Introduction

Feeding the 9 billion people expected to inhabit our planet by 2050 will be an unprecedented challenge for all mankind (Ash et al. 2010). Nevertheless, producing enough food for the world’s population in 2050 will be easy, but doing it at an acceptable cost to the planet will depend on research into everything, from high-tech seeds to low-tech farming practices (Anonymous 2010). There has been accelerated development through the application of science and technology in the world since the second half of the 20th century. It is also true that the advances in science and technology had significant impacts on agriculture, first in Europe and North America, later in Asia and South America, where it was known as “Green Revolution”. Green Revolution refers to a historic period 1966 to 1990 in the development of modern agriculture of developing countries in Asia and South America, characterized by massive adoptions of improved cereals, mainly wheat and rice. The introduction of high-yielding varieties of seeds after 1965 and the increased use of fertilizers and irrigation are known collectively as the Green Revolution, which provided the increase in production needed to make India self-sufficient in food grains, thus improving agriculture in India. On a global basis sufficient food is produced to adequately feed everyone. Yet, the pains of hunger continue to be a common experience of many people in the world today. Food shortages in developing countries are aggravated by rapid population growth. The primary role of agriculture is to produce a reliable supply of wholesome food to feed the burgeoning world population safely and without adverse effects on environment. The Green Revolution has created some problems mainly to adverse impacts on the
environment. The increasing use of agrochemical-based pest and weed control in some crops has affected the surrounding environment as well as human health. The intensified agriculture, in developing countries, has therefore dictated the increasing use of agrochemicals to meet growing food demands. About 30% of the food grown in the world is lost annually because of the effects of weeds, pests and diseases as reported by International Atomic Energy Agency (1987). In spite of the known difficulties caused as a result of pesticide usage, as yet the alternative to practical control measures employing pesticides are still in the developing stage. Crop losses would be doubled, if existing pesticide uses were abandoned. The use of pesticide chemicals has often a dramatic impact on food production. However, negative effects of pesticides on non-target organisms and their effect on the environment must be recognized (Ahmad et al. 2008). Contamination of the environment and food by pesticide residues is a radically current issue in many areas of the world. Any preventive act greatly depends upon international differences, but the common final goal is still to reduce air, water, soil and food contamination, as well as to prevent both acute and chronic adverse effects for manufacturers, consumers and the world community as a whole.

Rice (Oryza sativa L.), the most important food crop in the developing world and a staple food feeds nearly half of the planet’s inhabitant. More than 90% of the world’s rice is grown and consumed in Asia where 60% of the earth’s people live. Rice accounts for 35-75% of the calories consumed by more than 3 billion Asians. It is planted to about 154 million hectares annually or on about 11% of the world’s cultivated land. At present, rice is grown in all continents under various environmental conditions which can be separated into four main ecosystems: irrigated lowland, rainfed lowland, upland and flood prone (De Datta 1981; Ferrero and Tinarelli 2007). Any organism like insect, weeds, microorganism (fungi, bacteria), rodents, nematods and others, which caused economic loss or damage to the physical well being of human
being as well as plants and other organism, is known as pest. It may damage and destroy our crops, cause disease in them. Pests are considered as greatest enemies of modern agriculture. Any chemical that can kill or repel a pest is known as chemical pesticide. Pesticides are often used in agriculture to protect human beings from the insect vectors of disease-causing pathogens, to protect crop plants from competition with abundant but unwanted other plants species, and to protect crop plants and livestock from diseases and depredations by fungi, insects, mites, and rodents (Chen et al. 2007). Since rice is an irrigated crop, the use of pesticides such as insecticides, fungicides but especially herbicides during cereal growth may affect the quality of the surrounding aquatic environment (Pereira et al. 2000). Among the pesticides, herbicides are chemicals used to manage unwanted plants in the ecosystem, plants that are commonly referred to as “weeds”. Herbicide demand has unique characteristics compared with other common productive inputs in rice culture systems such as land, labour, seeds and chemical fertilizers (Yamamoto and Nakamura 2003). The goal of herbicide use is to kill or stunt weed infestation allowing the rice to grow and gain a competitive advantage (Monaco et al. 2002). Selective herbicides kill certain targets while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often based on plant hormones. Herbicides are generally classified as follows;

- Contact herbicides destroy only that plant tissue in contact with the chemical spray. Generally, these are the fastest acting herbicides. They are ineffective on perennial plants that are able to re-grow from roots or tubers.

- Systemic herbicides are foliar-applied and are translocated through the plant and destroy a greater amount of the plant tissue. Modern
herbicides such as glyphosate are designed to leave no harmful residue in the soil.

- Soil-borne herbicides are applied to the soil and are taken up by the roots of the target plant.
- Pre-emergent herbicides are applied to the soil and prevent germination or early growth of weed seeds.

