolytic anemia in experimental fish which exaggerates further disturbances in haemopoietic activities of fish. Similar finding were also observed by a number of studies in different fish [12, 13, 14 and 15].

Total WBCs count was significantly increased from 11.31 ± 0.184 103 /µL in control fish to 13.09 ± 0.657 103 /µL and 15.48 ± 0.213 103 /µL at low dose and high dose respectively. Associated with the increase in total WBC count was a noticeable percentage increase in small lymphocytes (S.L) and neutrophils (Nt). WBCs are important cells in the immune system, because of their main defensive function. The WBC will respond immediately to the change in medium due to xenobiotic transformation [16]. During exposure period of curzate the WBC counts got enhanced, indicating that the fish can develop a defensive mechanism to overcome the toxic stress. Our studies are in agreement with Lovell and Jantrarotai,(1991); Nanda, (1997); Wahbi, (1998); Hymavathi and Rao, (2000); Lebelo, et al.,(2001); Hassen, (2002) and Joshi, et al., (2002). .

Examination of Giemsa stained blood smears of control fish showed well developed erythrocytes and neutrophil (Nt) with bilobed nucleus, (fig: 2 A) while examination of Giemsa stained blood smears of treated fish showed increased number of lymphocytes and neutrophils with associated morphological alterations similar to clinical features of neutrophilia and lymphocytosis. It is indicative of compensatory and defensive reaction to the toxicant in a dose dependent manner. (Fig: 2 B and C).

The measurement of hematological parameters, which are used in this study, has provided valuable information which can contribute to the applied and basic research needs of aquatic toxicologists in the assessment of fish health and in monitoring stress responses. The present study suggests that the perturbations in the blood indices are a defense reaction against curzate toxicity. Whether these changes reflects compensatory mechanisms in the fish or biochemical results of the toxic action of the fungicide remains to be elucidated. Further biochemical and histomorphometry studies are required and will help in understanding the metabolic alterations.

4 ACKNOWLEDGEMENTS

The authors are grateful to the Department of the Zoology, The Maharaja Sayajirao University of Baroda for providing the facilities for carrying out the present work.

5 REFERENCE

[1] E. Lawrence, and T. Isioma, "Acute toxic effects of Endosulfan and Diazinon pesticides on adult amphibians (Bufo regularis)," *Journal of Environmental Chemistry and Ecotoxicology*, vol. 2, no. 5, pp. 73-78, 2010.

[2] S.B. Kumari, and M.C. Subisha, "Haematological responses in a freshwater fish Oreochromis mossambicus exposed to chlorpyrifos," *The Ekol.*, vol. 10, no. 1-2, pp. 83-88, 2010.

[3] M.J. Hemmer, B.L. Hemmer, C.J. Bawman, K.J. Croll, L.C. Formar, D. Marcovich, M.D. Hoglund, and N.D. Denslow, "Effect of pnonylphenol, methoxychlor and endosulfan on vitellogenin induction and expression in sheephead minnow (Cyprinodon variegates)," *Environ Toxicol Chem.*, vol. 20, pp. 336-343, 2001.

[4] S.O. Ayoola, "Histopathological Effects of Glyphsate on Juvenile catfish (Clarias gariepinus)," *American- Eurasian Journal of Agricultural and Environmental Science*, vol. 4, no. 3, pp. 362-367, 2008.

[5] J. Pant, H. Tewari, and T.S. Gill, "Effects of aldicarb on the blood and tissues of a freshwater fish. Bull. Environ." *Contam. Toxicol.*, vol. 38, pp. 36-41, 1987.

[6] O.B. Adedeji, V.O. Taiwo, and S.A. Agbee, "Comparative haematology of five Nigerian freshwater fish species," *Nig. Vet. Journal*, vol. 21, pp. 75-84, 2000.

[7] L.D. Zhitrineva, T.G. Poltavceva, O.A. Rudnickoja, "Atlas of normal and pathological cells in 23256526 the blood of fish." *Rostov-on-Don*., pp. 112, 1989.

[8] N.A Golovina, "Morpho-Functional Characteristics of the blood of fish as objects of aquaculture," pp. 53, 1996. (Doctorial thesis. Moscow)

[9] V. Luskova, "Annual cycles and normal values of haematological parameters in fishers." *Acta Sc. Nat. Brno.,* vol. 31, No. 5, pp. 70, 1997.

[10] O.M. Wahbi, S.M. Shyma, and A.Y. El-Dakar, "Effect of pulp and paper industrial effluent on some blood parameters, gonads and flesh proteins in experimentally exposed striped seabream Lithognathus mormyrus." *Egyptian journal of Aquatic research*., vol. 30, No. A, pp. 25-42, 2004.

