REVIEW OF LITERATURE

The survey of literature was carried out to investigate into the literature concerning paddy field cyanobacterial studies. This review was taken keeping in view taxonomic, ecological, physiological and to certain extent molecular studies of the photosynthetic prokaryotes.

Fogg G. E. (1956) studied comparative physiology and biochemistry of blue green algae. In his experiments various physiological parameters of blue green algae were studied while culturing the different forms blue green algae. Experimentation was done on various aspects of nutrition of these forms. Different factors responsible for the culture of blue green algae like resistance to extremes of temperature, pH, phototrophy, growth factor requirements were studied. The author mostly worked out the physiological and biochemical aspects of the culture media and analyzed the effects of different factors on the pigments and carbohydrate content of the cultured forms.

Whitton B. A. (1969) tried to keep account of new species of blue green algae created since 1959. He suggested that no one type of approach to the taxonomy of blue green algae is adequate but the simultaneous use of the different approaches can fulfill the needs of taxonomists.

Stanier et al (1971) made an attempt to purify and characterize unicellular blue green algae (Chroococcales). They for the first time attempted the purification of unicellular blue-green algae in their epoch making article. They described methodology for purification of unicellular blue-green algae and described various biochemical properties. This effort put forth line in cyanobacterial research taking into account not only the morphology but also the biochemical and cultural properties of blue green algae.

The fatty acids of 34 strains of unicellular blue green algae assigned to the genera- *Synechococcus, Aphnocapsa, Gloeocapsa, Microcystis* and *Chlorogloea* were chemically characterized by Kenyon C. N. (1972).

Stein J. R. (1973) edited a volume "Hand book of phycological methods". Different methods for the algal cultures- their isolation, purification, enrichment and biochemical characterization etc have been described with required protocols in that volume.

Bryant D. A. et al (1976) analysed the structural properties of the major biliproteins of *Anabaena* species. Two strains analysed were *Anabaena variabilis* and *Anabaena species* 6411 for allophycocyanin, c phycocyanin and phycoerythrene.

Biochemical studies on cyanobacteria during eighteen were not only restricted to the composition of macromolecules but were also diverted to nuclear studies Michael Herdmn et al (1979) analysed the mean DNA base composition of 176 strains of cyanobacteria by thermal denaturation or CsCl density gradient centrifugation. This data helped in analyzing the evolutionary trends in cyanobacteria.

On the basis of comparative study of 178 strains of cyanobacteria revised definitions of many genera were proposed by Rippka et al (1979). Revisions were designed to permit the generic identification of culture often difficult through use of the field base system of phycological classification. The differential characters proposed were both constant and redialy determinable in the cultured material. These authors recognized 22 genera which they placed in 5 sections. This attempt gave an impetus to the cultural studies of cyanobacterial strains and helped in assigning specific names to the different genera. This was a major attempt which linked the phycological and bacteriological classifications.

Effect of algal inoculation on soil aggregation in rice field soils was studied by Roychoudhary et al (1980). These authors co-related the degree of aggregation and aggregate stability in water by studying various characteristics of different soil samples with and without algal inoculations.

Puri and Groover (1981) studied the nitrogen fixation and release of extracellular nitrogen by a strain of *Anabaena* viz., *Anabaena* G.102 collected from rice fields of Punjab Agri. Univ. Ludhiana.

Distribution pattern of blue green algae from rice fields of Konkan area from Maharashtra state was studied by Sardeshpande and Goyal (1981).This was the first attempt from Maharashtra to investigate paddy field cyanobacterial flora from western part of Maharashtra. Soil samples from 3 districts viz., Ratnagiri, Raigad and Thane were analyzed for the cyanobacterial growth by the authors. They reported 19 blue green algal genera of which 11 were nitrogen fixers. Most of the nitrogen fixing blue green algae was restricted to Ratnagiri district. The authors co-related the abundance of blue green algae from Ratnagiri district to the high organic matter content of the soil. In another attempt

Sardeshpande and Goyal co-related the pH and growth of blue green algae and their nitrogen fixation ability from the Konkan area.