The use of rice herbicides has been expanding enormously worldwide over the past 20-40 years. However, herbicides are considered a “two-edged sword” (Kudsk and Streibig 2003) or the “reverse of the coin” (Jurado et al. 2010). Since the subsequent dispersion of herbicide compounds and their degradation products in rice fields and adjacent areas with strong ecological value still threatens the integrity of ecosystems, thus resulting in serious global environmental concern (Olofsdotter et al. 1998). One of the major issues about environmental herbicide contamination in wetland rice fields is its bioaccumulation in ecosystem primary producers and its subsequent propagation through trophic chain. Therefore, reliable legislation and risk assessment tools are needed to carry out the monitoring of herbicide residues in autochthonous living organisms inhabiting rice fields. The widespread use of pesticides has been suggested to be an important factor limiting its productivity (Qiu et al. 2002). Several herbicides may enter aquatic environments through spray drift or runoff events, drainage or leaching, resulting in contamination of surface waters and groundwater (Cerejeira et al. 2003; Faria et al. 2007), thus affecting phytoplankton and delicate autochthonous living organisms and also human health (Srivastava et al. 2010). The removal of these herbicides from soil and aquatic ecosystem has become a difficult problem and as a result of this, they persist in the ecosystem for a longer period of time (Singh 1973).
Beside this, photosynthetic organisms entirely depend on light as the ultimate source of energy for their survival. Light is an electromagnetic wave that propagates through space at the phenomenal velocity of 300 km/s, but it represents only a tiny fraction of what we term the electromagnetic spectrum. When one enters the world of radiation biophysics and the effects of electromagnetic radiation upon biological systems, it is frequently useful to separate the entire EM spectrum into ionizing and non-ionizing radiation. Ionizing radiation has enough photon energy to create ion pairs; that are, to make an atom gain or lose electrons. Examples of ionizing radiation include: X rays and gamma rays. Non-ionizing radiation includes optical radiation (ultraviolet, visible and infrared radiation) and radiofrequency radiation. The optical spectrum is frequently broken into smaller spectral bands. The band definitions depend upon whether one is interested in optical engineering, meteorological optics, photobiology or some other technical area. The division of ultraviolet spectrum by optical engineers could be divided by the transmission characteristics of soft glass materials (330-400 nm), quartz optics (middle ultraviolet: 180-320 nm) and the absence of transmission in quartz and air (far ultraviolet or vacuum ultraviolet at wavelengths below 180 nm). From the standpoint of meteorological optics, the bands are defined relative to the transmission of water bands in the infrared and ozone in the ultraviolet. However, from the standpoint of the photobiologist, the absorbance and transmission bands of proteins and water are critical. When considering photobiological effects, it is useful to employ the convention of the International Commission on Illumination (CIE) for spectral bands. The CIE (convention of the International Commission on Illumination) has designated 315 to 400 nm as UV-A, 280 to 315 nm as UV-B, and 100-280 nm as UV-C (CIE 1987).

Sun light provides to photosynthetic organisms the energy for life and, thus, is a key environmental factor. It is characterized by both the intensity
and the spectral composition. Its spectrum reaches from approximately 290 nm to 3000 nm. A considerable amount of the energy is contained in the ultraviolet band of which especially the short wave part below 315 nm is considered to be harmful for men, animals and plants (Xi-Da et al. 2010) The efficiency of light-induced damage increases with decreasing wavelength. Thus, from natural sunlight that reaches the Earth the UV-B (280-315 nm) spectral range has the highest damaging potential. The main absorbers influencing the UV range of the spectrum at the Earth’s surface are oxygen and nitrogen (both atomic and diatomic) and ozone. Stratospheric ozone (O₃) is created over low latitudes by the action of ultraviolet radiation of wavelengths shorter than 240 nm. An oxygen molecule (O₂) reacts with the high energy radiation and two oxygen atoms are formed in the reaction. A third molecule (M), e.g. another oxygen or nitrogen, is required to remove the excess kinetic energy in the following way:

\[ \text{O}_2 + \text{UV} (< 240 \text{ nm}) = \text{O} + \text{O} \]
\[ \text{O}_2 + \text{O} + \text{M} = \text{O}_3 + \text{M} \]

In the last three decades, the presence in the atmosphere of ozone-depleting substances (CFCs, HCFCs, halons, carbon tetrachloride, etc.) has been reducing the ozone concentration in the stratosphere (Peng and Zhou 2010) over high and mid-latitudes of both hemispheres. Notwithstanding ozone concentration in the atmosphere is very low, the stratospheric layer, which contains approximately 90% of the total ozone, has the function of a protective filter for the Earth’s surface, cutting-off solar radiation under 280 nm and greatly reducing UV-B radiation (280-315 nm). The reduction of stratospheric ozone has been recognized as the main cause of the increase of UV-B irradiance at the Earth’s surface. This increase has been estimated in the range 6-14% in the last twenty years. The destruction of ozone results in its breakdown to molecular oxygen and atomic oxygen. In equilibrium, these two events of synthesis and degradation have in the past resulted in an

[6]
average ozone content of 300 DU (Dobson unit, DU = 1 mm of ozone at STP).
However, with the loading of the atmosphere with halogen compounds containing Cl and Br from industrial activities, the balance is no longer in place, since Br, BrO, Cl and ClO take part in catalytic breakdown cycles involving ozone. Most of the stratospheric ozone occurs between 10 and 30 km above the surface of the earth, providing an effective filter against harmful ultraviolet radiation. The ozone layer typically removes 70-90% of the UV radiation. Therefore, Depletion of the stratospheric ozone layer by human activity produced ozone-depleting substances has been recognized as a global environmental hazard for more than three decades.

