Introduction

Food availability is a necessary prerequisite for food security. Ensuring food security ought to be an issue of great importance for a country like India where more than one-third of the population is estimated to be absolutely poor and one-half of all the children are malnourished in one way or another. Governments and agencies do their job by formulating policies and their implementation. There is a long history of study and debate about the interrelationship among population growth, food security and the environment. A growing population exerts pressure on agricultural land, causing environmental degradation and our agricultural lands become poorer and poorer in quality. This environmental degradation ultimately reduces agricultural yields and food availability. Environmental issues are the matter of concern, not only for environmentalists but also for intellectuals from all fields. Organizations often estimate environmental losses in monetary terms which are of billions of dollars. Dimensions of ecological degradation are many like—water, air, sound, soil, radioactive pollution 

etc. Impact of environmental degradation can be easily observed in natural disasters like—earthquakes, tsunamis, land sliding, cloud bursts, flood and drought, reduced productivity of plants, complete devastation of flora and fauna of a region, extinction of plant and animal species 

etc. The situation became the worst between 1947-1995. But post-liberalization, privatization and globalization India has made one of the fastest progresses in the world in addressing its environmental issues and improving its environmental quality. Pollution remains a major challenge and opportunity for India. Still, India has a long way to go to reach environmental quality similar to those enjoyed by developed economies.

Human population size has grown enormously over the last hundred years and the population of the world has reached 7 billion on 31st October, 2011. This warns us to
immediately work upon food security. According to different estimates, in developing countries like India more than 40% people are either hungry or living under severe starving condition and malnourished which obviously results into greater mortality rate. In last two decades, with the result of immense economic growth, the use of agricultural land in non-agrarian tasks like establishment of industries, townships, power plants, roads, railways and even for energy plantation has substantially increased. The changing scenario exerts tremendous pressure on our agricultural lands, peasants, intellectuals and agricultural scientists to ensure food security. The need of the hour is to check the degradation and depletion of our precious natural land resources and pollution without halting the process of development.

Attainment of self-sufficiency in food grains at the national level is one of the country’s major achievements in the post-independence period. After remaining a food deficit country for about two decades after independence, India became largely self-sufficient in food grain production at the macro level. There have hardly been any food grain imports after the mid-1970s. Food grain production in the country increased from about 50 million tons in 1950-51 to around 244.78 million tons in 2010-11. But the experience of the last two decades shows that growth rates of production and yield have declined for crop groups/crops during the period 1996-2008 as compared to the period 1986-97. The growth rate of food grain production declined from 2.93 per cent to 0.93 per cent during the same period. The growth rate of production was much lower than that of population in the later period. Similarly, growth rate of yields of food grains declined from 3.21 per cent to 1.04 per cent. There was also a decline in growth rates of production and yields for cereals, pulses, oilseeds, rice and wheat (Dev and Sharma, 2010).

Rice is the most important food crop of the developing world and the staple food for more than 60% of the Indian populace, who are also highly vulnerable to inflationary pressure due to high rice price. Rice production in India is an important part of the national economy. Being a tropical plant, rice flourishes comfortably in hot and humid climate. Rice is mainly grown in rain fed areas that receive heavy annual rainfall, that is
why, it is fundamentally a *kharif* crop in India. India has the biggest area under rice cultivation. India stands second after China in rice production accounting for 20% of all world rice production. Since 1950 the increase in rice production has been more than 350 percent. Most of the increase is due to both, the cover under rice fields has significantly increased as well as the yield per hectare has also significantly increased. Besides structural reforms in the agricultural sector, improved varieties of seeds, improved facilities of irrigation, use of fertilizers and pesticides had a tremendous role in enhancing productivity and yield. At present, the average productivity of rice in India is 2.2 tons/hectare, which is far below the global average of 2.7 tons/hectare. The productivity of rice is higher than that of Thailand and Pakistan but much lesser than that of Japan, China, Vietnam and Indonesia. In India, the annual compounded growth rate of rice production has declined from 3.55 per cent during 1981-90 to 1.74 per cent during 1991-2000 (CRRI, 2011). Now, rice production has been estimated upto 102.75 million tons for 2011-12 crop year. The country’s rice production declined to 89.13 million tons in 2009-10 crop year (July-June) from all-time record 99.18 million tons in the previous year with a productivity of 2.2 tons/hectare was achieved during the year 2008-09. India needs to produce 120 million tons by 2030 to feed its 1.5 billion plus population by then. A real-time analysis of this scenario provides sufficient justification for strengthening, intensifying and introducing cutting edge science and technology for increasing rice productivity in India (CRRI, 2011).

Development in industries and agriculture is taken as a general criterion for development of any country. This has resulted into imprudent and unlimited exploitation of natural resources. Unlimited exploitation of natural resources due to the anthropogenic activities is causing ecological imbalance, and biotic as well as abiotic components of our ecosystem are disturbed. The ecological balance is necessary for all life forms to live. Faster industrialization, urbanization and indiscriminate use of agrochemicals cause imbalance in both abiotic and biotic components of soil and water ecosystem which in turn, results into harmful effects, not only on entire soil and aquatic ecosystem but also on human beings.
One of the major environmental related issues is loss in agricultural productivity which in turn threatens food security of our country. Our lands get polluted due to excessive use of agrochemicals like– pesticides, fertilizers etc. In the wake of Green Revolution, use of chemical fertilizers and pesticides increased manifold for enhancing crop production. Steep drop in agricultural productivity may be due to one or more reasons among several: use of agricultural land in non-agrarian tasks, excessive and imprudent usage of chemical fertilizers and pesticides, which not only saturates the soil but also intoxicates the cereal crop by harming their overall physiology and biochemistry. In addition to this, non-target organisms that are important components of the soil ecosystem like soil microbes, bacteria, fungi and blue green algae (privileged to be associated with atmospheric nitrogen fixation, fertility of the soil and nutrient recycling) may be harmed, which may indirectly affect the productivity and food security.

Soil and water pollution due to pesticides have become a common concern among environmentalists. Use of pesticides became indispensable and an integral part of modern agriculture and their use under Integrated Pest Management Programme to save the crop losses becomes quite decisive in countries like India in wake of second green revolution likely to be experienced in next few years (Sheeba et al., 2011). Use of pesticides cannot be ruled out in our agricultural fields because of steady but continuous rise in population and lesser availability of agricultural fields. Pesticides that are used in agricultural practices are transported to water bodies through run-off, drift and leaching, and increase the risk of exposure to non-target organisms (Chen et al., 2007). Some of the pesticides (organochlorines e.g. DDT) are biomagnified in the terrestrial ecosystems, so they were banned worldwide according to the international treaties and conventions.

Organisms like insects, weeds, microorganisms (microfungi, bacteria), rodents, nematodes, weeds, unwanted plants and others, that cause economic loss or damage to the physical well-being of humans as well as other organisms are known as pests. They may damage and destroy our crops, cause disease in them and are considered as the greatest enemies of modern agriculture. Pesticides are often used in agriculture to protect
crop plants from weeds, diseases and depredations from insects, fungi, mites and rodents. Pesticides are also used to control the populations of rats, cockroach and to remove termites. Any chemical that can kill or repel a pest is known as chemical pesticide. Some prominent pesticides which are frequently used in these days by the farmers in paddy fields include— 2, 4-D, aldrin, atrazine, butachlor, carbaryl, carbofuran, chlordane, chlorpyrifos, cypermethrin, DDT, dieldrin, dimethoate, endosulfan, glyphosate, heptachlor, lindane, malathion, monocrotofos, parathion, permethrin, phorate, triazophos, trifluralin etc. Most of the pesticides are non-degradable and accumulate in the environment.