[11]. M.S. Zaki, O.M. Fawzi, and El-Jackey, J., "Pathological and biochemical studies in Tilapia nilotica infected with Saprolegnia parasitica and treated with potassium permanganate." *American-Eurasiun J. Agric. & Environ. Sci.,*vol. 3, No. 5, pp. 677-680, 2008.

[12] X. Chen, D. Yin, S. Hu, and Hou, "Immunotoxicity of penta chlorophenol on macrophage immunity and IgM secretion of the crucian carp (Carassius auratus)," *J. Bull. Environ. Contam and Toxicol.,* vol. 73, pp. 153-160, 2004.

[13] A. Murad, and S. Mustafa, "Blood parameters of catfish, heteropneuttstes fassilis (bloch) parasitized by metacercaria of Diplostomum species." *J. Fish Dis*., vol. 9, pp. 295-302, 1988.

[14] V.K. Garg, S.K. Garg and S.K. Tyabi, "Manganese induced Hematological and Biochemical anomalies in H. fossils," *J. Environ. Biol*., vol. 10 no. 4, pp. 349-353, 1989.

[15] A. Laurent, J.J. Durussel, J. Dufaux, C. Penhonet, A.L. Bailly, M. Bonneau, and J. Merland, "Effect of contrast media on blood rheology comparison in human, pigs and sheep." *J. Card. Vas. Intervent. Radiol.* ,vol. 22, pp. 62-66, 1999.

[16] J. Drastichova, Z. Svobodova, V. Luskova, and J. Machova, "Effect of cadmium on haematological indices of common carp Cyprinus Carpio," *J. Bull. Environ. Contam and Toxicol*., vol. 72, no. 4, pp. 725-732, 2004.

[17] A. Naveed, P. Venkateshwarlu, and C. Janaiah, "Impact of triazophos infestion on heamatological parameters of cat fish Channa punctatus (Bloch)." *International journal of pharmacy & life sciences*., vol. 1, No. 6, pp. 298-301, 2010.

[18] R.T. Lovell, and W. jantrarotai, "Subchronic toxicity of dietary aflatoxin B1 to channel catfish." *Journal of Aquatic Animal Health*., vol. 2, No. 4, pp.248-254, 1991.

[19] P. Nanda, "Haematological changes in the common Indian Catfish Heteropneustes fossilis under nickel stress." *J. Ecobiol*., vol. 9, pp. 243-246, 1997.

[20] O.M. Wahbi, "Effect of tanning processing wastewater in physiological characteristics of Solea sp." Ph.D. Thesis, Faculty of Science, Alexandria University, 1998.

[21] V. Hymavathi, and M. Rao, "Effect of sublethal concentration of lead on the haematology and biochemical constituents of Channa punctatus," *J. Bull. Pure and Appl. Science,* vol. 19, no. 9, pp. 1-5, 2000.

[22] S.L. Lebelo, D.K. Saunders, and T.G. Crawford, "Observations on blood viscosity in striped bass, Morone saxtilis (Walbaum) Associated with fish Hatchery conditions." *Kansa Acad. Sci.,* vol. 104, pp. 183-194, 2001.

[23] F.E.Z.M. Hassen, "Studies on diseases of fish caused by Henneguya infestation,"2002. (Ph.D Thesis, Faculty of Veterinary Medicine, Suez Canal University. Egypt.)

[24] P.K., Joshi, M. Bose, and D. Harish, "Haematological changes in the blood of Clarias batrachus exposed to mercuric chloride, Ecotoxicol." *Environ. Monit.,* vol. 12, pp. 119-122, 2002.

Figure: 1. Graphical representation of Blood indices in Control as well as treated fish.