The electromagnetic radiation emitted from the sun in the ultraviolet (UV) range (200–400 nm) constitutes about 7% of the total radiation. As it passes through the atmosphere, the total flux transmitted is greatly reduced and the composition of UV radiation is modified (Frohnmeyer and Staiger 2003). Increases in the UV-B radiation have been estimated since the 1980s (UNEP 2002) and present projections estimate a return to pre-1980 levels by 2050–2075 (UNEP 2008). In addition, stratospheric ozone recovery may possibly be delayed due to a number of uncertainties (UNEP 2008). Therefore, it is still essential to investigate the effects of enhanced UV-B radiation on various aspects of plant growth (Solomon 2008). Recent reductions in the stratospheric ozone layer, which enhances UV-B intensity on the surface of earth (Caldwell et al. 2003; Callaghan et al. 2004), and has initiated extensive research efforts to elucidate molecular mechanisms regulating responses of various organisms to UV-B radiation. It affects both positively and negatively. Short exposure to UV-B radiation generates vitamin D, but can also lead to sunburn depending on an individual’s skin type. Fortunately for life on Earth, our atmosphere’s stratospheric ozone layer shields us from most UV radiation. In sunlight, the ratio of UV-B to photosynthetically active radiation (PAR; 400–700 nm) fluctuates, primarily caused by changes in solar angle and
thickness of the ozone layer. Following the discovery of stratospheric ozone depletion caused by anthropogenic pollution, scientists became concerned about the effects of the resulting increasing UV-B radiation on life (Pouneva and Minkova 2010). UV-B (280–315 nm) radiation reaching earth’s surface is increasing because of depletion of stratospheric ozone layer primarily owing to continuous release of chlorofluorocarbons (CFCs) and other ozone depleting chemicals (Sharma et al. 1998; Barsig and Malz 2000; Agrawal and Mishra 2009; Rastogi et al. 2010). A lot of investigations show dramatic effects of UV radiation on plant molecular and morphological structures, as well as on the most important biochemical and physiological processes (Viñegla et al. 2006; Lesser 2008). The UV-B radiation is known to induce deleterious photochemical reactions in various biomolecules (pigments, proteins, structural changes in DNA molecules, etc.), and to damage photosynthesis, respiration and other cell metabolic processes (Agrawal and Rathore 2007). In cells of non-photosynthetic organisms, DNA absorbs ~50% of the incident ultraviolet radiation and it is the primary target of UV damage (Rinalducci et al. 2006). However, in oxygenic photosynthetic organisms, such as plants, algae, and cyanobacteria, chlorophyll, and other pigments may contribute significantly to shielding DNA from ultraviolet radiation. It has been calculated that the light-harvesting proteins (phycobiliproteins and chlorophyll proteins) account for >99% of the UV-B absorption, therefore it is not surprising that these pigmented complexes could represent the main target of UV absorption. However, in photosynthetic organisms chlorophyll and other pigments may contribute significantly to shielding DNA from UV radiation (Rinalducci et al. 2006). Therefore, besides damaging DNA a crucial part of the overall UV-B effect is related to the damage of pigments, lipids, amino acids as well as complex enzyme systems of the photosynthetic apparatus.
According to the Food and Agriculture Organization (FAO) of the U.N., there has been a major decline in world rice production since late 2007 due to many reasons including climatic conditions in many top rice producing countries. Over the last decades, the extensive agricultural and non-agricultural use of herbicides has elicited extensive research on their effects on non-target organisms. The indiscriminate use of herbicides may also have an effect on the production of rice because it affects on agronomic important biofertilizers.

Herbicides are one of the class of pesticides which are preferred to kill or slow the growth of unwanted herbs from agronomic fields. In recent years, numerous synthetic herbicides have been developed to control weeds in important agronomic crops. Because of increasing agricultural application and industrial production, pesticides, also named phytosanitory products, are becoming a major stress factor in the environment (Frankart et al. 2003). The mechanism of herbicide action has been reviewed comprehensively by several workers (Devine et al. 1993). Selective herbicides are those that kill various weed species without causing significant damage to crop species. Herbicide selectivity can be partially attributed to differences in herbicide uptake or in the method of application. Since the introduction of DDT as a pesticide in the late 1930s and the subsequent development of other organochlorine pesticides, the residues of these compounds have been found in many parts of the world. Because these compounds are lipophilic and have low chemical and biological degradation rates, they tend to accumulate in biological tissues and increase in concentrations at higher levels in the food chain (Moore and Walker 1964; Kennedy et al. 1970). Their persistence and accumulation of herbicides in aquatic organisms have led to their use being largely discontinued in the agricultural sector. Toxic tests for synthetic chemicals have demonstrated that higher plants are rather sensitive to herbicides as compared to phytoplankton (Miller et al. 1985). Most of the herbicides
interfere with the synthesis of essential amino acids, proteins, lipids, plant pigments and enzymatic activities and their regulation (Devine et al. 1993; Funke et al. 2006; Dayan et al. 2010). Herbicides may interfere with the structure, production, development and integrity of thylakoids by inhibiting the photosynthetic reactions. Inhibition of photosynthesis by herbicides was reported in several studies (Singh et al. 2011; Sheeba et al. 2011). Different herbicides differ in their mode of action in anabolic and catabolic processes such as photosynthesis, respiration, macromolecular synthesis, lipid metabolism, protein synthesis and hormone synthesis etc.

