Chapter-2
Review of Literature
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To present the thesis entitled “Impact of urea on plant growth promoting rhizobacteria with reference to rice crop” related available information in general has been reviewed and presented below.

2.1. PLANT GROWTH PROMOTING RHIZOBACTERIA

In rhizosphere large number of microorganisms such as bacteria, fungi, algae and protozoa exist. Bacteria are the most abundant among them. Plants release organic compounds through their root exudates and select those bacteria which fits them.(Lynch, 1990). PGPR promote plants growth by many mechanism such as they fix atmospheric nitrogen, solubilize insoluble phosphate, produce growth hormones, siderophores and also by producing ACC deaminase, HCN etc. (Glick et al., 1999). PGPR are free-living root colonizing soil bacteria which directly or indirectly enhance growth of plants.(Mayak et al.,1999). Those bacteria which colonize plant roots and enhance growth of plant by number of mechanism are known as plant growth promoting rhizobacteria (PGPR).(Dey et al., 2004). It is found that about 2-5% of rhizobacteria are PGPR. (Antoun and Prevost, 2005). The original concept of rhizosphere now include the soil which surrounds a root in which physical properties, chemical properties and biological properties is changed by growth and activity of root.(McCully, 2005). Bacteria being the most abundant microorganisms in the rhizosphere, influence plant physiology to much greater extent.(Antoun and Kloeper, 2001; Barriuso et al., 2008). Thus PGPR become potential tools for sustainable agriculture. Therefore there is a need for research to clear that what bacterial traits are important, useful and necessary for different environmental conditions and plants, so that those bacteria can used for bioferlization.
Plant Growth Promoting Rhizobacteria belongs to genera *Pseudomonas*, *Azotobacter, Azospirillum, Bacillus, Klebsiella, Enterobacter, Alcaligenes, Arthobacter, Burkholderia, and Serratia* etc.(Zhang, 1996; Misko and Germida, 2002). Many researchers observed significant increase in growth and yield of agronomical crops when inoculated with PGPR. (Glick *et al.*, 1996; Asghar *et al.*, 2000; Biswas *et al.*, 2000, Misko and Germida, 2002).

### 2.2. DIVERSITY OF PGPR

Although there has been considerable research on PGPR having growth promoting traits, relatively little is known about the diversity and population dynamics of PGPR in field soils.

Diversity of PGPR is studied by many researchers. The microbial population of soil was influenced by the environmental changes in soil. The relationship between diversity of soil microbes, plant and soil quality and soil sustainable environment has been observed (Gerbeva *et al.*, 2004).

Soil bacteria plays very important role in many biogeochemical cycles, influence plant health, fertility of soil and above ground ecosystem. Plant growth promoting rhizobacteria fix atmospheric nitrogen, solubilize mineral nutrients, decomposes the organic matter and protect plants from pathogen thus play significant role in maintaining plant health.(Gaind *et al.*, 2008).

Diverse PGPR were isolated and identified from sweet potato rhizosphere. They were identified as *Pseudomonas corrugate, Serratia ficaria, Klebsiella terrigena, Erwinia cypripedii, Acinetobacter radioresistens, Pseudomonas maculicola, Paenibacillus pabuli* etc. (Yasmin, 2009)

From wheat rhizosphere of Northern Himalayan region *Bacillus* is found to be the most dominant genera. It is followed by *Pseudomonas, Serratia, Flavobacterium,*
*Micrococcus, Klebsiella, Azotobacter, Enterobacter, Xanthomonas, Staphylococcus* and *Micrococcus.* (Joshi and Bhat., 2011).

Saline stress tolerant PGPR are isolated from Avocado tree showing phosphate solubilization, IAA, siderophore and hydrogen cyanide production. Out of the PGPR isolated dominant is *Pseudomonas* followed by *Bacillus* species. (Nadeem *et al.*, 2012).

