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Occurrence and Distribution of Nitrogen-Fixing Cyanobacteria in Local Rice Fields of Dera Ghazi Khan

Aroosa Arzoo and Allah Bakhsh Gulshan*

[1]*Department of Botany, Ghazi University, Dera Ghazi Khan, Pakistan*

Abstract

Sulfur is an essential nutrient for achieving profitable crop production as it plays a crucial role in chlorophyll formation and protein synthesis. It also contributes to the composition of chemical substances and enhances seed oil content. This study was conducted during the winter season of 2018-19 at University of Agriculture, Faisalabad and the impact of sulfur on seed yield and oil content of canola was investigated. The treatments consisted of four levels (0, 10, 20, and 30 kg ha^{-1}) and three sources (single super phosphate, ammonium sulfate, and gypsum) of sulfur. The experiment followed a Randomized Complete Block Design (RCBD) with a split plot arrangement, and the data were analyzed using Fisher's analysis of variance (ANOVA) with a significance level of $p \leq 0.05$. The means of the treatments were compared using Tukey's HSD test at a 5% probability level. Results showed that the application of sulfur in the form of ammonium sulfate at 30 kg ha^{-1} had a significant impact on the growth, yield, and quality of canola. The highest values for plant height (165 cm), number of branches (18), number of siliqua (291), 1000 seed weight (5.8 g), seed yield (2299 kg ha^{-1}), biological yield (8368.0 kg ha^{-1}), protein content (13.7%), and oil content (37.5%) were observed ammonium sulfate treatment at 30 kg ha^{-1}. It is concluded that ammonium sulfate emerged as the most effective sulfur source, significantly enhancing the yield characteristics and quality traits of winter canola followed by single super phosphate and gypsum. While, the protein and oil contents, as well as grain yield, showed a progressive increase by increasing concentration level of sulfur source.

Keywords: Sulfur; Winter Canola; *Brassica napus;* Nitrogen Fixing

1. Introduction:

Photosynthetic Prokaryotic bacteria are the largest group of kingdoms Monera and their existence in number of diversity and distribution across the globe (Lee *et al.,* 2021). The application of cyanobacteria in diverse aspects like feed, fuel, fertilizer, food, colour and production of various important substances like vitamins, enzymes pharmaceutics and pharmacological types of metabolites (Żymańczyk-Duda *et al.,* 2022). They were involved in research day by days and the interest was increased in probing the various types of activities of cyanobacteria in the soil habitat. Soil is the best habitat of various terrestrial cyanobacteria species which are beneficial organisms for soil fertility by fixing atmospheric nitrogen (N) into the soil and they were helping in binding soil particles, and to maintain and retain moisture content. The cyanobacteria were also helping and preventing any type of soil erosion. Increase of major and essential macroelements/ major elements in soil which are necessary for plant growth

*Corresponding author Tel.: 000000000
E-mail address: agulshan@gmail.com

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and development (Joshi et al., 2020).

Plants which were grown in rich organic matter soil, they were enhanced the uptake of ions and increase of N content of the surface soil, as well as production of plant growth promoting substances such as plant growth promoters or phytohormones. The plant growth promoter's substances were made up of various types of amino acids, sugars and vitamins are the most important factors that are suggested for plant growth stimulating effects in the presence of these microorganisms. These studies were done in various habitats by various groups of researchers such as (Karthikeyan 2006; Karthikeyan *et al*., 2007; Obana *et al*. 2007).

Cyanobacteria, formerly called "Blue-Green Alga" are relatively simple and primitive life forms. They are closely related to bacteria and bacteria like microbes. They are morphologically and taxonomically diverse group of exclusive photosynthetic organisms of great importance because of their very long existence for well over 3.5 billion years and cosmopolitan distribution in terrestrial, freshwater and mesic habitats (Chellapa *et al,* 2004; Maurya *et al.,* 2023). They are naturally important gift to mankind and their needs for staple food, as they possess various natural properties that make them ideal organisms with potential for versatile biotechnological applications. As a basic research tool, they are largely known to provide critical insights into the origin of life, photosynthesis, nitrogen fixation and primary and secondary metabolism (Rucker *et al.,* 2023).