Apart from herbicides, the UV-B effects on plants have been studied during the last thirty years in growth chamber, green houses or in field mainly by irradiating plants with supplemental artificial UV-B radiation simulating different ozone depletion scenario. Such work has been extensively reviewed by Jordan et al. (1996), Allen et al. (1998), Jordan (2002), Paul and Gwynn-Jones (2003), Kakani et al. (2003), Hidema and Kumagai (2006), Julkunen-Titto et al. (2005), Rastogi and Sinha (2011) etc. The effects of UV-B radiation have extensively studied on plant communities, plant species, isolated chloroplasts and thylakoids (Teramura and Sullivan 1994; Murthy and Rajagopal 1995; Caldwell et al. 1998; Mishra et al. 2008). Enhanced UV-B radiation can affect many aspects of the plant growth and metabolism. UV-B radiation has been shown to affect plants from the molecular to the ecosystem level (Caldwell et al. 1998) and multiple target sites for UV-B action have been known (Bornman 1991; Rastogi et al. 2010). Plants species that are sensitive to UV-B radiation often exhibit changes in morphological traits, e.g., reduction in height, biomass accumulation and leaf area when exposed to increased radiation (Gonzalez et al. 1998; Correia et al. 1999; Yang et al. 2005) and physiological properties (Zhao et al. 2003). Adverse plants responses to enhanced UV-B depend on the level and duration of the stress. Responses of enhanced UV-B range from growth
inhibition and accelerated leaf senescence under moderate stress to permanent wilting of shoots with subsequent plant death under severe stress. Extensive studies on the effect of UV-B radiation on plant physiology, growth and development have shown that there are interspecific and intraspecific differences at the levels of UV-B tolerance, which differ considerably between genera, species and even closely related species, and even other factors. Lizhe et al. (2003) based on his experiment on two soybean cultivars grown under enhanced UV-B radiation reported variable responses. One of the two cultivars was more sensitive to UV-B, which was evident at the level of biomass and photosynthetic pigment reduction. The variable UV-B responses in cultivars of different plants such as maize (Correia et al. 1999), soybean (Lizhe et al. 2003), rice (Teramura et al. 1996) and some other leguminous plants (Singh 1996) have also been reported.

Plants under stress show unusual growth patterns and coloration, and UV-B radiation has no exception producing these symptoms. Apart from visible manifestations such as chlorosis and necrosis, growth characteristics are altered in plants showing UV-B sensitivity. Reduced stem growth, leaf area and biomass accumulation are often regarded as reliable indicators of plants sensitivity to enhanced UV-B radiation (Gao et al. 2003). Reduction in biomass accumulation due to UV-B exposure was found in several trees (Liu et al. 2005) and crop plants (Kakani et al. 2003). Mishra et al. 2008 also reported the reduction of growth and biomass accumulation in Vigna unguiculata, when exposed to enhanced UV-B radiation. Enhanced UV-B radiation exposure reduced the chlorophyll content (Smith et al. 2000; Correia et al. 2005; Juozaityte et al. 2008), photosynthetic rate as well as other plant processes (Rathore et al. 2003). Damage to genetic material has also been reported (Mazza et al. 1999; Hidema et al. 2000).

Since, photosynthesis is dependent on the light harvesting properties of the chlorophylls, a UV-B induced reduction in chlorophyll may be expected
to result in lower levels of biomass accumulation (Smith et al. 2000). Apart from the chlorophylls, the carotenoids have also been reported to be UV-B sensitive to enhanced UV-B radiation. The essential function of carotenoids in protecting the photosynthetic system from photo-oxidative damage in well documented and carotenoids of the xanthophylls cycle play a major role in photoprotection (Carletti et al. 2003). UV-B can also increase level of protective compounds such as flavonoids (Damme et al. 2009). However, when the protection mechanism is over taxed detrimental effect of elevated UV-B radiation on photosynthesis do occur (Bassman et al. 2001; Feng et al. 2003; Tapia et al. 2010). Reduction in rate of photosynthesis is one of the frequently reported responses to UV-B. The photosynthetic system by its nature is vulnerable to UV-B damage, and a number of targets to UV-B damage within the chloro plast and photosynthetic apparatus have been identified (Mackerness 2000). It is evident that UV-B can potentially impair the performance of all the three main component processes of photosynthesis, the photophosphorylation reactions of the thylakoids membranes, the CO2 fixation reactions of the Calvin cycle and stomatal control of CO2 supply (Allen et al 1998; Keiller and Holmes 2001; Kakani et al. 2003; Juozaityte et al. 2008). In photosynthesis particularly, photosystem II (PSII) has been found to be UV-B sensitive (Noorudeen and Kulandaivelu 1982; Olsson et al. 2000; Tevini 2004; Prasad et al. 2005). It is well established that the redox components of PS II are affected by UV-B. From previous studies, it has been assumed that ultraviolet radiation acts on either the reaction centre itself or the reducing side of PS II (Vass et al. 2000). Most observations support the notion that UV-B preferentially inactivates the water-oxidizing complex with additional effects on the QA and QB acceptors, as well as on the TyrZ and TyrD donors (Tyystjarvi 2008). It has been also observed that UV-B modifies the binding sides on the PSII with a simultaneous blocking of pheophytin, the primary electron acceptor (Teramura and Ziska 2004). Essential UV-B targets
in photosynthetic organisms include photosystem II (PSII), whose electron transport is inhibited and its D1 and D2 subunits damaged (Vass et al. 1996; Campbell et al. 1998; Viczian et al. 2000). Within PSII, QA and QB quinone electron acceptors (Greenberg et al. 1989) as well as the catalytic Mn cluster of the water oxidizing complex are damaged (Renger et al. 1989). Strid et al. (1990) showed that activity of PSII, ATP-synthetase and ribulose 1, 5-bisphosphate carboxylase was drastically decreased upon exposure of Pisum sativum to enhanced UV-B radiation.

