Introduction

The current population of India is almost 1.4 billion as based on Worldometer elaboration of the latest United Nations data and equivalent to 17.7% of the total world population (Riggs et al., 2018; World Population Prospects 2019, 2019).The global population is expected to cross 10 billion by 2050 Rising population has led to increase food demand. To meet the food and nutrition needs of a growing population, a country requires a sustainable approach that put thrust on increasing productivity against the background of lower yields in a definite land. However, increase in food production faces with the ever-growing challenges especially the new area that can be increased for cultivation purposes is limited (Soheil et al., 2011; FAO, 2020). A high emphasis on achieving food grain self-sufficiency along with rapid population growth has compelled farmers to resort to the substantial use of pesticides. India comprises nearly 17% of the total world's population, but has just less than 2% of the total landmass, whose economy primarily depends on agriculture. Pesticides are widely used to guarantee increased crop production and meeting the constantly escalating food demand (Raza et al., 2019). In order to increase crop production, herbicides, insecticides, fungicides, nematicides, fertilizers and soil amendments are now being used in higher quantities than in the past (Gill & Garg, 2014; Riggs et al., 2018; Sharma et al., 2019)

Pesticides are the chemicals (natural or synthetic) employed in various agricultural practices to control pests, weeds and diseases in plants. Pesticides include a wide range of herbicides, insecticides, fungicides, rodenticides, nematicides, etc. In the process of agricultural development, pesticides have now become a vital tool for plant protection and for enhancing crop yield. Approximately, 45% of the annual food production is lost due to pest infestation; therefore, effective pest management by using wide range of pesticides is required to deal with pests and to increase the crop production

(Abhilash & Singh, 2009). However, in the last half of the century, vigorous growth in the world economy including both industrial and agricultural sectors have led to the progressive rise in the generation and utilization of agriculture-based chemicals which often induce disastrous effects on the environment. Imprudent use of pesticides and other persistent organic pollutants in agricultural soils have overwhelmed future impact. The persistent and ubiquitous nature of various agriculture-based pesticides and other organic pollutants has posed disaster to the mankind due to their bioaccumulation properties and high toxicity (UNEP 2007).

The top ten pesticide consuming countries in the world are China, the USA, Argentina, Thailand, Brazil, Italy, France, Canada, Japan and India World-atlas (2018). India is one of the major pesticides producing countries in Asia with annual production of 90,000 tonnes, and it stands at twelfth position in the world in the manufacturing of pesticides. In the past, India used and exported organochlorine pesticides on large scale including DDTs and HCHs (Sampath et al., 2014; Sharma et al., 2019). The success of the Green Revolution in India is largely due to the usage of high-yielding variety seeds and chemical fertilisers, thereby boosting the agriculture and agri-input sectors with increased output and demand, respectively. The agri-input sector, including agrochemicals, has grown steadily and is supported by increasing commercialisation of agriculture, growing area of land under cultivation of high-value crops and increasing cropping intensity and farm mechanisation. However, the industry faces the following challenges: 1) Lack of awareness and non-scientific usage 2) High reliance on generic molecules (FICCI, 2020).

However, on the flip side the discovery of pesticide residues in various sections of the environment has raised serious alarms regarding their use; concerns of which have outweighed the overall benefits derived from them (Ali et al., 2014). The potentially deleterious effect on various components in the

natural environment has elevated a great deal of concern in scientific community for pesticide management (Reddy & Kim, 2015). Due to low cost and broad-spectrum toxicity, it is estimated that more than 100,000 tons of pesticides have been applied in India alone, primarily for agricultural pest control (Arora et al., 2013). The abundant use of these chemicals, under the adage, "if little is good, a lot more will be better" has played important role in increasing the consumption. The annual application of agricultural fertilizers and pesticides is over 140 billion kilograms which is a massive source of pollutant through agricultural runoff (Arora et al., 2013). Agricultural pollution is the biotic and abiotic waste products of agriculture that contribute to pollution, degradation, and/or injuries to human beings and their economic interests, of the environment and surrounding ecosystems. Food and drinking water may be polluted by agrochemicals, and human health may be at risk (Nathiga Nambi et al., 2017). Application of such agrochemicals directs towards potential health hazards and has becomes a major concern for aquatic habitat due to their toxicity, persistency and tendency to accumulate in the organisms (Joseph & Raj, 2010). Fishes are most important and highest interacted species of aquatic ecosystem and have become a bridge between aquatic and terrestrial ecosystem as consumed as primary source of food.

