International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 05, 2020
ISSN: 1475-7192

Polyfunctional Properties of Cyanobacteria Isolated from the Saline Soils of Uzbekistan

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Abstract--- *Salt-tolerance of the local cyanobacterial strains of genus* Nostoc, Anabaena, Gloeothece *and* Synechococcus *was studied. The strains were subjected to grow and develop at NaCl concentrations ranging from 100 to 500mM. 200 mM NaCL was found not to affect the nitrogen-fixing activity of the cultures. Production of indole acetic acid by* N. calcicola *25 at salinization (300mM NaCl) with concentrations of tryptophan 0.5 and 2.5 mg/ml was 26.6 and 48.6 mg/l, respectively. The polychlorinated biphenyls' destructive activity in the samples of* N. pruniforme *20 and* A. variabilis *21 after 4 months was 66.78 and 73.28%, respectively.*

Keywords--- *Culture, Cyanobacteria, Indole-3-acetic Acid, Polychlorinated Biphenyls, Salinization.*

I. INTRODUCTION

The production of safe and eco-friendly food products, the conservation of natural resources, the ecosystem restoration and preservation are the primary goals of the agriculture's steady progression [1- 3]. Accordingly, cyanobacteria may play a prospective role in the reclamation of the saline, dry and arid soils, because the saline soils negatively affect plants' growth, development and crop productivity. According to global projections, the soil salinization may result in 30% loss of farm lands in upcoming coming 25 years and 50% lead to of crop loss by 2050 [4].

As cyanobacteria are all-pervesive, frequently occurring in the extreme environmental conditions, among other cultures they may greatly contribute to the improvement of soil productivity in the saline soils as active fixators of atmospheric nitrogen and carbon. The soil microflora, including cyanobacteria, is important for stimulation of growth and development of agricultural plants. Cyanobacteria are known to produce a wide spectrum of compounds, including amino acids, auxins, gibberellins and cytokinins [3, 5].

At the present time, there are many experimental data on biodegradation of these chemical agents with some microorganisms, including cyanobacteria [6]. Singh et al. [7] demonstrated that *Synechocystis* sp. PUPCCC 64 decompose anilofos and chlorpyrifos in the laboratory setting.

This work aimed to study poly functional peculiarities of active nitrogen-fixing local strains of cyanobacteria in the soil salinization and pesticide pollution.

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DOI: 10.37200/IJPR/V24I5/PR2020736
Received: 18 Apr 2020 | Revised: 26 Apr 2020 | Accepted: 16 May 2020

II. MATERIALS AND METHODS

Microorganisms and Culture Medium

Local strains of cyanobacteria, such as genus *Nostoc*, *Anabaena*, *Gloeothece* и *Synechococcus* isolated from the saline and pesticide polluted sierozem soils of Kashkadarya, Namangan and Sydrarya regions of Uzbekistan [8-10]. The strains were cultivated on the BG-11_0 (nitrogen-free) medium [11] at 26-28°C and illumination of 2500-3000 lx with a 12:12 hour light: dark photoperiod. The axenic cultures were stored in the BG-11_0 agar solid medium. The whole procedure was performed in the aseptic conditions.

Salt-tolerance of Cultures

To study growth and development of the cyanobacteria in salinity conditions, the cultures were inoculated with the initial titer of $4.8x10^8$ CFU /ml into the liquid medium with NaCl concentration ranging from 100 to 800 mM. The cultures were incubated in the optimum conditions for 8-10 days. Upon completion of the experiment the cell titer per 1 ml was calculated by the microbiological limiting dilution method.

Acetylene Reduction Activity of the Cultures

The nitrogen-fixation of the cyanobacteria strains was determined by the acetylene reduction assay. Seven-day cyanobacteria cultures cultivated on the 5 ml of nitrogen-free medium were collected into the serum vials. The vials were closed with the tight rubber cocks to introduce acetylene up to the final concentration of 10 vol. % of the gas phase. Coming up to be ethylene was determined by means of a flame ionization detector on gas chromatograph (Laboratory Universal Chromatograph-80, Russia) [12].