Pesticides are the only toxic chemicals deliberately released into the environment in large amounts. By the time use of pesticides became indispensable and an integral part of modern agriculture. The excessive use of pesticides has a serious impact on many beneficial microorganisms resulting in greater loss in crop productivity. Since most of the pesticides are non-biodegradable, they have long residence time in water and soil and thus may enter and magnify at various trophic levels (Yadav, 2010). Their potential to cause adverse effect to human and wildlife populations has been the subject of intense study and has led to the development of increasingly stringent regulations for the risk assessment of novel formulations and to control the use of existing compounds (Galloway and Handy, 2003).

The organophosphorus pesticides were introduced in the 1970s as replacements for the persistent organochlorines after the tendency of DDT and its metabolites to accumulate in ecosystems and to cause health hazards, particularly in top predators (Murphy, 1986). The increased use of organophosphorus pesticides originally seen as lesser threat to the environment but by the time organophosphorus pesticides have become a serious environmental concern due to their high acute toxicity despite their low persistence. They form the largest group of chemicals used to control the pests including invertebrates, vertebrates and to a lesser extent, plants. There are some 200 organophosphorus pesticides available which have been formulated into literally
thousands of different products (Hill, 2003). These products are used in agriculture, forests, gardens, home and industrial sites etc.

Organophosphorus pesticides are ubiquitous in the environment and are highly toxic to animals like fish, amphibians, rats etc. They inhibit acetylcholinesterase (AchE) enzyme of animals by binding it, resulting in neurological dysfunction and normal nerve impulse is checked. Inhibited AchE results in repeated and uncontrolled firing of neurons leading to death, usually by asphyxiation as respiratory control is lost. Most chemicals in this group require oxidative desulfuration to achieve their greatest cholinesterase-inhibiting potencies. Oxidative desulfuration is mediated by mixed-function oxidases (MFO) residing in the liver and results either in an oxon or a sulfon degradate, depending on the active moiety of the molecule (Tahara et al., 2005). These MFOs are also involved in cellular wastes metabolism but, in the case of organophosphorus pesticides, increase the toxicity of the pesticide involved.

Organophosphorus insecticides are widely used in the direct soil application for the control of mosquitoes, flies, various crop pests in soil and on foliage, household pests and aquatic larvae (Bicker et al., 2005; Goel et al., 2005). While there may be ancillary effects of pesticides (Schuh et al., 2002; Sparling, 2003), acetylcholinesterase depression remains the primary mechanism. The rate of inhibition is related to exposure concentration which is due to the rate of assimilation of the pesticide, rate of conversion to the oxon form, affinity of the insecticide molecule for cholinesterase and rate of cholinesterase regeneration. Organophosphates are specific to a target animal, similarly animals are also specific to organophosphates, as many organophosphates do not kill a particular pest even if their highest concentrations are used while few of them can kill those particular pests at their recommended doses.

Many studies prove that oxon derivatives of organophosphates are significantly more toxic, sometimes upto 3000 times than their respective parental forms. Since the rate of cholinesterase depression was much more rapid for the oxon derivatives than their
parental counterparts, conversion to oxon forms was an important factor in the relative toxicity of these pesticides. It is not unusual for pesticides to vary in their potency to reduce cholinesterase within a given species (Richardson et al., 2001; Kousba et al., 2004). In addition of being metabolized internally in liver cells, bacteria and other microorganisms can convert pesticides into sulfons or oxons (Hill, 2003), thereby making them available in the environment (Schomburg et al., 1991; Domagalski, 1996; Whitehead et al., 2005). There is a wide range of adverse environmental effects linked to organophosphorus pesticides, include toxicity to domestic animals, freshwater fish, other aquatic organisms, birds, beneficial insects, a variety of plants and soil organisms etc. It has been shown to bioaccumulate in fish and synergistically reacts with other chemicals.

One of the most important organophosphate pesticides is chlorpyrifos (molecular formula \( \text{C}_9\text{H}_{11}\text{Cl}_3\text{NO}_3\text{PS} \); IUPAC name \( O, O\)-diethyl \( O\)-3, 5, 6-trichloro-2-pyridyl phosphorothioate) which is a well-known home and garden insecticide. Brodan, Detmol UA, Dowco 179, Dursban, Empire, Eradex, Lorsban, Paqeant, Piridane, Scout and Stipend are some of the prominent formulations under which chlorpyrifos of various effective concentrations are sold. Chlorpyrifos is currently used in more than 100 countries worldwide and have been registered in India since last two decades in both agricultural and household pest control (http://www.chlorpyrifos.com/worldwide-use.htm, http://www.chlorpyrifos.com/worldwide-use.htm). Chlorpyrifos is a white, granular, crystalline solid. Its melting point is 42-43°C and vapor pressure is \( 1.87 \times 10^{-5} \) mm Hg at 25°C and \( 8.87 \times 10^{-5} \) mm Hg at 35°C. Its molar mass is 350.59 and specific gravity is 1.398. Chlorpyrifos is soluble in the solvents like– benzene (7900 g kg\(^{-1}\)), acetone (6500 g kg\(^{-1}\)), chloroform (6300 g kg\(^{-1}\)), ethyl acetate (2000 g kg\(^{-1}\)) and methanol (450 g kg\(^{-1}\)). It is slightly soluble in water (0.4 µg ml\(^{-1}\)). Chlorpyrifos is stable in air (non-volatile), forms weakly acidic solutions with water, and hydrolyzed by strong bases. Depending upon the type of application and formulation, chlorpyrifos residue may be detectable in water, soil and on surface for months to years (Jin and Webster, 1997; Phillips et al., 2007).
Chlorpyrifos is moderately toxic to animals and chronic exposure has been linked to neurological effects, developmental disorders and autoimmune disorders. Being an organophosphate, it binds with acetylcholinesterase, an enzyme that breaks down the neurotransmitter acetylcholine so that subsequent impulses are transmitted across the synapse. Chlorpyrifos is a neurotoxin and suspected endocrine disrupter (Das and Barone, 1999). Its poisoning usually affects many organs of the body. Among the most commonly affected are the central and peripheral nervous system, eyes, respiratory system and the digestive tract. Symptoms of acute and chronic chlorpyrifos exposure in humans include headache, dizziness, vomiting, weakness, twitching, sweating, stomach cramps and hypertension and at high doses cause unconsciousness, coma and even death. The estimated accepted daily intake (ADI) of chlorpyrifos for humans was set at 0-0.1 mg kg\(^{-1}\)bw d\(^{-1}\) by the World Health Organization (WHO)/Pesticide Specification and Quality Control Standards (PSC) and by the Food And Agricultural Organization (FAO)/WHO.

In vitro studies have shown that the oxygen analogue of chlorpyrifos to be 10\(^6\) times more active inhibitor of cholinesterase than chlorpyrifos itself (\(I_{50}\) values: chlorpyrifos approx. 2.5X10\(^{-3}\)M; oxygen analogue approx. 2.5X10\(^{-9}\)M) (Smith, 1966). The median lethal concentration (LC\(_{50}\)) for Rana boylii (yellow-legged frog) was found to be 3.005 mg L\(^{-1}\) by Sparling and Fellers (2007). Cowman and Mazanti (2000) reported LC\(_{50}\) (96 h) from 1 mg L\(^{-1}\) in Bufo americanus to 3 mg L\(^{-1}\) in Rana pipiens. As with other phosphorothioate esters, chlorpyrifos is rapidly absorbed, metabolized and excreted by
mammals following oral administration. Some mammals like rat can sustain even at 50 mg kg\(^{-1}\) and these animals can eliminate even the radioactive chlorpyrifos. The LD\(_{50}\) for chlorpyrifos in rat has been reported to be 82-270 mg kg\(^{-1}\) body weight (Berg, 1986; US Environment Protection Society, 1984). The LD\(_{50}\) in a number of avian species has been determined by Tucker and Crabtree (1970). The toxicity values range from 8 mg kg\(^{-1}\) in male ring necked pheasant to 167 mg kg\(^{-1}\) in mallard ducklings. LD\(_{50}\) for mammals is reported to be 94 mg kg\(^{-1}\) (Berteau and Deen, 1978).