Historically, the approach to hazard identification and risk assessment for new chemicals has been largely dependent on costly and time-consuming in vivo animal experiments. These experiments, which include the widely used 28-day repeat dose or 90-day sub-chronic studies, the two-year carcinogenicity study, and the multi-generational reproductive toxicity study, requires hundreds of animals and is highly expensive for a single candidate compound (McMullen et al., 2018). As environmental risk assessment of chemicals in traditional toxicity testing is mostly based on in vivo single compound experiments and has been well explored on all representatives of the tropic levels viz. producer and consumer level, *In-Vivo* testing is extremely time-consuming and costly,

requiring much maintenance and a high number of animals, which is ethically debated. However, the prohibitive animal and financial costs of in life toxicity studies ensure that it would be impossible to use these traditional approaches for addressing the large number of commercial compounds. This reality is one of the motivations to "develop a strategic plan to promote the development and implementation of alternative test methods and strategies to reduce, refine, or replace vertebrate animal testing and provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment of chemical substances or mixtures in general and agrochemicals in particular" (U. S. Congress, 2016). On the other hand, moving towards chemical safety decisions underpinned exclusively by *In-vitro* study results with many challenges, including defining an appropriate tiered testing and evaluation framework, designing suitable *In-vitro* assays, validating test systems, and securing public and regulatory acceptance of these new methods.

The increasingly restrictive legislation surrounding the use of animal research for toxicology testing has created a global pressing need to identify alternate methodologies for toxicity testing. There are initiatives in place that are attempting to address this dearth of information, including ToxCast/Tox21, the American Chemistry Council Long-range Research Initiative (ACC-LRI), the European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM), Safety Evaluation Ultimately Replacing Animal Testing (SEURAT), and EU-ToxRisk (Leist et al., 2012; Attene-Ramos et al., 2013; Tice et al., 2013; Kleinstreuer et al., 2014; Daneshian et al., 2015). While the methodologies for each of these initiatives differ, the goal remains the same: to utilize human cells/ cell lines to determine the effect of agrochemical perturbations on biological pathways and ultimately human health. Prior to registering and marketing any new agrochemical product manufacturers by law, generate safety data. These are assessed by regulatory agencies to determine the potential hazards to human health and/or the environment.

Tests performed on living animals (*In-vivo*) have traditionally been regarded as the "gold standard" for deducing the hazardous effects of any manufactured and/or accidentally produced component. However, industries are increasingly moving away from using animal models in safety testing, particularly toxicity testing for scientific, business, and ethical reasons. From a scientific perspective, I*n-vitro* testing/models can help unpick the mechanistic information as to how a substance may exert adverse biological effects, which although an *In-Vivo* model may identify, do not generally give information on how this may occur. There will always be the issue of cross-species extrapolation with using in vivo models; using *In-vitro* models with cell lines may overcome some of these issues. These ethical and scientific concerns have led to an increasing desire to apply the 3Rs principles; replacement, reduction, and refinement of animals in research. In practical terms, animal studies can be technically demanding, laborious, and expensive, especially when considering long-term exposure studies. By applying the 3Rs to the regulatory requirements of safety testing, in vivo testing can be minimized in favor of robust and predictive in vitro methodologies which do not affect the rigor of scientific safety tests (Maestri, 2021).

Thus, interest in *In-vitro* methods has been growing greatly in the recent years for economical, practical and ethical reasons, and the use of cell lines as alternatives to *In-vivo* testing is being seriously considered (Kasi Elumalai, 2012; Nagpure et al., 2016; Schug et al., 2020).The use of cell lines has many advantages. It avoids the testing of contaminants on living animals or even the regular sampling of cells for primary cultures. Their maintenance is less demanding since the only requirements are cell medium and an incubator at the right temperature and CO_2 concentration which is even unnecessary in the case of piscine cell lines. These methods are cost affecting and non-invasive, and the testing in itself uses very limited amounts of the test chemicals and creating little toxic waste. Results present little variability since the cell lines are

relatively homogeneous and used in a very controlled environment, the complex interactions happening in a whole organism being avoided.