Distribution of *Nostoc* and *Anabaena* in relation to soil properties from the cultivated soils of Karnataka state was studied by Bongale (1987).Soil samples where *Anabaena* was recorded had significantly higher pH and organic content. Significantly lower chlorine content, over the soils without *Anabaena* and *Nostoc* was recorded from the soils with the significantly higher values of pH and PO_4, Na and Mg. In their observations they concluded that lateritic soils did not appear to favour the growth of both the genera.

Effect of cyanobacterial strains on the rice seed germination has been investigated by Pedurand, P. and Reynaud P. A. (1987)

Methods for isolation and purification of cyanobacteria were proposed by Ferris M. J. and C. F. Hirsch (1991). In this method they used nutrient saturated glass fiber filters which allowed the isolation of cyanobacteria from fresh water. This was efficient method which reduce the load of heteromorphic bacteria 2-15 folds as compared to previous methods. Addition of broad spectrum antibiotic like β-lactum was also recommended to facilitate the production of axenic cyanobacterial cultures.

Kulik, Martin M. (1995) studied the potential for using cyanobacteria and algae in biological control of plant pathogenic bacteria and fungi. Cyanobacteria are best known for production of toxins by certain species that grow in fresh water. Present author studied the effect of some terrestrial cyanobacteria on the aerial parts of plants.

Santra, S. C. (1993) published a volume of rice field blue green algae. He reported 240 taxa belonging to 42 genera from the paddy field soils of Western Ghats. A detailed account regarding the individual species, its distribution along with the previous report of species from the area under investigation has been given. The Camera lucida drawings along with the photographes help in identifying the paddy field soil cyanobacterial flora. After Desikachary (1959) and (Anand 1989) this is the sole account on cyanobacterial biodiversity from paddy field soils.

Taxonomy of cyanobacteria is a universally debatable problem. The available literature shows clearly two distinct thoughts- One stresses the morphological characters while other emphasizes cultural characters. In order to confirm the phenotypic variability

of identical genotypes Palinska et al (1996) worked on the taxonomy of *Merismopedia* like isolates from the soil. Different criteria such as microscopical investigations, Spectroscopy, phycobilisome isolation, SDS, PAGE, DNA extraction, PCR amplification etc. were employed by the authors to differentiate between the various strains of the isolates. They concluded that molecule method should be used parallel to phenotypic characterization but never replace it. Otherwise more precise and probably more reliable molecular data would produce confusing results concerning the occurrence on cyanobacterial genera and species in the nature.

Phycobilisomes are the important pigments in cyanobacteria giving them the characteristic features. Isolation and characterization of these pigments was done by Ducret et al (1996) in *Anabaena* species PCC7120. Phycobilisomes were characterized with the help of spectroscopic measurement and SDS PAGE supported with amino acid sequencing. Pigments characterized these strains showed some deviation from the usual phycobilisomes as have been described by Bryant D. A. (1976).

Goyel et al (1997) studied the effect of salinity on nitrogen fixation and protein profile halotolerant *Nostoc muscorum* strain isolated from saline soils of rice fields and salt sensitive strains of the same species.

Shrivastava Renu and D.V. Amala (1997) worked out the modified procedure for purification of Glutamine synthatase from *Nostoc muscorum.*

The whole cell protein and 16s rRNA gene characterization of cyanobacterial strains was done with the help of SDS PAGE by Lyra Christina (1997). RFLP study of *Anabaena* showed that the neurotoxic strains were identical but the hepatotoxic once formed a heterogeneous group.

Devi Amita et al (1999) studied the Cyanobacterial flora of rice field soils of Manipur. A total of 703 soil samples were collected from different rice fields of Manipur comprising 8 districts. Altogether 110 blue-green algal forms belonging to 34 genera were identified by the authors. In their conclusion they have inferred that pH of soil proned to be important factor affecting the growth and distribution of blue green algae. These authors have concluded that lower pH restricted the cyanobacterial distribution.