During the recent and past, studies on Cyanobacteria, they have emphasized their important role in the arable land ecosystem. They grow at any place and in any environment where sufficient moisture and sunlight are available. However, they show specific growth pattern in a specific environment and therefore their distribution, ecology, periodicity, qualitative and quantitative occurrence differ widely (Muthukumar *et al*., 2007; Saadatnia & Riahi, 2009). On account of their immense applied biotechnological potentials, they are being explored widely. Recently, Cyanobacteria have gained significance as sources of wholesome food materials, fixed atmospheric nitrogen, bioplastics, biofuels, fine chemicals, bioactive substances (Subramanian & Shanmugasundaram, 1986 and Kumar *et al.,* 2011), fine chemicals like lipids, pigments, enzymes, polysaccharides (Pal, 2008), and other novel biologically active compounds involve in remediation of polluted environments (Kumar *et al.*, 2011 and Sri Kumaran *et al*., 2011). Active research in the field of Cyanobacterial bioremediation technology has opened up vast opportunities, whereby, these microbes could be used for eliminating human sufferings through pollution free environment (Vijaykumar *et al*., 2012).

1.1. Objectives:

- To collect and identify of cyanobacteria from local rice fields.
- To study of abundance and diversity of cyanobacteria at various locations of rice fields in Dera Ghazi Khan.
- To study of physico-chemical properties of rice field soils and their correlation with cyanobacterial population.

2. Materials and Methods:

2.1. Study Area:

Different locations of the arable lands from the surroundings of Dera Ghazi Khan were observed visually. The northern side along both sides of Indus Highway of Dera Ghazi Khan to choose to fit for this study, because this site was rich for the cultivation of rice. Thus, the various agricultural fields were selected for the proposed study. Because the grower of this area is frequently cultivated the rice annually. This study area is prominent for the rice growing.

2.2. Field Methodology:

10 rice fields were selected at the 20 km transect. At each and every 4 km the two rice fields containing one hectare land along the western and eastern side of Indus highway road at the vicinity of Dera Ghazi Khan were selected. The selection of the rice fields was made randomly. The approximate distance of each field from the roadside of Indus Highway was 10-20 meters away. All the selected fields of rice have cultivated for this crop about 10-15 years, this information was collected from the grower of the study area. Almost the study area was a prominent for the cultivation of rice crop of Dera Ghazi Khan over a decade.

2.3. Soil Sample Collection and Analysis:

For the proposed study, the soil samples were collected from several rice paddy fields located in the south to north of the study area. The sampling methods were followed by Rangaswamy (1996). Samples were collected from the rice cultivated fields in the year 2016. The samplings were done randomly from both soil and water of the paddy fields. Temporary slides were prepared for each sample for identification. The strains were identified based on their morphological features and cell structure following the monograph of Desikachary (1959) and Anand (1989). The collected samples were maintained by culturing in freshly prepared BG- 11±N medium (Roger *et al*. 1978) and incubated at 28±2°C with illumination at 25-30 µmol photon $m^{-2}s^{-1}$ white continuous light and aeration. A part of each collected cyanobacterial samples were preserved in 4% formaldehyde solution and deposited in the Department of Botany, Ghazi University Dera Ghazi Khan for future references. Moreover, the density, frequency of each species was calculated by using following formulae (Hussian 2007):

$$\text{Density (\%)} = \frac{\text{Total no. of individuals of a species in a paddy field}}{\text{Total no. of individuals of all species in all paddy fields}} \times 100$$

$$\text{Frequency (\%)} = \frac{\text{Number of a species occurred in paddy fields.}}{\text{Total number of all species occurred in all paddy fields.}} \times 100$$

2.3.1. Physio-chemical Characterization of Water and Soil Samples:

The pH and conductivity of the soil samples were determined by using digital pH meter and conductivity meter, respectively. The organic carbon (%), available soil phosphorus and potash were determined by colorimetric method, Bray's I method and Ammonium acetate extraction method, respectively. Moreover, the collected samples were processed for determination of colour, odour, BOD, COD, TDS, Chloride, Nitrate and Phosphate by using standard methods like APHA (1985).

2.3.2. Isolation of Cyanobacterial Species and Selection of Best Growth Media:

Microscopic examination of collected samples was initially done to assess the diversity. Collected samples were transferred to suitable culture medium like BG-11 medium for isolation and incubated for about two weeks in culture room at 25°C under controlled continuous illumination. Standard plating / streaking techniques will be used for purification of cyanobacterial strains (Nayak & Prasanna, 2007). The plates will be examined, and the best colonies will be selected, picked up and re-streaked to new agar plates to obtain mono algal cultures. Identification of individual isolate was done with the help of relevant literatures and monographs provided by Desikachary (1959); Prescott (1970). Pure monocultures will be further cultured using different media like BG-11 medium, SE (Soil Extract) medium, Chu 10 medium, Allen and Arnons medium, Pringsheim's to select the best growth medium.