In vitro fish cell assays are considered to be a promising alternative to fish bioassays to replace or reduce the use of fish in toxicological testing. Chemicals or water samples can be applied to fish cells at temperatures more typical of the temperatures to which fish would be exposed. Moreover, fish cells are largely easier to maintain and more tolerant to simple culture conditions. A large number of research has been done for toxic chemicals to compare *In-vitro* cytotoxicity in fish cell lines with *In-vivo* fish toxicity and confirmed its widespread applicability. Schirmer, (2006) proposed several routes for advancing fish cell line-based toxicity assays to overcome the hurdle like selecting cell lines derived from tissues that reflect the specific mode of action of a particular chemical; increasing sensitivity of the cellular response by modification of the culture environment to more closely resemble the *In-vivo* exposure; and by accounting for the chemical fraction available to the cells. Many scientists are known to develop new ways to detect the toxicity using various cell lines.

The application of in vitro techniques for questions related to fish toxicology started as early as ecotoxicology emerged as scientific discipline. Rachlin & Perlmutter (1968) published a very first study using an in vitro assay with fish cells to assess metal toxicity to fish. From the middle of the 1990s, fish cell systems became a commonly used tool for Eco toxicological research. Babich & Borenfreund (1991) are considered to be pioneers for evaluating the cytotoxic potential of various toxicants on fish cells. Later on, it was the laboratory of Niels Bols succeeded in establishing diverse fish cell lines such as the RTL-W1 from liver and the RTgill-W1 from gills of rainbow trout (*Oncorhynchus mykiss)* which was then used to detect specific toxicant responses (Behrens et al., 2001, Bols & Dayeh, 2005). In addition, fish cell lines were also used for purposes like the assessment of genotoxic or immunotoxic

activities of chemicals or for the toxicity screening of complex environmental samples such as water effluents or sediment extracts (Bols & Dayeh, 2005; Rehberger et al., 2018). Earlier fish hepatocytes cell lines were preferred due to its central role in toxicokinetic and toxicodynamic processes and xenobiotic biotransformation (Segner & Cravedi, 2001). Toxic potential of fluoroacetate pesticide was studied for the first time on two fish cell lines- RTG 2 and PLHC1 (Zurita et al., 2007). Later on number of scientist have explored the toxic potential in fish muscle cell line *Wallago attu* muscle (WAM) in *In- vitro* system (Nagpure et al., 2016). However, there is a dearth of information with regards to different classes of agrochemicals for In-Vitro studies compared to *In-vivo* condition. In the present study an attempt is made to prove the advantage of *In-vitro* assays for toxicity studies.

The first fish cell line RTG-2 was developed in 1962 using the ovary of a cold water fish, rainbow trout (Wolf & Quimby, 1962). Since then, an increasing trend in fish cell line development has been observed from a wide variety of tissues representing fish species from both tropical and temperate waters. A comprehensive review by Lakra & Swaminathan, (2011) has reported 283 fish cell lines globally. The latest information enlisting 517 fish cell lines in Cellulosaurus; a knowledge resource on cell lines has been reported by (Bairoch, 2018). Fish comprise around half of all vertebrate species together, yet very few cell lines have been established and characterized with specific biomarkers from piscinid species in comparison to mammals. Cell line research has gained momentum in the last decade and a number of cell lines from different organs of different fish species have been established in India, such as the SICH cell line from heart of *Catla catla* (Ahmed et al., 2009); two cell lines, RE and CB from the eye of *Labeo rohita* and brain of *Catla catla*, respectively (Ahmed et al., 2009), three cell lines RF, RH and RSB from heart, fin and swim bladder of *Labeo rohita*, respectively (Lakra & Swaminathan, 2011) cell lines from the fin tissue of *Tor tor*; two cell lines from fin and eye tissue of *Tor*

