The use of pesticides has become an integral part of modern agricultural systems. Irrigation water and agricultural runoff are the main sources of contaminating aquatic environment, where they subsequently deteriorate water quality. Alterations in the chemical composition of natural aquatic environments can affect the fresh water fauna, particularly fish. These xenobiotics even at low concentration can interfere with the metabolism of the organism (Farmer et al., 1972; Weber, 1977; Metelev et al., 1983). Prolonged exposure to these contaminants caused a number of alterations in the physiological, biochemical processes. The literature has been reviewed on pesticide toxicity to fish with emphasis on locomotory behavioral, growth, carcass composition, haematological, biochemical, chromosomal aberrations and genotoxicity in fish.

2.1 Locomotory and behavioural changes

Locomotion and behavioural modifications are among the most sensitive indicators/ measures of toxic stress for a wide range of environmental contaminants (Olla et al., 1983; Little and Finger, 1990; Cohn, 1996; Byrne and O'Halloran, 2001). These alterations represent a higher organizational level of biomarker than any other considered (Walker et al., 2003). Since behaviour serves as the link between physiological and ecological processes, it may be ideal for studying the effects of environmental pollutants. Any change in the behavior of fish indicates the deterioration of water quality, as fish are the biological indicator and hence index of environmental suitability and the cost of survival. Physiological effects of toxicants include disruption of sensory, hormonal, neurological, and metabolic systems, which are likely to have profound implications for many fish behaviours. The perception of motion is important for the survival and reproduction of many animals especially fish (Albenski and Powell, 1998). Studies concerning the effects of pesticides on the locomotor behavior of fish are lacking, due to methodological difficulties involved however diverse methods have already been developed and used to measure the locomotor activity of exposed organisms (Tadehl and Hader, 2001). The literature on various behavioural responses of fish to certain aquatic pollutants which include preference/avoidance, coughs, social hierarchies and body tremors has been reviewed by many authors (Marcucella and
Abramson, 1978; Little et al., 1985; Rand, 1985; Atchison et al., 1987; Beiting, 1990; Little and Finger, 1990; Doving, 1991; Blaxter and Hallers-Tjabbes, 1992; Scherer, 1992; Atchison et al., 1996; Kasumyan, 2001). Among the various behavioural effects of toxicant disorder in central nervous system, peripheral nervous system and especially the disturbance in lateral line complex has been opined by Mount (1962), Anderson (1968), Chebbi and David (2010) and Tilton et al. (2010). However, bulging in the eyeballs and sluggish movement of aquatic organisms due to the disturbance in hormonal equilibrium of hypophysis was earlier suggested by Van Dijjni (1967). The widespread use of DDT in agriculture has a long tradition so the effects of sublethal doses of DDT has been done to explore the changes in brook trout Salvelinus fontialis, goldfish Carassius auratus and Colisa fasciatus. Various behavioural alterations observed in the fishes were loss of locomotor activity, sometimes rapid and jerky movements of body and fins, increased rate in ventilation, jumping movements towards the surface, sometimes very slow and backward swimming followed by convolutions. All these studies revealed that both the central and peripheral nervous system were affected (Anderson and Peterson, 1968; Pimental and Goodman, 1974; Verma et al., 1975).

Toor and Kaur (1974) and Gopal et al. (1981) reported the excessive release of mucous in Cyprinus carpio on exposure to carbonyl and in Clarius batrachus in response to endosulphan. Pandey et al. (1990) suggested that it may be due to the dysfunction of pituitary gland under the stress of toxicants. With the addition of some toxic chemical into the aquatic environment certain changes in the behaviour of aquatic organisms appeared (Rath and Misra, 1981; Brewar et al., 2001). They attributed these changes due to the stress conditions caused by the toxic substances. Sudden decline in rate of opercular movements paralleled to the oxygen uptake in Labeo rohita and Mystus cavasius with increasing concentration of fenitrothion has been observed by Murty (1986). It is generally accepted that increased muscle tone lead to altered locomotion and increased oxygen consumption. These results have also been inferred in silver carp and common carp on exposure to both chlordane and malathion by Ravikumar and Gupta (1988). They opined that exposed fishes caused excitement due to the disturbance in the central nervous system which further led to increased oxygen consumption. Similarly, various alterations in locomotion behaviour in fish exposed to
various toxicants has been reported by Ram et al. (1990) and Ram and Gopal (1991). Anbu and Ramaswamy (1991) observed the direct effect of pesticide carbaryl on the behaviour and locomotory pattern of the fish *Channa punctatus*. The fish exhibited erratic and irregular movements with impaired swimming and also showed increased air gulping and opercular movements. Jonsson and Toledo (1993) were of the view that if zebrafish (*Brachydanio rerio*) and yellow tetra (*Hyphessobrycon bifasciatus*) were exposed to endosulphan, it reduces the activity of movement with erratic swimming, convulsions and sometimes hyperactivity. The findings of Saglio et al. (1996) while working on *Carassius auratus* on exposure of sublethal carbofuran revealed the alteration in behaviour and reported that short term exposure of carbofuran can affect behavioural responses of fish both directly and indirectly by altering the chemical perception of natural substances of ecological importance.

Oyewo (1998) also tested some prominent metals found in the industrial effluents against five animal species namely; *Cypris sp.*, *Mugil sp.*, *Tilapia sp.*, *Nerita senegalensis*, and *Clibanarius africanus* that normally inhabit the Lagos Lagoon. The author reported that these test metals affect the behaviour and locomotion patterns in the fish. Also, atrazine and diuron was found to have potential in decreasing grouping behaviour, sheltering and abrupt swimming reactions in goldfish, *Carassius auratus* (Saglio and Trijasse, 1998). They also inferred that both the pesticides affected the behaviour of fish by altering their chemical perception for natural substances. Metal ions can also act as toxicants to the aquatic organisms like pesticides. Otitoloju (2001) showed that sublethal doses of metal copper on Nile tilapia caused difficulty in breathing and abrupt swimming. Physiological changes in several invertebrates due to copper hazards have been extensively studied and reported by Hogstrand and Wood (1996), Khangarot (1989) and Eisler (1998). Sarikaya and Yilmaz (2003) observed anxiety, loss of balance, swimming upside down or vertical manner, sudden jerks, respiratory difficulties, lightening in color, excessive mucosal secretion, coming to the surface for breathing and hitting to the side walls of the aquaria in *Cyprinus carpio* on exposure to different concentrations of 2,4-D. Jindal and Jha (2005) conducted an experiment to delineate the impact of organophosphorous pesticide monocrotophos on the behaviour of *Cyprinus carpio*. They observed several alterations including erratic swimming, increased mucous secretion and scale loss. Similarly, the sublethal effects
of monocrotophos on locomotor behaviour, structural integrity of gill, enzyme acetylcholinesterase, gill articheture of the mosquito fish, *Gambusia affinis* were observed by Rao *et al.* (2005). The exposed fish were found to be under stress and paraprphastic locomotion. Similar emended changes in locomotion of fishes has been reported by various workers; Kane *et al.* (2004) exposed *Gambusia affinis* to monocrotophos; Yildirim *et al.* (2006) exposed *Oreochromis niloticus* to deltamethrin; Ismail *et al.* (2009) exposed *Cyprinus carpio* to profenophos; Halappa and David (2009) exposed *Cyprinus carpio* to chlorpyrifos and to dimethoate by Singh *et al.* (2010). Acute toxicity of endosulphan for 96 h can exhibit imbalanced position, restless movements, erratic swimming, flashing, tremor and lethargy in *Monopterus albus* (Siang *et al.*, 2007). Likewise, Patil and David (2008) assessed behaviour dysfunction as an index of malathion induced toxicity in *Labeo rohita* and reported that exposed fish showed irregular, erratic and darting swimming movements, loss of equilibrium, hyperexcitability and sinking to the bottom. They attributed the behavioural dysfunction due to the inhibition of AChE activity which resulted in excessive accumulation of acetylcholine at cholinergic synapses leading to hyper stimulation.