To Screen the Cyanobacterial Strains by the Indole-3-acetic acid (IAA) Production

The cyanobacterial culture with the titer of $4.2 \cdot 10^6$ CFU /ml was inoculated into the respective medium with NaCl concentration of 300 mM in the presence of tryptophan (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) and into a tryptophan-free medium [13]. The cultivation was conducted for 21 days at the illumination of 3000 lx. The culture was centrifuged for 30 minutes; 2 ml of the supernatant was mixed with 8 ml of the Salkovskii's reagent (50 ml, 35% $HClO_4$, 1 ml 0.5M $FeCl_3$). The photoelectric colorimeter (Russia) with green filter was used to measure the absorbency at 530nm in 30 minutes. The pink color was considered as a sign for indole acetic acid formation the concentration of which was calculated by the IAA standard values calibration curve (Sigma). The experiments were conducted in the salinization conditions (300 mM NaCl) thrice. The data were processed with PC statistical program Excel.

To study degradation of the tritium labeled polychlorinated biphenyls by the cynobacterial cultures we conducted the lasting experiment in the soil model.

Sierozem, a typical soil of our area, was sterilized by preheating in a thermostat at 180°C for 4 hours. 200 mg weight samples were prepared. 0.1 mg of the tritium labeled polychlorinated biphenyls in the 80 μl of hexane solution were introduced into each to be dried out for hexane to eliminate completely. The radioactivity of the

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Received: 18 Apr 2020 | Revised: 26 Apr 2020 | Accepted: 16 May 2020

polychlorinated biphenyl's dose was 17 872 counts/10 seconds. The samples were damped with water and incubated at the room temperature and constant soil humidity. The samples of the sterile soil containing the labeled polychlorinated biphenyls were used as the control. In two and four months 100 mg weight samples of the dry soil were selected from the preliminary dried out samples. The leftover soil in the samples was dampened and incubated at the room temperature.

Polychlorinated biphenyls were extracted from the samples by hexane: acetone (1:1) mixture three times by 1ml; the extracts were subsequently dried out. The dehydrated extracts were dissolved in 100 µl of hexane; 25 ml were selected for radio assay. The radioactivity was measured by means of a liquid scintillation counter RZhS-05 (Russia) in the toluene scintillation mixture containing one l of toluene, 4g of 2,5-dipheniloxasole (PPO) and 0.05 of 1,4 –di 2-(5-phenyl oxazolyl) benzene (POPOP).

III. Results And Discussion

The local cyanobacterial strains of *N.linkcia* 4, *N.muscorum* 14, *N.pruniforme* 20, *N.calcicola* 25, *A.variabilis* 21, *Gl.rupestris* 15 and *S. cedrorum* 12 cultivated on the medium with NaCl concentration ranging from 100 to 800 mM turned out to be resistant to the salinization of various degrees.

At the NaCl concentration of 100 mM the numbers of cyanobacterial cells were found not to decline as compared with the control, i.e. this degree of salinization appeared not to affect growth and development of local cyanobacterial strains. Virtually all cultures under study preserved their productivity for growing at NaCl concentrations ranging from 100 to 400 mM. At NaCl concentration of 200 mM cell titers remained comparable with one of the control samples in *N.calcicola* 25 and *A.variabilis* 21, more salt resistant cultures. Thus, salt-resistant *N.calcicola* 25 grew satisfactorily producing abundant biomass at NaCl concentrations ranging from 100 to 500 mM. Weight of biomass of the initial *N.calcicola* 25 was 148mg/100ml, reducing by 14% at NaCl concentration of 500 mM.

Cyanobacteria are known to grow and develop satisfactorily at a very low water potential resisting to drying and growing in the dry land (desert) or saline soils conditions, withstanding hypersalinity [14].

To clarify productivity of the cyanobacteria to grow and develop at the salinity stress we study nitrogen-fixing of the local cyanobacterial strains.

The initial nitrogen-fixing productivity of the strains was found ranging from 3 180 to 4 100 C_2H_2nmol/vial/day (Fig. 1). 200 mM NaCl was found not to affect the nitrogen-fixation of the cultures, while 300 mM NaCl reduced the activity of *A.variabilis* 21, *N.calcicola* 25, *N. pruniforme* 20 и *Gl.rupestris* 15 by 1.5, 2.0, 5.7 and 3.4%, respectively. The inhibition of nitrogen-fixing activity of the cyanobacteria under study was found to start at NaCl concentration of 400 mM. The NaCl concentration increased to 800 mM caused decline in the activity of *A.variabilis* 21 and *N.calcicola* 25 by 51.3 and 56.0%, respectively.