About ~2.7 billion years ago, the evolution of photosynthetic organisms resulted in a gradual, but massive, increase in the level of atmospheric molecular oxygen (O₂) that enabled aerobic life to blossom on earth. All life on earth is based on redox reactions (reduction; the gain of an electron and oxidation; the loss of an electron), using reductive processes to store energy and oxidative processes to release it. As a result of its unusual chemistry, it was possible to integrate highly reactive oxygen in life-giving redox metabolism. It is well documented that various abiotic stresses lead to the overproduction of AOS in plants which are highly reactive and toxic and ultimately results in oxidative stress. Overall, the involvement of AOS in various metabolic processes in plant cells might have general implications. The total reduction of oxygen produces water; however partial reduction produces active oxygen species (AOS) including superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and the hydroxyl radical (·OH). AOS are produced as a consequence of electron transport processes in photosynthesis and aerobic respiration. AOS, as their name suggests, are active and potentially harmful to cells, causing oxidation of lipids, proteins and DNA. High levels of AOS production lead to a process that is often referred to as 'oxidative stress'. Oxidative stress is a condition in which AOS or free radicals are generated extra- or intra-cellularly, which can exert their toxic effects to the cells. These species may affect cell membrane properties and cause oxidative damage to
nucleic acids, lipids and proteins that may make them nonfunctional. However, the cells are equipped with excellent defense mechanisms to detoxify the harmful effects of AOS. As aerobic life evolved, so did mechanisms to scavenge the AOS and it has been a long-held view that a major purpose of such scavengers is to protect the organism from the damaging effects of AOS. These scavengers known as antioxidants (Haliwall 2006) have received much media attention in recent years as potential anti-aging compounds and agents that protect the cellular system. AOS are generated at very high rates in plants. They are produced by organelles with a high oxidising metabolic activity or intense rate of electron flow, e.g. chloroplasts, mitochondria and peroxisomes (Rio et al. 2006; Navrot 2007), or by the oxidases (Foreman et al. 2003), a large class of enzymes. It is well established that organelles such as chloroplast, mitochondria or peroxisomes with a highly oxidizing metabolic activity or with intense rate of electron flow are a major source of AOS in plant cells.

In higher plants and algae, photosynthesis takes place in chloroplasts, which contain a highly organized thylakoid membrane system that harbours all components of the light-capturing photosynthetic apparatus and provides all structural properties for optimal light harvesting. Oxygen generated in the chloroplasts during photosynthesis can accept electrons passing through the photosystems, thus forming \( \text{O}_2^- \) (Temple et al. 2005). Under steady state conditions, the AOS molecules are scavenged by various antioxidative defense mechanisms (Foyer and Noctor 2005). The equilibrium between the production and the scavenging of AOS may be perturbed by various biotic and abiotic stress factors such as salinity, UV radiation, drought, heavy metals, temperature extremes, nutrient deficiency, air pollution, herbicides and pathogen attacks. These disturbances in equilibrium lead to sudden increase in intracellular levels of AOS which can cause significant damage to cell structures and it has been estimated that 1-2% of \( \text{O}_2 \) consumption leads to
the formation of AOS in plant tissues (Bhattachrjee 2005). Accumulation of AOS as a result of various environmental stresses is a major cause of loss of crop productivity worldwide (Mittler 2002; Apel and Hirt 2004; Tuteja 2007).