Halappa and David (2009) reported the behavioural response of freshwater fish, *Cyprinus carpio* (Linnaeus) following sublethal exposure to chlorpyrifos and reported that the fish in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom. Caudal bending was the main morphological alteration during the exposure periods. The investigations made by Marigoudar *et al.* (2009) on cypermethrin demonstrate behavioural modifications in *Labeo rohita* and reported that the carp manifested erratic, irregular and darting swimming movements, loss of equilibrium and hyper excitability attributed to inhibition of acetylcholinesterase enzyme activity. Kochhann *et al.* (2010) studied the effect of different crude oil fractions on the swimming performance of Tambaqui (*Colossoma macropomum*) and observed decreased swimming activity in fish exposed to insoluble crude oil. According to Lawal and Samuel (2010), exposure of *Poecila reticulata* to actellic water resulted in aggressive behaviour, rapid gulping of water, increased opercular movement and abnormal swimming movements. Shahi and Singh (2010) observed various forms of abnormal behaviour in *Channa punctatus* when exposed to different concentrations of rutin,
taraxerol and apigenin. Susan and Sobha (2010) studied the toxic effect of fenvalerate on Indian major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* and observed anomalous behavioural activities like swimming at the water surface, hyper excitation, loss of equilibrium, flaring of gills and increased mucus production attributed to inhibition of acetylcholinesterase enzyme activity. Several behavioral anomalies (locomotor response) were studied as indication of toxic effects of the metals. Ezeonyejiaku *et al.* (2011) studied the toxicity of copper sulphate on the behavioural response of tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) species. Behavioral changes, mostly locomotor responses (avoidance) were observed among the test animals on exposure to the different concentrations of copper sulphate. Fish exhibited a number of behavioural changes on exposure to different pesticides as neural paralysis, imbalance, abnormal swimming, sever impatience and paleness in body colour (Mishra *et al.*, 2011; Far *et al.*, 2012, Kamble *et al.*, 2012). Biopesticides which are considered much more beneficial for agricultural produce can also interfere with the physiology of the exposed fishes. The use of azadirachtin and biopesticide kethrin has been reported to cause deportal responses in *Labeo rohita* such as erratic swimming, decreased rate of opercular movement, increased surfacing, reduced agility and inability to maintain normal posture and balance with increasing exposure period and concentration (Bhatt *et al.*, 2012). Nwani *et al.* (2013) worked on toxic effects of chlorpyrifos based pesticide termifos on behavioural responses of *Clarius gariepinus* and reported similar abnormalities. Okechukwu *et al.* (2013) evaluated the impact of short term exposure to waterborne chlorpyrifos ethyl on *Clarius gariepinus* through changes in behavioural parameters and mortality. This is in line with the earlier findings of Omorogie and Ufodike (1991) and Lloyd (1992).

Studies of Scott *et al.* (2013) showed the effects of environmental pollutants metals, pesticides, and other organics on complex fish behavior and reported that toxicant exposure often completely eliminates the performance of behaviours that are essential to fitness and survival in natural ecosystems. Earlier studies on brain neurotransmitter levels and enzyme function correlate with behavioural states (Alanara *et al.*, 1998; Elofsson *et al.*, 2000; Hofmann and Fernald, 2000; Hoglund *et al.*, 2001), so it is likely that neurological dysfunction induced by toxicant exposure results in locomotory behavioural changes. Verma and Saxena (2013) found that acute toxicity of
chlorpyrifos exert certain behavioural modifications on *Puntius chola* (Hamilton-Buchanan). They observed change in skin colour, skin became brownish with a red spot in the head region, and slimy body was noticed. Fishes showed hyper activity and frequent surfacing to gulp air. It was also noticed that as time passed, they continued to swim near the water surface and tried to jump out from the holding tanks. The behavioural observations made were similar to the observations studied by Hulya *et al.* (2006) in *Oreochromis niloticus* following sublethal exposure to diazinon. Similarly, Devi and Mishra (2013) reported the effect of chlorpyrifos toxicity to behavioural and morphological anomalies on fresh water teleost, *Channa punctatus*. Panigrahi *et al.* (2014) studied monocrotophos impact on behaviour of *Cyprinus carpio* and observed abnormal swimming, loss of equilibrium, dispigmentation, coughing and opercular movement. Das and Gupta (2014) exposed *Esomus danricus* to malathion and the exposed fish were found to have faded color along with copious mucus secretion, irregular, erratic and jerky movements. Jindal and Kaur (2014) opined the effect of chlorpyrifos on *Ctenopharyngodon idellus* and reported that the fish showed erratic, speedy and jerky movements hyperactivity. They suggested that violent behaviour was related to toxicant concentration and time dependent. As reported by Jin *et al.* (2015), after prolonged exposure of the fish to the toxicant, it became sluggish and showed escape tendency with decreased swimming activity. Similar behavioural changes on the impact of different pesticides on other fish have been studied by various workers (Far *et al.*, 2012; Huang *et al.*, 2014; Walsh-Monterio *et al.*, 2014; Naserabad *et al.*, 2015).

### 2.2 Growth performance and carcass composition

Although effects of organophosphates pesticides on biological functions of fish have been widely studied, information on the effects of these pesticides on growth of fish is very limited. Comprehensive studies were conducted on various effects of OP exposure in fish, including growth performance, inhibition in acetylcholinesterase (AChE) activity (Sancho *et al.*, 1997; Gul, 2005; Chandrasekara and Pathiratne, 2007; Kavitha and Rao, 2008) and alteration in metabolism (Lal and Singh, 1986; Hai *et al.*, 1997; Tripathi *et al.*, 2003; Kavitha and Rao, 2008). Elchelberger and Liehleuberg (1971) reported that water quality attributes are prime factors that influence fish survival, reproduction, growth performance and over all biological reproduction. Survival was observed to be a more sensitive end point than growth and mortality.
mainly occurred during the initial stages of the exposure. Jarvinen and Tanner (1982) studied the negative effects of pesticides exposure on fish growth in fathead minnow juveniles, *Pimephales promelas*, in rainbow trout *Salmo gairdneri* by Cleveland and Hamilton (1983), in channel catfish, *Ictalurus punctatus* and in zebrafish *Brachydanio rerio* by Nagel et al. (1991). The reduction in fish growth and survival rate when exposed to metasystox for a prolonged period was reported by Sasikalaa (1984). Similar results were also reported by Chandrasekara and Pathiratne (2007) and Sun and Chen (2008). Various water quality parameters play an important role in the growth of fish. Bettoli et al. (1985) reported that general trend in growth of fish in pesticide treatment was temperature dependent. Maximum growth was found in the month of July and the least was found in the month of December, when temperature was lower. They inferred that better growth was found in the range of 26-32°C. Pesticide phosphamidon hampered the growth of *Labeo rohita* (Gopalakrishnan, 1990). The exposed fish exhibited retarded length and weight gain with increase in exposure period and concentration of the toxicant. Similar findings were reported by various authors in different fish species due to the effect of phosphamidon (Kurup et al., 1990; Govindan et al., 1994). Gill et al. (1991) found a significant elevation of HSI and growth of freshwater fish *Barbus conchonius* after exposure to endosulfan for a prolonged period. The same impact was also reported in *Anguilla anguilla* by Holmberg et al. (1972) and in the rainbow trout, *S. gairdneri* by Lidman et al. (1976). Gill et al. (1991) also correlated the enlargement of fish liver to an increase in liver protein and total lipid. Acute toxicity of pesticide like endosulfan, malathion and copper sulphate at different concentration to *Macrobrachium rosenbergii* were reported (Natarajan et al. 1992). There are also some reports of the effects of the herbicide 2, 4-D on the bottom dweller fishes of fish ponds (Sarkar, 1991). Exposure of the common carp *Cyprinus carpio* to sublethal concentration of endosulphan showed that fish responded with decrease in growth rate, survival ratio, levels of haemoglobin and haematocrit, significant elevation in blood glucose and little variation in the serum protein (Chandrasekar and Jayabalan, 1993). Several studies have documented the importance of micronutrients, zinc deficiency in fish leads to physiological perturbation of growth, reproduction, vision, and immunity (Eisler, 1993; Watanabe et al., 1997; De Schamphelaere and Janssen, 2004; Shukla and Pandey, 2006). Various studies reported that sublethal concentrations
of zinc over a long-term exposure had an inhibiting effect on the growth of guppies *Poecilia reticulata* (Crandall and Goodnight, 1962), fathead minnow *Pimephales promelas* (Brungs, 1969), minnow *Phoxinus phoxinus* (Bengtsson, 1974), rainbow trout (Watson and McKeown, 1976), and freshwater murrel, *Channa punctatus* (Shukla and Pandey, 2006). Mohanty *et al.* (2009) reported the effect of waterborne zinc on survival, growth, and feed intake of Indian major carp, *Cirrhinus mrigala* (Hamilton). Tawwab *et al.* (2013) also investigated the changes in growth and biochemical status of common carp, exposed to sublethal water-born zinc toxicity and reported that the growth performance was reduced significantly with increasing Zn concentrations revealing deterioration of fish health by Zn toxicity.

