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Characterization of Cyanobacterial Strains Isolated from Soils Polluted with Insecticides

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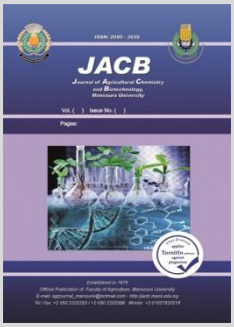

ABSTRACT

This research aimed to isolate and characterize cyanobacteria that have the ability to grow in the polluted soil with insecticide and evaluation of their efficiency in fixing nitrogen and dry weight. Cyanobacteria were isolated from samples of soil site polluted with insecticides in Kafr El- Sheikh, Governorate. Cyanobacteria were purified by different purification methods. Twelve cyanobacterial isolates were identified according to standard methods based on cultural and morphological characters of cyanobacteria as following: color of culture, morphology of thallus, vegetative, reproductive cells and heterocyst. Heterocyst from isolates of cyanobacteria were observed in *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp. and *Chroococcus* sp. *Nostoc* sp. was of the highest frequency (66.6 %) among the isolated cyanobacteria. While, *Oscillatoria* sp. and *Chroococcus* sp. were less frequency (8.3%). Dry weight and nitrogen fixation of cyanobacteria were determined for efficiency evaluation of these isolates. The superior cyanobacterial isolates, which isolated from soil polluted with insecticides were identified by 16S rRNA as *Nostoc muscorum* and *Anabaena oryzae*.

Keywords: cyanobacteria, nitrogen fixation, insecticides .

INTRODUCTION

Recently, cyanobacteria have been discovered to be a main source of many active compounds such as extracellular products and secondary metabolites (Afify and Ashour 2018) such as vitamins, enzymes, carbohydrates, peptides and amino acids, which have been found to increase plant crops (Singh *et al.* 2016). Zulpa *et al.* (2003) and Sammauria *et al.* (2020) found that cyanobacteria are different communities of photosynthetic prokaryotes and abundant in soil, water, as well air ecosystems (Seckbach, 2007). Currently, the farmers extensively used insecticides for the protection of plants from insects (Parte *et al.* 2017). While, insecticides has enhanced in product yield and prevented insect-borne diseases (Verma *et al.* 2014). But there are, the problems associated with the use of these chemicals have also increased. It has become a major cause of environmental pollution such as soil and water (Rani and Dhania 2014). For cyanobacterial process and an adequate population biological N_2 fixation is very important. Cyanobacteria are group of photosynthetic prokaryote and gram negative bacteria (El-Saadny, 2013 ; El-Zawawy, 2016; Abou Elatta, 2018; Zaki, *et al.* 2021). In addition, insecticides effects on growth, nitrogen fixation and photosynthesis in cyanobacteria (Mohapatra *et al.* 2003; Jha and Mishra 2005; Prasad *et al.* 2005; Chen *et al.* 2007). Insecticides represent the greatest proportion of pesticides used in developing countries. Chlorpyrifos used since 1960s, for the control of crop from insects (Bicker *et al.*, 2005) but contaminated aquatic and terrestrial ecosystem and public health because it has long half-life and high residual concentration (Nawaz *et al.*, 2011). Chlorpyrifos is remains biologically active in soil for periods ranging from twenty to eighty days and is moderately persistent, from ten to sixty days (Lakshmi *et al.* 2008). Residues of applied pesticides stay in the environment (air, soil, ground and surface water) for variable periods of time (Gavrilescu, 2005 ; Tariq *et al.*, 2007). This research aimed to isolate and identify cyanobacteria that have the ability to grow in the polluted soil with insecticides and evaluation of their efficiency in fixing nitrogen and dry weight.

MATERIALS AND METHODS

Source of soil samples :

Soil samples were collected from different locations at Kafr ElSheikh Governorate cultivated with rice and polluted with insecticides. The collected soil samples were used as a source of cyanobacterial isolates. Some chemicals and physicals analyses of soil (Piper 1950 and Jackson 1973) are previously presented in Abou Elatta *et al.*,(2023).