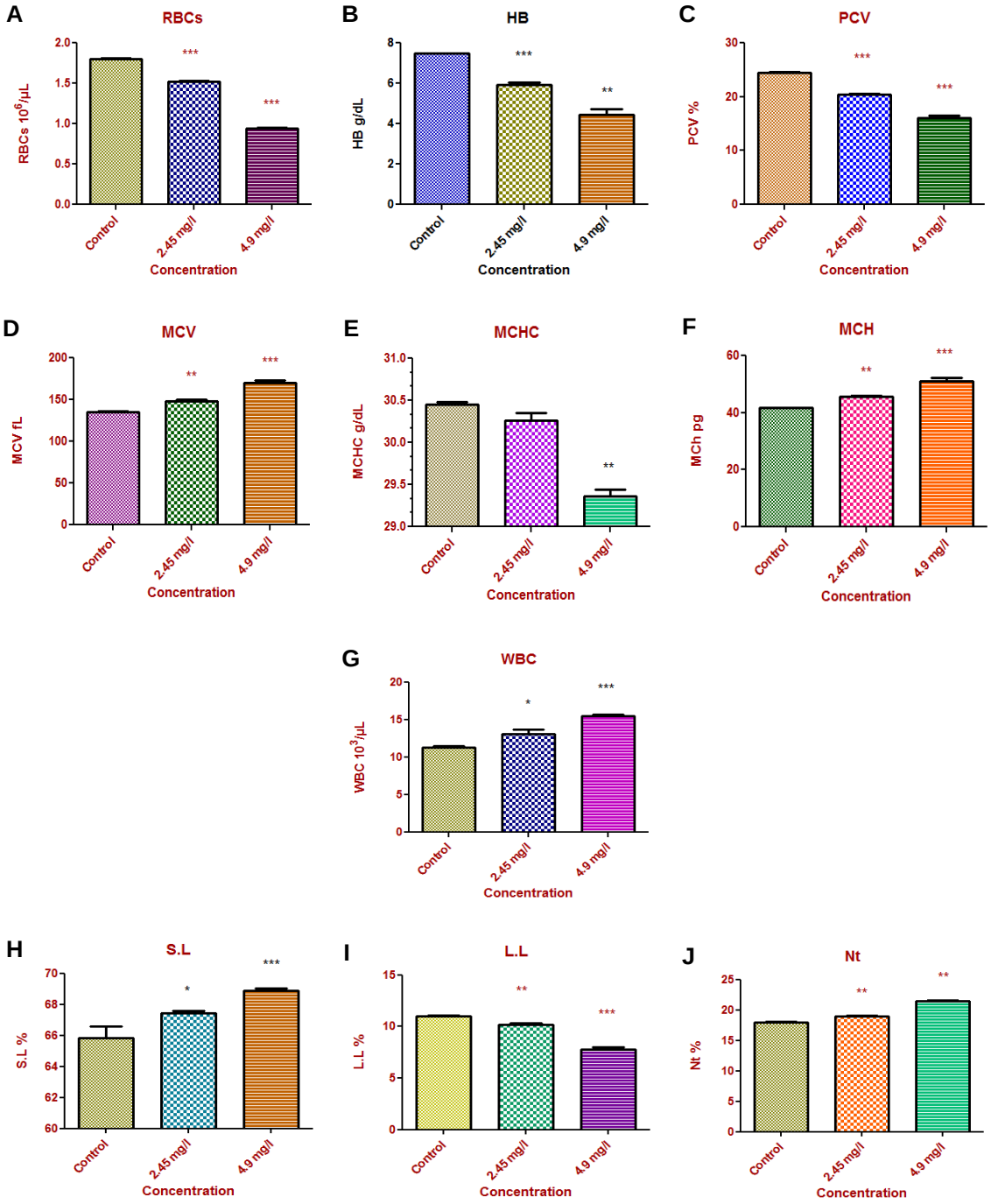


Table: 1. Blood indices in Control and treated fish.

Parameters	Concentration mg/l		
	Control (0 mg/l)	**Low Dose (2.45 mg/l)**	**High dose (4.9 mg/l)**
RBCs 10^6 /µL	1.807±0.006	1.523±0.013***	0.938±0.014***
HB g/dL	7.475±0.030	5.922±0.111***	4.457±0.287**
PCV (Htc) %	24.50±0.063	20.47±0.069***	16.04±0.505***
MCV fL	135.0±1.00	148.2±0.881**	170.2±2.557***
MCHC g/dL	30.45±0.028	30.27±0.088	29.37±0.074**
MCH pg	41.66±0.172	45.67±0.346**	51.15±1.011***
Total WBC 10^3 /µL	11.31 ± 0.184	13.09 ± 0.657*	15.48 ± 0.213***
Small Lymphocytes %	65.82 ± 0.745	67.42 ± 0.144*	68.88 ± 0.170***
Large lymphocytes %	11.03 ± 0.051	10.20 ± 0.152**	7.798 ±0.170***
Neutrophils %	18.02 ± 0.063	19.01 ± 0.129**	21.46 ± 0.158**