Jayakumar (2000) studied imidacloprid impact on growth and pattern of accumulation in the body tissue of *Clarias batrachus* and investigated lesser growth and significant variations in the patterns of accumulation of imidacloprid. Firstly, the exposed fish rejected the food provided, after that they started consuming the food and gradually resumed feeding activity to near normal situation. Reduction in survival and growth rate with significantly shorter body length and eye diameters in developing zebrafish on exposure of pesticide malathion was reported by Cook *et al.* (2005). The results revealed that malathion had teratogenic effects on zebrafish embryos. According to Petri *et al.* (2006) the toxic effects of organochlorines pesticide endosulfan includes reduced growth, lesser weight gain, low specific growth rate as well as decrease in haematological and biochemical parameters like total erythrocyte count, haemoglobin concentration, total protein content and a rise in total leucocyte count and glucose level confirming a direct risk of the presence of organochlorine pesticides in water column to the biota therein. Reports on the exposure of sublethal doses of dimethoate and malathion on Nile tilapia (*Oreochromis niloticus*) suggest that exposed fish showed reduced growth performance, survival rate, haematological parameters and total production. The intensity of reduction in growth, haematology and total production was found related to toxicant concentration and time dependent (Sweilum, 2006). Afzal *et al.* (2007) evaluated growth performance in bighead carp (*Aristichthys nobilis*) exposed to organic and inorganic fertilizers in relation to the temperature and found that the mean weight gain of bighead carp was significantly higher from other Chinese and major carps, Similar trend was observed between *Labeo rohita* and *Cirrhinus mrigala*,
whereas growth rate in *Catla catla* was found to be significantly higher than other major carps.

Yaji and Auta (2007) studied the effects of sub lethal effects of *monocrotrophos* on growth and food utilization of african catfish *Clarius gariepinus*. Various growth alterations observed in the fish were decreased weight gain, specific growth rate, gross feed conversion efficiency (GFCE), feed efficiency (FE), protein efficiency ratio (PER) and nitrogen metabolism (NM) in exposed fish while feed conversion ratio (FCR) increased with increasing concentration of the toxicant and also opined that these parameters provide an integrated measure of the health condition of the organism. Diazinon exposure has been shown to be able to inhibit brain cholinesterase acivity and growth parameters of snakehead fish (*Channa striata*) and showed a significant reduction in the growth and brain acetylase activity with increase in the sublethal dose of diazinon (Cong et al., 2009). Boss (2011) observed the effect of thiamethoxam on the growth and metabolism of liver total protein of exotic fish *Oreochromis niloticus* (Trewavas). The study revealed that various sublethal doses of thiamethoxam had significant impact on growth and liver total protein of this fish. Mean weight of the fish was gradually decreased with the increase of doses of Thiamethoxam. Length gain and breadth gain also showed a gradual decrease in measurement in comparison to the control fish. A significant decrease in the protein level was observed with the increase in the doses of pesticide. Huynh (2012) opined that both the growth and hepatostomatic index were affected in Australian catfish, *Tandanus tandanus*, exposed to sublethal doses of chlorpyrifos. Reduced growth in terms of live weight gain, gain per day (%), specific growth rate (%) and higher hepatostomatic index followed by lesser survival ratio. The intensity of the retarded growth and higher HSI of fish was found to be related to toxicant concentration and time dependent. Similar results were also reported by Sancho et al. (1997) evaluating the effect of pesticides fenitrothion in eel. Further, the work of Shalaby (2012) showed the toxic effects of EDTA on growth of Nile tilapia (*Oreochromis niloticus*) and reported reduction in growth rate and feed utilization however, these parameters were improved when EDTA was applied along with Cadmium. The study showed that the addition of EDTA to cadmium contaminated media, reduced significantly the cadmium level in water and helped to eliminate cadmium from the fish body, which in turn improved the growth, haematological and
biochemical parameters as compared to fish exposed to cadmium alone. Xing et al. (2012) investigated the growth performance, oxidative stress responses and histopathological changes in the liver and gill of common carp after exposure to chlorpyrifos and atrazine, alone or in combination and reported reduced growth, decreased feed intake, decrease in antioxidant enzyme (SOD, CAT and GSH-Px) activities and increase in MDA content in a dose-dependent manner in the liver and gill of common carp. Growth and survival of juveniles of Pacific coho salmon smolts to pesticides within urban streams in Western Washington USA was studied and the results inferred that exposure to pesticides in urban streams does not directly impair early life stages of coho salmon (King et al., 2014). Muralidharan (2014) investigated chronic toxicity studies on proximate composition of *Cyprinus carpio* exposed to fenthion and a significant decrease in glycogen, protein and increase in lipid and moisture contents were observed.

### 2.3 Haematological Parameters

Haematological characteristics are tools for screening pathological status. The haematological parameters constitute a good indicator of physiological responses (Blaxhall, 1972; Koundinya and Ramamurthi, 1979; Sharma and Gupta, 1984; Thakur and Pandey, 1990). Many reports have been published on the toxic effects of some pesticides on haematological parameters of several fish species, dichlorvos in *Cyprinus carpio* (Svobodava, 1975), ekolux organophosphorus preparation on *Oreochromis mossambicus* (Sampath, 1995), formothion (Singh and Srivastava, 1994), methylparathion based pesticide, and cypermethrin (Nath, 1993) in *Heteropneustes fossilis*, malathion in *Cyprinion wabsoni* (Khattak, 1996) and trichlorphon in *Piaractus mesopotamicus* (Tavares et al., 1999).

A significant decrease in red blood cell count, haemoglobin (Hb) content and packed cell volume (PCV) has been observed in fish exposed to different pesticides (Koundinya and Ramamurthi, 1979) and such decreasing effect has been primarily attributed to a condition of hypochromic microcytic anemia (Bhai and Nath, 1971; Raja Rishi, 1986). Shankar (1975) reported a significant increasing trend in the number of white blood cell (WBC), in catfish exposed to sublethal concentration of phosphamidon. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin
concentration (MCHC) showed appreciable decrease in exposed fish. Padmaja et al. (1985) and Ghosh and Chatterjee (1989) conducted studies on the impact of sublethal dose of nuvan on snail *Bellamya dissimillis* and fish *Channa punctatus*. Major alterations included significant decrease in the haematological parameters. These results were found to be similar to the study of various workers (Borthwick *et al*., 1985; Mayer and Ellersieek, 1986; Tilak *et al*., 2004). Effects of exposure on the blood chemistry of fish include decreased arterial oxygen, haemocrit and haemoglobin levels (Bradbury, 1991). Similarly, the study of impact of toxicant on *Cyprinus carpio* resulted in alterations in the values of total blood ammonia content and total erythrocyte counts (Jeney *et al*., 1992). With increase in the concentration of toxicant, total ammonia content, haemoglobin and serum protein content were found to reduce and total glucose levels were found to increase. Likewise, Svobodova *et al*.* (1994) reported significant increase in the erythrocyte count and haematocrit value in carp after toxic exposure to organophosphorus pesticides. The noticed necrotic changes and alterations were increased volume of mean corpuscular haemoglobin concentration (MCV) and a decreased level of mean corpuscular haemoglobin concentration (MCHC). Impairment to physiology and health status of fishes was disturbed at cellular levels has been observed by various workers (Tripathi *et al*., 2003; Banaee *et al*., 2008; Wang *et al*., 2009; Banaee *et al*., 2011; Banaee, 2012). They inferred these changes were due to the alteration in the haematology and biochemistry of the fish.