Belonging to phosphorothioate class, chlorpyrifos is one of the most used insecticides in the world. However, no report is available for India, it was the most widely used insecticide during 90s in U.S., estimating total use at almost 30 million pounds per year (Cox, 1994). Besides its usage in our agricultural fields, it is also used to control cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, mosquitoes and lice in households. It is a widely used insecticide effective against broad spectrum insect pests of economically important crops. Chlorpyrifos is characterized by P-O-C linkage, as in diazinon, parathion, methyl parathion and fenitrothion. The crops with the most intense chlorpyrifos use are grain, cotton, corn, almonds, vegetables, nuts and fruit trees including oranges and apples besides its usage in lawns and ornamental plants (Knezevic and Serdar, 2009). In paddy fields, chlorpyrifos is intensively used for effective control of gall midge (Orseolia oryzae), leaf folder (Nappalocroas medinalis) and leaf hopper (Nephotettix virescens) (Mallick et al., 1999).

Mineralization/half-life of pesticides depends upon many factors viz. form of the pesticide (i.e. analytical grade or commercial grade), dispersion phase (e.g.– soil, water, nutrient media, other organic solvents etc.). The granular formulation of chlorpyrifos has been found to be more persistent and may persist as long as 180 days. Chlorpyrifos is sensitive to light, alkaline substances such as bleach and microbial degradation. Eventually, it degrades completely in response to carbon dioxide and water. The half-life of aqueous methanolic solution at pH 6 is 1930 days. The rate of hydrolysis increases with both, pH and temperature. Dissipation of chlorpyrifos follows probably the first order reaction which varies with analytical form and commercial formulations. The half-
life of chlorpyrifos in water is relatively short, from a few days to two weeks. After hydrolysis, it liberates 3,5,6-trichloro-2-pyridinol which undergoes further decomposition to diols and triols and ultimately cleavage of ring to fragmentary products (Racke et al., 1996). Hydrolysis occurs least readily at about pH 6 and very readily above pH 8. Chlorpyrifos is thermally sensitive to temperature over 50°C and undergoes violent exothermic decomposition at 130°C.

As common with the most organophosphates, chlorpyrifos has a relatively short biological half-life, roughly 24 hours in blood and 60 hours in fat (assuming that multiple or continuous exposure does not occur). Chlorpyrifos remains biologically active in soil for periods ranging from 20 to 90 days depending upon the soil type, climate and other prevailing conditions and is moderately persistent, with half-life varying from 10 to 60 days (Lakshmi et al., 2008) with degradation being primarily due to the microbial action (Environment Canada, 1986). This range in half-life is due to the fact that degradation of chlorpyrifos in soil is affected by its initial concentration, soil moisture, temperature and pH (Racke et al., 1994; Awasthi and Prakash, 1997; Singh et al., 2011), cell density of the microorganism (if any microorganism is present in the vicinity). Major routes of chlorpyrifos degradation are volatilization, microbial degradation and chemical hydrolysis on dry soil surfaces (Getzin, 1981a, b; Racke et al., 1988). Residues remain on plant surfaces for approximately 10 to 14 days. Data indicate that this insecticide and its soil metabolites can accumulate in certain crops. The metabolic fate of chlorpyrifos has been investigated in plants (Smith et al., 1967a, b) and data indicate an oxidative and/or hydrolytic breakdown, yielding des-mono-ethylchloropyrifos; desethylchlorpyrifos; 3,5,6-trichloro-2-pyridinol and further degradation products. Chlorpyrifos may be degraded very efficiently by the Flavobacterium sp. ATCC 27551 (isolated from diazinon-retreated rice fields) (Sethunathan and Yoshida, 1973) and the Arthrobacter sp. (isolated from a flooded soil retreated with methyl parathion) (Misra et al., 1992).

The inhibition of physiology through organophosphates (e.g. chlorpyrifos) is completely different in plants in comparison to animal pests. Studies with Alcaligenes faecalis (gram-negative bacterium) demonstrated its effectiveness to withstand and
metabolize chlorpyrifos in soil (100 mg kg\(^{-1}\)) (Yang et al., 2005). Phormidium
valderianum BDU 20041, a marine species of Phormidium could sustain as much as 55
ppm (55 µg ml\(^{-1}\)) was reported by Palanisami et al. (2009). Singh et al. (2011) showed
through their experiment that Synechocystis sp. could survive in chlorpyrifos upto 15 mg
L\(^{-1}\) and exhibited 20%, 50% and 77% inhibition of growth in 5, 6.5 and 10 mg L\(^{-1}\) chlorpyrifos treated samples, respectively. The highest recommended dose of the
chlorpyrifos as given by the producer (Dow Agro Science India Pvt. Ltd., Ratnagiri,
Maharashtra, India, in paddy field (for the removal of leaf roller insect, Cnaphalocrocis
medinalis) is 375 µg ml\(^{-1}\). In case of plants, chlorpyrifos at 105 mg L\(^{-1}\) has been reported
to be stimulatory on Vigna radiata (Parween et al., 2011). Further, studies showed that 5
mg kg\(^{-1}\) soil and 10 mg kg\(^{-1}\) soil of chlorpyrifos did not significantly influence growth of
rape oilseed and wheat seedlings, respectively and at the same doses of chlorpyrifos
microbial activity was also higher (Wang et al., 2007).

Pesticides appear to be very effective in controlling a wide range of pests that
infect crop plants including paddy. But the application of pesticides in agricultural
systems may also exert side effects on non-target organisms, particularly soil microflora.
The effects of a few pesticides have been investigated on cyanobacteria with respect to
growth, photosynthesis, nitrogenase activity and carbon fixation etc. Suresh Babu et al.
(2001) studied the effect of lindane on growth and metabolic activity of cyanobacteria.
Furthermore, several reports have been published on the comparative toxicity of
herbicides and fungicides towards various organisms such as blue-green alga (Abou-
Waly et al., 1991).