DOI: 10.37200/IJPR/V24I5/PR2020736
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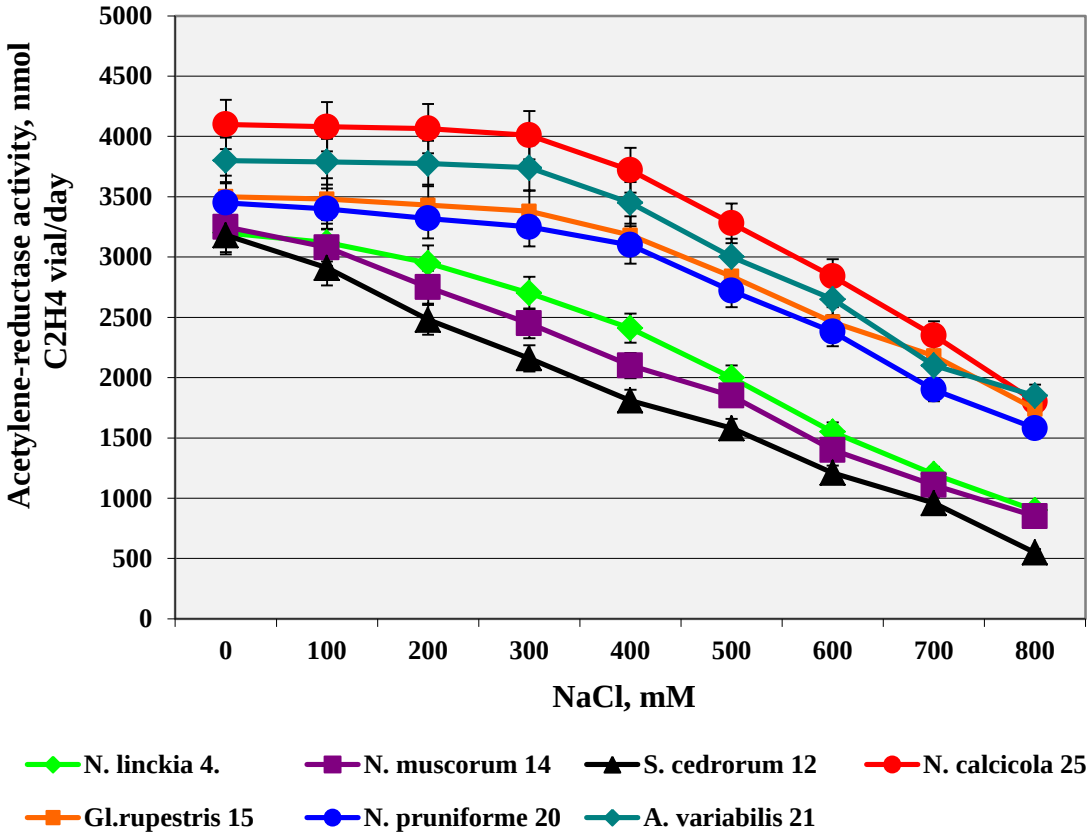

Figure 1: Acetylene-reductase Activity of the Cyanobacteria in Salinization

The nitrogen-fixing cyanobacteria were found to survive in the highest levels of salinization [15]. Contrary to other soil algae, heterocystic blue-green algae are able not only to fix atmospheric molecular nitrogen, but also to produce various biologically active compounds confirming their importance in generation of an organic matter [16-19].

We studied the phytohormone-producing intensity of *A.variabilis* 21 and *N.calcicola* 25 upon salinization with 300 mM NaCl in the presence of tryptophan in various concentrations in the culture medium. Tryptophan is known as an indole acetic acid precursor, the addition of this amino acid to the culture medium resulted in higher IAA production [13, 18, 19]. Added to the culture medium, tryptophan significantly increased tryptophan-dependent pathways of IAA production (approximately in 15-20 times). IAA biosynthesis without tryptophan as a precursor was previously found in plants, but seems unusual in the bacteria [13, 20].