From the plethora of mechanisms that can generate AOS it is clear how plants are able to produce them in large quantities and that this generation can be flexible. This flexibility has enabled plants to utilize AOS in a wide variety of physiological processes such as the regulation of photosynthesis, cell wall metabolism and for defense against pathogens and more recently they have been shown to play important roles in the control of gene expression and plant development. Moreover, AOS are part of the repertoire (store house) of signals that facilitates interactions between plants and other organisms such as bacteria and fungi in order to form beneficial structures such as mychorriza and N-fixing nodules. For AOS to be effective in these roles Graham Noctor of the University of Paris, notes that “the production and concentration of AOS requires effective regulation by a powerful antioxidant system”. The plant antioxidative system is continuously processing AOS, by acting as electron donors the antioxidants are themselves oxidized in the process of neutralizing the AOS. AOS are natural products of cell metabolism; however under conditions of environmental stress their generation may be greatly increased. Stress-induced AOS accumulation is counteracted by enzymatic antioxidant systems that include a variety of scavengers, such as SOD, APX, GPX, GR, GST, and CAT and non-enzymatic low molecular metabolites, such as ascorbate, glutathione, carotenoids and flavonoids (Gill and Tuteja 2010; Mittler et al. 2004). In addition, proline can now be added to an elite list of non-enzymatic antioxidants that microbes, animals, and plants need to counteract the inhibitory effects of AOS (Chen and Dickman 2005). Superoxide dismutase (SOD) is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to AOS mediated
oxidative stress. SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of AOS. The SOD remove O$_2$•$^-$ by catalyzing its dismutation, one O$_2$•$^-$ being reduced to Hydrogen peroxide (H$_2$O$_2$) and another oxidized to O$_2$. It removes O$_2$•$^-$ and hence decreases the risk of *OH formation via the Habere Weiss-type reaction. Hydrogen peroxide in plant tissues may be scavenged by catalases and peroxidases. Catalases are tetrameric heme containing enzymes with the potential to directly dismutate H$_2$O$_2$ into H$_2$O and O$_2$ and are indispensable for AOS detoxification during stressed conditions (Garg and Manchanda 2009). CAT has one of the highest turnover rates for all enzymes: one molecule of CAT can convert ~6 million molecules of H$_2$O$_2$ to H$_2$O and O$_2$ per minute. CAT is important in the removal of H$_2$O$_2$ generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catabolism, whereas ascorbate peroxidase (APX) is thought to play the most essential role in scavenging AOS and thus, protecting the higher plants. APX is involved in scavenging of H$_2$O$_2$ in water-water and ascorbate-glutathione cycles and utilizes ascorbate as the electron donor. Other peroxidases, including guaiacol peroxidase (POD), eliminate H$_2$O$_2$ using various reductants, e.g. phenolic compounds. PODs are involved in several processes of cell wall strengthening like lignification, cross linking of hydroxyproline rich wall proteins and feruloylated polysaccharides (Gajewska and Skłodowska 2008). Glutathione S-transferase (GST) catalyzes the conjugation of electrophilic substrates to reduced glutathione (GSH) and the resulting complexes are transported to a vacuole for further processing or degradation (Marrs 1996). Apart from participation in the metabolism of natural plant secondary compounds and detoxification of xenobiotics GST plays an important role in the removal of toxic products of lipid and protein peroxidation (Edwards and Dixon 2004). Induction of GST has been found in plants in response to herbicide treatment as well as pathogen attack,
wounding, drought, UV radiation, heavy metals, pesticides and other stress factors (Marrs 1996; Edwards and Dixon 2004). Other than as an osmolyte, now proline is considered as a potent antioxidant and potential inhibitor of plant cell damage. Accumulation of free proline has been observed in plants subjected to a wide range of stress factors like drought, salinity, extreme temperatures, UV radiation, herbicide toxicity and excess concentrations of heavy metals (Hare and Cress 1997; Gajewska and Skłodowska 2008; Gill and Tuteja 2010). This amino acid is known to take part in osmoprotection, regulation of cytosolic acidity, membrane stabilization and protection of enzymes from denaturation. In addition, proline is involved in the regulation of intracellular redox potential, possesses antioxidative activity, and serves as a storage compound and nitrogen and carbon reservoir in a cell (Kishor et al. 2005). Under stress conditions an antioxidative system can be overwhelmed and therefore not able to maintain balance between generation and removal of AOS, which may result in oxidative injuries within a plant cell.

Higher plants function as one of the essential producers in ecosystems, with important roles in sustaining the integrity of ecosystems. The herbicide and UV-B induced alterations in the growth and photosynthetic pigments have been shown in number of studies. Among these studies growth rate, pigment contents and particularly chlorophyll fluorescence were used to elucidate the mode of action of herbicide on plant physiology. Ahluwalia et al. (2002) have observed the inhibitory effect of rice field herbicide benthiocrab on the growth and pigment of *Nostoc muscorum* and *cylindrospermum* species. However, the import of various herbicides to the system results in the abnormal biochemical/physiological metabolisms in plants (Wang and Zhou 2006; Song et al. 2007). Herbicides and in general, cause toxicity and cellular disruption in plant species through induction of oxidative stress (Arora et al. 2002; Song et al. 2006), and subsequently accelerated peroxidation of lipids and oxidation of proteins (Morelli and
Scarano 2004; Gajewska and Sklodowska (2010). However, in general oxidative stress results deleterious effects include production of active oxygen species and free radicals, DNA damage and, for plants, partial inhibition of photosynthesis. As a consequence of the inhibition, plants will die or stop growing. Formation of toxic active oxygen species is generally considered to be detrimental to cellular functions, but these molecules are also formed in normal cell metabolism and their destruction is regulated (Gey 1994). In order to avoid AOS induced damage, plants evolve a complex antioxidant defense system (Kondo and Kawashima 2000; Mahalingam and Federoff 2003; Zhou et al. 2005). Herbicides induced increase in level of SOD, CAT, GPX, APX and other antioxidants were reported in several studies (Holst et al. 1982; Hassan and Alla 2005; Alla and Hasan 2006; Sood et al. 2011; Martins et al. 2011). Similar to herbicides enhanced UV-B radiation has also been reported to enhance production of antioxidants in cyanobacteria and higher plants (Häder et al. 1998; Sinha et al. 2001; Turunen and Latola 2005; Ren et al. 2006; Xu et al. 2008; Mishra et al. 2008). UV-B induced formation of AOS may proceed by multiple pathways. The probable electron transfer from the electron transport chain, especially in photosystem I (PSI) to molecular oxygen is an alternative source of AOS. Photoreduction of molecular oxygen by the primary electron acceptor in the PSI complex is thought to be the main source of $O_2$· in chloroplasts (Asada 1994).