***Significant p < 0.001; **Significant p <0.01; *Significant p< 0.05; ± S E

Fig: 2 Pathological observations of blood smear of curzate treated fish

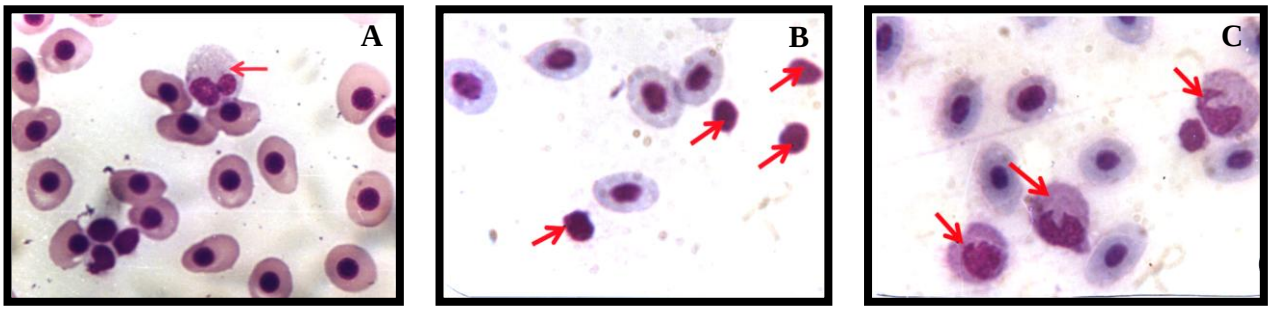


Fig 2 A shows well developed erythrocytes and neutrophil (↑) with bilobed nucleus, Fig 2 B and C shows increased number of lymphocytes and neutrophils with associated morphological alteration (↑).

Biohelica: Vol.1 (2): 16 - 21, 2010 **ISSN 0976-5204**
A Scientific Journal of Biological Sciences

Acute toxic and behavioural responses of *Oreochromis mossambicus (Peters, 1852)* exposed to three agrochemicals

Pragna Parikh*, Shalaka Sadekarpawar and Bhavika Desai
Department of Zoology, Faculty of Science,
The Maharaja Sayajirao University of Baroda,
Vadodara – 390 002

(Received: September 23, 2010, Revised & accepted: December 16, 2010)

Present study was undertaken to assess the dose-response of *O. mossambicus* to different concentrations of a insecticide, a fungicide and a plant nutrient and to estimate the LC50 of each using probit analysis. The mortality data obtained was based on Finney's Probit Analysis statistical method. The LC50 values of the insecticide (Imidacloprid), the fungicide (Curzate) and the plant nutrient (Librel) were found to be 0.7442 mg/L, 49.61.0 mg/ L and 4932.16 mg/L respectively. Behavioral responses to all the three tested chemicals exhibited profound changes.

Key words: Acute toxicity, Fungicide, Insecticide, Nutrient, *Oreochromis mossambicus*

INTRODUCTION

Agricultural areas have the potential to pollute the aquatic ecosystem via the popular use of pesticides (chemicals), fertilizers (nutrients), salts and sediments. Agricultural pesticides are indispensable in contemporary agriculture as they are provide reliable, persistent and relatively complete control against harmful pests with less cost and effort. But their effects are less than desirable when they leave the target compartment of the agricultural ecosystem. Up to 90% of the pesticides applied never reach the intended targets (Sparling et al., 2001); as a result, many other organisms sharing the same environment as pests are accidentally poisoned. One of the non-target biological groups mostly affected by pesticides is fishes; (Velmurugan, 2006; Omitoyin, 2007). Agrochemical fertilizers and their effluents have been shown to have devastating effects on aquatic biota (Bobmanuel *et al.*, 2006; Ekweozor, *et al.*, 2001; Chukwu and Okpe, 2006; Yadav, *et al.*, 2007; De Solla and; Boone, *et al.*, 2007). Increased use of chemical pesticide results in the excess inflow of toxic chemicals, mainly into the aquatic ecosystem (Kalavathy *et al.*, 2001). The aquatic flora and fauna are affected by the toxic substances which eventually enter in to their systems or bring about external damages (Pant and Singh, 1983; Hodson, 1988; Johal and Dua, 1995).

Toxicity tests are conducted to measure the effects of one or more pollutants on one or more species of organisms (Reish & Oshida, 1987). Data obtained on the concentration of selected individual pollutants which are lethal to fish provide very necessary information, apart from identifying a boundary limit above which fish are likely to be killed (Lockwood, 1976). One of the commonly used measures of toxicity is the LC_{50}, i.e. the lethal median concentration that causes mortality in 50% of test organisms. Probit Analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms.