3. Results:

A total of 20 various species of cyanobacteria was recorded in 10 different cultivated fields of rice. From the recorded data from the study fields, the families had shared 17%; Genera 26% and species contribution in biofertilizers were about 57% (Figure 3). The highest percentage of families in terms of species was about 45% Nostocaceae family followed by Oscillatriaceae family having 30% and Chroococaceae 10% number of species were recorded in the present investigated fields of rice cultivation of Dera Ghazi Khan. The remaining families were little share in the biological nitrogen fixation of paddy soil (Figure 5). The same trends were found in terms of genera, e.g. the highest number of genera were belonged to Nostocaceae family, which was 34% followed by Oscillatoriaceae was 22% and the remaining families had equal number of genera in this current investigation (Figure 2 and 4). The total spectrum of cyanobacteria in the 10 different paddy fields of investigated area had showed significant variability in terms of total number of species of the 6 families. The highest percentage of species belonging to Nostocaceae and Oscillatoriaceae families was found, which was about 46% and 34% respectively (Figure 1).

Relative frequency of the various types of species of cyanobacteria was also measured. The results were showed the abundance of cyanobacteria in the investigated area of rice cultivation. It was cleared from the results the good fertility of the soil in the observed fields due to abundance of various types of cyanobacteria. The cultivation of rice plants (*Oryza sativa*) in the soil is a good sign for the grower, because the rice cultivation rather than the absorption of nutrients from the soil more nitrogenous substances added into the soil and soil become more fertile for the further cultivation. *Nostoc communae* had the highest relative frequency (8.74) and followed *Oscillatoria animalis* which was about 7.77 relative frequencies measured in the paddy fields (Table 2).

The highest relative density was measured in paddy fields number 4 and 7, which was about 13.59 %. And lowest was found in field number 2 which is almost 6.79%. Paddy fields number 5; 6 and 9 had equal shared as 7.77% in the soil as a biofertilizer and in the field number 10, has a second one in terms of relative density in relation to cyanobacteria present in the soil (Table 3). The role of cyanobacteria towards fertility of soil was much more increased due to cultivation of rice crops in the soil. The grower could save his money in terms of use of synthetic fertilizers in arable land due to cultivation of rice.

Overall interesting sketch was observed during this first-time investigation of cyanobacteria in the arable land of Dera Ghazi Khan. During this study Nostoc communae and Oscillatoria animalis was frequently found in the soil of Dera Ghazi Khan. The positive sign in table 4.1 showed the maximum abundance of cyanobacteria in the various fields of rice crops. The negative sign showed that the cyanobacteria not found in the fields of cultivated crops of rice.

The abundance of cyanobacteria is attributed to favorable contents of soil nutrients. The physic-chemical parameters are given in Tables (4 and 5). Maximum pH was observed in fields number four (7.84) and minimum was in field number one (6.40) Table 4.4. pH is one of the important parameters as it plays an important role in the acid-base neutralization and water softening. Available phosphorus and potash in all the observed 10 fields of rice was fluctuated in the range of 8.43±0.65 and 296.30±31.89 respectively. The organic carbon and electrical conductivity were observed in the investigated paddy fields in the range of 0.67±0.06 and0.79±0.13 respectively (Table 5). Overall results of physico-chemical properties of the observed fields were the inclined figures from acidic condition to basic condition of the soil. The growth and development of cyanobacteria in the rice paddies was good in such type of soil environment. More and more growth of cyanobacteria in the fields, the fertility status of the soil is increased.

Table 1: Various species of Cyanobacteria found in 10 various fields of rice cultivation.