chelynoides and fin tissue of *Scizothorax richardsonii* (Goswami et al., 2012; 2014). These *In-vitro* cell culture systems have proved to be essential tools for studying cellular biology, biotechnology and toxicology (Goswami et al., 2014; Sarath Babu et al., 2012; Taju et al., 2014). Fish cell lines have been successfully used to evaluate the cytotoxicity potential of more than 50 aquatic pollutants, such as heavy metals, pesticides and nanoparticles and *In vitro* data obtained from fish cell lines have shown good correlation with *In vivo* toxicity data (Goswami et al., 2014; Dubey et al., 2015).

The physiology and blood plasma constituents of teleost's are similar with those of terrestrial vertebrates; therefore, the methodology for culture of cells is also similar. Fish cell lines are more advantageous over mammalian cell lines in terms of its maintenance and versatile applications. Because of lower metabolic rates than eurythermic cells, fish cells can be maintained with little care for long periods of time. Thus, permanent fish cell lines, in contrast to the mammalian cells, are easier to maintain and manipulate, the physiology and blood plasma constituents of teleost are similar with those of terrestrial vertebrates; therefore, the methodology for culture of cells is also similar. Fish cell lines are more advantageous over mammalian cell lines in terms of its maintenance and versatile applications. Because of lower metabolic rates than eurythermic cells, fish cells can be maintained with little care for long periods of time. Thus, permanent fish cell lines, in contrast to the mammalian cells, are easier to maintain and manipulate (Goswami et al., 2014; Mukunda Goswami & Education, 2018).

Tissue culture and the development of cell lines from fish are of priority interest for pathogen detection, toxicology, carcinogenesis, cellular physiology and genetic regulation and expression. The first fish cell line was developed in 1962 from gonad of rainbow trout (*Oncorhynchus mykiss*) and designated as RTG-2, and even now this cell line has tremendous applications in virological and toxicological studies (Taju et al., 2014). Since then the work on developing

fish cell lines is progressing and the number of fish cell lines have increased tremendously, a comprehensive global list of freshwater and marine fish cell lines was last published in 1994 by Fryer & Lannan, and reported some 159 fish cell lines, established from 74 species or hybrids representing 34 families of fish. Lakra & Swaminathan, (2011) have further reported the 124 new established fish cell lines (including 59 cell lines from 19 freshwater, 54 from 22 marine and 11 from 3 brackish water fishes) from the year 1994 to 2010. Among the fish cell lines listed, more than 60% were established from Asian region, which contributes more than 80% of total fish production (Pandey, 2013).

Up to 2010, out of over 3400 cell lines deposited at the American Type Culture Collection (ATCC) only 43 cell lines could be found that are of aquatic animals, and only 17 fish cell lines are usable and available for dissemination to the researchers globally. The European Collection of Cell Cultures (ECACC) holds over 40000 cell lines representing 45 different species and 50 tissue types. The reluctance to use cell lines stems from researcher's misconception that cell lines are mostly derived from transformed cells and that differentiated characteristics of the tissues of origin are not maintained. This may be the case for many mammalian cell lines, but most cell lines derived from fish tissues have been from normal tissues with a few exceptions, most notably EPC and RTH-149 cells which were derived respectively from an epithelioma and ahepatoma. Fourteen out of 159 fish cell lines reported up to 1994 were initiated from tumorigenic tissues, which is less than 10%. Further among the fish cell lines listed at ATCC, only three were derived from tumorigenic tissues. This contrasts with mammalian cell lines where over 50% of listed cell lines at the ATCC were derived from cancerous tissues or transformed cells. Altogether about 283 cell lines have been established from finfish around the world but only 43 fish cell lines are being listed in the international cell repository like ATCC, ECACC. If all the established cell lines would have been deposited in

that repository, it would be beneficial to the international research community in order to use those cell lines as they are the best alternative to the whole animal research (Lakra & Swaminathan, 2011; Goswami et al., 2012, 2014).