The purpose of this study is to establish the LC_{50} for some agrochemicals – Imidacloprid (insecticide), a Imidacloprid, Curzate M8™ (fungicide), a systemic fungicide, and Librel™, a rapidly soluble chelated micronutrient mixture. Toxicity studies on Rainbow trout, Gold fish, catfish, and carp have reported that Mancozeb is highly toxic

Corresponding author: *php59@yahoo.co.in

whereas, Cymoxanil has been proved to be slightly toxic on carp, sheepshead minnow, mysid shrimp and blue gill sunfish. (Edwards *et al.*, 1991; Kidd and James, 1991). However, there effects on *Oreochromis mossambicus* are lacking. Hence, the present study was designed to determine the LC_{50} of the three tested chemicals and the behavioral changes caused by these in *Oreochromis mossambicus*.

MATERIALS AND METHODS

The specimens of freshwater fish, *O. mossambiccus* of similar size in length (12 ± 2 cm) and weight (25 ± 1.9 g) were brought from a local pond of Baroda district. The fishes were acclimatized in laboratory conditions in well aerated dechlorinated tap water. The acute fish bioassay experiments for 24, 48, 72 and 96 hours were conducted. Pilot experiments were conducted to determine the concentrations causing 10 to 90% mortality of the test fish.

Preliminary tests were conducted to provide guidance on range of concentration of pesticide to be used in the bioassay. The nominal test concentrations used in this study were 0.5mg/l, 0.55 mg/l, 0.6mg/l, 0.65mg/l, 0.7mg/l, 0.75mg/l, 0.8mg/l, 0.85mg/l, 0.9mg/l and 0.95 mg/l; for Imidacloprid, 36mg/l, 37 mg/l, 38 mg/l, 39 mg/l, 40 mg/l, 41 mg/l, 42 mg/l, 43 mg/l, 44 mg/l and 45 mg/l for Curzate and 4600 mg/l, 4700 mg/l, 4800 mg/l, 4900 mg/l, 5000 mg/l, 5100 mg/l, 5200 mg/l, 5300 mg/l, 5400 mg/l and 5500 mg/l for Librel with three replicates each The behaviour of specimens in response to each dose of each test chemical was observed and death if any was recorded for the 96-h test period of each experiment.

Probit analysis (Finney, 1971) was used to calculate the median lethal concentration and time with their upper and lower confident limits. Data (OBF, TBF and mortality) were subjected to analysis of variance (ANOVA) for difference between means of both the group using statistical programme (Biostat 2009 Professiojnal 5.8.1 and Graphpad Prism 5). Other abnormal behaviours were noted and the extent of mucus production on the skin and gills of exposed fish was assessed by feeling with the fingers. Opercular beat frequency (OBF), tail beat frequency (TBF) and cumulative mortality was recorded. A fish was considered dead when it failed to respond to simple prodding with a glass rod. Death was defined as complete immobility with no flexion of the abdomen upon forced extensions (Lockwood, 1976).

RESULTS AND DISCUSSION

Of the three agrochemicals, Imidacloprid was found to be most toxic compared to Curzate and Librel. The order of toxicity of agrochemicals to fish was Imidacloprid > Curzate > Librel. The behavioral responses of the fish varied in accordance with the test concentrations. Relatively reduced activity was exhibited during early hours of exposure at all the concentrations of Imidacloprid and Curzate, but same was not true for Librel. Probably Librel, being a plant nutrient, was not as lethal as the other two. No mortality or morphological changes were observed in the control experiment for the 96 h acute toxicity test. Fishes in the control experiment appeared active and healthy throughout the test period. However, the proportion of abnormal avoidance response in the control was less than 10%. The test organisms exposed to varying agrochemical concentrations for 96 hrs, recorded mortality for each concentration. It was found that relatively Librel was relatively least toxic as the mortality of test fish was found at the higher concentration of the chemical. Most of the fish which died during the experiment exhibited symptoms of poisoning such as change in colour as well as behavior. Initially their colour darkened and they swam erratically with their body inclined downwards.

The LC_{50} values observed for Imidacloprid, Curzate and Librel with their LCL and UCL are presented in Table – 1 and Fig 1(A, B, C). Different behavioral responses were seen in the fishes exposed to the agrochemicals. Behavioral effects were more in case of Imidacloprid and Curzate compared to the control group. Decreased swimming activity and decreased abnormal hyperkinetic activity was more pronounced at higher concentrations, suggesting sensitivity to the agrochemicals. One of the most common behavioural responses to biotic stressors, however, is reduced movement (Chivers & Smith, 1998), and the alternative hypothesis of decreased opercular movements would be consistent with overall reductions in activity. OBF rate has been used to provide a measure of response to stress in fishes. (Gibson and Mathias, 2006). Fishes exposed to

Imidachloprid and Curzate exhibited decreased OBF. This may be due to the gill damage, where the toxicant acts as respiratory poison possibly affecting the gills, impairing respiration and leading to various abnormal behaviour and eventually death. Gabriel and Okey (2009) have studied the effect of aqueous leaf extracts of *Lepidagathis alopecuroides* on the behaviours and mortality of hybrid catfish fingerlings and have reported similar observation.