**2.3. ISOLATION AND CHARACTERIZATION OF PGPR**

The root surface bacteria known as rhizobacteria can be isolated more from the rhizospheric region than from the bulk soil. (Curl *et al.*, 1986). In soil ecosystem bacterial community forms an important component of biological characteristics. (Kent *et al.*, 2002). Bacterial population differs in different soil types depending upon the ecosystem and environment of soil. Among soil microbial population bacteria are the most abundant microorganism. They form association with the plants either in their root surface or inside the root. (Larcher *et al.*, 2003).

Several researchers isolated and characterized diverse rhizobacteria from different plant rhizospheres.

Twenty three bacteria out of 31 isolated from wheat rhizosphere were found most efficient in producing auxin in presence of L-tryptophan. (Khalid, 2003).

Thirty rhizobacteria from wheat rhizosphere were isolated showing auxin production in different range and also observed to increase plan growth. (Khalid *et al.*, 2004).

Twenty one rhizobacterial isolates were isolated from different crops such as mustard, cauliflower and wheat from some parts of Uttar Pradesh region. Out of these ten were *Azotobacter* and eleven were found to be *Pseudomonas*. Each strain of both the bacteria were auxin producer increasing the growth of plants. (Ahmad *et al.*, 2005).
Twenty nine diazotrophic rhizobacteria were isolated from the rhizosphere of oil palm, tested for nitrogen fixing activity. Some of the species of Paenibacillus, Azospirillum and Acetobacter were found to fix atmospheric nitrogen.(Azlin et al., 2005).

Fifty fluorescent Pseudomonas were isolated from soil, 72% of them were found producing auxin. (Khakhipour et al., 2008).

Thirty fluroscent Pseudomonas were isolated from different plant rhizosphere. All showed the growth promotion of plants by producing auxin at different concentration of tryptophan.(Karnwal, 2009).

From sweet potato rhizosphere 15 bacteria were isolated producing auxin, 6 were able to solbilize phosphate and 3 were siderophore producing bacteria.(Farzana et al., 2009).

Bhavnath and coworkers were isolated 170 nitrogen fixing rhizobacteria from rice rhizosphere. All were found to increase plant growth in various parameters. (Jha et al., 2009).

Prakash Nathan and coworkers isolated fourteen Pseudomonas species from rice rhizosphere. They observed all the Pseudomonas tolerating low temperature and some tolerate high temperature.(Nathan et al., 2011).

Bacillus species were isolated from rice rhizosphere and screened for auxin production. Different species of Bacillus were found to produce different intensity of auxin. (Karuppiah and Rajaram, 2011).

From Uttarkashi district 133 isolates were isolated. Some of them were able to produce auxin, some siderophore and some solubilize phosphate. (Joshi and Bhatt, 2011).

Bacillus megaterium and Pseudomonas florescence were observed to show positive result for solubilization of phosphate.(Sharma et al., 2011).
Eight species of efficient plant growth promoting Pseudomonas are isolated, characterized and screened for auxin production and phosphate solubilization from rice rhizosphere. (Lavkush and Verma, 2012).

Twenty stains of three different species of Pseudomonas were isolated from rice rhizosphere in Malaysia. They were screened for their plant growth promoting activity. (Noori and Saud, 2012).

Seven rhizobacteria were isolated and evaluated in rice field of Haryana, for plant growth. They were able to grow at pH range 5 to 13 and tolerate 20°C to 40°C temperature. (Subramaniam et al., 2012).

Kurthia species with auxin producing and phosphate solubilizing properties were isolated from tea rhizosphere. (Sharma, 2012).

2.4. GROWTH PROMOTING FEATURES OF PGPR

Plant growth promoting rhizobacteria possess various growth promoting features such as nitrogen fixation, mineral solubilization, phytohormone production, siderophore production and antagonism against various bacterial and fungal pathogens.

Rhizobacteria increase the growth of crop plants by auxin synthesis which is a plant growth regulators. (Frankenberger and Bunner, 1983).

They are able to solubilize inorganic and organic phosphates present in soil. (Liu et al., 1992) and produce auxin, gibberellins, siderophore, HCN and antibiotics (Arshad et al., 1992).