S.No.	**Species of Cyanobacteria**	**Sampling sites of Rice fields of Dera Ghazi Khan**										**Total No. of Species**
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
1	*Anabaena aequalis*	+	-	+	+	-	-	+	-	+	-	05
2	*Anabaena constricta*	-	-	-	+	-	-	+	+	-	-	03
3	*Anabaena oryzae*	-	-	+	-	-	+	-	+	-	+	04
4	*Anabaena variabilis*	-	-	-	+	+	-	+	+	-	+	05
5	*Chroococcus cohaerens*	-	-	-	+	+	-	+	-	-	-	03
6	*Chroococcus minor*	+	+	-	+	-	+	-	+	-	+	06
7	*Cylindrospermum indicum*	-	+	-	+	-	+	+	-	-	+	05
8	*Cylindrospermum stagnale*	+	-	+	-	-	-	+	-	+	-	04
9	*Lynbya infixa*	-	+	-	-	+	-	-	-	-	+	03
10	*Microcoleus lacustris*	-	-	-	+	-	-	-	-	+	-	02
11	*Microcystis bengalensis*	+	-	+	-	-	-	+	+	-	+	05
12	*Nostoc calcicola*	+	-	+	+	+	-	+	+	-	+	07
13	*Nostoc communae*	+	+	+	+	+	+	+	-	+	+	09
14	*Nostoc spongiaeforme*	+	-	+	+	-	+	+	-	+	-	06
15	*Oscillatoria acuminate*	+	+	+	+	-	-	+	+	-	+	07
16	*Oscillatoria acuta*	+	-	-	+	-	+	-	+	+	+	06
17	*Oscillatoria animalis*	+	+	+	+	-	+	+	-	+	+	08
18	*Oscillatoria homogenea*	-	-	+	+	+	-	+	+	-	+	06
19	*Oscillatoria trichoides*	+	-	-	-	+	+	-	+	+	-	05
20	*Spirulina gigantea*	-	+	-	-	+	-	+	-	-	+	04
Total No. of various species in 10 rice paddy fields		11	07	10	14	08	08	14	10	08	13	103

Table 2: Relative frequency of various species of cyanobacteria found in 10 different rice fields of cultivated soil of Dera Ghazi Khan

S.No.	**Species of Cyanobacteria**	**Family Name**	**Relative Frequency with actual no of sp.**	
1	*Anabaena aequalis*	Nostocaceae	4.85	(5)
2	*Anabaena constricta*	Nostocaceae	2.91	(3)
3	*Anabaena oryzae*	Nostocaceae	3.88	(4)
4	*Anabaena variabilis*	Nostocaceae	4.85	(5)
5	*Chroococcus cohaerens*	Chroococcaceae	2.91	(3)
6	*Chroococcus minor*	Chroococcaceae	5.82	(6)
7	*Cylindrospermum indicum*	Nostocaceae	4.85	(5)
8	*Cylindrospermum stagnale*	Nostocaceae	3.88	(4)

9	*Lynbya infixa*	Oscillatoriaceae	2.91	(3)
10	*Microcoleus lacustris*	Microcoleaceae	1.94	(2)
11	*Microcystis bengalensis*	Microcystaceae	4.85	(5)
12	*Nostoc calcicole*	Nostocaceae	6.79	(7)
13	*Nostoc communae*	Nostocaceae	8.74	(9)
14	*Nostoc spongiaeforme*	Nostocaceae	5.82	(6)
15	*Oscillatoria acuminate*	Oscillatoriaceae	6.79	(7)
16	*Oscillatoria acuta*	Oscillatoriaceae	5.82	(6)
17	*Oscillatoria animalis*	Oscillatoriaceae	7.77	(8)
18	*Oscillatoria homogenea*	Oscillatoriaceae	5.82	(6)
19	*Oscillatoria trichoides*	Oscillatoriaceae	4.85	(5)
20	*Spirulina gigantea*	*Spirulinaceae*	3.88	(4)

Table 3: Relative density of various species of cyanobacteria found in 10 different rice fields of cultivated soil of Dera Ghazi Khan

No. of sites/fields observed For cyanobacteria	**Total no of species in each sampling site**	**Relative Density**
Paddy Field 1	11	10.68
Paddy Field 2	07	6.79
Paddy Field 3	10	9.71
Paddy Field 4	14	13.59
Paddy Field 5	08	7.77
Paddy Field 6	08	7.77
Paddy Field 7	14	13.59
Paddy Field 8	10	9.71
Paddy Field 9	08	7.77
Paddy Field 10	13	12.62