Another aspect of environmental degradation is enhanced level of UV-B radiation
is of course, due to the increased human interference with environment which would
ultimately lead to the climate change. Solar UV-B radiation comes on the earth’s surface
due to depletion of stratospheric ozone layer. Ozone layer is found in the upper part of
the atmosphere, the stratosphere and it acts as shield for ultraviolet radiation coming from
the sun. It is the thinnest in the tropics (around the equator) and denser towards the poles.
The thickness of the ozone in a column of air from the ground to the top of the atmosphere is measured in terms of Dobson unit (DU); typically ~260 DU near the tropics and higher elsewhere, though there are large seasonal fluctuations. UV rays are highly injurious to living organisms since DNA and proteins of living organisms preferentially absorb UV rays and its high energy breaks the chemical bonds within these molecules. Ozone gas is continuously formed by the action of UV rays on molecular oxygen and also degraded into molecular oxygen in the stratosphere. There should be a balance between the production and degradation of ozone in the stratosphere. Rapid industrialization in the past few decades has resulted in an increase in anthropogenically released chlorofluorocarbons, chlorocarbons and organobromides causing depletion of the stratospheric ozone layer (Crutzen, 1992). Considerable amounts of reactive nitrogen species such as nitric oxide (NO), peroxynitrite (ONOO⁻) and nitrous oxide (N₂O), produced naturally from unpolluted terrestrial and aquatic ecosystems or from anthropogenic sources (biomass or fuel burning and chemical fertilizers), also contribute to the depletion of the ozone layer (Kramlich and Linak, 1994; Singh et al., 2010). CFCs find wide use as refrigerants. CFCs are discharged in the lower part of the atmosphere, move upward and reach stratosphere. In stratosphere, UV rays act on them and release chlorine atoms. Chlorine degrades ozone and releases molecular oxygen, with these atoms acting merely as catalysts, chlorine atoms are not consumed in the reaction. Hence, whatever CFCs are added to the stratosphere, they have permanent and continuing effects on ozone levels. In the past 50 years, the concentration of ozone has decreased by about 5% (Pyle, 1996). The process of ozone depletion has been reported at mid-latitudes and especially in the Antarctic where ozone levels have been reported to decline by more than 70% during late winter and early spring in the polar vortex (Smith et al., 1992). This has resulted in the formation of a large area of thinned ozone layer, commonly called as the ozone hole.

Due to the ozone depletion, there is an increase in the amount of harmful ultraviolet radiation (UVR; 280-400 nm) on the earth surface. Ultraviolet radiation can be divided in three categories on the basis of energy level i.e. UV-A (320-400 nm), UV-B
(280-320 nm) and UV-C (less than 280 nm). Among these UVR, UV-C is highly energetic and damaging and does not come on the earth surface followed by UV-B and UV-A. UV-B and UV-A both are coming on the earth surface due to the ozone depletion. In past few decades, UV-B has attracted more and more attention of scientists due to its harmful impact on flora and fauna. UV-B radiation is an integral component of the sunlight. Seven percent of the electromagnetic radiation emitted from the sun falls under UV range (200-400 nm). As it passes through the atmosphere, the total transmitted flux is greatly reduced and the composition of the UV radiation is modified. Wavelengths shorter than UV-C radiation (200-280 nm) are completely absorbed by atmospheric gases. UV-B radiation (280-320 nm) is additionally absorbed by the stratospheric ozone and thus only a very small proportion is transmitted to the earth surface, whereas UV-A radiation (320-400 nm) is hardly absorbed by ozone.

The increased exposure to UV-B is detrimental to human health, food production and the ecosystem. It causes aging of skin, damage to skin cells and various types of skin cancers. In human eye, cornea absorbs UV-B radiation, and a high dose of UV-B causes inflammation of cornea, called snow-blindness, cataract etc. Such exposure may permanently damage the cornea. Recognizing the deleterious effects of the ozone depletion, an international treaty, known as the Montreal Protocol, was signed at Montreal (Canada) in 1987 (effective in 1989) to control the emission of ozone depleting substances. Subsequently, many more efforts have been made and protocols have laid down definite roadmaps, separately for developed and developing countries for reducing the emission of CFCs and other ozone depleting chemicals. It has been assumed that chlorofluorocarbons, which can deplete the ozone layer can remain in the upper atmosphere for 40-150 years, hence, the global UV-B radiation will not recover to the levels of the pre-industrialization era by 2050, even if all the nations implement the Montreal Protocol (World Meteorological Organization, 2007; Mohammed and Tarpley, 2011).

Stratospheric ozone is important to protect terrestrial life from potentially lethal solar radiation, and as a trace gas layer, it extends between 10 and 40 km above sea level
(Phoenix, 2000). Ozone concentrations are found maximum between 20-40 kilometers, where they range from about 2-8 ppm. Ozone (O\textsubscript{3}) is created by the dissociation of oxygen (O\textsubscript{2}) by short wavelength ultraviolet (UV; less than 280 nm) radiation in the stratosphere. When oxygen free radical (O\textsuperscript{•}) combines with molecular form of oxygen (O\textsubscript{2}), ozone is formed. Absorption of UV at the wavelength upto about 320 nm converts the O\textsubscript{3} back to the O\textsubscript{2} and O (Chapman, 1930).

The atomic oxygen quickly combines with further oxygen molecules to form ozone:

\begin{align*}
O\textsubscript{2} + h\nu & \rightarrow O\textsuperscript{•} + O\textsuperscript{•} & (1) \\
O\textsuperscript{•} + O\textsubscript{2} & \rightarrow O\textsubscript{3} & (2)
\end{align*}

\((h\nu = \text{wavelength} < \sim 280 \text{ nm})\)

Presence of ozone in the stratosphere is essential for our survival but, ozone is a pollutant. On the earth surface, it is a major constituent of photochemical smog. Up in the stratosphere, it potentially absorbs harmful ultraviolet radiations from the sun which can cause skin cancer and damage vegetation. Although the UV radiation splits the ozone molecule, ozone can reappear through the following reactions resulting in no net loss of the ozone:

\begin{align*}
O\textsubscript{3} + h\nu & \rightarrow O\textsubscript{2} + O\textsuperscript{•} & (3) \\
O\textsuperscript{•} + O\textsubscript{2} & \rightarrow O\textsubscript{3} & (4)
\end{align*}

Ozone is also destroyed by the following reaction:

\begin{align*}
O\textsuperscript{•} + O\textsubscript{3} & \rightarrow O\textsubscript{2} + O\textsubscript{2} & (5)
\end{align*}

The above reactions are known as the “Chapman reactions” as these photochemical reactions were first discovered by the British scientist Sidney Chapman in 1930. Reaction (2) becomes slower with increasing altitude while reaction (3) becomes
faster. The concentration of ozone is balanced by these competing reactions. The layer of ozone formed in the stratosphere by these reactions is sometimes called the ‘Chapman layer’. In the upper atmosphere, atomic oxygen dominates where UV levels are high. Moving down through the stratosphere, the air gets denser, UV absorption by air particles increases and ozone level reach its peak at roughly 20 km. From the stratosphere to the ground, UV level decreases and ozone level also declines.

Elevated UV-B radiation (UV-B) has pleiotropic effects on plants’ development, morphology, physiology and biochemical composition (Jansen et al., 1998; Xiong and Day, 2001; Brosche´ and Strid, 2003; Caldwell et al., 2003; Frohnmeyer and Staiger, 2003). UV-B can generate reactive oxygen species and cause damage to macromolecules, including DNA. Because UV-B affects the growth, development, reproduction and survival of many organisms; there is growing concern that any further increases in ambient levels of UV-B may have a significant impact on natural and agricultural ecosystems (Caldwell et al., 2003; McKenzie et al., 2003; Paul and Gwynn-Jones, 2003; UNEP, 2005). Hence, it is important to understand how plants and other organisms protect themselves against the potentially damaging effects of UV-B.

The morphological consequences of UV-B-supplemented white-light treatment include reduced growth, thickening of leaves and of cuticular wax layers. In addition, a lower photosynthetic capacity due to the degradation of the D1 protein of photosystem II and reduced pollen fertility have been described for various plant species (Jansen et al., 1998; Caldwell et al., 2003). Sessile life style of plants forces them to adapt to changing environmental conditions. The most common protective mechanism against potentially damaging irradiation is the biosynthesis of UV absorbing compounds (Hahlbrock and Scheel, 1989). These secondary metabolites, mainly phenolic compounds, flavonoids and hydroxycinnamate esters, accumulate in the vacuoles of epidermal cells in response to UV-B radiation and attenuate the penetration of the UV-B range of the solar spectrum into deeper cell layers. Recent genetic approaches have shed some light on novel components that might be involved in the perception of UV-B and in the transduction of signals generated by UV-B. DNA is particularly sensitive to UV-B radiation because
absorption of UV-B causes photo-transformations, resulting in the production of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidinone dimers (6-4 PPs). Because DNA and RNA polymerases are not able to read through these photoproducts, their elimination is essential for DNA replication and transcription and thus for survival (Britt and May, 2003).