A comprehensive account on cyanobacterial potential as biochemicals has been given by Fatma Tasneen and L. V. Venkataraman (1999). The potential of cyanobacteria

for production of various biochemicals viz., pigments, hydrocarbons, vitamins, antibiotics, polysaccharides, essential fatty acids, biofloculents, biosurfactant, enzymes, plant growth regulators, sterols, waxes etc has been recognized in the modern days. Review of all these have been presented in their article.

Kannan (1999) studied the effect of short term Argon flushing in 3 species of cyanobacteria viz., *Tolypothrix tenius, Mastigocladus laminosus* and Westiellopsis iyengarii.The effect of argon flushing was studied in terms of chlorophyll content, oxygen evolution, heterocyst frequency and ARA activity.

Anand and Gayathri (1999) have studied ammonia release by a non-heterocystous filamentous cyanobacterium *Lyngbya spiralis* Gietler. In their experiment the ammonia release from the algae was studied in the different cultural stages co-related with the chlorophyll content. The intra and inter day fluctuations were studied by the authors. This was the first report on release of ammonia by non-heterocystous cyanobacteria.

Large numbers of experiments were carried out on the occurrence and corresponding ecological variation in rice field cyanobacteria. Adhikari and Sahu (2000) studied the survival strategies of cyanobacteria occurring as crust in rice field under drought conditions. The organisms in crust absorb water rapidly and become saturated within 10 minutes. Resumption on metabolic activities and reappearance of filamentous structures of cyanobacterium *Aluosira* sp. started soon after wetting. However the species synthesized cytonemin pigment only when desiccated. Thus these experiments have helped in analyzing the responses of various factors in the yield of pigments during fluctuations of ecological conditions in the environment.

Floristic abundance and relative distribution of cyanobacterial genera in rice field soils at different crop growth stages has been studied by Nayak S. et al (2001). These authors estimated abundance of heterocystous and non-heterocystous blue green algal forms in paddy fields during different stages after plantation. Various modifications in the medium and manuring patterns have been co-related with the abundance of different blue green algal forms. Surprisingly their observations indicate relative abundance of non-heterocystous forms in the paddy fields rather than heterocystous forms.

The distribution pattern of cyanobacteria in rice field soils of Hojai sub-division of Assam was studied by Ahmed S. U. (2001). 59 species of cyanobacteria belonging to

10 genera were reported by the author. Among these Nostoc, Anabaena, Calothrix and Aulosira were found dominant within the rice fields.

Lyra Christina et al (2001) worked out the molecular characterization of planktonic cyanobacteria viz., *Anabaena, Aphanezomenon, Microcystis* and *Planktothrix* isolated from European countries, America, Japan, Netherlands etc. These authors do not agree with the names assigned by J. Kaumarek and Anagnostedis (1989) to the cultured forms. They mostly relied on the phenotypic and genotypic classification of cyanobacteria on the studies of Stanier. A comparative account of the strains from various collections (PCC, ATCC etc.) was also given.16s r-RNA analysis of different strains yielding various toxic substances has also been done by them.

Balakumar and Ravi (2001) studied the catalytic degradation of the herbicide glyphosate by the cyanobacterial strains isolated from paddy field soils. In their analysis they have relied mostly on the pigment and protein content of the two strains of two genera of *Nostoc* and *Westeillopsis*.

Nitrogen fixing potential of local heterocystous isolates from paddy fields of Uttar Pradesh was studied by Mishra Upasana (2001). Along with the nitrogenase activity, Chlorophyll a, protein and carbohydrate was also studied to co-relate the efficiency of isolates. The surprising observations were noted that the nitrogen fixation was independent of growth and isolation showing less growth fixed higher amount of atmospheric nitrogen.