Table 4: Physico-chemical properties of rice paddy fields at different sites

Paddy fields	pH	EC $mmol^{-1}$	Organic Carbon %	Available Phosphorus %	Available Potash %
1	6.40	0.60	0.58	7.84	280
2	6.54	0.80	0.64	8.42	324
3	6.68	0.90	0.69	8.23	230
4	7.84	1.0	0.72	8.64	340
5	6.56	0.68	0.59	7.64	332
6	6.94	0.74	0.68	7.39	300
7	7.64	0.85	0.76	8.92	299
8	6.69	0.72	0.64	9.42	286
9	7.60	0.93	0.72	8.96	276
10	6.68	0.65	0.69	8.88	296

Table 5: Mean values with SD of Physico-chemical properties of rice paddy fields of 10 different sites

Variables	**Level of sites**	**Means with SD**
Fields	10	5.50±3.03
pH	10	6.95±0.53
EC m mol^{-1}	10	0.79±0.13
Organic Carbon%	10	0.67±0.06
Available Phosphorus %	10	8.43±0.65
Available potash %	10	296.30±31.89

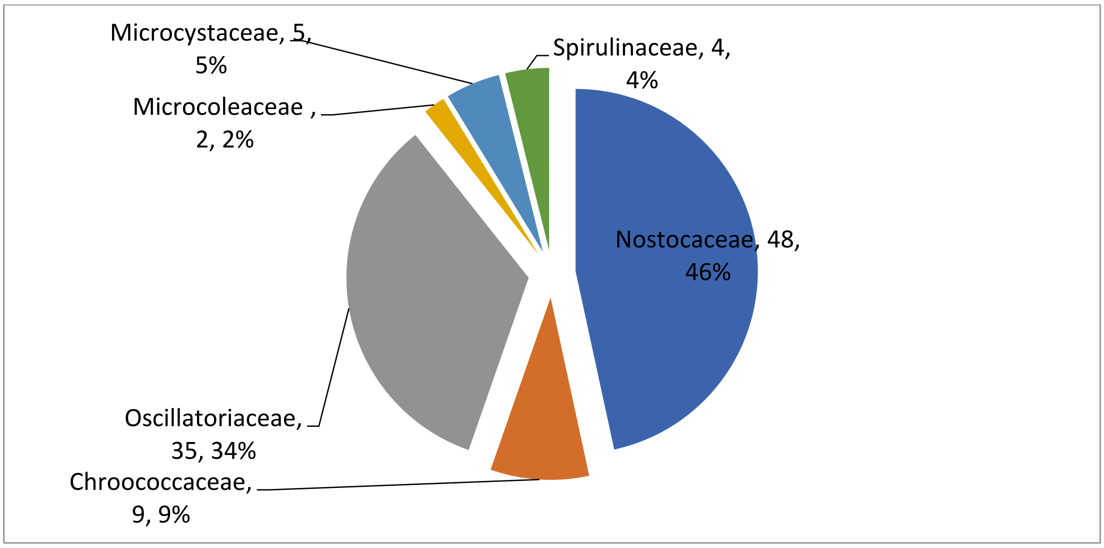

Figure 1: Spectrum of various species of cyanobacteria (%) of different Cyanobacteria families.