PGPR are involved in increase nitrogen uptake, synthesis of phytohormones, solubilize minerals such as phosphorus, and produce siderophores that chelate iron and make it available to the plant root (Lalande et al., 1989; Glick, 1995).
Functionally the Plant Growth Promoting Rhizobacteria has been categorized into two groups:-

(i) Those showing direct activity in plant growth by nutrient cycling and phytostimulating and

(ii) Those showing indirect activity over plant growth by bio control of plant pathogens.(Bashan, 1998).

Inoculation of plants with beneficial bacteria is not a new concept; it has been already applied from centuries. From their experience, farmers says that they mix the soil of previous legume crop to the soil in which non-legumes were to be grown by which yields often improved. For a long period *Rhizobium* have been used as biofertilizer produced around the world, primarily by small companies.(Bashan, 1998).

Among the plant growth promoting rhizobacteria, genus *Pseudomonas* is significantly important since it widely distributed in the soil. They are able to colonize plants rhizosphere and produce wide range of metabolites.(Penrose and Glick, 2003).

PGPR have a wide range of plant growth promoting properties such as auxin production, phosphate solubilization, production of ACC deaminase enzyme, production of siderophore, chitinase salicylic acid and hydrogen cyanide which are directly or indirectly involved growth of plant.(Rashid et al., 2004).

### 2.4.1. Auxin production

Thus PGPR having IAA producing ability was largely used in stimulating seed germination and accelerating root formation. Soil and rhizospheric bacteria synthesis IAA which promote growth of plant and also some bacteria with this ability are antagonist for various disease causing plant pathogens.(Comai and Kosuge, 1982). It is a common product of L-tryptophan metabolism by PGPR.(Frankenbeger and
Bunner, 1983). It is found that more than 80% of the rhizospheric bacteria able to produce auxin and enhance root growth.(Salisbury, 1994). IAA is simple metabolite that is derived from tryptophan (Trp) by many enzymatic pathways in plants and in bacteria. Thus a pathway for IAA biosynthesis is common to plants and many microorganism including PGPRs which may be tryptophan dependent or tryptophan independent.(Glick et al., 1996). The production of auxin by members of genera *Azospirillum, Pseudomonas, Erwinia* etc is beneficial for plant growth.(Pattern et al., 1996).

Various PGPR secrete auxins and gibberlins which regulate and promote plant growth. (Vidhyasekaran, 1998; Lebuhan et al., 1997). The Dutch botanist Frits W. Went discovered auxin in 1926. In 1935 K. V. Thimann isolated IAA from fungus *Rhizopus sinus*. Since then number of research related to auxin is reported. Many bacteria present in rhizosphere of various plants synthesis and release auxin as secondary metabolites. Indole Acetic Acid is most physiologically active auxin. It regulates plant growth and development from cell division, cell elongation and cell differentiation to root initiation, apical dominance, flowering, fruit ripening and senescence.(Guilfoyle et al., 1998). Auxin regulate growth of plant by affecting morphological and physiological processes at very low concentration.(Arshad and Frankenbeger, 1998).

Plant growth promoting rhizobacteria highly promote growth of roots and help in establishing young seedling to soil by promoting the primary and lateral roots.(Pattern et al., 2002). Auxin has been much extensively studied plant growth hormone which regulates cell division and cell elongation.(Berleth and Sachs, 2001).

*Pseudomonas flourescence* is able to produce auxin, plays an important role in plant growth and increase plant production.( Benizri et al., 2001). Several
microorganisms are capable of producing plant growth hormones such as auxin, cytokinins, gibberlins etc. (Tilak et al., 2005). Tilak and coworkers also analyzed many soil bacteria producing auxin and other growth hormones. Auxin mediates plant growth by various mechanisms such as apical dominance, cell tropism, root formation and elongation. (Tilak et al., 2005). Pseudomonas and Azotobacter isolated from different crop plants and screened for Indole acetic acid production with different concentration of tryptophan. Both found to be good auxin producer at high level of tryptophan. Root length significantly increased by their inoculation. Thus such PGPR must be used as biofertilizers for crop yield.(Ahmad et al., 2005). It is reported that growth rate and root elongation in seed germination of *Sesbania aculeate* and *Vigna* inoculated with *Pseudomonas* increased due to the production of indole -3 - acetic acid.(Ahmad et al., 2005). Different strains of Azotobacter and Pseudomonas were able to produce auxin in various ranges at different concentration of tryptophan. (Ahmad et al., 2005).