To avoid the cytotoxic effects of UV-induced DNA damage, most organisms have developed a complex set of repair mechanisms including photo-reactivation, excision and recombination repair. Photo-reactivation is a light-dependent enzymatic process using UV-A and blue light to monomerize pyrimidine dimers: photolyase binds to the photoproducts and then uses light energy to initiate electron transfer to break the chemical bonds of the cyclobutane ring and restore integrity of the bases. In addition, plants respond to DNA-damaging treatments such as high doses of UV with repair by homologous recombination (Ries et al., 2000a). Under high UV-B irradiance, cells cannot survive for a long time. High doses of UV-B induce the formation of CPDs which are the most lethal and directly result into the cell death. During UV-B radiation, the increased homologous recombination frequency correlates with the amount of CPDs formed. Although homologous recombination in plants is generally classified as a dark repair process, it is stimulated by red (but not by far-red) exposure after UV-B treatment. These observations indicate that photosynthetic activity or other as yet undefined processes dependent on photosynthetic active radiation (400-700 nm) may promote UV-B induced homologous recombination in plants (Ries et al., 2000b).

In general, different plants respond differentially to UV-B irradiation with low or high doses of UV-B, either by stimulating protection mechanisms or by activating repair mechanisms (Kim et al., 1998; Brosché and Strid, 2003; Caldwell et al., 2003; Frohnmeyer and Staiger, 2003). Exposure to UV-B is obligatory for the photoautotrophs as they need to maximize light capture for photosynthesis. In analogy to phytochromes and cryptochromes, distinct low- and high-fluence responses could also be sensed by different UV-B receptors. Frohnmeyer and Staiger (2003) worked on dose dependent gene expression and characterization on putative signaling elements linked to gene
expression. Although low-fluence, longer wavelength UV-B responses appear to be photoregulatory rather than damaging or stress causing, no UV-B photoreceptor has been identified and little is known about the signaling components. There is strong evidence from studies of gene expression and growth responses that the longer wavelength, low-fluence UV-B responses are not mediated by DNA damage signaling (Kim et al., 1998; Frohnmeyer et al., 1999; Boccalandro et al., 2001; Ulm et al., 2004). At low fluence rates, UV-B is capable of promoting metabolic and developmental changes, such as morphogenesis, biosynthesis of phenolic secondary metabolites and mycosporin-like amino acids (MAAs), inhibition of hypocotyl elongation (Kim et al., 1998; Jenkins et al., 2001; Suesslin and Frohnmeyer, 2003; Ulm and Nagy, 2005; Jenkins and Brown, 2007). It is well documented that the responses to low UV-B fluence rates are in part due to transcriptome changes. The molecular underpinnings of UV-B perception and the proposed signaling cascade by the UV-B photoreceptor(s) have been reviewed in detail (Jordan, 1996; Jansen et al., 1998; A.-H.-Mackerness, 2000; Brosche´ and Strid, 2003). It has been demonstrated that low fluence rates of UV-B stimulate expression of a range of genes that help in protecting plants against UV damage or to ameliorate its damaging effects (Jenkins et al., 2001; Frohnmeyer and Staiger, 2003; Ulm et al., 2004; Brown et al., 2005; Jenkins and Brown, 2007). These include genes concerned with the production of flavonoids and other phenolic compounds that accumulate in the epidermal layers and provide a UV-absorbing sun screen (Hahlbrock and Scheel, 1989; Li et al., 1993). Mutants lacking UV-protective components, such as the flavonoids and sinapic acid esters, are highly sensitive to ambient levels of UV-B (Li et al., 1993; Landry et al., 1995). Although plant responses to low ambient fluence rates of UV-B are keys to survival, the underlying mechanisms of UV-B perception and signal transduction are very poorly understood, but some recent researches pertain with specific receptors and thereby signal transduction (Brown et al., 2005), however, no UV-B-specific signaling pathway could be defined. In addition to this, the underlying physiology has also not been understood. It has often been speculated that UV-B may be perceived by a novel class of photoreceptor, but no such molecule has ever been identified.
Thus, it is clear that lower dose of UV-B can initiate protective responses while higher dose can initiate damaging responses based on its fluence rates. It has been reported that UV-B induces decrease in growth and photosynthesis by damaging lipids, proteins and nucleic acids (He and Häder, 2002b). The most effective protection mechanism stimulated under such light regime is the biosynthesis of flavonoids and other UV-B-absorbing phenolic components. A similar strategy is employed by cyanobacteria to withstand deleterious UV-B radiation impinging on them. They are thought to use a special class of compounds with absorption maxima between 310 and 360 nm as UV protector. In the filamentous and heterocystous N\textsubscript{2}-fixing Anabaene sp., Nostoc commune and Scyttonema sp. Shinorine (a representative of these mycosporin-like amino acids which are defined by the presence of a cyclohexenone or cyclohexenimine chromophore conjugated with an amino acid or its imino alcohol) accumulates in response to solar UV-B radiation, mostly during the daily light period (Sinha et al., 2001).

High doses of UV-B or UV-C are damaging to cellular components and the energy of the radiation is sufficient to cause photochemical changes to a certain set of molecules. Studies have demonstrated that high UV-B fluence rate produces excess reactive oxygen species (ROS) and thus causes damage to DNA, proteins, membranes and lipids and does not involve specific receptors (A.-H.-Mackerness et al., 2001; Brosche´ and Strid, 2003; Frohnmeyer and Staiger, 2003; Ulm and Nagy, 2005; Jenkins and Brown, 2007). These higher fluence rates can cause leaf curling and growth inhibition, wound signaling and initiate the expression of genes characteristic of stress responses via signaling pathways which are not specific to UV-B. Several lines of evidence indicate that these pathways overlap with wound and defense-signaling pathways (Conconi et al., 1996; A.-H.-Mackerness et al., 2001; Stratmann, 2003; Jenkins and Brown, 2007). Ultraviolet-B radiation affects plants including cyanobacteria from the molecular level to the ecosystem level (He and Häder, 2002a). It is well established that enhanced UV-B radiation damages nucleic acids, proteins and lipids that are essential for genetic, biochemical and physiological functions such as growth, pigmentation, photosynthesis and nitrogen metabolism within the cyanobacterial cells (Karentz et al.,
1991; Sinha et al., 1996; Rai et al., 1998; He and Häder, 2002a; Gao et al., 2007). Especially the DNA molecule itself has been considered an attractive candidate for a UV-B receptor, and a number of responses in plants and animals were related to UV-B absorption by DNA because they were maximally stimulated by wavelengths between 250 and 280 nm (Herrlich et al., 1997). However, action spectra of UV-B responses in plants revealed their maximal stimulation between 290 and 310 nm, whereas wavelengths below 290 nm inhibited these responses (Herrlich et al., 1997). In addition, a lack of correlation between the increase in DNA damage (caused by UV-B) and UV-B elicited changes in transcript profiles contradicts the theory that damaged DNA serves as a UV-B receptor (Kim et al., 1998; Frohnmeyer et al., 1999; Kalbin et al., 2001).