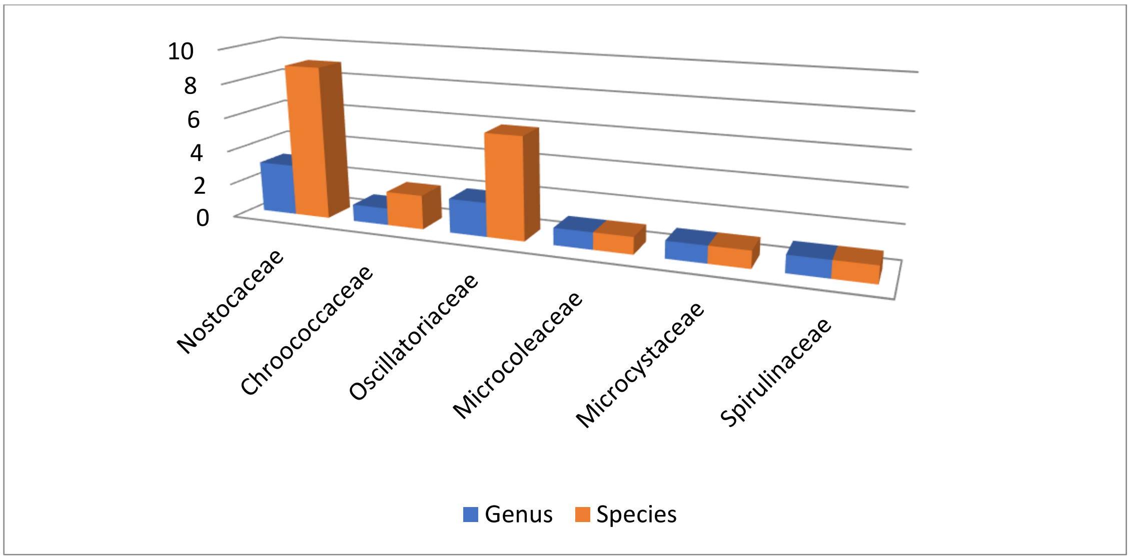

Figure 2: Graph among the various families containing no of genus and species found in study fields of cultivated rice

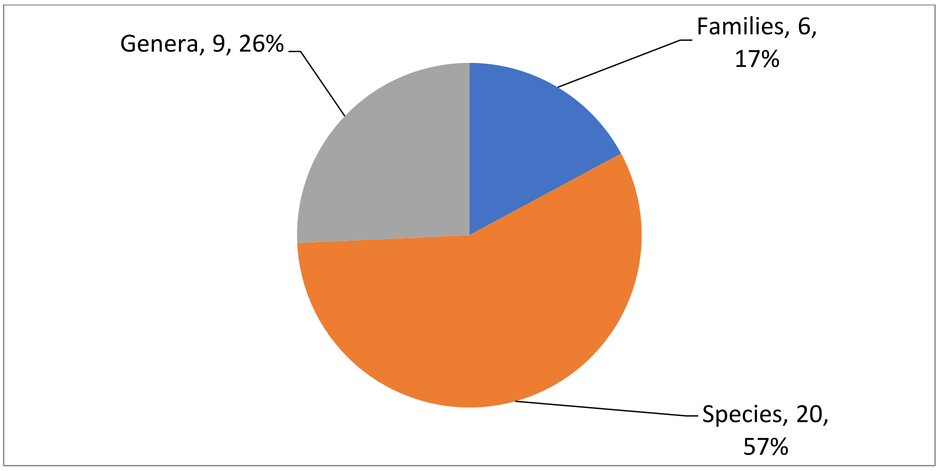

Figure 3: Graph among the various families, genus and species (%) found in study fields of cultivated rice

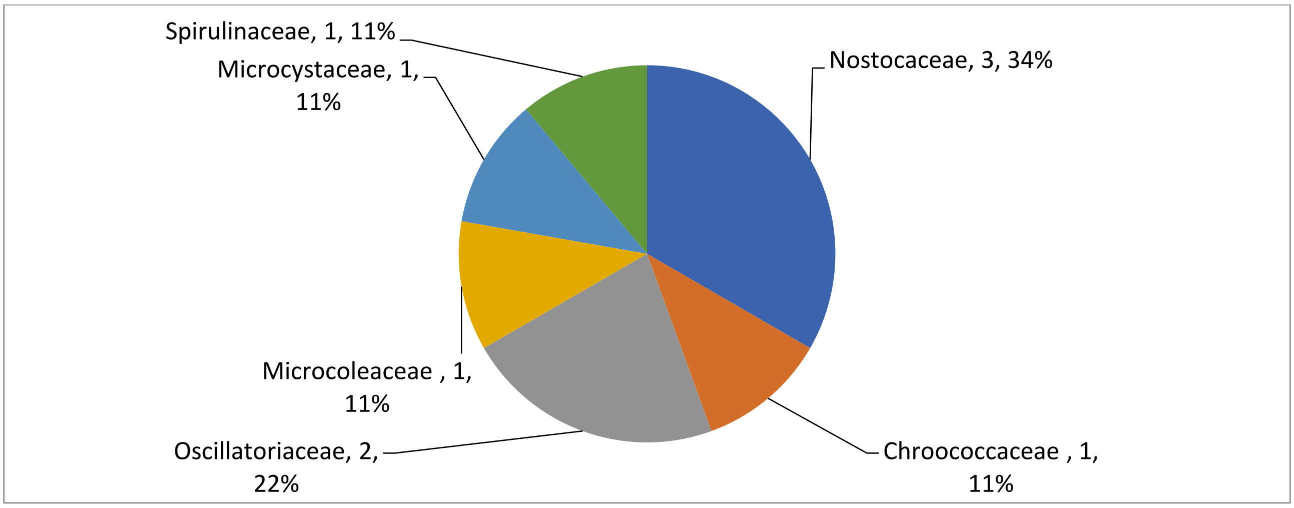

Figure 4: Graph of Percentage and number of Genera of different families found in study paddy fields.