*Pseudomonas* florosence and *P. aeriegnosa* isolated from different plant rhizosphere shows growth promotion of plant by producing IAA at different tryptophan concentration, as the concentration of tryptophan increased more IAA is produced.(Karnwal, 2009). PGPR isolates showing auxin production remarkably increase seed germination of rice plant. These PGPR results in increase plant height and root length.(Ashrafuhammad, 2009). With the increase in the concentration of tryptophan from 50µg/l to 500µg/l increased production of indole acetic acid was observed by two species of *Pseudomonas*. (Karnwal, 2009).

Different species of Bacillus isolated from rice fields were able to produce auxin in different ranges in presence of 100 µg/l of tryptophan. (Karuppih and Rajaram, 2011). Different species of *Pseudomonas* isolated from rice field of
Malaysia were found to produce siderophore, hydrogen cyanide, auxin and also solubilize phosphorous. (Noori and Saud, 2012). Significant production of indole acetic acid in presence of 100µg/l tryptophan was observed by Kurthia species. (Sharma et al., 2012). Species of Pseudomonas and Bacillus were found to produce comparatively more indole acetic acid in presence of tryptophan then in its absence. (Jarak et al., 2012).

Thus seeds inoculated by auxin producing bacteria is important for enhanced seed germination, increase root length, increase plant height since auxin induce the tissues to redifferentiate

2.4.2. Phosphate solubilization

Although soil has sufficient phosphate but it is mostly unavailable to plants, since they are less soluble and less mobile. The phosphorous applied as fertilizer also reprecipitate into the various insoluble forms of phosphate both in alkaline and acidic soils thus they were also not observed by plants. (Pikovskya, 1948). Phosphorous comprises of 0.2% of plants dry weight. It is an essential component of nucleic acid, phospholipids and ATP.(Theodorou and Plaxton, 1993). Phosphorus is an essential macronutrient required for growth and development of plant after nitrogen. Phosphorous cycle in atmosphere is described as open cycle since there is no interchange with the atmosphere.(Richardson,1994). These bacteria solubilize the insoluble phosphate by various mechanisms. (Bagyaraj and Varma, 1995).

Plants absorb phosphorous in soluble form. Microorganisms are involved in transformation of insoluble phosphate into soluble form. They are effective in releasing phosphate from total soil phosphorous by the mechanism of solubilization and mineralization and play a vital role to make them available to plants.(Daniel et al., 1998). Inorganic phosphorous is solubilized due to the formation of organic acid
and phosphatase enzyme. These enzymes are present in various soil microorganism including bacteria. Microorganism release phosphorous from mineral phosphate by producing organic acids such as oxalic acid, lactic acid, tartaric acid etc. (Kim et al., 1998).

Different strains of bacteria were found to synthesis and release different organic acids which help to free the phosphates from insoluble phosphatic forms and make available for plants. (Kucey et al., 1989; Gyaneshwar et al., 1998; Leinhos, 1994; Chakoskaya et al., 2001). Due to this less availability of phosphorous to the crops, the farmers are directed to apply more and more phosphate than the requirement. The application of over dose of phosphorous to particular crops cause over fertilization which disturbs the soil nutrient balance and leads to imbalance in soil environment. (Lal, 2002). It plays an important role in cell division, photosynthesis and many other physiological activities of plants. Its deficiency results in browning of leaves, weak stem and slow development of plant. Ancient common agriculture practice to provide phosphorus to plant is use of animal manure. (Kannaiya et al., 2004).

A wide range of plant growth promoting rhizobacteria also known as phosphate solubilizing bacteria (PSB) are found to solubilize phosphorous and make it available for plants, thus enhance plant growth. (Sharma, 2005, Tripura, et al., 2005). Many soil bacteria solubilize phosphorous and make it available to plants in several ways. The most efficient phosphate solubilizing bacteria (PSB) belongs to genera Bacillus and Pseudomonas. (Gaind et al., 2008).