IAA production by *N.calcicola* 25 and *A.variabilis* 21 was found increased on the 9th day of cultivation regardless of tryptophan concentrations (Fig.2). Maximum IAA synthesis by *N.calcicola* 25 and *A.variabilis* 21 could be observed on the 14th day of cultivation. The IAA production by *N.calcicola* 25 with tryptophan concentrations 0.5 and 2.5 mg/ml was 26.6 and 48.6 mg/l, respectively. Thus, IAA synthesis with tryptophan concentration of 2.5 mg/ml increased by 1.8 times as compared with the one with tryptophan concentration of 0.5

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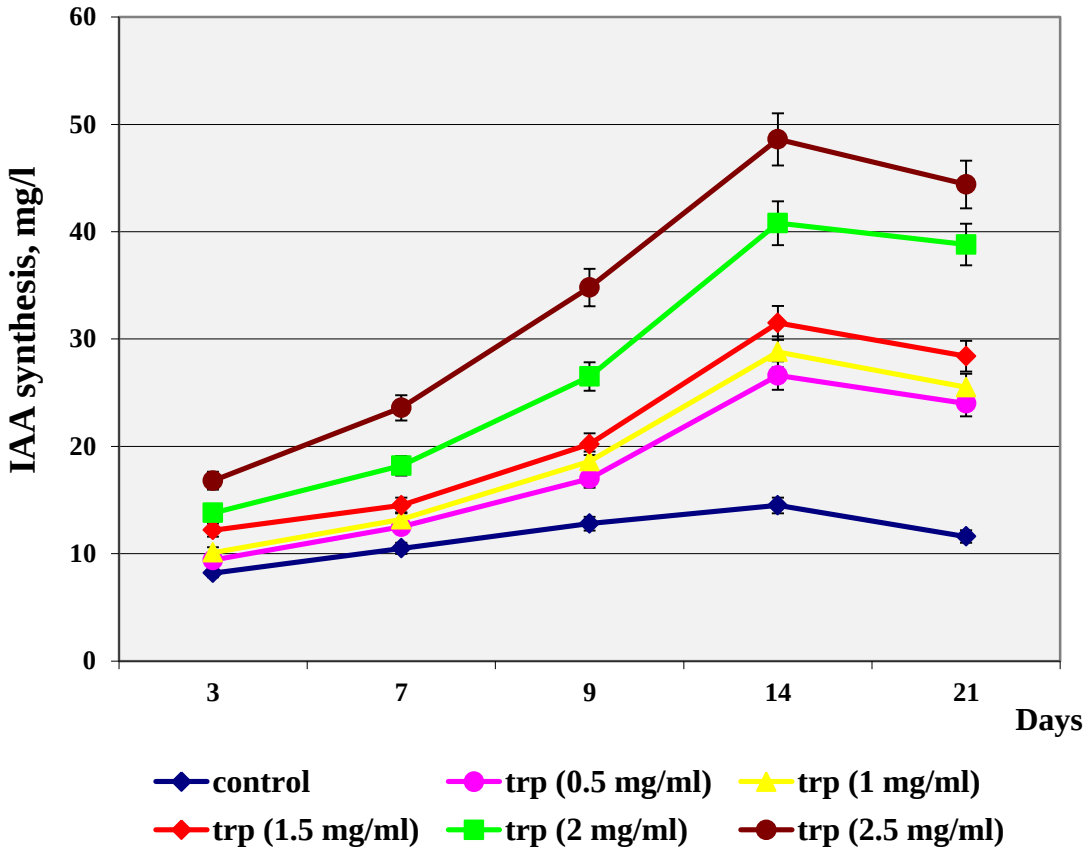
mg/ml. It is noted that at concentration of tryptophan of 2.5 mg/ml, the IAA production by *N. calcicola* 25, as compared with the control, increased by 3.3 times upon salinization within 14 days of cultivation. Similar results were obtained with *A.variabilis* 21. Maximum IAA produced by *A.variabilis* 21 in the presence of tryptophan in concentrations of 0.5 and 2.5 mg/ml was 19.8 and 38.8 mg/l, respectively, on the 14th day of cultivation regardless of tryptophan concentration. IAA production in *N.calcicola* 25 and *A.variabilis* 21 was observed regardless of cultivation conditions both upon salinization and presence of tryptophan. Thus, in 7 and 9 days of cultivation with 300 mM NaCl the IAA production was 10.5 and 14.5 mg/l and 6.8 and 12.1 mg/l, respectively.