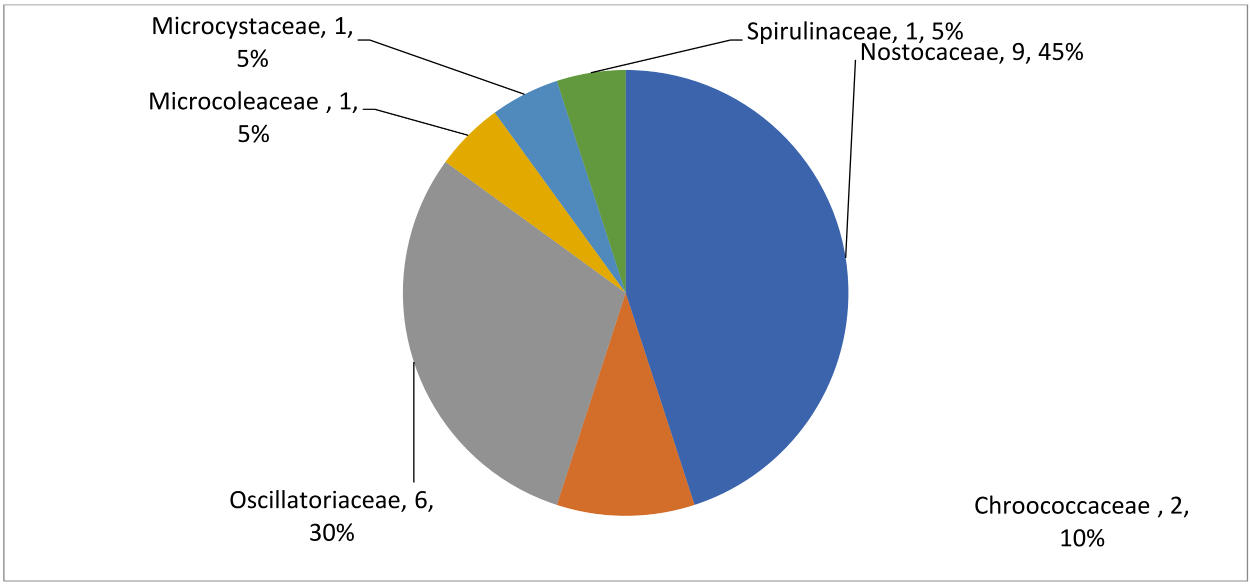

Figure 5: Graph of Percentage and number of Species of different families found in study rice fields of Dera Ghazi Khan

4. Discussion:

Cyanobacteria previously known as blue green algae identify a small taxonomic group of photosynthetic prokaryotes which some of them are able to nitrogen fixation and the cyanobacteria had also possess a notable potential for producing a wide range of secondary metabolites. Cyanobacteria had overwhelmed much attention as ultimate and rich sources of biologically active components and have been identified as one of the most promising groups of organisms capable of producing bioactive compounds and this study followed the findings of Parmar *et al.* (2011). These microorganisms had shared in the production of secondary metabolic substances which improving soil structure and porosity. Due to appropriate porosity of soil and good soil aggregation micelles, the growth of plants is vigorous and ultimate to production of high yields of crops. These types of studies already discussed by the various workers in the paddy fields like our findings about cyanobacteria were closed to the findings of Karthikeyan *et al.* (2007). The fertility of soil was increased due to abundance of cyanobacteria and maintained the biological nitrogen fixation, and this agreed the findings of Abinandan *et al.* (2019).

This was the first-time report, which recognized the agronomic potential of cyanobacteria in paddy fields of Dera Ghazi Khan Punjab Pakistan. The widespread application by the grower of single element fertilizers (especially nitrogen in Asian countries) in the cultivation of major economical as well as staple crops had led to accelerated exhaustion of other macro and micronutrients leading to nutrient imbalances and poor soil fertility. In the present study therefore, a significant need has been felt to categorize microbial biological fertilizers which are wide-ranging such as cyanobacterial bio-fertilizer. As yet for partial fulfill of chemical fertilizers by microbial bio-fertilizers many studies had been done. Blue green algae somehow the influence from the seed germination to growth and development of the cultivated crops and vegetables. The yields and yields contents of the crops are also better in the presence of cyanobacterial growth and these findings agreed with the previous findings of Pereira *et al.* (2009) and Bidyarani *et al.* (2016).