Soil contains wide range of organic substrates as a source of phosphorous for plants, but are in unavailable form. This form can be made available as nutrition by hydrolyzing them to inorganic phosphorous. Plant growth promoting rhizobacteria
expressing phosphatase includes the species of genera *Pseudomonas*, *Serratia*, *Enterobacter*, *Bacillus* etc. These are reported as phosphate solubilizing bacteria.

### 2.4.3. Siderophore production

To survive in soil many bacteria synthesize and secrete low molecular iron binding siderophores. This compound binds iron in rhizosphere and prevents the spreading of fungal pathogen by depriving the iron availability. (Kloepper, *et al.*, 1980). Siderophore producing *Pseudomonas* strains colonize roots of several crop plants and resulted in increased yield. (Schroth and Hancock, 1982). The siderophore binds to ferric iron in the external environment and then iron siderophore complex is recognized by the outer membrane receptor protein. Binding of the ferric-siderophore complex induces changes, perhaps signaling to initiate TonB interaction. Using energy provided by the TonB complex the ferric-siderophore complex is transported actively into the periplasm where it get bind to a periplasmic binding protein that transports the complex to the ABC-type transporter in the cytoplasmic membrane. This transports the iron siderophore complex into the cytoplasm utilizing energy from the hydrolysis of ATP. Iron is released from the siderophore by either reduction by ferric reductases, acetylation or by breakdown of ferric siderophore complexes by esterases. (Neilands, *et al.*, 1987).

The bacteria synthesizing the siderophores takes up iron siderophore complex by using receptor located in the outer cell membrane of bacteria. As the iron is released inside the cell it supports the microbial growth. Iron is an important and essential micronutrient for bacterial metabolism. Bacteria directly cannot assimilate iron present in soil because it is unavailable and also very low in concentration to support their growth. (Rachid, *et al.*, 2005, Jurkevitch, *et al.*, 1992). The microbial is siderophores widely varied in structure but most contain hydroxamate and catechol
groups. These are involved in chelating the ferric ion (Neilands, 1995). The environmental factors including pH, iron level and iron ions forms, presence of other trace elements, and an adequate supply of carbon, nitrogen and phosphorous also modulate siderophore synthesis.(Duffy and Defago, 1999). Siderophore are low molecular weight compound that chelate iron (Fe\(^{3+}\)) and transport it into the cell across the cell membrane.(Wandersman, et al., 2004).

Generally they are categorized into two groups, Hydroxamates and Catecholates. The siderophore synthesized by bacteria is regulated fur protein, global regulators, sigma factors, autoinducer N-acyl homoserine lactone and many site specific recombinase.(Compant, et al., 2005). Species of much plant growth promoting rhizobacteria such as Bacillus, Azotobacter, Pseudomonas etc isolated from various crop rhizosphere are able to produce different types of siederophore. (Suttiviriya et al., 2008). Thirty six siderophore producing bacteria were isolated from the rhyospheric region of wheat plant in Uttarkashi district. (Joshi and Bhatt, 2011). Pseudomonas putida isolated from rhyospheric region of mustard plant was found to produce siderophore. (Ahmad and Khan, 2011). Pseudomonas isolates were found to produce siderophore depending upon the iron content, pH and temperature.(Sulochana et al., 2014).

Thus siderophore producing PGPR improve plant health at various levels. They improve iron nutrition; inhibit the growth of pathogens by limiting the iron available for them, generally fungi, which are unable to absorb the iron siderophore complex.