Exposure to Librel showed different behavioural responses. Hyperkinetic activity was not seen and the OBF and TBF were increased (Fig 2: (2004), Chukwu and Okpe, (2006) and Omitoyin *et al*. (2006).

Behavioural changes are the most sensitive indication of potential toxic effects of a chemical studied. From the findings of the present investigation, one can conclude that of the three tested agrochemicals had profound impact on the behaviour of fish. As Thompson and Schuster (1968) noted, the study of toxic effects on the behavioural level offers ecologists and environmentalists two major advantages. First, chemical agents that produce only behavioural changes and have serious and possibly irreversible deleterious effects on the animals' ability to adapt can be identified and controlled. Second, the behaviourally toxic effects of chemical agents can be considered as an early warning system for the detection of the toxicity before irreversible structural and biochemical damage are caused by them.

The study suggests that the all the three agrochemicals (nutrient fertilizer, fungicide and pesticide) have toxic effects and in fish. However, further studies on the toxicity of these agrochemicals using various other test systems/ animals are required to corroborate the findings of this study.

Table 1 LC_{50} values (mg/L) with their fiducial limits used in acute toxicity tests for *Oreochromis mossambicus*

Agrochemicals	Application	Duration	LCL	LC_{50}	UCL
Imidacloprid	Insecticide	48 hrs	0.6896	0.7319	0.7742
Curzate	Fungicide	48 hrs	38.67	39.84	40.78
Librel	Plant Nutrient	24 hrs	4868.19	4985.63	5079.54

Note: LCL = Lower Confidence Limit, UCL = Upper Confidence Limit, LC_{50} = Lethal Concentration for 50 percent of the exposed fish

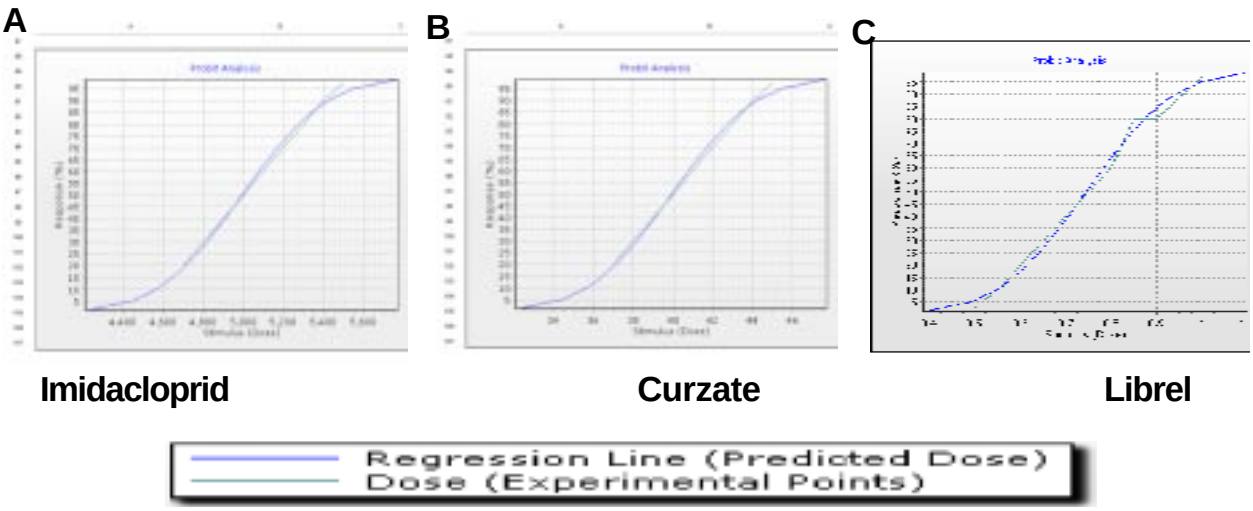


Fig 1: Plot of adjusted probits and predicted regression line for three agro-chemicals to *Oreochromis mossambicus*