Long-term exposures to sub-lethal diazinon concentrations had adverse effects on different biological and physiological aspects of fish. Balini *et al*.* (1995) observed an increase of glucose in common carp after exposure to deltamethrin. The findings of Svoboda *et al*.* (2001) while working on common carp exposed to sublethal concentration of diazinon revealed lower values of erythrocyte count (RBC), haemoglobin content (Hb) and haematocrit (PCV), MCV, MCH, MCHC, leucocyte count whereas developmental forms of neutrophile granulocytes, myelocytes and metamyelocytes were found to increase in count. Atamanalp *et al*.* (2002) found changes in the concentrations of calcium and phosphorus in rainbow trout (*Oncohynchus mykiss*) following cypermethrin exposure. Likewise, Das *et al*.* (2004) suggested that both haematological and biochemical parameters were affected in fingerlings of *Cirrhinus mrigala* exposed to sublethal concentration of nitrite (NO$_2^-$).
Various alterations observed were decrease in total erythrocyte count, total leucocyte count, decrease in haemoglobin, increase in blood glucose and decrease in serum proteins. Similarly, lower count of erythrocyte, leucocyte, haemoglobin, haematocrit and other haematological indices as MCV, MCH and MCHC values in *Cyprinus carpio*, on exposure to diazinon were also reported by Koprucu *et al.* (2006). Swelium (2006) observed the effects on sublethal doses of dimethoate and malathion on haematological properties of Nile tilapia and results showed decrease in total erythrocyte count, haemocrit value and haemoglobin content with increasing concentration of pesticides. In addition, serum protein was found to decrease while values of serum glucose and lipid increased. Abo-Hegab and Kamel (1987) reported the same results and were supported by Mourad and Abd-Allah (1999) on *Tilapia zillii* exposed to organochlorines pesticide lindane. Velisek *et al.* (2006) opined a significant increase in AST level in carp after acute exposure to the deltamethrin.

El-Sayed *et al.* (2007) noted the haematological and biochemical changes in the fish, *Oreochromis niloticus* after exposure to acute and subacute concentrations of deltamethrin. From their findings, they inferred increased total leucocytes cell count, low serum protein, albumin and globulin levels. Changes in haematology and biochemistry in other fish with the impact of cypermethrin have been studied by various workers (Das and Mukerjee, 2003; Jee *et al.* 2005). Likewise, Saravana (2007) reported the significant decrease in the values of haemoglobin, haematocrit, RBC and increase in WBC count. The haematological indices like MCV, MCH, MCHC were found to decrease with increase in plasma glucose level and decreased plasma protein level. Rodrigues *et al.* (2007) studied the acute toxicity of ammonia and nitrite to juvenile cobia (*Rachycentron canadum*) and observed ammonia could be problematic at relatively low levels for the intensive rearing to juvenile cobia. The significant decrease in haematological indices including MCH, MCHC and MCV have been observed in *Carassius auratus* to intraperitoneal injection of microcystins with the possible mechanisms of anemia by Zhang *et al.* (2007). Velisek *et al.* (2008) evaluated the biochemical and haematological effects in common carp exposed to bifetethrin and the results showed significantly higher values of plasma glucose, ammonia, aspartate aminotransferase and creatinine kinase as well as monocyte count in exposed fish when compared to the control. Tilak and Kumar (2007) worked on the toxic effects of Nuvan
to the grass carp Ctenopharyngodon idella using static and continuous flow through 96 h and reported reduction in oxygen consumption when the fish were exposed to the toxicant. Similarly, the studies of Saravanan et al. (2011) inferred a significant decrease in entire haematological parameters including haemoglobin, haemocrit, red blood cell, MCV, MCH and MCHC while an increase in WBC count was observed along with the elevated levels of plasma glucose and protein levels when the fish were exposed to neem leaf extracts.

On exposure of hybrid catfish to sublethal concentration of tobacco (Nicotiana tobaccum) leaf dust, Adamu et al. (2011) reported the significant differences in albumin, glucose, triglycerides, cholesterol, urea and uric acid of serum, liver and kidney. Also, the effects of the plant dust on test fish was dose dependent. Narra et al. (2014) reported the sub lethal effects of chlorpyrifos on the food fish Clarius batrachus and reported the sublethal concentrations of chlorpyrifos on protein metabolism in gills, kidney, liver, and muscle. Total protein, amino acid, and ammonia contents were decreased and recovery was observed. Urea and glutamine levels were elevated, except in kidneys, and recovered at the end of the exposure period. Stalin and Das (2012) opined the impact of incipient lethal level of fenthion on brain, gill, intestine and liver and observed decrease in glycogenesis in liver and intestinal tissue. Maximum decrease in protein levels were observed in brain tissue in Nile tilapia. The experiment was conducted by Prabhakar et al. (2012) to analyze the toxic effects of cadmium containing pesticides on biochemical parameters of fresh water fish Cirrhinus mrigala and investigated the effect on histology of gill, liver, kidney and found significant decrease in glycogen content in liver, gill and kidney. Similar trend was found in the decrease of total protein contents. Similar alterations in haematological and biochemical changes in Catla catla fingerlings exposed to cypermethrin were observed by Vani et al. (2012). They reported that the fingerlings under exposure showed decrease in total erythrocyte count, TLC, haemoglobin content and haematocrit. All the serum parameters viz., total serum, protein, albumin, globulin contents and albumin globulin ratio were also found to decrease with increase in the dose of the toxicant. The similar findings were reported when different species of fish were exposed to cypermethrin i.e., in Cyprinus carpio (Dorucu and Girgin, 2001), Labeo rohita (Das and Mukherjee, 2003), Korean rockfish Sebastes schlegeli (Jee et al., 2005). Banee et
Veeraiah et al. (2013) performed the biochemical diagnosis induced by cypermethrin to the fresh water fish *Cirrhinus mrigala* (Hamilton). The fish were exposed to lethal and sublethal concentration of the pesticide for 96 h and the biochemical changes of total glycogen, total protein, and nucleic acids, were estimated in the tissues of fish exposed to the toxicant. A continued decrease in total glycogen, proteins and nucleic acids were observed with the increase in the period of exposure. Rauf and Arain (2014) determined the effect of acute toxicity of diazinon on haematological parameters in the Indian Carp, *Cirrhinus mrigala* and revealed that after 96 h, fish exhibited lower values of all the haematological parameters except MCHC which remained the same. These findings were consistent with the findings of some other authors who studied the responses of the others fishes exposed to malathion in *Cyprinodon watsoni* (Khattak et al., 1996), formothion in *Heteropneustes fossilis* (Singh and Srivastava, 1994), trichlorfon in *Piarctus mesopotamicus* (Tavares et al., 1999), chlorpyrifos in *Cyprinus carpio* (Ramesh and Saravaran, 2008), and phoslone in *Oreochromis mossambicus* (Jha and Rani, 2009). Similarly, lowering in values of entire haematology except MCHC after acute exposure to diazonin have been reported in fingerlings of European catfish *Silurus glanis* (Koprucu et al., 2006), male brood stock *Rutilu*, African cat fish *Clarias gariepinus* (Adedeji et al., 2009; Shamoushaki et al., 2012), common carp *Cyprinus carpio* (Svoboda et al. 2001) and European catfish *Silurus glanis* (Rao, 2010). Banee et al. (2014) reported about altered biochemical parameters of freshwater fish, *Alburnus mossulensis* exposed to the sub lethal concentration of fenthopathrin and reported that fish in toxic media showed elevation in the biochemical parameters. Sudden increase in AST, ALP, LDH, lipid peroxidation and MDA levels was observed in the study. CAT and ALT activities were found to decrease in toxic environment.

Richterova et al. (2014) determined the effects of cyhalothrin based pesticide on early life stages of *Cyprinus carpio*, and reported that larvae died soon after hatching and histological examinations of livers of larvae revealed dystrophic changes. The values of intoxication enzyme GST were significantly higher. The results of
investigations confirmed that contamination of aqueous environment by pesticides containing cyhalothrin may impair growth and development of early life stages of carp and cause disturbance in haematology and biochemistry of cells as well as disbalance of defensive enzymes. Mariappan and Karuppasamy (2014) studied the alterations of biochemical parameters as total glucose, total protein, acid and alkaline phosphate enzyme activities in gill, liver and kidney tissue of freshwater fish *Cyprinus carpio* exposed to sublethal concentration of binary mixture of copper and cadmium for short term and long term and reported about the ACP and ALP activities with increase in exposure time.