### 2.4.4. Other properties

Protection of cucumber plant by Pythium ultimum pathogen and tomato plants by Fusarium oxysporum was reported by invitro production of hydrogen cyanide by
Pseudomonas species. (Ramteke et al., 2003). Pseudomonas species is ubiquitous in agriculture soil. Many species of it possess various plant growth promoting activities and also act as biocontrol agent. (Weller, 2007). Pseudomonas also suppress plant parasitic nematodes by producing compounds such as hydrogen cyanides. (Aly et al., 2007). Various plant growth promoting rhizobacteria in the rhizosphere of plants, which are growing under trace metal contaminated soil were reported to play an important role in phytoremediation. These rhizobacteria mitigates the heavy metal toxicity on plants by secreting acids, proteins, phytoantibiotics etc. (Denton, 2007)

Some of the plant growth promoting rhizobacteria also regulates ethylene production in plants to enhance root elongation by hydrolysis of ACC (1 aminocyclopropane-1-carboxylic acid). Three of the rhizobacteria named S5, S7 & S9 isolated from wheat rhizosphere were found to contain ACC deaminase activity and promote growth of wheat plant under axenic condition. (Naveed et al., 2008). Plant growth promoting rhizobacteria can also stimulate growth of plant in stress conditions by reducing ethylene level since it is a gaseous hormone and the gas usually inhibits plant growth. (Kang, et al., 2010). Bacillus species are one of the most common soil bacteria. Many species of Bacillus due to their resistant endospore can tolerate heat, cold, extreme pH, pesticides fertilizers and heavy metals. Out of 63 Bacillus species isolated from rice rhizosphere of Erode district of Tamil Nadu 8 exhibit plant growth promoting activities such as auxin production, hydrogen cyanide production, phosphate solubilization and chromium reducing activity. They also possess antifungal activity against species of Fusarium, Penicillium and Cercospora. (Karuppiah and Rajaram, 2011). Plant growth promoting rhizobacteria such as Azospirillum, Azotobacter, Pseudomonas and Bacillus generally increase 10-25 %
yield of rice crop by production of growth substances and fixation of 20-30 kg nitrogen per hectare. (Bajpai and Bachalaiya, 2012).

Some of the plant growth promoting activity and responses of PGPR to various plants had been reviewed and tabulated in table 2.1

Table 2.1
PGPR and plant their growth promoting activities in different plants.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant</th>
<th>PGPR</th>
<th>Growth promoting activity and responses</th>
<th>References</th>
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<tbody>
<tr>
<td>2.</td>
<td>Cajanus cajan</td>
<td>Different PGPRs</td>
<td>IAA production, phosphate solubilization, enzyme like urease, cellulose, etc production, Siderophore production, HCN production, increase root and shoot length</td>
<td>Rani <em>et al.</em>, 2012.</td>
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2.5. **RICE PLANT**

Plant growth results from interaction of roots and shoots with the environment. The environment for root is the soil which provides water, nutrients and structural support to the plant. Roots also support the growth and function of complex of microorganism which constitute rhizospheric microorganisms and which is beneficial or neutral with respect to plant health.

Large amounts of mineral nutrients including nitrogen is required by rice plant for their growth, development and grain production. Rice crops remove 16-17 kg N for the production of each ton of rough rice including straw. (Saharawat, 2000).

Chhattisgarh state is largely based on agriculture despite of having vast mineral deposit. About 80% of the total population of Chhattisgarh depends on agriculture. Rice is the major crop produced in the region. The state has often known as the ‘rice bowl’ of India. Chhattisgarh used to produce over seventy % of total paddy production in the state.

Rice productivity was 1.4 tons per hectare due to inadequate irrigation in the state. However, according to the Agriculture University, rice production in Chhattisgarh had increased due to increased acreage under High yielding varieties (HYVs), increased use of fertilizers and improved production technology. Despite this, average yields remain at 1.6 tons per hectare. Compare this to the 1960s when the yield was around one tone with low fertilizer inputs and no institutional support. Although the use of HYVs increase the productivity, but it has also led to increased use of fertilizers, loss of biodiversity and farmers becoming dependent on the government machinery for seeds, agricultural inputs and loans. There seems to be a total negation of indigenous varieties except as a source of genetic material. (Menon, 2002).
Rice is the most important staple food of Chhattisgarh and to increase its productivity and growth rate chemical fertilizers mostly urea as nitrogen source is used in cultivation of rice crop. Excess and regular use of urea may lead to the depletion of soil organic matter which is a source of nutrition for several PGPR and plants also. A sustainable cultivation which is less dependent on the chemical fertilizer is required for plants as well as for environment since due to the excessive use of chemical fertilizer the soil environment is getting polluted. The cultivation can be made sustainable by the use of bio-fertilizers. The use of PGPR as bio-fertilizer that can fix N biologically, solubilize P and induce IAA to improve rice growth is studied extensively. (Ashrafuzzaman, et al., 2009).