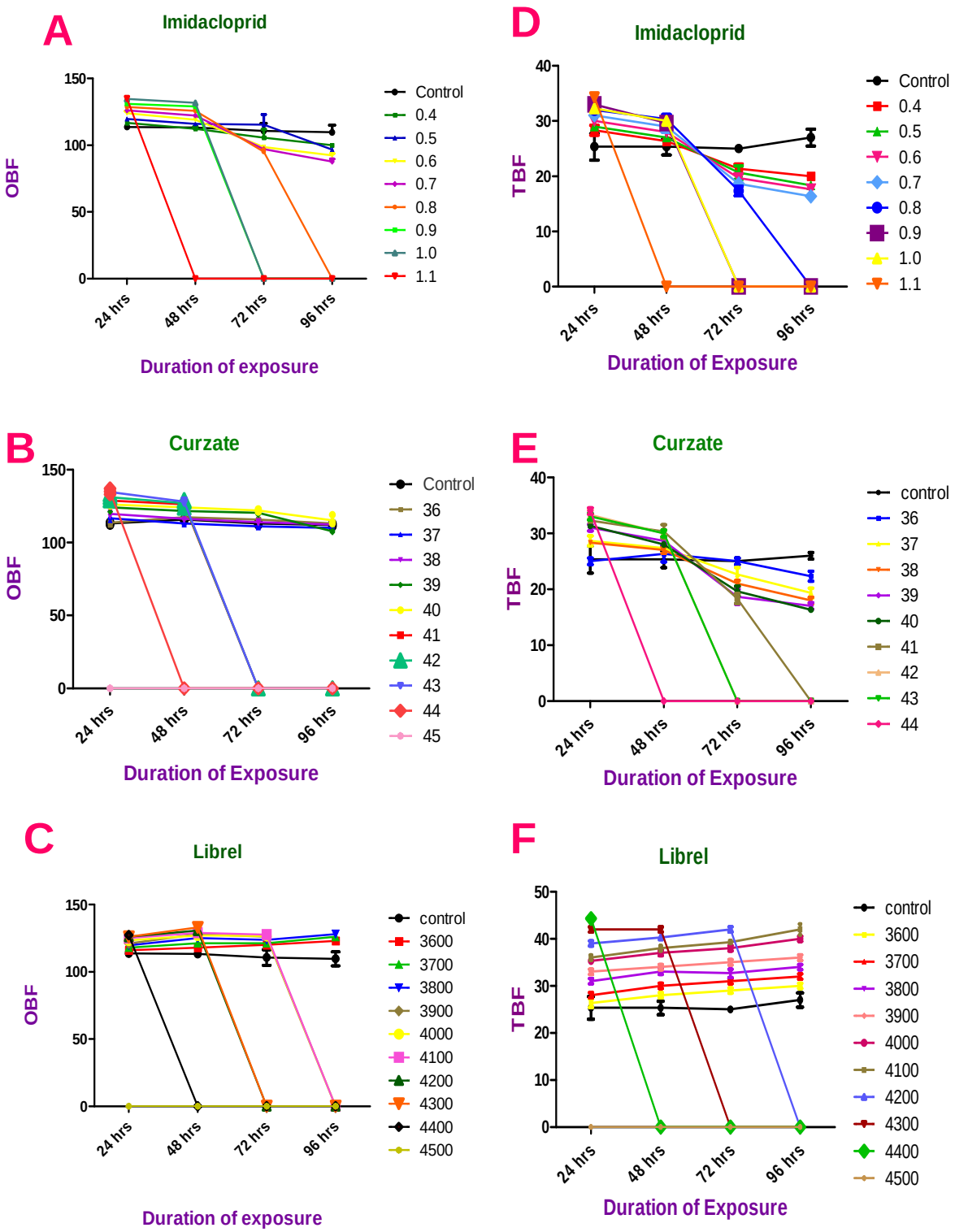


Fig 2. Graphs showing the Opercular Beat Frequencies and Tail Beat Frequencies exposed to three agrochemicals depending on the duration of exposure.

REFERENCES

Bobmanuel NOK, Gabriel UU and Ekweozor IKE (2006) Direct toxic assessment of treated fertilizer effluents to *Oreochromis niloticus* and catfish hybrid (*Heterobranchus bidorsalis* % x *Clarias gariepinus* &). Afr J Tech **5**(8): 653-642.

Boone MD, Semlitsch RD, Little EE and Doyle MC (2007) Multiple stressors in amphibian communities: effects of chemical contamination.Bullfrogs Fish Ecol Appl , **17**(1): 291-301.

Chindah AC, Sikoki FD and Vincnet-Akpu I (2004) Toxicity of an Organnophosphate pesticide (Chloropyrifos) on a common Niger Delta wetland fish – *Tilapia guineensis* (Blecker 1862). J Appl Sci Environ M gt **8**(2): 11-17.