Extensive confirmation of the predictive ability of the RTgill-W1 cell line assay for fish acute toxicity was first presented by Tanneberger et al., (2013), who explored 35 industrial chemicals and pesticides with a wide range of physicochemical properties , modes of action (i.e., narcotic, reactive, uncoupler and neurotoxic), and acute toxicity to fish . They found a very good agreement between the in vitro effective concentrations causing a 50% decline in cell viability (EC_{50}) and the in vivo lethal concentrations (LC_{50}). Indeed, for 73% of the test chemicals, the differences between EC_{50} and LC_{50} values were less than five-fold. In addition, Andreas et al., 2017 tested 38 fragrance chemicals with a considerable range of physicochemical properties with the RTgill-W1 cell line assay. They also found a very good agreement between EC_{50} cell line and LC_{50} fish toxicity, confirming the predictive capacity of the cell line-based assay. The RTgill-W1 cell line-based assay has recently been adopted as ISO guideline 21115 (ISO, 2019). Further, Kolarova et al., (2021) in their studies have proposed that in vitro (fish cell lines) is a cost-effective, very rapid, and informative tool for toxicological assessments. Using the neutral red assay, they have compared the *In vitro* acute toxicity of twenty-six chemical substances on a rainbow trout gonad cell line (RTG-2). The authors recommend the use of the Neutral red assay on the RTG-2 cell lines as a screening protocol to evaluate the toxicity of xenobiotics in aquatic environments to narrow the spectrum of the concentrations for the fish toxicity test.

Neonicotinoids

Neonicotinoids are members of a relatively new class of neuroactive insecticides that are used as seed coatings in large quantities to protect crops against pest. Neonicotinoids are additionally used as sprays in crop production, in managing household pests, and in deterring pests on domesticated animals

(Goulson, 2013). Neonicotinoids derive their toxicity from agonistically binding to nicotinic acetylcholine receptors (nAChRs) on the post-synaptic nerve membrane and fring nerve impulses in a manner that is uncontrollable and uninterrupted (Sánchez-Bayo et al., 2016; Sanchez-Bayo & Goka, 2014). Neonicotinoids were first developed in the 1990s (Tokumoto et al., 2013), gained popularity from 2003- 2011 (Douglas et al., 2015) and are now the most widely used pesticides in the world (Maloney et al., 2017; Berheim et al., 2019).

Neonicotinoids are widely found in the environment for numerous reasons. First, only a small quantity (2–20%) of the seed-coated insecticide is absorbed by the developing plant; the remainder is released into the environment through leaching, drainage, run-of, or snowmelt (Mason et al., 2013). Neonicotinoids are highly water soluble (Morrissey et al., 2015), they are prevalent in diverse water bodies in the United States, Canada, Australia, Europe, and Asia (Berheim et al., 2019). Moreover, under the right conditions, neonicotinoids can persist in the soil, sometimes for many years. Finally, untreated plants associated with cropland are often contaminated by neonicotinoids due to the systemic nature of these chemicals (Mogren & Lundgren, 2016). The widespread use of neonicotinoids provides numerous opportunities for exposure to non-target, beneficial species via the water, soil, and contaminated plant tissues. Various environmental and ecotoxicological aspects related to applications of neonicotinoid insecticides are assessed. The first neonicotinoid insecticide, imidacloprid (IMI) - (E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine; CAS No. 138261-41-3) was introduced in 1991 as a result of Japanese and European research activities, followed by nitenpyram and acetamiprid in 1995, thiamethoxam in 1997, thiacloprid in 1999 and clothianidin in 2002 (Jeschke et al., 2011). Globally, neonicotinoid insecticides comprise the most widely used insecticide class in agriculture (Simon-Delso et al., 2015; Wang et al., 2020) and have largely replaced a variety of older chemistries (e.g., organophosphates, carbamates, and

organochlorine pesticides). With their increased use, neonicotinoid insecticides have increasingly been included in surface water monitoring programs, with a number of publications citing detections of these compounds.