Growth and yield of rice plant can be obtained by inoculating seeds or roots with certain specific root colonizing plant growth promoting rhizobacteria. (Gupta, et al., 2000).

PGPR commonly used as inoculants for improvement of growth and yield of rice offers an attractive way to replace chemical fertilizers and pesticides. Over the years the PGPR have gained worldwide importance and acceptance for agricultural benefits. They become the potential tools for sustainable agriculture and the trend for the future.

2.5.1. Importance of rice

Thousands of varieties of rice are grown throughout the world making it rich in genetic diversity. Rice is highly nutritious and medicinally important food. It is the excellent source of carbohydrate which our body needs and can be included in balance diet since it almost does not contain fat and cholesterol also it is free of sodium. Rice is a good source of vitamins, minerals, proteins containing eight amino
acids and starch. Thus rice grain is very important nutritive source. (Umadevi, et al., 2012).

The medicinal importance of rice has been described in Ayurveda. It is considered as tonic, aphrodisiac, fattening, diuretic etc. (Caius, 1986). Rice is also used as medicine by traditional healers of Chhattisgarh in treatment of various diseases. (Oudhia, 2008). One of the medicinally important varieties of rice is known to prevent the skin disease named ‘Laicha’. Similarly some of the medicinal varieties of rice which are cultivated and used as medicine by traditional healers are Alcha, Udan, Shyam Lal, Kanthi, Tenduphool etc. (Oudhia, 2000).

Along with the nutritional and medicinal value of rice its by-products are of equal important. Some of the useful by-products are rice husk, rice bran, rice starch, rice vinegar etc.

2.6. UREA

Urea also known as carbamide is an organic compound. Its chemical formula is CO(NH₂)₂. It is solid, odorless, white crystalline and highly soluble in water. Dutch scientist Herman Boerhaave in 1727 for the first time discovered urea in urine. It get synthesized either from amino acid oxidation or from ammonia in the body of many organism. It is present in the urine of mammals and amphibians and some fishes. (Urea-Wikipedia). The German chemist Friedrich Wohler in 1828, success synthesizing in artificial urea by the treatment of silver isocynate with ammonium chloride. (Meessen and Peterson, 2005 ).

As the urea is applied to the soil it gets converted to ammonia and carbon dioxide by the soil bacteria involved in nitrogen cycle and it is well known that various soil bacteria play a significant role in the nitrogen cycle in different steps.
Thus the ammonia is then oxidized to nitrite by some ammonia oxidizing bacteria. (Carow, 2008). The nitrite is oxidized to nitrate by nitrite oxidizing bacteria.

2.6.1. Physical form of urea

Urea is available in granular form for fertilizer, since granules are large, hard and resist moisture. It is applied to soil as solid form as well as a liquid in the form of a foliar spry in some crops.

2.6.2. Uses of Urea in Agriculture

Urea is widely used as nitrogen fertilizer. Out of all the commonly used solid nitrogenous fertilizer urea posses the highest nitrogen content. It contains 46% nitrogen and thus widely used in the agriculture sector as fertilizer. (Curtis, et al., 1991). Due to the high nitrogen content even spread of urea in the field is very important. Usually urea is speeded at rate between 40 to 300 kg/hectare, but the rate may vary according to the crop plants.

2.6.3. Harmful effect of Urea

Urea causes germination damage if placed close to the seeds. Therefore it is applied in the fields generally as spray by dissolving with water.