There is large agreement that a UV-B receptor consists of a protein with a bound pterin or flavin as chromophores (Galland and Senger, 1988; Ensminger and Schäfer, 1992). Investigations of other UV-B-induced events indicated that reactive oxygen species (ROS) serve as signaling components. UV-B radiation on plant tissue itself causes the generation of ROS such as singlet oxygen. Besides the ROS-responsive pathway and calcium sensitive pathways, a third non-specific pathway activated by high doses of UV-B and/or UV-C has been proposed (Brosche´ and Strid, 2003). This pathway might be activated by deleterious effects of high energy radiation and is possibly linked to wound signal transduction in plants (Conconi et al., 1996). UV-B has also been shown to stimulate a complete biosynthetic pathway consisting of more than a dozen genes. An added complication is that, whereas many UV-B-induced responses are pertinent with reducing the levels of damage that UV-B inflicts on a plant, some are triggered directly by the light signal and others may arise as a reaction to the molecular damage caused within cells. Therefore, it may not be intuitively obvious which responses are regulated by which UV-B pathway.

Nitrogen-fixing cyanobacteria are known to be a prominent component of the microbial population in wetland soils, especially rice fields, contributing significantly to the fertility as a natural biofertilizer (Kumar and Kumar, 1998). Cyanobacteria are known as major biomass producers both in aquatic as well as terrestrial ecosystems and represent
more than 50% of the biomass in many aquatic ecosystems (Häder et al., 2007). Cyanobacteria are valuable sources of various natural products of medicinal and industrial importance (Cardozo et al., 2007). Besides this, some of them have inherent capacity to fix atmospheric nitrogen which makes them ecologically important for rice-growing countries including India where they add to fertility of rice field soils as natural fertilizer (Vaishampayan et al., 2001). They use the enzyme nitrogenase to convert atmospheric molecular nitrogen into ammonium ($\text{NH}_4^+$; a central ion of ammonium assimilation pathway) through which nitrogen enters the food web. It has been estimated that cyanobacteria fix over 35 million tons of nitrogen annually and thus play major role in nutrient cycling of ecosystems (Häder et al., 1989). In India, it has been shown long before that the introduction of cyanobacteria (blue-green algae) to saline and alkaline soils in the state of Uttar Pradesh increases the soils’ nitrogen content and organic matter and also their water holding capacity (Singh, 1961). This treatment has enabled formerly barren soils to grow crops. Watanabe and Kiyohara (1960) found that the introduction of *Tolypothrix tenuis* resulted in a 120% increase of rice crop. Booth (1941) noticed that cyanobacteria coating the land surface maintain a high water content in soil and reduce erosion.

In filamentous cyanobacteria, three levels of cellular differentiation are found: vegetative cells for photosynthesis, heterocysts for nitrogen fixation and akinetes which are developed under stress conditions and store phosphate. Cellular differentiation in cyanobacteria has been reported to be affected by UV-B (Gao et al., 2007). It was found that exposure of cells to UV-B radiation delayed the differentiation of vegetative cells into heterocysts and akinetes in *Anabaena aequalis* (Blakefield and Harris, 1994). In *Anabaena* sp. PCC 7120, differentiation of heterocysts has been suppressed by UV-B (Gao et al., 2007) and alteration in the carbon:nitrogen ratio following UV-B exposure suggested that UV-B to be responsible for the altered spacing pattern of heterocysts in the filament (Sinha et al., 1996). The spiral filaments of *Arthrospira platensis* have been reported to be broken and compressed under solar UV-B (Wu et al., 2005; Gao et al., 2008). It has been reported that the number of motile filaments in a population of
*Phormidium uncinatum* was found to be decreased to zero within a few minutes after UV-B exposure (Donkor and Häder, 1991). The growth of the cyanobacteria *Oscillatoria priestleyi* and *Phormidium murrayi* was drastically decreased flowing UV-B exposure (Quesada and Vincent, 1997). Similarly, reduction in growth of *Anabaena* sp. was also recorded following UV-B exposure (Han et al., 2003; Gao et al., 2007). Complete devastation of several cyanobacteria was reported within 120-180 min of UV-B exposure (Tyagi et al., 1992; Sinha et al., 1995).

Cyanobacteria also called as blue green algae (BGA) are phylogenetically primitive group of gram-negative prokaryotes having cosmopolitan distribution ranging from hot spring to the Arctic and Antarctic regions (Singh et al., 2010). They were probably the first photosynthetic oxygen-evolving organisms appeared during the Precambrian era and thus provided favorable conditions for the evolution of current aerobic life (Fischer, 2008). They can also grow under very low water potential, arid environments and such species can resist desiccation or can tolerate high salinity. Their diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colorless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to basophilic, planktonic to barophilic, fresh-water to marine including hypersaline (Brock, 1976; Bullerjahn and Sherman, 1986; Castenholz, 1996). They are found both, free living and endosymbionts. Therefore, changes in their community may have indirect but significant effects on the rest of the fresh water communities, nitrogen status and organic matter accumulation (Li et al., 2010). It is known that organisms differ in their sensitivity to different stress factors. Therefore, for better exploitation of cyanobacteria as biofertilizer their degree of tolerance and tolerance strategies against various stresses must be known.

Cyanobacteria possess various photosynthetic pigments such as chlorophyll *a*, carotenoids and phycobiliproteins to optimize the harvesting of solar radiation. Among these pigments, light harvesting is primarily carried out by phycobiliproteins (phycoerythrin; \( \lambda_{\text{max}} \) 540-570 nm, phycocyanin; \( \lambda_{\text{max}} \) 610-620 nm and allophycocyanin; \( \lambda_{\text{max}} \) 650-655 nm) which are arranged in a light-harvesting complex called phycobilisome.
A decrease in phycobiliproteins and disassembly of phycobilisomal complex following UV-B radiation has been reported in a number of cyanobacteria (Sinha et al., 1995, 1997). Exposure of cyanobacterial cultures to intense UV-B radiation causes bleaching of phycocyanin and phycoerythrin (Prasad and Zeeshan, 2004; Sinha et al., 2005). There are several studies indicating that UV-B radiation also influences the chlorophyll and carotenoids contents in cyanobacteria (Han et al., 2003; Prasad and Zeeshan, 2004, 2005; Lesser, 2008; Sheeba et al., 2011). In addition to the photosynthetic pigments, UV-B also decreases other photosynthetic parameters such as \(^{14}\text{CO}_2\) uptake, \(\text{O}_2\)-evolution and ribulose-1, 5 bisphosphate carboxylase (RUBISCO) activity in cyanobacteria (Prasad and Zeeshan, 2004, 2005; Sinha et al., 2008). It has been also observed that UV-B modifies the binding sites on the PS II with a simultaneous blocking of pheophytin, the primary electron acceptor (Renger et al., 1986). The D1 and D2 proteins, major constituents of PS II reaction centre are also degraded by UV-B exposure (Sass et al., 1997; Campbell et al., 1998). Several families of transcripts including mRNAs specifying proteins involved in light harvesting and photosynthesis were also down-regulated by UV-B treatments (Huang et al., 2002).

The process of nitrogen fixation is also severely inhibited either directly or indirectly by UV-B radiation due to the extreme sensitivity of the nitrogenase enzyme (Tyagi et al., 1992; Kumar et al., 2003; Lesser, 2008). Complete loss of nitrogenase activity within 25-40 min of UV-B exposure was reported in several rice field cyanobacteria (Kumar et al., 2003). Further, enzymes of nitrogen metabolism such as nitrate reductase, nitrite reductase and glutamine synthetase have been reported to be affected by UV-B radiation (Appenroth et al., 1993; Rai et al., 1998).