From this study twenty different types of cyanobacteria were observed from the various collected samples of rice paddy fields of Dera Ghazi Khan. The highest members of cyanobacteria were belonged to Nostocaceae and Oscillatoriaceae families. The contribution of Nostoc spp and Oscillatora spp. in biological fertilizers in the studied area are more and more. Beneficial effects of cyanobacterial inoculation were reported, not only for *Oryza sativa*, but for other crops such as *Triticum aestvum*, *Avena sativa*, *Lycopericon esculentum*, *Raphnus sativus*, *Gossypium hirsutum*, *Saccharum officinarum*, Zea mays and *Capsicum frutescens* were very prominent and these studied had done by the various workers such as (Thajuddin & Subramanian 2005, Saadatnia & Riahi 2009, Maqubela *et al*. 2008). Several reasons have been proposed for beneficial effects of cyanobacteria on the growth and development of different plants. The potential for biosynthesis of growth regulatory substances such as auxins, amino acids, sugars and vitamins (Vitamin B12, Folic acid, Nicotinic acid and Pantothenic acid) was reported by Misra & Kaushik (1989 a, b) that can enhance growth of plant. Additionally, cyanobacteria excrete complex organic carbon compounds that bind to the soil micelles and improve soil aggregation, hence improve soil structure, soil permeability and water holding capacity of soil, and all these studied by Karthikeyan (2007).

However, todate, the effect of single species cyanobacteria act as bio-fertilizer for plant growth has not yet been fully investigated. The primary aim of this research was to study cyanobacteria species isolated from soil samples of rice fields and the second aim was pointing out the role of cyanobacteria as biological fertilizers in various crops especially in rice cultivation.

The review of various literatures showed that, there are only a few studies on cyanobacteria, especially on staple crops and vegetables; yet results of other studies on other plants confirm the results of this study. The results obtained in the first time this work of the study area showed that the production of rice in rice cultivated area of Dera Ghazi Khan due to the virtue of Cyanobacterial growth in the respective cultivated fields of rice. These findings are also the confirmation of the previous findings of Nanda *et al*. (1991) and their findings were showed that, pre-soaking of pumpkin and cucumber seeds in *Westiellopsis prolific* extract can accelerate seed germination and spraying extracts of this cyanobacterium to emerged seedling during their subsequent cultivation led to significant increase in growth and development of both crops. They suggested that the supply of nitrogenous nutrients to the seeds is important.

Statistical analysis confirms that there is a significant difference in plant height, root length, number of leafs, fresh and dry weight of root, leaf and stem in treated plants as compared to control. Venkataraman & Neelakantan (1967) showed that the production of growth substances and vitamins by the algae may be partly responsible for the greater plant growth and yield.

The capacity for biosynthesis of growth promoting substances such as auxins, amino acids, sugars and vitamins (Vitamin B12, Folic acid, Nicotinic acid and Pantothenic acid) also can enhance plant growth. The other reason that can suggest for increased plant growth by using cyanobacterial extract is that the growth of BGA in soil seems to influence the physical and chemical properties of soil. The water stable aggregate significantly increases as a result of algal growth and thereby improves the physical environment of the plants.

5. Conclusion:

The quantity of nitrogen fixed by Cyanobacteria was supported in the growth and development of the rice plant. Cyanobacteria previously known as blue green algae increase in grain production was due to the nitrogen fixation of the algae which cause the increment in the plant height and grain quality of rice plant. The application nitrogen fertilizer could increase the yield of rice, chemical fertilizer resulted the poor performance than combined with blue green algae. The considerable of nitrogen fixed by blue green algae is almost certainly released only in death and decay of blue green algae. The results suggest that application of cyanobacteria with lower level of applied urea-nitrogen was more effective in enhancing the growth characteristics of rice plant. This indicates the better efficiency of cyanobacteria in promoting the growth of rice plants in that soil, which low in nitrogen fertility. The presence of cyanobacteria could increase the yield of rice grain significantly.

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