2.4 Chromosome aberration Assay

In India, the work on the fish chromosome was initiated by Sharma *et al.* (1960). Thereafter, Nayyar (1962, 1964, 1965 and 1966) published more data on Indian fish species. Since then, a large number of fish species have been analyzed cytogenetically by various workers (Manna and Parshad, 1971a,b; 1973a,b; 1974; 1977; Prasad and Manna, 1971, 1974; Chatterji and Majhi, 1974, 1975; Patro and Prasad, 1979; Sharma and Tripathi, 1981 a,b; Sharma and Aggarwal, 1978, 1980, 1981; Khuda Baksh *et al*., 1980, 1995; Rishi, 1971, 1973 a,b; 1975, 1976 a,b, 1978a,b 1979, 1980, 1981 a,b; Rishi and Singh, 1982; Rishi and Haobam, 1987,1990; Rishi and Shashikala, 1994; Rishi *et al*., 1977,1993a,b; Bhatnagar *et al*., 2014).

Alex Fraser (1966) opined various types of chromosomal anomalies in fish exposed to metal ions. Chromosomes of some cells were so fragmented that it seems nearly impossible for them to undergo a normal mitosis. Tsio (1970) and Tsoi *et al.* (1975) observed that dimethyl sulphate and nitrosomethyl induced chromatid and chromosome breaks in fish *Puntius puntius*. Chromosomes of *Cirrhinus mrigala* have been studied by Manna and Prasad (1971a) and Zhang and Reddy (1991) and reported 50 number of normal diploid chromosomes in fish species. Kligerman *et al.* (1975) reported an increase in the frequency of aberrant cells (Chromatid gaps, breaks) in fish after exposure to X-radiations. Longwell (1976) observed that eggs of Atlantic Mackereomatid exchangel, collected from highly polluted area, were abnormal and observed disoriented spindles, chromosome stickiness, breakage and loss. Klingerman and Bloom (1976) demonstrated the usefulness of *Umbra limi* as a test model for
chromosome aberration test. Further, Klingerman (1979) observed a highly elevated rate of SCE (Sister Chromatid Exchange) in fish tissue after exposure to methylmethane sulphonate, cyclophosphamide, neutral red. Prein et al. (1978); Alink et al. (1980) and Hoofman and De Raat (1981) studied chromosome aberration in *Notobranchinus rachowii* to study chromosome aberration induced by ethyl methane sulphonate and benzopyrene. Also, chromosomal aberrations in some cichlid species due to the effect of radiations were reported by Manna and Som (1982) and Som and Manna (1984).

Many water-borne pollutants have cytogenetic properties which cause enhanced frequency of chromosomal aberrations in fish (Kligerman et al., 1975; Prien et al., 1978; Kligerman, 1979; Barker and Rackam, 1979; Alink et al., 1980; Sofradzija et al., 1980; Refstie, 1981; Landolt and Kocan, 1983; Al-Sabti et al., 1984). Several authors (Das and Nanda, 1986; Al-Sabti and Metcalfe, 1995; Kushwaha et al., 2003) reported genotoxic effects in different species of fish using cytogenetic analysis. Exposure of fish to pollutants and toxicants for prolong period, even at low levels, leads to chromosomal aberrations including gene changes (Barker and Rackham, 1979). Chromosomal studies revealed a measure of sublethal effects of xenobiotics in vivo. Lakra and Rishi (1991) had reported that chromosomal study of 175 fresh water fish species. Similarly, Mathew and Srinivash (1999) investigated various chromosomal aberrations in fish exposed to lead nitrate. Besides the chromosome damage due to the penetration of pollutants into the fish body, retarded growth and population depletion of fishes was also observed. Similarly, retarded growth with consequent population depletion due to the genetic damage was also noticed earlier by Devi (1988). Genotoxic effects of some pesticides and other pollutants on mammals and fish using chromosomal aberration and sister chromatid exchange has been observed by various workers (Rishi and Grewal, 1995; Arockia-Rita and Selvanayagam, 1998; Chauhan et al., 2000; Devi et al., 2001; Aboul, 2002; Poli et al., 2003; Saxena and Rana, 2005; Ali et al., 2008 and Yadav et al., 2010) and have reported about various types of chromosomal aberrations. Magtoon and Arai (1990) opined the karyotypes and distribution of Nucleolus Organizer Regions in cyprinid fishes from Thailand and reported that diploid chromosomes number of *Cirrhinus jullieni*, *Cirrhinus mrigala*, *Osteochilinus waanderni*, *Cycloheilichthys enoplos*, *Labeo rohita* were same, 2n= 50,
Tor sorotozysron \(2n=100\) and Puntioplies proctozyron \(2n=76\) chromosomes and the karyotypes were reported for first time excepting for Cirrhinus mrigala (Rishi, 1981) and Labeo rohita (Gui et al., 1986) and localization of the NORs were also reported for the first time. The study of various authors (Hayashi et al., 1998; Hose and Brown, 1998; Amanuma et al., 2000; Diekmann et al., 2004) on genotoxic effects of pollutants in early developmental stages of fish and inferred highest number of aberrant metaphases on exposure of highest doses of cyanobacterial extract as well as microcystins. Anitha et al. (2000) evaluated the genotoxic effects of heat shock by using chromosomal aberration assay at different temperatures on gold fish, Carassius auratus. The results showed various types of chromosomal anomalies in exposed cells. Also, analysis of toxicity of synthetic pyrethroids using chromosomal aberration and sister chromatid test has been observed by various workers (Saxena and Seth, 2002; Seth and Saxena, 2003; Sirohi and Saxena, 2006; Saxena and Sirohi, 2007) and reported that these chemicals were highly toxic to fish because these compounds were absorbed strongly by the gills even at very low concentration in water due to their high lipophilicity. Cestari et al. (2004) evaluated the genetic damage induced in the neotropical fish Hoplias malabaricus (Characiformes, Erythrinidae) exposed to various doses of lead by using the comet assay and chromosomal aberrations. The exposed fish showed significant increase in frequency of chromosomal aberrations in metaphase spread and increase in frequency of tailed cell nuclei indicating DNA damage. This study was confirmed in H. malabaricus, which has a low diploid number and relatively large, biarmed chromosomes, but no heteromorphic sex chromosomes.

Various authors dealt with genotoxicity of fertilizers containing cyanotoxins using chromosomal assay for \textit{in vitro} studies on mammalian cell lines (Rao et al., 1998; Humpage et al., 2000; Žegura et al., 2003; Fessard and Bernard, 2003; Lankoff et al., 2003; Lankoff et al., 2004; Lankoff et al., 2006), in bacterial cells (Ding et al., 1999; Mankiewicz et al., 2002) and \textit{in vivo} using mice and brown rats (Rao and Bhattacharya, 1996; Rao et al., 1998; Shen et al., 2002; Bouaicha et al., 2005). Kumari and Ramkumaran (2006) reported the cytogenetic changes in an air breathing fish, Channa punctatus inhabiting in the polluted water of Hussainsagar lake. The fish showed normal number of chromosomes (2n=32) consisting of 14 metacentric, 8 submetacentric, 6 subtelocentric and 4 acrocentric which is in full agreement with the
findings of Manna and Som (1982) but exhibited chromatid exchanges, dicentric and ring type of chromosomes. Mathew and Srinivash (1999) also reported similar chromosomal aberrations in fish exposed to lead nitrate. Besides the chromosome damage due to the penetration of pollutants into the fish body, retarded growth and population depletion was recorded in the fish species. Retarded growth with consequent population depletion was also noticed earlier by Devi (1988). Palikova et al. (2007) reported the chromosomal aberrations in early embryonic stages of weather fish (Misgurnus fossilis L.) exposed to crude cyanobacterial and semipurified compound of microcystins and reported that the highest number of aberrant metaphases was recorded after the application of the highest concentrations of cyanobacterial extract and microcystin. Changes in the genetic material caused by genotoxins using chromosomal aberration test has also been studied by Saxena and Chaudhary (2010). Their study further revealed that these compounds were highly toxic to fish and other aquatic invertebrates whereas these were moderately toxic to mammals (Eisler, 1993). Further, Saxena and Chaudhary (2010) conducted genotoxic trial by exposing Channa punctatus to synthetic pyrethroid fenvalerate by using chromosomal aberration test and inferred that exposed fish exhibited normal diploid cells in both sexes with no sex chromosome. Changes in the structure of chromosomes, such as decrease in length, increase in breadth and over all compression in size was observed. Different chromosomal abnormalities such as chromatid break, fragment, gap, chromatid separation, deletion and ring type chromosomes were observed. These observations were in agreement with the earlier data available (Rishi and Haobam, 1990; Rishi and Grewal, 1995; Saxena and Rana, 2005). Anbumani and Mohankumar (2011) investigated the nuclear and cytoplasmic abnormalities in the fish Catla Catla (Hamilton) exposed to chemicals and ioning radiations and reported the rings, chromosomal breaks, chromatid gaps, nuclear buds, lobed nucleus, and nuclear pycnosis.