Chivers DP and Smith RJF (1998) Chemical alarm signaling in aquatic predator prey systems: a review and prospectus. Ecoscience **5**: 338–352.

Chukwu LO and Okpe HA (2006) Differential responses of *Tilapaia guineensis* fingerlings inorganic fertilizer under various salinity regime Lockwoods. J Environ Biol **27**: 687-690.

De Solla SR and Martin PA (2007) Toxicity of nitrogenous fertilizers to eggs of snapping turtles (*Chelydra serpentina*) in field and laboratory exposures. Environ Toxicol Chem **26**(9): 1890-1895.

Edwards IR, Ferry DG and Temple WA (1991) Fungicides & related compounds, In Handbook of Pesticide Toxicology. Hayes, W. J. and Laws, E. R., Eds. Academic Press, New York, NY,.42.

Ekweozor IKE, Bobmanuel NOK and Gabriel UU (2001) Sublethal effects of ammoniacal effluents on three commercial fish species from the Niger Delta, Nigeria. J Appl Sci Environ M gt. **5**(2): 9-11.

Fafioye OO, Adebisi AA and Fagade SO (2004) Toxicity of *Parkia biglossa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. Afr J Biotech **3**(11): 627-630.

Finney DJ (1971) Probit analysis, Cambridge University Press, London, New York, Edinburgh. pp. 333.

Gabriel UU and Okey IB (2009) Effect of Aqueous Leaf Extracts of *Lepidagathis alopecuroides* on the Behaviours and Mortality of Hybrid Catfish (*Heterobranchus bidorsalis* , *Clarias gariepinus* &) Fingerlings. Research Journal of Applied Sciences, Engineering and Technology **1**(3): 116-120.

Gibson AK and Mathis A (2006) Opercular beat rate for rainbow darters *Etheostoma caeruleum* exposed to chemical stimuli from conspecific and heterospecific fishes. Journal of Fish Biology **69**: 224–232.

Graham JB (1997) Air breathing fishes: Evolution, Diversity and Adaptation. Academics Press, San Diego.

Hodson PV (1988) The effect of metal metabolism on uptake, disposition and toxicity in fish. Aquat Toxicol **11**: 3-18.

Johl MS and Dua A (1995) Elemental lepidological and toxicological studies in *Channa punctatus* (Bloch) upon exposure to an organocholorine pesticide, endosulfan. Bull Environ Contam Toxicol **55**(6): 916-921.

Kalavathy K, Sivakumar AA and Chandran R (2001) Toxic effect of the pesticide Dimethoate on the fish *Sarotherodon mossambicus*. J Ecol Res Bioconserv **2**(1-2): 27–32.

Kidd H and James DR (1991) (Eds) The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, pp.4-40.

Kind PK, Grigg GC and Booth GT (2002) Physiological responses to prolonged aquatic hypoxia in the Queensland. lungfish, *Neoceratodus forsteri*. Resp Physiol Neurobiol **132**: 179-190.

Lockwood APM (1976) Effects of Pollutants on Aquatic Organisms. Cambridge University Press, Cambridge. pp.193 .

Omitoyin BO, Ajani EK, Adesina BT and Okuagu CNF (2006) Toxicity of lindane to *Clarias gariepinus* (Burchell 1822). W J Zool **1** (1): 57-63.

Oti EE (2002) Acute toxicity of cassava mill effluent to the African catfish fingerlings. J Aquat Sci **17** (1): 31-34.

Pant JC and Singh T (1983) Inducement of metabolic dysfunction by corbomate and organophosphorus compounds in a fish, *Puntius conchonius*. Pestle Biochem Physiol **20**: 294-298.

Reish DL and Oshida PS (1987) Manual of methods in aquatic environment research- part 10 short-term static bioassays. FAO Fisheries Technical Paper pp.247- 64.

Shah SL (2002) Behavioural abnormalities and *Cyprinion watsonic* on exposure to copper and zinc. Turk J Zool **26**: 137-140.

Sparling DW, Fellers GM and McConnell LS (2001) Pesticides and amphibian population declines in California, USA. Environ Toxicol Chem **20**: 1581-1595.

Thompson and Schuster (1968) Effects of certain metallic salts upon fishes. Trans Am Fish Soc **44**: 20-124.

Val AL, Silva MNP and Almeida-Val VMF (1998) Hypoxia adaptation in fish of the Amazon: a never ending task. S Afri J Zool **33**(2): 107-114.

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

Yadav A, Neraliya S, and Gopesh A (2007) Acute toxicity levels and ethological responses of *Channa striatus* to fertilizer industrial waste water. J Environ Biol **28**(2): 159-62.