The protein contents and profile of cyanobacteria is also affected by UV-B radiation (Kumar et al., 1996; Bhargava et al., 2007). Kumar et al. (1996) reported complete loss of protein bands between 14.2 and 45 kDa after 90 and 120 min of UV-B exposure in \textit{Nostoc calcicola}. Ehling-Schulz et al. (2002) monitored the proteomic change following UV-B exposure using two-dimensional gel electrophoresis in the cyanobacterium \textit{Nostoc commune} and observed that out of 1350 protein spots, 493 were changed by UV-B radiation.
Any situation in which cellular redox homeostasis is disturbed can lead to the generation of reactive oxygen species (ROS) and oxidative stress (Asada, 1994). ROS generation is excessively increased during stress condition (He and Häder, 2002a, b; Mallick, 2004; Prasad and Zeeshan, 2005; Wang et al., 2008). The increased production of ROS and the resultant oxidative stress are considered to be the initial event and act as an alert signal for the organisms under several environmental stresses *i.e.* metals, UV-B, drought, salt, temperature *etc.* Enhanced production of ROS during environmental stress is one of the main causes for decrease in productivity, injury and death which accompany stresses. ROS are repeatedly reported to be the inevitable by-products of biological redox reaction and normal metabolism in humans, animals, plants and algae and pose a constant threat to all aerobic organisms. ROS are produced in both unstressed and stressed cells, and in various locations (Halliwell and Gutteridge, 1989). Although some of them may function as important signaling molecules that alter gene expression and modulate the activity of specific defense proteins, however, all ROS can be extremely harmful to organisms at their high concentrations (Dietz et al., 1999; Pinto et al., 2003).

The bulk of the earth’s present oxygen concentration (*i.e.* 21%) is, in fact, derived from the photosynthetic activities of cyanobacteria and plants. Cyanobacteria, the oxygenic photosynthetic organisms, are prone to the oxidative stress due to the facts that they contain an array of photosynthetic pigments which are also potential photosensitizers under UV-B radiation and that they both produce oxygen during photosynthesis and consume oxygen during respiration. It is quite impressive that cyanobacteria are capable of adapting various stress conditions such as excess light, heat, salinity and cold by the induction of relevant anti-stress proteins or molecules. Several strategies have been thrived to allow cyanobacteria to survive under UV-B stress including migration into mat communities, induction of UV-absorbing compounds such as mycosporine-like amino acids (MAAs) and scytonemin, induction of antioxidant defense and efficient repair system.

UV-B radiation has been reported to enhance the production of ROS in cyanobacteria (Häder et al., 1998, Ehling-Schulz and Scherer, 1999). UV-B induced
formation of ROS may proceed by multiple pathways. The photosynthetic pigments such as chlorophyll and phycobiliproteins in cyanobacteria can act as photosensitizers under UV-B exposure since they may also absorb in the UV-B range (Quesada and Vincent, 1997; Ehling-Schulz and Scherer, 1999). Furthermore, photosynthetic electron transport chain in the life of cyanobacteria promotes the possibility of subjecting to oxidative stress under UV-B stress (Prasad and Zeeshan, 2005). The probable electron transfer from the electron transport chain, especially in PS I to molecular oxygen is an alternative source of ROS. Photoreduction of molecular oxygen by the primary electron acceptor in the PS I complex is thought to be the main source of superoxide radical (SOR, \(\text{O}_2^{•−}\)) in chloroplasts (Asada, 1994). UV-B induced oxidative stress might also be result of enhanced production of hydroxyl radical (\(\text{•OH}\)) in Fenton and Haber-Wiess reactions, if \(\text{O}_2^{•−}\) and hydrogen peroxide (\(\text{H}_2\text{O}_2\)) are not properly scavenged. In the presence of increased concentrations of transition metals such as Fe\(^{2+}\) or Cu\(^+\), there is a risk of generation of highly reactive \(\text{•OH}\) and non-selective oxidation of cellular constituents (Bienfait and Van der Mark, 1983; He and Häder, 2002a, b). Generation of ROS does depend upon the duration, amount and the fluence rates of UV-B but not proportional to the UV-B dose. The formation of ROS increases with the increase in UV-B dose. Under high UV-B radiance, cells cannot survive for long time and the formation of ROS decreases when the cells die. It was found that ROS formation was higher when photosynthetically active radiation (PAR, 400-700 nm) was included with UV-B than without PAR. This suggested that the photosynthetic electron transport chain and photosynthetic processes, both contribute to the formation of ROS (Halliwell and Gutteridge, 1989; Asada, 1994; Foyer et al., 1994).

Application of agricultural pesticides for improving crop productivity has become necessary in the present day agricultural practices. Pesticides may also generate ROS via their toxic effects on photosynthesis. This has resulted in either stimulatory or inhibitory effects on the soil microflora including nitrogen-fixing cyanobacteria (Kumar et al., 2012). Any adverse effect of pesticide may severely affect metabolic functions and overall growth performance of the cyanobacteria. Some of the of chlorophyll biosynthesis enzymes are hindered and thereby ROS are generated which in turn damage to
macromolecules such as lipids, proteins and nucleic acids (Sheeba et al., 2011). There are several studies which show the worst effect of pesticides on growth and photosynthesis, nitrogen metabolism and amino acid metabolism in higher plants. However, effect of pesticides on cyanobacterial physiology is still poorly known. As stated above that UV-B may play dual role based on its fluence rates, other toxicants may also have dose dependent responses on cyanobacteria. Hormesis is a dose-response phenomenon characterized by a low dose stimulation and high-dose inhibition (Calabrese and Baldwin, 2003). Hormesis is seen upto 40% of toxicological experiments (Calabrese and Baldwin, 2001). Calabrese and Baldwin (2003) have carried out the dose-range finding studies under National Toxicology Program and suggested 5 doses of such types for each toxicant. However, the study was done on animals. They argued that the enhanced efficiency in the utilization of consumed nutrients was the primary cause of low dose-stimulation. Hammouda (1999) observed in his experiment with Anabaena doliolum that the sheathless heterocystous cyanobacterium was initially able to utilize low concentrations of carbofuran (a carbamate pesticide), whereas higher concentrations and the subsequent formation of hydrolytic breakdown products were toxic. Growth inhibition reached more than 50% when treated with 80 and 100 ppm of the insecticide. Rajendran et al. (2007) have observed that lower concentrations of bavistin (a fungicide) supported good growth of Tolypothrix scytonemoides with maximum protein and pigment contents.

Cells respond to oxidative stress by removing ROS and maintaining antioxidant defense system at levels that reflect ambient environmental conditions (Scandalios, 1997). Metabolic mechanisms for ROS scavenging involving antioxidant genes and associated processes are likely to have predated or co-evolved with the appearance of aerobiosis and represent fundamental adaptations of aerobic systems to an oxygen dependent metabolism (Grene, 2002). Under unstressed conditions, the formation and removal of ROS are in balance. However, when the defense system faces increased ROS under stress conditions can be overwhelmed when it is unable to remove the toxic ROS with increased antioxidant processes (Apel and Hirt, 2004). Under stressful conditions, organisms develop efficient antioxidant systems to scavenge ROS (Costa et al., 2002;
White and Jahnke, 2002; Fedina et al., 2003; Mallick, 2004; Gong et al., 2005; Wang et al.
2008). Antioxidative system may be enzymatic or non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione-S-transferase (GST) of which SOD is a major scavenger of $O_2^\cdot$ and converts them into $O_2$ and $H_2O_2$. The $H_2O_2$ is then scavenged by CAT and variety of POD into $H_2O$ and $O_2$. In addition to SOD, CAT and POD, other major components of the enzymatic defense system are enzymes of ascorbate-glutathione cycle that involve four enzymes including ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) (Noctor and Foyer, 1998). Among these four enzymes, APX is the major scavenger of $H_2O_2$ which requires reduced ascorbate (AsA) as electron donor provided by ascorbate-glutathione cycle for the reaction to occur (Noctor and Foyer, 1998). DHAR and GR regenerate reduced form of ascorbate and glutathione from their oxidized forms while MDHAR catalyzes reduction of monodehydroascorbate into reduced ascorbate using NADPH as an electron donor.