Morrissey et al., (2015) recently reported that 29 studies from 9 countries have published detectable concentrations of neonicotinoid insecticides in puddles, streams, rivers, wetlands, and irrigation channels. Government monitoring appears to be expanding in recent years, with surveillance programs now reporting findings from Canada and the United States (Struger et al., 2017). While not systematically monitored, the presence of neonicotinoid insecticides in marine environments has also been reported (van der Sluijs et al., 2015). These detections have led to suggestions that aquatic ecosystems may be impacted by neonicotinoid insecticides (Anderson et al., 2015; Sánchez-Bayo et al., 2016). *In-vivo* and *In-vitro* studies have been reported to misbalance the antioxidants on exposure of IMI (Wang et al., 2020). A number of studies have assessed the toxic effects of IMI on several endpoints of various fish species in different parts of the world. IMI is reported to affect survival (Qadir & Iqbal, 2016), behavioural responses (Patel et al., 2016; Sadekarpawar, S., et al., 2010), embryogenesis biochemical alterations and haematological profile (Mastan et al., 2009; Patel B, 2016) ; oxidative stress (Ge et al., 2015; Patel B, 2016), cytotoxic stress and histopathological alterations in tissues (Sadekarpawar, e al., 2010; Sadekarpawar, et al., 2015; Qadir & Iqbal, 2016) of different fish species. Further, the genotoxic potential of the IMI has been well explored in *Oreochromis niloticus* (Ansoar-rodríguez et al., 2015), where they have proved primary DNA damage at the chromosomal level confirming the potential risk of IMI. IMI exposure cause the histopathological changes, activation of TNF-α, iNOS, 8-OHdG biomarkers, and alteration of caspase 3, iNOS, CYP1A, MT1 gene expression levels in common carp (*Cyprinu scarpio* L) (Özdemir et al., 2018). Further, Su et al., (2007) have also reported the cell growth inhibition in FG cell line by IMI.

Earlier in our lab the *In-vivo* studies with reference to IMI exposure on Freshwater Teleost, *Oreochromis mossambicus* has been thoroughly studied, however, there is a paucity of the information as far as the *In-vitro* studies for IMI toxicity fish cell line is concerned. Hence, in the present studies an attempt is made to investigate the potential molecular mechanism for the toxic effects of IMI.

Fungicide (CZ)

As fungal diseases are a major threat to crop production,1 the application of fungicides to control fungal infestations is often considered indispensable to secure global food supply (Zubrod et al., 2019). Fungicides are agents that are used to prevent or eradicate fungal infections from plants or seeds. Most of the fungicides have low to moderate toxicity. However, several fungicides are known to cause developmental toxicity and oncogenesis. More than 80% of all oncogenic risk from the use of pesticides derives from a few fungicides; some fungicides are known to disrupt the endocrine system and lead to reproductive and developmental abnormalities (Gupta et al., 2017) . Fungicide use is regionally predicted to increase because of changes to climatic conditions, development of fungicide resistance, and invasive fungal species (Fisher et al., 2012). Following their use, fungicides enters aquatic ecosystems through point and nonpoint sources (Kahle et al., 2008; Bereswill et al., 2012).

In aquatic systems, fungicides can be toxic to a wide range of no target organisms as they act on basic biological processes that are not specific to fungi and are known to occur in surface water bodies in agricultural catchment areas. Frank et al., (2014) in their studies have suggested that Mixtures of two different compounds is one of the Fungicide Resistance Management Tactic. Curzate is one of the fungicide a mixture of cymoxanil (8%) and mancozeb (64%); it is a systemic and contact disease control solution for crop disease control in grape downy mildew and late blight of potato and tomato. Toxicity of Mancozeb at a

individual level have proved to be toxic (Axelstad et al., 2011; Runkle et al., 2018; Palmerini et al., 2018; Sprovieri et al., 2020). Further, earlier studies on cymoxanil have mainly focused on its preparation, efficacy, disease- and insect-control properties, degradation in soil, residues, metabolites, etc. (Gan et al., 2019; Huang et al., 2020), and their toxicities in aquatic animals or even in humans are rarely reported. There has only been one article published on their effects on immunity and neurotoxicity (Cheng et al., 2020). However, the toxicity of the mixture is not well explored, baring the work of Sadekarpawar, et al (2010), where they have proved the toxicity of curzate on fresh water fish *O. mossambicus*. Moreover, there are no reports on the toxic effect of curzate on cell line.