During the warm weather if urea remains in soil its nitrogen gets lost to the atmosphere. As soon as urea is applied to the soil its breakdown begins. Unless it rains urea must be incorporated into the soil to avoid urea loss. Percentage of urea lost increase with high temperature causing pollution. When urea gets dissolved in the soil moisture, the pH of the area of nearby urea becomes high and also the ammonia concentration increases in this area. This high pH and high ammonia concentration zone for few hours is toxic to the seeds and seedling roots which comes in contact of this zone. But this toxicity gets neutralized by conversion of ammonia to ammonium.
Use of urea increases crop productivity but at the same time it causes some harmful effect not only to plant and environment but also to the plant growth promoting rhizobacteria. Most of the rice field soil is nitrogen deficient therefore nitrogen fertilizer applications are necessary to meet the crops nitrogen demand. Generally, urea is used as a source of nitrogen for rice production. (Choudhary, et al., 2001).

Adding excess urea results in ammonia volatilization and denitrification that cause atmospheric pollution through the production of greenhouse gases. (Reeves, et al., 2002).

In addition to these environmental problems, the long term use of urea decreases the rich source of organic matters present in soil. These problems are of great concern to soil and environment. Thus it is now necessary that alternate nitrogen source must be applied to minimize these problems. Biological nitrogen fixation technique can play an important role in substituting chemical nitrogen fertilizer use in rice cultivation. This may reduce these environmental problems to some extent. Use of biofertilizers can prevent the soil organic matter depletion. (Jeyabal, et al., 2001).

Results of slightly reduced cell wall constituents of rice straw at different level of urea were presented and recommended to replace some of the urea by other compounds. (Nguyen, et al., 1998).

2.6.4. Effect of Urea on soil microorganisms

The beneficial microorganisms of soil including plant growth promoting rhizobacteria are greatly influenced by various factors involving the agrochemicals which are applied in modern agricultural practices to offset the noxious weeds and to improve the productivity of crops. (Ahmed, et al., 2009). Different concentrations of urea show variable effect on plant growth promoting rhizobacteria. Low concentration of
urea stimulated the growth of the two plant growth promoting rhizobacteria - *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* but higher concentration was found lethal for *Sinorhizobium meliloti* and *Pseudomonas aeruginosa*. Urea adaptive bacterial variants were reported to show better growth rates at sub-lethal concentrations as compared to non-adaptive variants. They retained plant growth promoting attributes similar to non adaptive variants (Maheshwari, *et al.*, 2010).

### 2.6.5. Effect of Urea on plant growth in combination with PGPR

Various studies on the growth of plants and crop yield by applying of chemical fertilizer such as urea and biofertilizer such as plant growth promoting rhizobacteria has been done by various researchers. Application of two of the plant growth promoting rhizobacteria *Pseudomonas fluorescence* and *Azospirillum lipoferum* in combination with different doses of urea had significant effect on yield and yield component. (Yaser, *et al.*, 2011). Effect of application of urea and *Azotobacter* on maize plant under nursery condition has been studied. Their combination with the recommended dose of urea results in the better growth of plant then urea used alone. But as the dose of urea is increased the growth of plant lowers. (Moses, *et al.*, 2011). Phosphate solubilization activity of *Pseudomonas* species is found minimum in presence of urea as compared to ammonium chloride, potassium nitrate, sodium nitrate and ammonium sulphate in which it is found maximum. (Pallavi *et al.*, 2013).

In another experiment it was observed that in presence of urea the phosphate solubilizing activity of PSB decreases. (Sagervanshi *et al.*, 2012).
2.7. EFFECT OF CHEMICAL FERTILIZER ON PGPR

The insecticide tolerant and phosphate solubilizing *Pseudomonas putida* strain PS9 was isolated from the mustard rhizosphere. As the concentration of insecticide was increased from the recommended dose rate to the higher one, a progressive decline in all plant growth promoting properties of the *Pseudomonas putida* strain PS9 was observed. (Ahmad, 2011).

Different combination of chemical fertilizer, compost and PGPR inoculants were applied in wheat plant for growth improvement and yield. Maximum increase in plant height, number of tillers and number of spikelet, grain and straw yield were recorded with the use of PGPR inoculated seeds in combination with compost and chemical fertilizers. Maximum grain yield and 1000 grain weight were observed where PGPR inoculated seeds were used in combination with recommended chemical fertilizers. Higher N content in grain and straw were recorded with the application of seed inoculation with PGPR along with compost and recommended chemical fertilizers. (Akhtar *et al.*, 2009).