Mahboob et al. (2013) investigated the genotoxicity of mercuric chloride to freshwater Clarias gariepinus using Chromosomal aberrations and sister chromatid exchange. Mercuric chloride was earlier reported as a novel compound to cause toxicity in the kidney of the teleosts (Nagarani et al., 2012). Karim et al. (2013) investigated the effect of oxytetracycline and florifenicol on the chromosomal structure and micronuclei induction in RBC’s of Nile tilapia (Oreochromis niloticus) and
reported the induction of micronuclei and certain types of chromosomal anomalies as gaps, breaks, deletions, fragmented and centromeric attenuation in the chromosomes of exposed fish. Kaur et al. (2013) assessed chromosomal aberration as an index of dyeing industry effluent in *Cirrhinus mrigala* and reported exposed fish showed various erythrocyte abnormalities as Nuclear Extrusion (NE), Blebbed (B), Binucleate (BN), Lobed (L), Notched (N) nuclei and cellular abnormalities included Enucleated (EnC), Vacuolated (VC), Deformed (DC), Echinocytic (EC), Spindle shaped (SC) and Apoptotic (AC) Cells. Erythrocyte abnormalities indicated that effluent induced clastogenic effects on the erythrocytes of *Cirrhinus mrigala* and may have similar effects on the human population located around the river and consume fishes.

Yadav et al. (2013) evaluated the aberrations in chromosomes of *Cirrhinus mrigala* (Hamilton) exposed to butachlor. The exposed fish exhibited normal diploid number of chromosomes (2n=50) from the somatic cell along with various types of chromosomal aberrations *i.e.*, stickiness, clumping and end to end joining. Frequencies of these revealed a significant (P<0.05) time dependent response. Srivastava and Singh (2013) reported the induction of chromosomal aberrations by carbamate fungicide in fish *Clarius batrachus* (Asian Catfish). Incidence of chromosome aberrations (CA) was positively correlated with duration of exposure. The most common abnormalities were categorized as chromosome and chromatid break, acentric fragments, chromatid and sub-chromatid exchanges, chromatid gaps (achromatic lesions), heterochromatic regions and sister chromatid exchanges at metaphase and also chromatid and chromosome bridges and side arm bridges and fragments at anaphase. Chromosome breaks, fragments, chromatid exchanges and dicentric chromosomes were generally consider as unstable aberration while deletion, inversions, duplications and translocations were considered as stable aberrations.

### 2.5 Genotoxicity studies

Genotoxic pollution of aquatic ecosystem refers to the introduction of contaminants with mutagenic, teratogenic, and/or carcinogenic potentials into its principal media and genome of the resident organisms (Fagr et al., 2008). Genotoxicity is a deleterious action, which affects a cell's genetic material affecting its integrity (Smith, 1996). Genotoxicants include certain chemical compounds like heavy metals
(Matsumoto et al., 2005; Igwilo et al., 2006), microbial toxins (Smith, 1996), and polycyclic aromatic hydrocarbons including pesticides (Fernandez and L'Haridon, 1992; Germain et al., 1993). These genotoxicants have been reported to cause mutations because they form strong covalent bonds with the DNA, resulting in the formation of DNA adducts preventing accurate replication (Hartwell et al., 2000). Since there is a growing concern over the presence of genotoxins in the aquatic environment, the development of sensitive biomarkers for detection of genotoxic effects in aquatic organisms has gained importance (Hayashi et al., 1998). The genotoxic effects of environmental pollutants can be monitored using a broad range of both in vitro and in vivo biomarker assays, but the comet assay is gaining popularity over others since its advantages include sensitivity for detecting low levels of DNA damage (Gedic et al., 1992) and the short time needed to complete a study. The micronucleus (MN) assay is another useful and popular technique for showing clastogenic and aneugenic effects (Norppa and Falck, 2003) and has been extensively used in situ (Al-Sabti and Metcalfe, 1995). Several studies have shown that the MN test and comet assay (CA) are the two sensitive, rapid, and extensively used methods in the detection of mutagenicity and genotoxicity of chemicals and xenobiotics under field and laboratory conditions (Ateeq et al., 2002; Ateeq et al., 2005; Abdul-Farah et al., 2003; Cavas and Ergene-Gözükara, 2005; Pandey et al., 2006; Sharma et al., 2007; Ergene-Gözükara et al., 2007; Nagpure et al., 2008; Ventura et al., 2008; Xiao et al., 2008; Ali et al., 2008; Ali et al., 2009; Nwani et al., 2010).

Several authors dealt micronucleus test to assess the frequency of micronucleated cells in the fish mud minnows Umbra pygmaea exposed to ethyl methane sulphonate under laboratory conditions (Hooftman and De Raat, 1981), likewise micronuclei test was performed in amphibians such as Pleurodeles waltl, Ambystoma mexicanum and Xenopus laevis (Fernandez et al., 1993) and tadpoles of the anurans Rana catesbeiana and Caudiverbera caudiverbera (Krauter et al., 1987; Venegas et al., 1987). Al-Sabti (1986) tested several chemicals (aflatoxin B1, Arochlor 1254, benzidine, benzo(a)pyrene and 20-methylcholanthrene) for their ability to induce micronuclei under laboratory conditions in three cyprinids (common carp, Cyprinus carpio; tench, Tinca tinca; grass carp, Ctenopharyngodon idella). Das and Nanda (1986) opined an increase in MN frequency in the erythrocytes of the common Indian
catfish (*Heteropeustes fossilis*) exposed in the laboratory to mitomycin C and to paper mill effluent. Report on induction of MN in the erythrocytes of mudminnows (*Umbra limi*) and brown bullheads (*Ameiurus nebulosus*) when exposed to ethyl methanesulphonate (EMS) and benzo(a)pyrene (BaP) were given by Metcalfe (1988). Morbidity among employees engaged in the manufacture or formulation of chlorpyrifos was studied by Brenner *et al.* (1989). The study showed that buccal cells of the subjects had various cell anomalies as micronuclei induction and broken eggs. Schultz *et al.* (1993) made investigation on genotoxicity in the erythrocytes of rainbow trout (*Oncorhynchus mykiss*) exposed to X-ray. Micronuclei and other related anomalies as broken egg, multiple micronuclei, binucleated cell were observed to increase after each exposure of X-ray. Al-Sabti *et al.* (1994) investigated the cytogenetic effects of chromium (Cr [VI] and Cr [III]) in prussian carp (*Carassius auratus gibelio*) using the erythrocyte MN assay and reported similar results. Again, Al-Sabti and Metcalfe (1995) investigated the clastogenic effects of various chemical and physical agents on fish cells, with emphasis on the induction of micronuclei in teleosts and the peripheral erythrocytes of fish showed a high incidence of micronuclei after exposure to different pollutants. These findings were in agreement to the previous reports of various authors (Das and Nanda, 1986; Cross and Hose, 1986; Hose *et al.*, 1987; Metcalfe, 1988; Al-Sabti and Hardig, 1990; Hughes and Hebert, 1991; Ueda *et al.*, 1992; Schultz and Norgren, 1993).