Treatment of plants with the cyanobacteria is known to increase the synthesis of auxin, gibberellins and cytokinins [16]. The cyanobacteria are able to synthetize almost all the phytohormones, including auxins, cytokinins, gibberellins, dormin (ABA), ethylene and others [17, 18].

At present time, the attempts to use destructor microorganisms for detoxification of pesticides and other toxic agents in the soil are being made in the agriculture worldwide [21, 22]. Organochlorine pesticides, such as polychlorinated biphenyls, used for the cotton seed treatment are the most harmful ones. Polychlorinated biphenyl is highly persistent, as its retention in the soil exceeds 5-10 years.



a

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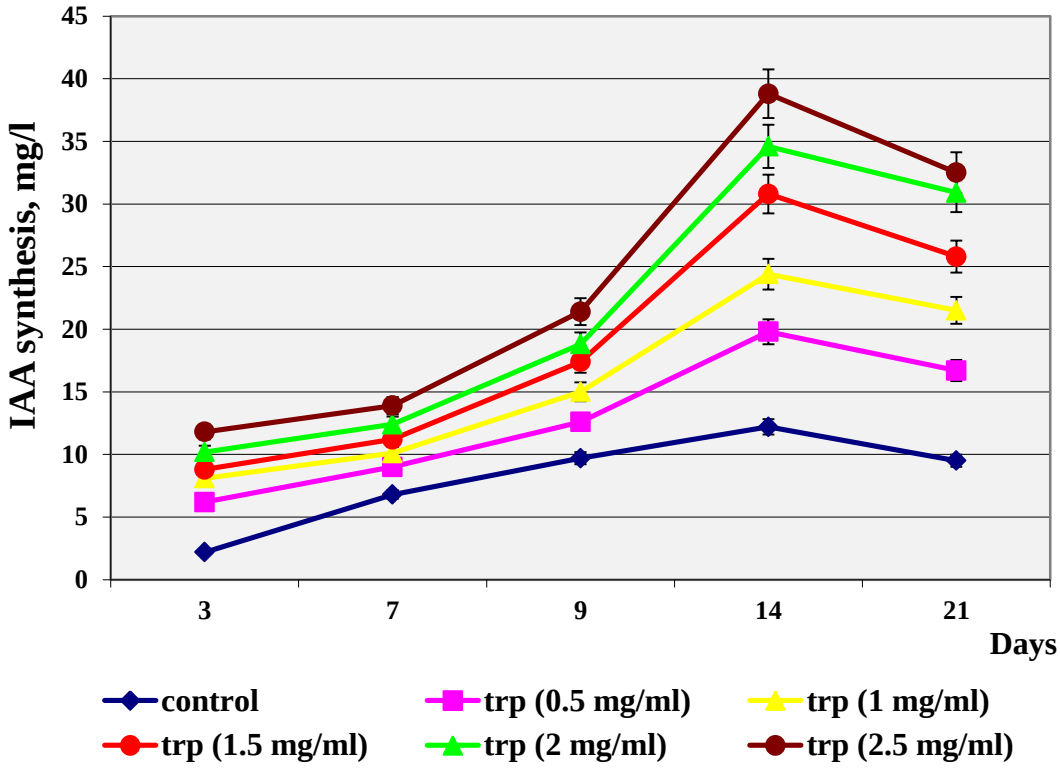

b

Figure 2: IAA Production by *N.calcicola* 25 (a) and *A.variabilis* 21 (b) at NaCl Concentration of 300 mM in the Presence of Tryptophan in Various Concentrations

We have conducted the lasting experiment in the soil model to study destructive activity of the local salt resistant cyanobacteria for the polychlorinated biphenyl. The data on destruction of tritium labeled polychlorinated biphenyl by the local salt resistant cyanobacteria taken place in two months of incubation can be seen in Table 1. Compared to the control, significant reduction of radioactivity in soil samples incubated with *N. pruniforme* 20 and *A.variabilis* 21 was observed by 40.51 and 36.03%, respectively, to be evidence of active destruction of tritium labeled polychlorinated biphenyls (Table 1). The reduction of radioactivity by *N.calcicola* 25 was not so significant, but, still, the destructive activity reduced by 21.31%. In the control samples the radioactive label degree remained unchanged.