Non-enzymatic antioxidants *i.e.* reduced ascorbate (AsA), reduced glutathione (GSH), non-protein thiols (NP-SH) and cysteine are also crucial compounds involved in defense against oxidative stress (Noctor and Foyer, 1998; Mishra et al., 2009; Shan and Liang, 2010). It is known that organisms can adjust AsA and GSH contents by modulating the regeneration and biosynthesis of AsA and GSH. Ascorbate-glutathione cycle is the recycling pathway of AsA and GSH regeneration. Thus, the AsA-GSH cycle plays an important role in maintaining the contents of AsA and GSH. Of the many functions ascribed to AsA, relatively few are well characterized. Being major antioxidant, AsA directly reacts with $'OH$, $O_2^\cdot$ and singlet oxygen (Buettner and Jurkiewicz, 1996). In addition to its importance in photoprotection and regulation of photosynthesis (Foyer and Harbinson, 1994; Forti and Elli, 1995), AsA also plays an important role in preserving the activities of enzymes that contain prosthetic transition metal ions (Padh, 1990). Ascorbate is also a powerful secondary antioxidant reducing the oxidized form of $\alpha$-tocopherol, an important antioxidant (Padh, 1990). Ascorbate may also be involved in regulation of cell cycle (Kerk and Feldman, 1995). When the AsA pool is diminished
(during oxidative stress), cells are arrested in G1-phase preventing replication of damaged DNA (Kerk and Feldman, 1995). Recently, it has been reported that cadmium toxicity in rice seedlings was associated with declined level of AsA (Chao et al., 2010). Glutathione is involved in both the direct and the indirect control of ROS (May et al., 1998; Foyer and Noctor, 2005). As a component of the ascorbate-glutathione cycle, GSH takes part in the removal of excess H$_2$O$_2$ (Noctor and Foyer, 1998). The high ratio of GSH and its oxidized form (GSH/GSSG ratio) occurring inside cell under optimal growth conditions can be restored by means of higher GR activity, increased GSH synthesis, decreased GSH degradation or the transport of GSH. Besides the ascorbate-glutathione cycle, GSH may also participate in H$_2$O$_2$ degradation in a reaction catalyzed by glutathione peroxidase. Besides H$_2$O$_2$, GSH removes lipid peroxides, methylglyoxal and herbicides (Moons, 2005; Yadav et al., 2008). The reaction is catalyzed by glutathione-S-transferases (GSTs). Glutathione not only participates in the direct detoxification of ROS, it may also protect cells against unfavorable stress effects through activation of various defense mechanisms due to its involvement in redox signaling (Foyer et al., 1997; Apel and Hirt, 2004; Mullineaux and Rausch, 2005; Pitzschke et al., 2006). Recently, it has been noticed that glutathione protects cell metabolism during stress condition (Shan and Liang, 2010).

Non-protein thiols (NP-SH) are low molecular weight non-enzymatic antioxidants, contain a high percentage of cysteine-sulfhydryl residues and play a pivotal role in heavy-metal detoxification by oxidation of sulfhydryl moieties to disulfides (Morelli and Scarano 2004; Stoiber et al., 2007). Cysteine has also been considered as an important amino acid imparting tolerance in plants against various stresses (Rausch and Wachter, 2005; Grill et al., 2006). Cysteine is synthesized at final step of sulphate assimilation by the enzyme cysteine synthase and it is involved in the synthesis of GSH and phytochelatins for controlling metal toxicity (Dominguez-Solis et al., 2001).

In natural condition, generally, cyanobacteria encounter multiple stresses simultaneously. Thus, it is possible that simultaneous exposure of cyanobacteria to UV-B radiation and pesticide contamination in natural condition may lead alteration in growth of cyanobacteria which could have adverse impact on productivity and biomass
production in ecosystems. Thus, it became necessary to evaluate whether the toxicity of pesticide will be enhanced or weakened due to the presence of low and high fluence rates of UV-B. Although much of the works have been done on the effects of pesticides and UV-B separately on the physiology and biochemistry of plants, however, rarely of them have dealt with the interactive effects of these two stresses on plants (Mishra et al., 2008, 2011) and the studies pertaining to the impact of these twin stresses on cyanobacteria are the rarest. No report is available which could show impact of low (non-damaging) and high (damaging) UV-B fluence rates on growth, pigments, photosynthesis, nutrient uptake, nitrogen metabolism, phosphate mobilization, more importantly on oxidative stress and antioxidant system despite their ecological and economic importance.

In modern agricultural technological programs, cyanobacteria have been used to increase plant available nitrogen and organic matter in soil for better production of rice in south Asian countries including India. *Nostoc muscorum*, a heterocystous cyanobacterium, fixes molecular nitrogen in aerobic condition. Non-heterocystous cyanobacteria which are predominantly found in paddy fields (Desikachary, 1959) may also fix atmospheric nitrogen under aerobic conditions as Ohki et al. (1992) demonstrated the potential of a non-heterocystous cyanobacterium *Trichodesmium* in nitrogen fixation. *Phormidium foveolarum*, a non-heterocystous cyanobacterium, fixes nitrogen in microaerobic condition particularly in darkness. Therefore, in order to study the impact of low and high UV-B fluence rates on alterations in physiological and biochemical processes of two cyanobacteria *viz. Nostoc muscorum* and *Phormidium foveolarum* which lead to oxidative stress and to further to understand the response of antioxidant system under low and high doses of chlorpyrifes the following objectives were set forth:

- To study the level of sensitivity of the two cyanobacteria exposed to low and high doses of UV-B radiation under low and high doses of chlorpyrifes.
- To study the growth behavior, photosynthetic pigments, photosynthesis and respiration in two cyanobacteria exposed to low and high doses of UV-B radiation under low and high doses of chlorpyrifes.
- To study the pattern of nutrient uptake, enzymes of nitrogen metabolism and phosphate mobilization in two cyanobacteria exposed to low and high doses of UV-B radiation under low and high doses of chlorpyrifos
- To study the influence of UV-B radiation on oxidative stress in two cyanobacteria under low and high doses of chlorpyrifos:
  (a) **Generation of reactive oxygen species:**
      (i) Generation of superoxide radical
      (ii) Generation of hydrogen peroxide
  (b) **Oxidative damage to macromolecules**
      (i) Damage to lipids as lipid peroxidation
      (ii) Damage to proteins as protein oxidation
- To study the influence of low and high doses of UV-B radiation on antioxidant defense system under low and high doses of chlorpyrifos:
  (a) **Enzymatic antioxidants**
      (i) Superoxide dismutase activity
      (ii) Catalase activity
      (iii) Peroxidase activity
      (iv) Glutathione-S-transferase activity
      (v) Ascorbate peroxidase activity
      (vi) Glutathione reductase activity
      (vii) Monodehydroascorbate reductase activity
      (viii) Dehydroascorbate reductase activity
  (b) **Non-enzymatic antioxidants**
      (i) Total ascorbate, reduced ascorbate, dehydroascorbate and reduced/oxidized ascorbate ratio
      (ii) Total glutathione, reduced glutathione, oxidized glutathione and reduced/oxidized glutathione ratio
      (iii) Cysteine content
      (iv) Non-protein thiols content