The first line of the UV-B defense is to limit the penetration of UV-B within the leaf tissue. Exposure to enhanced UV-B radiation results in the accumulation of flavonoids, synaptic esters and anthocyanin contents, which selectively attenuate the penetration of UV-B radiation (Mackerness 2000). The potential of flavonoids to act as UV-B screen has been demonstrated by examining protection of the photosynthetic apparatus by UV-B damage (Watanabe et al. 2006). Flavonoids act as catalyst in the light phase of the photosynthesis and/or as regulator of iron channels involved in
phosphorylation (Pieta 2000). They can also function as stress protectants in plants in plant cells by scavenging AOS produced by the photosynthetic electron transport system (Pinto 2003). Several studies showed that herbicides indirectly affect the flavonoids content in plants (Hoagland and Duke 1981; Braidot et al. 2008). The levels of UV-B absorbing compounds such as anthocyanin accumulated in the plants correlated with UV-B tolerance in several studies (Gonzales et al. 1996; Gruber et al. 2010). Hirose et al. (2008) reported that the anthocyanin accumulation in transgenic rice plants was induced by a slightly higher concentration of herbicides alachlor and metolachlor.

Nitrogen is an essential mineral element plants required mostly, and nitrate is the most important source of nitrogen for plants (Crawford and Glass 1998). In nitrogen metabolism, nitrate is taken up and transported in plant, then NO$_3^-$ is reduced to NO$_2^-$, and finally NO$_2^-$ is converted to NH$_4^+$. Internal source of ammonia is photorespiration and amino acid catabolism (Srivastava and Singh 1987; Rajasekaran et al. 2009). However, high level of ammonia is toxic; therefore it has to be converted to amino acids, nucleic acids, proteins, chlorophylls, and other metabolites (Stitt et al. 2002), in plants to maintain its low level (Miflin and Lea 1980; Rajasekaran et al. 2009). Enzymes namely Glutamine synthetase (GS) / Glutamate synthase (GOGAT) and Glutamate dehydrogenase (GDH) play a vital role in ammonia assimilation, detoxification and regulation of nitrogen metabolism in plants. Among three enzymes, GDH occupies a key role in plant metabolism. Obviously, nitrogen influences all levels of plant function, from metabolism to resource allocation, growth, and development. The process of nitrogen fixation is also severely inhibited either directly or indirectly by herbicides (Singh and Wright 1999; Drew et al. 2007) and enhanced UV-B radiation (Wu et al. 2005; Krywult et al. 2008) due to the extreme sensitivity of the nitrogenase enzyme (Kumar et al. 2003; Lesser 2008).
Most of Indian agricultural lands are deprived of some of the essential nutrients for growth and development of crop plants. One of the major essential elements for growth of plants is nitrogen. Nitrogen is required in large quantities for plants to grow, since it is the basic constituent of proteins, and nucleic acids. Nitrogen is a fundamental constituent of plant structure, particularly which are associated with the photosynthetic apparatus, including carboxylating enzymes, chlorophyll, carotenoid-containing membrane proteins and its metabolism. Since nitrogen composes about 80% of the earth’s atmosphere, the plant world may literally be said to be submerged in a sea of nitrogen. Yet nitrogen in this form is unavailable to most of the plants. For plants, the most available form of this element is nitrate (NO$_3^-$) (Gajewska and Sklodowska 2009). Plants get nitrogen from the soil, by absorption of their roots in the form of either nitrate ions or ammonium ions. If nitrate is absorbed, it is first reduced to nitrite ions and then ammonium ions for incorporation into amino acids, nucleic acids, and chlorophyll (Smil 2000). After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers (Malik et al. 2001). The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water. These problems can be tackled by use of biofertilizers (Choudhury and Kennedy 2005; Rai 2006). Biofertilizers are agriculturally important, particularly in tropical rice field soils, because of the capacity of some of these to synthesize organic substances and also to fix atmospheric nitrogen (De 1939; Stewart et al. 1987). Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen in the available form for plants either by living freely in the soil or being associated symbiotically with plants (Subba Rao, 1993; Shaheen et al. 2007). Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non usable form to usable form
through biological processes (Tien et al. 1979). Biological nitrogen fixation is carried out by both symbiotic and free living bacteria and blue green algae. Symbiotic nitrogen fixation provides 80% of the biologically fixed nitrogen on land. It has been estimated that on global level nearly 200 million ton nitrogen is fixed annually and approximately two third of it on the earth comes from biological nitrogen fixation. In the biological nitrogen fixation process, N\textsubscript{2} is reduced to ammonium and the ammonium is converted to the organic form. Various biofertilizers like *Rhizobium*- legume symbiosis, *Azolla-Anabaena* symbiosis, associative and free living bacteria and blue green algae have been used for crop production (Vaishampayan et al. 2001). The estimated range of nitrogen fixation per crop (and maximum potential) was reported to be 1-7 kgN/ha (potential 40 kg) for rice rhizosphere associated bacteria, 10-80 kgN/ha (potential 170 kg) for blue green algae, 20-150 kgN/ha (potential 224 kg) for *Azolla*, and 20-190 kgN/ha (potential 212 kg) for legumes by Roger and Ladha (1990). These figure shows that *Azolla* can provide nitrogen comparable to legumes and much more than free living bacteria and blue green algae. The agricultural importance of *Azolla* in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. *Azolla*, a genus of small, fast-growing aquatic ferns that has a symbiotic association with a nitrogen-fixing cyanobacterium floats freely on the surface of water and is widespread in fresh water habitat of India, Sri Lanka, Japan, China and Philippines. The floating sporophyte phase of the plant generally called a frond has tiny adventitious roots, short-branched stem (rhizome) covered with small alternate overlapping leaves with dorsiventral organization. In the dorsal leaf lobe of the sporophyte exists an ellipsoidal cavity formed by the enfolding of the adaxial epidermis and contains the cyanobiont has often been classified as *Anabaena azollae* (Strasburger) (Uheda et al. 2004). However, it has been assigned to the genus *Nostoc* (Meeks et al. 1988) or *Trichormus* (Grilli Caiora et al. 1992). The genus *Azolla* Lamarck
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(established by Lamarck 1783) belonged to the family salviaceae and consisted of two subgenera and six living species. a- subgenus **Euazolla** included four species: *Azolla filiculoides*, *Azolla caroliniana*, *Azolla microphylla* and *Azolla mexicana*. b-the subgenus Rizosperma included two species: *Azolla pinnata* with simple glochidia and *Azolla nilotica* with no glochidia (Lumpkin and Plucknett 1980). The name of *Azolla* is derived from the two Greek words, Azo (to dry) and Ollyo (to kill) thus reflecting that the fern is killed by drought. *Azolla–Anabaena* symbiosis has proven to be the most promising and efficient system in sustaining production in low input cultivation or where little or no combined nitrogen is available, especially in paddy fields (Watanabe 1987; Rai et al. 2006). In mature frond of *Azolla*, the cyanobiont actively reduces atmospheric nitrogen and release the fixed nitrogen in the form of NH\(_3\) (Peters and Meeks 1989). The nitrogen released into the leaf cavity is rapidly incorporated into the host fern and transferred to the apical region where nitrogenase activity of the cyanobiont is absent (Kaplan and Peters 1981). *Azolla* and Cyanobacteria have been identified as ecofriendly natural nitrogen fixers in the rice field ecosystem. Widely cultivated in the Asian regions, *Azolla* is either incorporated into the soil before rice transplanting or grown as a dual crop along with rice.