Commercial production of carotenoid using *Spirulina platensis* and optimizing the low cost nutrient media was studied by Tri-Panji and Suharyanto (2001). The identification of different carotenoids was done with the help of HPLC while various modifications in the culture medium were assumed for the production of quality carotenoid pigments. Out of 11 compositions of formulated low costed media was tested by these authors. The optimum medium for growth and carotenoid production was medium containing latex serum supplemented with mineral C, N, P, and Mg at the ratio of 1.0:3.0:0.3:0.2. The composition medium supported carotenoid in cellular biomass higher than the synthetic medium.

An account of non-neretocystous filamentous cyanobacteria from paddy field soils of Uttar Pradesh was given by Tiwari et al (2001). In all 58 strains were encountered from 8 districts under study.

A note on Nostocales from 24 paraganas from West Bengal was given by Singh et al (2001).

Mishra Upasana et al (2001) described heterocystous forms from Terai belt of Uttar Pradesh. They reported 30 heterocystous strains of cyanobacteria. In that area species of *Nostoc* showed dominance followed by *Hapalosiphon.*

Cyanobacterial diversity of rice fields from Lakhimpur district Assam was given by Hazarika et al (2001). They described 25 heterocystous filamentous cyanobacteria out of 52 strains.

Kumawat and Jawale (2001) enumerated 8 taxa of *Spirulina* from fish ponds of Anjali from Maharashtra. A new species viz., *Spirulina Anjalensis,* Kumavat and Jawale was reported by these authors.

Cyanobacterial association with paddy fields at various growth stages of the paddy was studied by Sen C. R. (2001) from West Bengal. The cyanobacterial flora from different fields during field preparation, transplantation, crown root, tellering, pre-flowering, flowering, grain setting and grain maturing, grain ripening and harvesting stage was studied by the author. In this effort author also tried to co-relate pH and temperature with the cyanobacterial flora.

Biochemical composition and biomass production of acid tolerant cyanobacteria was done by Gopalswamy et al (2002). Pigments like chlorophyll a, phycocyanin, allophycocyanin, phycoerythrin along with polysaccharide protein ARA were estimated from the strains of *Anabaena, Nostoc, Oscillatoria* and Weistellopsis isolated from soil collected from Tamil Nadu. According to these authors acid tolerant cyanobacteria are capable of producing higher biomass than normal non-acid soil isolates. Even these strains showed higher pigments and protein content. Their experiments also confirmed the higher ARA than normal non-acid soil cultures.

Interspecific variation for N_2 fixing potential in the genus *Tolypothrix* was studied by Prasanna R. et al (2002). For this paddy field soils from different regions of India were collected and the isolated strains of *Tolypothrix* have been used for the study. The data recorded for the three parameters viz., protein, ARA and chlorophyll in various strains was analyzed with the help of ANNOVA, DMRT and MSTAT-C. In this experiment evaluation of 20 selected *Tolypothrix* accessions belonging to 4 different species has been done for the N_2 fixation ability.

Cyanobcterial diversity in different ecosystems from Kashmir area was explored by Ara Shokat et al (2002). In their studies they reported 101 cyanobacterial taxa belonging to 25 genera from rice field of Kashmir Valley. In their observation genus *Oscillatoria* found to be most common member of rice fields.

Pandey Usha (2002) studied soil cyanobacteria from arable land of southern Rajasthan. From around 240 soil samples 25 species were reported by the author.

An account of genus Oscillatoria Voucher from Lakhimpur district, Assam has been given by Hazarika et al (2002). 17 species of *Oscillatoria* were reported by this author.

Nitrogen fixing potential of cyanobacteria isolated from rice field soils of Nagaon, Assam was studied by Ahmed and Kalita (2002). 53 cyanobacterial strains of 9 genera were isolated and their nitrogen content was determined to co-relate with their nitrogen fixing ability.

The widely used genus of cyanobacterium viz., *Spirulina* is a point of interest by many research workers. Protein profiles of *Spirulina fujiformis* around the year were studied by Sharma and Shrivastav (2002). This work recommended 12 polypeptide bands but the author could not come to the conclusion regarding seasonal variatios.