Herbicide (PE)

Herbicides are the most used pesticides and in European surface waters are the most frequently detected pesticide (Moschet et al., 2014; Booij et al., 2015; Lopez-Antia et al., 2016). Herbicides are often well soluble in water to increase the systemic uptake by plants. This increases the chances of transport and discharges into water, and consequently, a wide variety of herbicides often exceed environmental quality standard and regulatory acceptable concentrations (Moschet et al., 2014; Casado et al., 2019). Hence, herbicides are expected to have a significant effect on aquatic ecosystem functioning (Moschet et al., 2014; Knauer, 2016). Herbicides can enter surface waters from several sources through various processes, with the main source being runoff and drainage from agricultural fields (Knauer, 2016). Compounds ranking at the top of global herbicides use are amides, phenoxy hormone products bipyridyls, urea derivatives, dinitroanilines, carbamate herbicides, sulfonylureas and uracil (Ayanda et al., 2018). The unintentional as well as intentional sources of herbicides in the aquatic environment are numerous, evidently leading to the widespread presence of herbicides, inevitably leading to the exposure of non-target aquatic organisms. Fish serve as bio-indicators of environmental

pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem (Nwani et al., 2010).

Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops. Among sulfonylurea products, pyrazosulfuron ethyl (PE) herbicide is widely used for selective post-emergence control of annual, perennial grasses and broad-leaved weeds in cereals, and is currently recommended for use on some relevant crops in over 30 countries (Upadhyay etal., 2014). Pyrazon Sulfonylurea herbicides inhibit biosynthesis of branched chain amino acids, such as valine, isoleucine and leucine. Production of such amino acids is dependent on acetolactate synthase (ALS), the target of these herbicides. In spite of this herbicide group shows very low animal toxicity, when used on arable lands it gets transported to waterways. As a result, many other non-target organisms sharing the same environment are in disguise unintentionally poisoned. Recently, the residues of some common and widely used herbicides (acetochlor, bispyribac-sodium, bentazon, bensulfuronmethyl, halosulfuron-methyl, and quinclorac) were detected in the surface water, soil, sediments, and fish tissues as the agricultural drainage problems (Fathy et al., 2019). Water bodies became illegally the end point of the discharge of such chemicals. Public concern about the adverse effects of pesticides on aquatic organisms, and bioaccumulation in fish and other aquatic invertebrate is increasing; therefore, there have been many monitoring surveys and research on pesticides in freshwater system.

Furthermore, residues of herbicides and other toxicants have been found to accumulate in fish that comprise hazards on fish health consequently threatening human health (Drishya et al. 2016; Pereira et al. 2013). Indeed, many herbicides (i.e., acetochlor, quinclorac, bensulfuron-methyl, halosulfuron-methyl, and bentazon) and their metabolites have been persistent

as residues (hazardous levels) in major surface water sources (i.e., river, lakes, canals, and aquaculture), soil, sediments, and tissues of some fish worldwide (Yang et al., 2018). Alterations in behaviour, histology, hematobiochemical parameters, and genetic disruption in Nile tilapia (*O. niloticus*) after exposure to many herbicides have been reported (Burgos-Aceves et al., 2019) and in fresh water teleost *O. mossambicus* (Upadhyay et. al., 2016) as well as other fish species (Babatunde & Oladimeji, 2014; Lutnicka et al., 2018; Burgos-Aceves et al., 2019). Bensulfuron-methyl has also been reported to induce genotoxicity and disrupt embryonic development in *D. rerio* (Jixin et al., 2017). The exposure to these herbicide residues may jeopardize the health of tilapia species as well as the health of human consumers; therefore, the application of these herbicides in weed management in rice needs to be considered carefully. However, to our knowledge there is a paucity of information with reference to *In-vitro* work on herbicide

Micronutrient (MN)