The work on the assessment of genotoxicity of chlorpyrifos was started in the 1995 when Gollapudi *et al.* (1995) reported the genetic toxicity of chlorpyrifos in some mammals along with fishes. The organisms were exposed to various sub-lethal doses of chlorpyrifos and the result showed micronuclei induction, chromosomal anomalies only in the highest available concentration. Similarly, lethal toxicity of monocrotophosphos on the juveniles of rohu and mrigala were inferred by Sulekha *et al.* (1999). Studies on the effect of malathion and phosphamidon on the cultivable species of carps in the paddy fields using genotoxic tests have been done by many scientists. Ayllon and Garcia-Vazquez (2000) reported the induction of micronuclei and other nuclear abnormalities in European minnow *Phoxinus phonixus* and mollie *Poecillia latipinna*. Gustavino *et al.* (2001) reported dose dependent increase in the micronucleus frequency in fish *Cyprinus carpio* exposed to X-rays and colchicine. The exposed fish showed higher
number of micronuclei induction. The count of MN also served as an index of chromosome breaks and mitotic spindle dysfunction (Bombail et al., 2001). Ateeq et al. (2002) reported about the induction of micronuclei and erythrocyte alterations in the catfish *Clarius batrachus* exposed to 2, 4-dichlorophenoxyacetic acid and butachlor. A wide range of altered cells, pyncnotic and granular micronuclei (MN) were observed in the experimental fish. Schimmel et al. (2002) reported the acute toxicity, bioconcentration, and persistence of benthio carb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment. Evaluation of the relative hazards of these chemicals to aquatic environments showed genotoxic damage to the aquatic vegetation and organisms. Similarly, the micronuclei and comet assay were also used to evaluate the genotoxic effects of pyrethroid insecticide lambda-cyhalothrin on micronuclei induction in *Rana catesbeiana* tadpoles. The effects were found to be concentration and exposure time dependent (Campana et al., 2002). This result was also found similar in four species of fishes exposed to various pesticides observed by Bolognesi et al. (2003).

Lee and Steinert (2003) reported the use of single cell gel electrophoresis for detecting DNA damage in aquatic animals and recommended it as most suitable way to assess the DNA damage in aquatic organisms. The study of Freeman and Rayburn (2004) showed that atrazine has no genotoxic effects on anuran larvae whereas Srivastava and Mishra (2009) worked on the toxic effects of commercial formulation atrazine (Gesaprim) on *Allium cepa* and *Vicia faba* test systems and reported abnormalities like micronucleus formation, chromosome aberrations, and mitotic aberrations. Similarly, Bolle et al. (2004) observed significant increases in genotoxic damage in *Allium cepa* treated with atrazine. Koprucu and Aydin (2004) studied the toxic effects of pyrethroid deltamethrin on the common carp embryos and the larvae and reported more number of dead larvae in increased dose of pesticide. Whitehead et al. (2004) evaluated the genotoxicity in native fish in ponds associated with agricultural runoff events. The exposed fish supported a linkage between induction of DNA strand breakage and timings of agricultural runoff. Mamaca et al. (2005) studied the styrene impact on mussels (*Mytilus edulis*) and fish (*Symphodus melops*) and reported DNA damage assessed by comet and lysosomal assay. Significant biological responses were observed in the studied period in both organisms with these two tests. Wong et al.
reported about the comet assay as a biomonitoring tool for nutraceutical research. Bolognesi et al. (2006) opined the assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Chromosomal damage was determined as micronuclei (MN) frequency in fish erythrocytes. Nuclear anomalies such as blebbed, notched and lobed nuclei were also recorded. Significant increase in MN frequency was observed in erythrocytes of fish exposed to bisphenol A and tetrabromodiphenylether. Donatella et al. (2006) observed the genotoxicity of pesticide treated vegetables extracts using chromosome aberration and micronucleus tests for monitoring mutagens in edible vegetables. The exposed extracts showed various anomalies in twenty one species of vegetables. Zeljezic et al. (2006) opined that the atrazine was not genotoxic or capable of inducing apoptosis or necrosis in human lymphocytes whereas Gesaprim treatment showed significant increase in DNA damage. Herbicide butachlor and 4-nitrophenol were also reported toxic to freshwater fish *Channa punctatus* (Bloch) by viewing more frequency of micronuclei induction by Tilak et al. (2007). Some changes in the procedures of comet assay in the fourth International Workgroup on Genotoxicity testing were suggested by Burlinson et al. (2007). Cavas and Konen (2007) observed the cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to glyphosate formulation using micronuclei and comet assay and revealed significant dose dependent increase in the frequencies of micronuclei, nuclear abnormalities as well as DNA strand breaks. The formation of morphological nuclear abnormalities (NAs) was also described in fish erythrocytes by Carasso et al. (2006). Gautam and Gupta (2008) reported about the toxicity of cypermethrin in the juveniles of freshwater fish *Poecilia reticulate*. Kirkland et al. (2008) reported about the recommended lists of genotoxic and non-genotoxic chemicals for assessment of the performance of new or improved genotoxicity tests and categorized the chemicals in four groups as highly genotoxic, moderately genotoxic, toxic and non-genotoxic. Nagpure et al. (2008) performed comet assay for genotoxicity assessment in fishes from Gomati River and the results indicated DNA damage in the cells of fish exposed to various pollutants in river water when compared with the fish kept in clean and fresh water.

Srivastava and Rao (2008) reported the acute and genotoxic studies of carbaryl in an Indian Major Carp, *Labeo rohita* (Ham.) using micronuclei and comet assay. The
MN frequency and DNA damage observed was found time and dose dependent. This work was also related to the study of Konen and Cavas (2008) who observed the genotoxic effects of the active substance trifluralin on fish *O. niloticus* using MN and comet assay and reported similar increase in DNA damage and higher frequency of micronuclei induction with time and dose. Ali *et al.* (2009) studied the genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* using MN and alkaline single cell gel electrophoresis. They reported that short as well as long term exposure to chlorpyrifos and cause the induction of micronuclei and DNA damage. The frequency of micronuclei induction and DNA damage was found to be related to toxicant time and concentration dependent.

Nwani *et al.* (2009) reported the mutagenic and genotoxic effects of carbosulphan in freshwater fish *Channa punctatus* (Bloch) using MN and alkaline single cell gel electrophoresis. The results revealed that the MN induction was highest on 96 h at all the concentrations in the peripheral blood. Similar trend was observed for the DNA damage measured in terms of the percentage of tail DNA in the erythrocyte and gill cells. The findings of the study were also similar to the findings of Nagarani *et al.* (2009). They reported the genotoxicity assessment of mercuric chloride in the marine fish *Therapon jarbua* using micronuclei and comet assay. Evaluation of genotoxic potential of herbicide glyphosate in erythrocytes of broad-snouted caiman (*Caiman latirostris*) after *in vivo* exposure and reported the significant increase in DNA damage was observed at higher concentration of toxicant (Polleta *et al*., 2008). Nwani *et al.* (2010) made genotoxic studies using comet and micronuclei test on fresh water fish *Channa punctatus* (Bloch) exposed to various sublethal concentrations of carbosulphan and reported higher frequency of MN induction and large sized tail DNA in case of fishes exposed to higher dose of pesticide when compared to the control. Yadav *et al.* (2010) reported the assessment of genotoxic effects in butachlor in fresh water fish, *Cirrhinus mrigala* (Hamilton) and results showed the frequency of micronuclei induction was found maximum at 48 h and the broken egg were found maximum at 72 h. Fenech *et al.* (2011) reported the molecular mechanism of micronucleus; nucleoplasmic bridge and nuclear bud formation in mammalian and human cells and reported that micronuclei (MN) and other nuclear anomalies such as
nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) were biomarkers of genotoxic events and chromosomal instability.