Table 1: Radioactivity in the Soil after Two Months of Incubation of the Cyanobacteria Under Study

Culture	Radioactivity in 25 µl, counts/10 seconds	Radioactivity in 100µl, counts/10 seconds
Control	2234	8 936
Nostoc pruniforme 20	1329	5316
Nostoc calcicola 25	1758	7032
Anabaena variabilis 18	1834	7336
Anabaena variabilis 21	1429	5716
Gloeothece rupestris 15	1454	5816

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Received: 18 Apr 2020 | Revised: 26 Apr 2020 | Accepted: 16 May 2020

It was found that the destruction of the tritium labeled polychlorinated biphenyls in the soil took place within 4 months (Table 2). Radioactivity of the control tritium labeled polychlorinated biphenyl in 100 µl was 8 936 counts/10 seconds. As it can be seen in Table 2, the values of residual radioactivity of

N.pruniforme 20, *N. calcicola* 25 and *A. variabilis* 21 were 2 968, 2848 and 2387 counts/10 seconds, respectively. It is noted that the polychlorinated biphenyls' destructive activity in the samples of *N. pruniforme* 20, *N. calcicola* 25 and *A. variabilis* 21 within 4 months was 66.78, 68.12 and 73.28%, respectively.

Table 2: Radioactivity in the soil after 4 Months of the Cyanobacteria's Incubation

Culture	Radioactivity in 25µl, counts/10 seconds	Radioactivity in 100µl, counts/10 seconds
Control	2234	8 936
Nostoc pruniforme 20	742	2968
Nostoc calcicola 25	712	2848
Anabaena variabilis 18	955	3821
Anabaena variabilis 21	596	2387
Gloeothece rupestris 15	799	3196

By reference to data received, the destruction of polychlorinated biphenyls in *N. calcicola* 25 within two months of incubation was 21.31%; further incubation up to 4 months increased the destructive activity to 68.12 %. As to *A. variabilis* 21, the destructive activity in question increased almost twofold. Consequently, the cultures under study are virtual destructors of the polychlorinated biphenyls. Active destruction of the tritium labeled polychlorinated biphenyls was observed within 4 months of the experiment, which can be evidence of prolonged active biodestruction of polychlorinated biphenyls by the cyanobacteria. Received data suggest that the cyanobacterial strains under study degraded or transformed the tritium labeled polychlorinated biphenyl to volatile compounds or completely utilized it. It is noted that the concentration of the introduced tritium labeled polychlorinated biphenyl was quite high, 0.5 mg/g of the soil. Accordingly, considerable reduction in the amounts of polychlorinated biphenyl within 4 months in the samples could be the evidence of a polychlorinated biphenyl-destructive activity of the local cyanobacterial strains under study.

IV. CONCLUSION

Cyanobacteria are not only photosynthetic nitrogen-fixers, but also biostimulators of growth and development of higher plants, and other heterotrophic microorganisms used as biofertilizers in many countries, such as China, Japan, India, Vietnam and others. The potential of these organisms as biofertilizers for paddy fields to sustain fertility has been proved. Eco-friendly bioproducts on the basis of the nitrogen-fixing and phytohormone-producing cyanobacteria are widely used in the agriculture worldwide. Introduction of the cyanobacteria under study into the soil not only result in increased degradation of some toxic compounds in the soil, but also contribute to increase the crop productivity to considerably compensate expenditure for restoration of the saline and polluted farmlands.

REFERENCES

[1] Singh, J. S., Abhilash, P. C., and Gupta, V. K. Agriculturally important microbes in sustainable food production. *Trends Biotechnol.* 2016a; 34, P.773–775.

[2] Singh, J.S., Kumar, A., Rai, A.N., and Singh, D.P. () Cyanobacteria: a precious bio-resource in agriculture, ecosystem and environmental sustainability. 2016, *Front Microbiol* 7: 529; P.1–19.

DOI: 10.37200/IJPR/V24I5/PR2020736
Received: 18 Apr 2020 | Revised: 26 Apr 2020 | Accepted: 16 May 2020

[3] Singh, J. S., and Strong, P. J. Biologically derived fertilizer: a multifaceted bio-tool in methane mitigation. 2016. *Ecotoxicol. Environ. Saf.* 124, P. 267–276.