Micronutrients, or trace elements, are those elements required by crops in relatively small quantities ranging from a few grams to a few kilograms per hectare. They include iron, manganese, copper, zinc, boron, molybdenum, cobalt and chlorine. Any soil may become deficient in one or more trace elements after intensive cropping for many years. Temporary deficiencies may be induced by unusually heavy applications of liming materials or phosphate fertilizers. Obtaining the maximum yield has a fundamental importance in the current global scenario. In this relentless pursuit of higher productivities, farmers use technologies related to various areas, such as new forms of fertilizer and pesticide application, different fertilizers, crop breeding, equipment and techniques of planting and harvesting. In order to increase the efficiency in this activity, due to the increased requirement for competitiveness of economic globalization, one of the tools found by farmers is the use of fertilizers containing micronutrients in their crops. In recent years, copper and copper-

based nanoparticles (CuNPs) have been used for industrial purposes, electrical equipment, construction materials, antimicrobial agents, and alloy formation with other metals. CuNPs are increasingly used in various sectors, including as catalysts in organic synthesis, for drug delivery, sensors, agriculture and food preservation and paint and water treatment (Förstner & Wittmann, 1979; Inkinen et al., 2017)

There is an abundant supply of copper in the earth's crust. Metals react based on their soluble properties in an aquatic medium. The free ions or complexes generated by metals can be absorbed on suspended particulates in the aquatic medium. Metal constituents might behave differently in an aqueous system. The ever-increasing metal usage in different forms around the world is a matter of great concern in present times, as it eventually affects all forms of life in our ecosystem. Therefore, it is important to understand the underlying chemistry and mechanism of these metals to the environment and organisms at a basic phenomenal level (Salem, 2019). Fertilizer toxicity typically happens from over-fertilizing or using a fertilizer with too many nutrients and in theory run-off or fertilizer toxicity in a nearby area of the farmlands also are at risk of the toxic effect of fertilizers.

Agrochemical toxicity remains one of the major causes of morbidity and mortality around the world today (Mullin et al., 2016). The application of fish cell lines for toxicology goals can be evaluated by scrutinizing cellular response, such as cytotoxicity, cell growth, genotoxicity as well the xenobiotic metabolism, these endpoints often encompass overlapping cellular activities. Cell lines have been used extensively to study the cytotoxicity of substances to fish cells. Basal cytotoxicity evaluation is generally by cell viability assay. Cytotoxicity and cell proliferation assays are commonly used in the toxicological studies to assess a compound's ability to cause or block a biologic activity without having toxic effects on cells. Cell viability and cytotoxicity assays measure cellular or metabolic changes associated with viable or

nonviable cells. These assays can detect structural changes such as loss of membrane integrity upon cell death or physiological and biochemical activities indicative of living cells. Cell viability tests are numerous and have been grouped below into six types based on the cellular process being targeted. Although some tests preferentially measure damage at one site over another, in practice, the results can be due to events at several cellular sites, which can interact, making their relative importance to the overall loss of cell viability difficult to distinguish. In fact, most tests of cell viability focus either directly or indirectly on the integrity of the plasma membrane (Ermler et al., 2013).

Literature survey done till date has plethora of references for screening the toxic potential of agrochemicals which are limited to *In-vivo* conditions. That too with either single or in combination of the pesticides. Baring the previous *In-vivo* studies from our lab which has well established the toxic potential of all the classes of agrochemicals viz: IMI, CZ, MN and PE by reporting the alteration of Haematological, Histological, blood biochemical parameters, behaviour alteration and neuroendocrine response as well (Patel et al., 2016; Sadekarpawar,et al., 2010; Upadhyay et.al., 2016; Sadekarpawar, etal., 2015). However, there is a gap in our understanding with regards to the molecular mechanism. Thus to fill the gap the present study was undertaken, to unravel the genotoxic potential of agrochemicals (PE, CZ, MN and IMI) in *In-vitro* system. To evaluate these obscure aspects of the loss of normal cell orchestration, cell death, cell proliferation and other genetic markers which will make us to understand the perturbed machinery.

Keeping in view the above mentioned facts the present study is an attempt to unravel the process of proliferation and cell cycle (Chapter I), cell death (Chapter II) in ICG cell line on exposure to Agrochemicals and evaluating its Genotoxic potential (Chapter III) as well as In-silico analysis of target prediction and gene interactions of agrochemicals (Chapter IV).