Mural and Guner (2011) reported the induction of micronuclei and nuclear anomalies in the RBC of fish *Gambusia affinis* on exposure to pyrethroid insecticide. Cavas (2011) reported the genotoxicity evaluation of atrazine and atrazine–based herbicide on fish *Carassius auratus* using the micronucleus test and the comet assay and results revealed significant increase in the frequencies of micronuclei and DNA strand breaks in erythrocytes of *Carassius auratus*. Pandey et al. (2011) reported the DNA damage in *Channa punctatus* using alkaline single cell gel electrophoresis with the exposure of prefenofos. The intensity of DNA damage was concentration dependent and was recorded maximum in gill cells. Assessment of genotoxic impact of pesticides on farming communities in the Catarina state of USA, by micronucleus testing was also studied. All the fish species found in the nearby water bodies viz., *Cyprinus carpio, Hypostomus punctatus, Rhamdia quelen* and *Oreochromis niloticus* showed the evidence of induction in micronuclei frequency (Salvangni et al., 2011). The results were contradictory with the study of Liu et al. (2011). They reported about the impacts of herbicide butachlor on the larvae of a paddy field breeding frog (*Fejervarya limnocharis*) in subtropical Taiwan and reported that butachlor had no impact on *Fejervarya limnocharis* tadpole growth, but survival ratio, genetic damage whereas development and time of metamorphosis was negatively affected development. Malik and Ganie (2011) reported about the genotoxic effects of organophosphate insecticide thiometon in some exotic fishes of Kashmir and the genotoxicity of pesticide was confirmed by incidence of micronucleus in peripheral erythrocytes using three sub-lethal concentrations. Al-Sharif (2012) reported about the genotoxicity of 4-Nonylphenol (4NP) on fish *Oreochromus spilurs* and evaluated the number of micronuclei and cytogenetic aberrations increase with increase in dose of chemical.

All the pesticides used in agricultural fields were found hampering health of paddy farmers as well as environment, plants, fishes and livestock. Pesticide toxicity in fish with indiscriminate use of various pesticides, which usually enter into the aquatic environment and inferred indiscriminate use of these pesticides to improve agricultural production and yield had impacts on all non-target organisms, especially aquatic lives and environment. DNA damage in fish *Anguilla anguilla* exposed to a glyphosate based
herbicide and its role in oxidative stress. The results revealed that both liver and kidney displayed an increase in DNA damage for Roundup® concentrations and liver showed less susceptibility to the lower concentration (Guilherme et al., 2012). Kumar (2012) suggested the micronucleus assay as a sensitive indicator for aquatic pollution. *Channa punctatus* was exposed to chlorpyrifos and malathion at different concentrations. He inferred that both the pesticides had potential to cause genotoxicity but chlorpyrifos was found comparatively more toxic than malathion.

Khushwaha *et al.* (2012) studied the genotoxic and mutagenic effect of polluted river water in *Channa punctatus* and *Mystus vittatus*. The induction of DNA damage and micronuclei were determined in blood erythrocytes using comet assay and micronucleus test. The induction in micronuclei frequencies and DNA damage were found to be significantly elevated in exposed specimens. Likewise, assessments of genotoxic potential of the insecticide Dichlorvos using cytogenetic assay, *i.e.* micronucleus (MN) assay, mitotic indices (MI) and Chromosome abberation (CA) analysis *in vivo*. Yadav *et al.* (2013) reported about the aberrations in the chromosomes of *Cirrhisus mrigala* (Hamilton) upon exposure to butachlor. These aberrations in chromosome from kidney cell preparation illustrated the risk that butachlor possesses. Ismail *et al.* (2014) attributed the genotoxic damage due to the interference of toxicant with genetic material when chlorpyrifos in various doses was exposed to common Indus valley toad, *Bufo stomaticus* using alkaline Single cell gel electrophoresis. The exposed tadpoles showed different morphological abnormalities. Similar results of cytotoxicity of chlorpyrifos were inferred in the root meristematic cells of Srivastava and Singh (2014) observed the impact of changes in the hydrological properties of Maheshara lake on the cytogenetic changes in an air-breathing fish, *Channa punctatus* and noticed higher micronuclei formation in the fish which received lake water thus indicating induced mutation in fish living in Maheshara lake water.

### 2.5 Mitigation effect of high protein diet and vitamin C

Vitamin C also protects by preventing the development of nitrosamines, the cancer-causing chemicals that stem from the nitrates contained in many food (Gaby and Singh, 1991). Merchie *et al.* (1997) reported about the optimization of dietary vitamin C and crustacean larvae by adapting the HPLC techniques and quantification of vitamin
C and the amount required in the diet of the same because deficiency causes deformities during hatchery rearing, nursery stages and adult stage. Blom and Dabrowki (2000) opined the vitamin C requirement for normal growth and survival was quite low but a higher level was required to improve the stress resistance of fish (Garcia et al., 2007). Madhuban and Kaviraj (2003) observed that dietary supplementation of ascorbic acid can counter the stress of the pesticide deltamethrin in *Clarias gariepinus*. Similar results were also reported by other authors (Ortuno et al., 2003; Zhou et al., 2005; Azad et al., 2007). The roles of dietary high protein and vitamin C as antistressors have been studied in some fish species (Manush et al., 2005). Jana et al. (2006) reported about the effect of varying dietary protein levels on growth and production of *Chanos chanos* (Forsskal) in inland saline groundwater, laboratory and field. The studies revealed that irrespective of the protein source, fish fed on diet containing 40% protein showed significantly higher growth rate in terms of live weight gain, weight gain, biomass, specific growth rate, growth day, specific growth rate, low feed conversion ratio, high nutrient retention gross energy retention and digestive enzyme activity (specific protease and amylase). Hepatic damage induced by lead has been reported to be neutralized by a combination of ascorbic acid and thiamine by Wang et al. (2009). Protective effects of vitamin C against chlorpyrifos poisoning on haematological and biochemical changes were reported by Ambali et al. (2007). Kaleli et al. (2008) reported about the increase in total serum proteins levels of rats fed by hot smoked rainbow trout (*Onchorhynchus mykiss*) plus a vitamin diet were investigated.

El-Hossary et al. (2009) reported about the neutrotoxic effects of chlorpyrifos and the protective role of antioxidant supplements vitamin C and E. The optic nerve showed degenerative changes in the form of increased vacuolations, thick optical septa and abnormal myelin sheath. Treatment with vitamins C and E produced marked improvement in brain tissue. Sarma et al. (2009) investigated the dietary high protein and vitamin C mitigation effect on endosulphan toxicity in the spotted murrel, *Channa punctatus*. The fish exposed to endosulfan fed on high CP and vitamin C diet exhibited significant improvement in their growth performance and metabolic enzyme activities. Results indicated that vitamin C (0.2%) supplementation in high CP (50%) diet improves growth, metabolism, and reduced endosulfan bioaccumulation in *Channa punctatus*. The antioxidant vitamin C was suggested as major anti-stress substance.
(Misra et al., 2007; Norouzitallab et al., 2007). Most fish species cannot synthesize vitamin C, and has to depend on external sources to meet their needs (Chatterjee et al., 1975).

Uzun et al. (2009) reported about the malathion induced testicular toxicity in male rats and the protective role of vitamin C and the sperm counts, sperm motility, sperm morphology, FSH, LH, and testosterone levels. Co-treatment of malathion-exposed rats with vitamins E and C had a protective effect on sperm counts, sperm motility and abnormal sperm numbers, but not on plasma FSH, LH and testosterone levels. Ambali et al. (2010) investigated the attenuation of vitamin C on the haematological and biochemical alterations induced by acute chlorpyrifos exposure in wistar rats. Haematological evaluation revealed chlorpyrifos-induced alteration in packed cell volume, and levels of hemoglobin, red blood cells, absolute differential and total white blood cells count. Alteration in the levels of serum glucose, total proteins, albumin, globulin, electrolytes, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and erythrocyte and liver malonaldehyde. Innocent et al. (2011) studied the effect of vitamin C supplement diet on haematology of *Cirrhinus mrigala* and reported that vitamin C can activate the immune system in a non specific way, and providing resistance against pathogens. Issa et al. (2011) reported about the histological hazards of chlorpyrifos usage on gills and kidneys of *Tilapia nilotica* and protective role of vitamin E supplementation. It was concluded that vitamin E was partially able to ameliorate these effects. Pugazhendy et al. (2012) reported about the protective role of *Cardiospermum haliacabum* against the cypermethrin effect on the haematological parameters of *Cirrhinus mrigala* (Hamilton) and the analysis significant reduction in RBC’s count, Hb values, PCV, mean corpuscular Hb (MCH, MCHC, MCV) and increase in TLC were significantly increased.

The foregoing review reveals that the toxicity of pesticides is causing serious biochemical alterations and DNA damage in fishes. Chlorpyrifos is a toxic pesticide and no studies so far are available on the toxicity of this pesticide on *Cirrhinus mrigala* especially in Haryana. Further studies are necessary to study the effect of this chemical on DNA damage and biochemical aspects of this fish.