[4] Jamil A., Riaz S., Ashraf M., Foolad M.R. Gene expression profiling of plants under salt stress. 2011, *Crit. Rev. Plant Sci.;* 30 (5); P.435–458.

[5] Sood A, Singh PK, Kumar A, Singh R and Prasanna R (2011) Growth and biochemical characterization of associations between cyanobionts and wheat seedlings in co-culturing experiments. *Biology* 66 (1); P.104-110.

[6] Subashchandrabose S.R., Ramakrishnan B., Megharaj M., Venkateswarlu K., Naidu R. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation Environ. 2013, *Int.,* 51; P. 59–72.

[7] Singh D.P., Khattar J.I.S., Kaur M., Kaur G., Gupta M. and Singh Y. Anilofos tolerance and its mineralization by the cyanobacterium Synechocystis sp. strain 2013c. PUPCCC 64. *PLoS ONE* 8(1): 1-10.

[8] Kadirova G.Kh. Taxonomy and nitrogen-fixing activity of nonheterocystic local *Synechococcus cedrorum* cyanobacteria upon soil salinization // *Bulletin of National University of Uzbekistan*, 2011, P.196-198.

[9] Kadirova G.Kh. Taxonomy and some properties of the local nitrogen-fixing cyanobacteria of the genus *Nostoc // Uzbekistan Academy of Sciences Reports*, 2012, №1. P. 71-75.

[10] Kadirova G.Kh., Korobkova E.S. Identification of cyanobacteria genus *Anabaena* isolated from the cotton rhizosphere// *Ukrainian Microbiological Journal*, 2013. Vol.75, No.1, P.48-53.

[11] Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. 1979, *J. Gen. Microbiol.* 111 P. 1–61.

[12] Hardy D.W., Halstein R., Jecson E. C_2H_2-C_2-H_4 Assay to N2 Fixation Laboratory and system Field evaluation// *Plant Phys.*1968. Vol.43. P.9-13.

[13] Ahmad F., Ahmad I., Khan M.S. Indole Acetic Acid Production by the Indigenous Isolates of Azotobacter and Fluorescent Pseudomonas in the Presence and Absence of Tryptophan// 2005, *Turk. J. Biol.,* 29; P.29-34.

[14] Horacio G.P., Walter A.V., Graciela L.S. Structural characterization of the members of a polymer series, compatible solutes in *Anabaena* cells exposed to salt stress. 2007, *Plant Sci,* 172 (1); P. 29–35.

[15] Zhang W., Feng Y. Characterization of nitrogen-fixing moderate halophilic cyanobacteria isolated from saline soils of Songnen Plain in China. 2008, *Progress in Natural Science*, Volume 18, Issue 6; P. 769-773.

[16] Sergeeva E., Liaimer A., Bergman B. Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. 2002, *Planta*, 215; P. 229-238.

[17] Rodriguez A.A., Stella A.A., Storni M.M., Zulpa G. and Zaccaro M.C. Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in Oryza sativa L. // 2006, *Saline Systems,* 2 (7), P. 1186-1148.

[18] Spaepen S., Vanderleyden J., Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. 2007. *FEMS Microbiology Reviews* 31, P.425–448.

[19] Prasanna R., Joshi M., Rana A., Nain L. Modulation of IAA production in cyanobacteria by tryptophan and light. 2010, *Polish J. Microbiol.,* 59(2); P. 99-105.

[20] Idris E.E., Iglesias D.J., Talon, M., and Borriss R.. Tryptophan- dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42. 2007, *Mol.Plant. Microbe Interact.* 20, P.619–626.

[21] Chávez, F.P., Lunsdorf, H., Jerez, C.A. Growth of polychlorinated-biphenyl- degrading bacteria in the presence of biphenyl and chlorobiphenyls generates oxidative stress and massive accumulation of inorganic polyphosphate. 2004, *Applied and Environmental Microbiology* 70, P. 3064-3072.

[22] Cycoń Mariusz and Piotrowska-Seget Zofia Pyrethroid-Degrading Microorganisms and Their Potential for the Bioremediation of Contaminated Soils: A Review. 2016, *Front Microbiol.,* 7:1463.

DOI: 10.37200/IJPR/V24I5/PR2020736
Received: 18 Apr 2020 | Revised: 26 Apr 2020 | Accepted: 16 May 2020