The cyanobacterial diversity in field soils of Sambalpur district, Orissa was studied by Das Mihir (2002). Total 33 spescies belonging to 15 genera were recorded.

Shukla S. P. and A. K. Kashyap (2003) accessed the biopotential of three cyanobacterial isolates from Antarctica for carotenoid production. The isolated organisms were compared with the local strains available at BHU Waranasi, India for morphological, cultural and biochemical characteristics. The carotenoid production was measured for indoor mass culture as well as through semi outdoor unit. Authors have concluded that the isolates from Antarctic region were far superior to local strains. The semi outdoor culture showed higher carotenoid production than the indoor ones. However those isolates showed slower growth rate. It has been inferred that the slower growth of isolates may be because of the lower temperature.

The cyanobacterial diversity and their potential application in biotechnology were discussed by Thajuddin N. and G. Subramaniyam (2005).

Ecological diversification in cyanobacteria has been studied by Tiwari O. N. (2005). They have discussed in detail the ecology of rice fields and their soils with special reference to light, water and desiccation, salinity and pH of these soils.

In their discussion on 'Algal biodiversity from morphology to molecules', Radha Prasanna and B. D. Kaushik (2005) has given stress on modern approaches to algal identification and diversity analysis. Different parameters used in the biodiversity analysis of algae have also been discussed. The biochemical diversity can be studied with the help of absorption spectra, lipid and protein profiles, N_2fixation and assimilation ability etc. Various markers such as protein profiles, nucleic acids 16s r-RNA have also been suggested to access the biodiversity in blue green algae.

Trikey J. and S. P. Adhikary (2005) analyze the cyanobacteria in soil crust from southern parts of India. The cultures were raised from soil crust and then analyzed for chlorophyll, protein and N_2 fixing ability. The absorption spectra of methanolic extract of both soil crust and corresponding dominant cyanobacteria from the culture have been compared by the authors.

The role of cyanobacteria in organic scented rice farming has been described by Singh and Singh (2006). Potential of cyanobacterial in the detailed with future of organig farming has been also discussed.

Biodiversity analysis of selected cyanobacteria was made by Narayan K. P. et al (2006). Authors analyzed three genera *Nostoc, Anabaena, Calothrix* for their pigments, carbohydrates, total soluble proteins and enzyme glutamine synthatase. Based on the similarity matrix dendrogram has also been provided by the authors. Finally these authors have been concluded that cyanobacterial strains cannot be differentiated based on the physiological parameters.

Most of the cyanobacterial diversity analysis has been restricted to paddy field soils however a different experimentation was made by Karthikeyan N. et al (2006) to study the potential of plant growth promoting cyanobacteria as inoculants for wheat crop. It is concluded by them that biofertilizer such as blue green algae can provide a suitable supplement to the chemical fertilizers and organic farming can become a reality in the future. Rhizocyanobacteria can be used as suitable candidates in this regard as their close interaction with roots can make them ideal PGPR for not only rice but other crops including wheat.

Morphological, physiological and biochemical characters of different isolates of *Anabaena* from Indian soils has been done by Nayak S. et al (2007) around 24 strains of *Anabaena* from various physiographic divisions of northern India were isolated and used for the study. For the morphological comparison parameters like growth pattern, trichome shape, vegetative cell-shape and size, heterocyst-shape, size and frequency and akinetes-shape and size were used. For physiological characterization chlorophyll, carotenoid, phycobillins and phycocyanin to phycoerythrin ratios were also used.

Prasanna Radha and Saswati Nayak (2007) studied the influence of diverse rice soil ecologies on cyanobacterial diversity and their abundance. Soil samples from various locations especially from northern and eastern Indian parts were analyzed by these authors to study the cyanobacterial diversity from these areas. Around 166 cyanobacteria isolates were purified. Various diversity indices were applied to co-relate the occurrence of different forms in different soil samples. Abundance of genera viz., *Nostoc* and *Anabaena* has been shown by these authors. Their studies have shown that in India on an average blue green algae account to about 33% whereas reports showed that up to 50% of the total algae was blue green algae in some of the southern and eastern states.