Nina (1999) reported that, fast growing floating and submerged freshwater macrophytes are used commercially all over the world in aquaculture systems to produce protein rich feed for animals, green manure; remove nutrient in waste water treatment, and biogas production. *Azolla* have a symbiotic association with the N\(_2\) fixing cyanobacteria *Anabaena azollae*. It can fix 30-60 kg N ha\(^{-1}\) in 30 days. It has been also used as an important biological source to improve the nitrogen balance of rice fields. Where, it contains 3-6% N dry weight and it could double its biomass every 3-5 days (Watanabe 1982). The aquatic nature for *Azolla*, its rapid growth, high nitrogen content, and its ability to increase rice yield are main reason that
Azolla has been used as a green manure in lowland rice cultivation for centuries especially in countries like China and Vietnam (Kulasooriya et al. 1984).

Pretilachlor (chloroacetanilide) is a selective systemic herbicide, commonly used in the rice field, absorbed primarily by the germinating shoots, and secondarily by the roots, with trans-location throughout the plant, giving higher concentrations in vegetative parts than in reproductive parts. This is widely used as herbicide to control grasses, sedges, broad leaved weeds like Echinochloa, Cyperus iria, Cyperus difformis, Fimbristylis milliaceae, Ludwigia parviflora, Pannicum repens etc. in rice fields particularly in Asian and South American countries, both seeded and transplanted fields. It shows selectivity in rice, barley, cotton, peanuts, sugar beet, wheat and several Brassica crops.

Nowadays, environmental problems are multiple and complex, especially those arising from the identification and the assessment of the toxicity of such substances. Although, several studies have demonstrated individually, the effect of rice field herbicides and enhanced UV-B radiation on plants assessment of UV-B exposure and herbicide through biological monitoring offers one method to evaluate the magnitude of the potential risks of these abiotic stresses. Pretilachlor is a chloroacetanilide herbicide commonly used as rice field herbicide to eliminate the unwanted weeds. Extensive literature is available on the herbicide with their target organisms. However, no attention has been paid to observe their effect on non-target organism like Azolla, commonly used as a biofertilizer in the rice field. Therefore, it is highly essential to assess the effect of pretilachlor and enhanced UV-B radiation on physiology and biochemistry of Azolla species.

Keeping in view the above points, the present work has been undertaken and following objectives have been set forth:
The sensitivity level of two *Azolla* species (*Azolla microphylla* and *Azolla pinnata*), under pretilachlor (5, 10 and 20 µg ml⁻¹) and to enhanced UV-B radiation (UV-B₁ and UV-B₂), applied separately and in combination.

1. To study the biomass accumulation and photosynthetic pigments under the impact of pretilachlor and enhanced UV-B radiation.
2. To understand the impact of pretilachlor and enhanced UV-B radiation, on key physiological processes such as photosynthetic oxygen evolution, photosynthetic activity in isolated chloroplast, PSI, PSII and Whole chain.
3. To study the impact of pretilachlor and enhanced UV-B radiation, on nitrogen metabolism: NR activity, NiR activity, Glutamine synthetase (GS) / Glutamate synthase (GOGAT) and Glutamate dehydrogenase (GDH) activities.
4. To analysed the effect of pretilachlor and enhanced UV-B radiation, on oxidative stress: superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂) and cellular damage such as lipids, proteins and membrane leakage.
5. To understand the role of protective mechanism of *Azolla microphylla* and *Azolla pinnata* against oxidative stress induced by pretilachlor and enhanced UV-B radiation:
   (a) Enzymatic antioxidants such as SOD, CAT, GPX, APX, GR, GST and DHAR activity.
   (b) Non-enzymatic antioxidants such as Proline, Ascorbate (Oxidized, Reduced and Total), Non-protein thiol, UV-B absorbing pigments (Flavonoids) and anthocyanin content.