Above authors in (2009) also published an account on cyanobacterial diversity in rice field soil in relation to soil pH. Abundance of cyanobacteria from the soil samples was co-related with two parameters namely pH and EC of the soil. The effect of pH on algal flora is generally difficult to evaluate as it is often co-related with other factors. However these authors concluded a positive co-relation between pH and EC of the soil with relative abundance of cyanobacteria in various soil samples collected from different parts of the country.

A species of Mastigocladus was isolated and studied for its growth characteristics, N2 fixation and N2 assimilation at higher temperatures by Khumanthen N. et al (2007) from hot spring in the state of Meghalaya.

Studies on biodiversity of epilithic cyanobacteria from fresh water streams of Kakoijana reserve forest, Assam were carried out by Saha S. K. et al (2007). These types of habitats have been less investigated by the phycologists. Authors have reported 29 species belonging to 18 genera, 12 families and 4 orders.

Cyanobacteria have been classified using features of their morphology and development, but many phenotypic characters vary as environmental conditions change

and therefore molecular methods have also been used to describe diversity within this group. An attempt has been made to give insight to this difficult problem by Paul K. Hayes et al (2007).These authors have made an attempt to resolve the taxonomy of *Cyanococcus* and *Prochlorococcus*, the members of Pycocynobacteria.

Isolation and characterization of three new species of cyanobacteria from dessert soil has been done by Rehakova K. et al (2007). The separation of the new species has been supported on not only morphological basis but also on molecular characterization such as 16s r-RNA sequencing and comparing them with PCC and ATCC strains.

Kaushik P. and Abhishek Chauhan studied the antibacterial potential of aqueous and organic extracts of *N. commune* (2008). The aqueous and methanol extract were tried to access the antimicrobial activity. It was found that the methanol extract had more potent activity than other organic and aqueous extracts. No antimicrobial activity was detected in dichloromethane extract and hexane extract. Gram positive bacteria were found more susceptible as compare to Gram negative bacteria

The morphological observations on various isolates from a variety of cyanobacteria lead to the phycologists as well as bacteriologists to establish co-relation between various taxa of cyanobacteria. Recently Rama Kant et al. (2008) established a genus *Symphyonemopsis* as a link between two families Scytonemataceae and Mastigocladacead of order Nostocales. It is suggested by the author the genus could be reasonably placed in Nostocales, Scytonemataceae and not in Stigonematales, Mastigocladaceae as has been done earlier.

Description of the genus *Anabaena* from North 24-Paragana district, West Bengal has been given by Naskar and Naskar (2008).

Mishra P. K. et al (2008) have described the different species of genus *Oscillatoria* from Gorkhapur district of Uttar Pradesh.

Cyanobacterial diversity around Ahamdnagar region of Maharashtra has been studied by Suryavanshi et al (2010). They have reported 53 taxa of cyanophyceae around the Ahamdnagar region.

The use of blue green algae in the reclamation of saline soils has been discussed by number of workers the exact role of a genus or species was not determined by the phycologists. The experiments done by Jaiswal et al (2010) on the potential of *N. calcicola* and its bicarbonate resistant mutant in bioameleoration of 'usar soil' were mops

significant in this regard. The authors have simulated the experiment in laboratory using growth potential, the glutamine synthetase activity, heterocysts frequency and effect of pH on soil. Promising results have yielded through these experiments.

The literature cited above shows the cyanobacteria have been investigated on different lines. The survey of literature shows the paddy field soils of western Maharashtra have been studied for their cyanobacterial studies by vary few researchers (Sardeshpande andGoyal 1981, Ghadge and Karande 2008, Karande 2009). Besides there are no reports from this area. Hence to update the knowledge and investigate the paddy field associated blue-green algae this problem was worked out.