Isolation of cyanobacteria:

The method of preparation of the soil which were collected from rice fields polluted with the pesticides was carried out according to the Oxford Manual of Culture Media (Manual, 1990). BG11 medium (nitrogen – free medium) was used (Abdel-Razek *et al.*, 2019) the soil samples (little milligrams) were spread in the Petri dishes containing BG11 medium (Black *et al.*, 1965) and dishes incubated at 28-30°C with constant lighting of 2500 lux until appearance of cyanobacterial growth. After incubation cyanobacteria were purified by standard cyanobacterial purification techniques (Desikachary, 1959 & El-Ayouty and Ayyad, 1972). The purification techniques according to Watanabe liquid medium (Watanabe *et al.*, 1951) without nitrogen sources.

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Morphological identification of isolated cyanobacteria:

Cyanobacterial isolates were identified according to standard methods of Bergey's Manual of Systematic Bacteriology (2001) based on cultural and morphological characters of cyanobacteria, *i.e.* color of cultures, morphology of thallus, vegetative, reproductive cells and heterocyst.

Molecular identification of cyanobacterial isolates:

Only the most efficient cyanobacterial isolates were identified by molecular16S rRNA genes of approximately 1500bp length in Agricultural Genetic Engineering Research Institute (AGERI) Agriculture Research Center, PO Box12619, Giza, Egypt. RNA genes contain regions of variable DNA sequence that are unique to the species carrying the gene. The species identity of an unknown bacterium may therefore be deduced from its unique rRNA gene sequence.

Selection of the most efficient cyanobacterial isolates in fixing nitrogen and dry weight :

Selection of the most efficient cyanobacterial isolates in fixing nitrogen and dry weight. Each cyanobacterial isolate was cultivated separately for 21 days on suitable medium (modified Watanabe liquid medium) at 28-30ºC with constant lighting of 2500 lux. The dry weight and fixed nitrogen were determined for each cultivated isolate. The most efficient isolates were selected.

Preparation of cyanobacterial inoculum:

Liquid cultures of cyanobacterial isolates were prepared using Modified Watanabe liquid medium with incubation at 28-30ºC under continuous illumination (2500 lux).

Total nitrogen determination:

using the micro-kjeldahl method according to Jackson (1973), total nitrogen was determined for the cyanobacterial isolates .

RESULTS AND DISCUSSION

Distribution and morphology of cyanobacterial isolates:

Twelve cyanobacterial isolates were obtained from a soil sample polluted with insecticides in Kafr El- Sheikh as described by El-Gamal *et al*. (2008). These twelve isolates were found to be belonging to four genera (*Anabaena*, *Nostoc*, *Oscillatoria* and *Chroococcus*). Data presented in Table (1) indicate that these genera varied in their densities and frequncy in the collected soil sample either in broth or solid medium (Staub, 1961). Among the twelve isolates eight isolates were found to be belonging to *Nostoc* Genus, represinting 66.6% of the total isolates. Moreover, two isolates were identified as *Anabaena* genus aming the twelve isolates represinting 16.6% of the total isolates. Furthermore, each of *Oscillatoria* and *Chroococcus* genera was found to represent 8% of the total isolates. These results may indicate that *Nostoc* is the most dominant genus among the detected genera in the insecticides polluted soil. Similar results were obtained by Venkataraman (1981) & Roger and Ardales (1991).

Table 1. Densities of the isolated cyanobacterial genera in the collected soil sample polluted with insecticides

Origen soil samples	Cyanobacteria genera	No. of Isolates	Genera Frequency (%)
Kafr El-Sheikh Governorate, Egypt	*Anabaena* sp.	2	16.6
	Nostoc sp.	8	66.6
	Oscillatoria sp.	1	8.3
	Chroococcus sp.	1	8.3
Total number of isolates	4	12	100

The morphological characters of the twelve cyanobacterial isolates were studied based on the phenotypic properties, appearance and color of cultures in addition to the microscopic examination. The characteristics of the isolated cyanobacterial genera are presented in Table (2). In the Bergey's Manual of Systematic Bacteriology (2001) cyanobacteria contain five groups or subsections. According to the dichotomous key: the morphotype is unicellular or trichome; presence or absence of differentiated cells (Naz *et al.* 2004; Pinevich 2008; Shariatmadari & Riahi 2010; and Komárek *et al.* 2014). Individual cyanobacteria of all isolates were identified by cultural appearance (color, shape of colonial aggregates) in addition to its distinct of cells or filaments, heterocysts and akinetes.

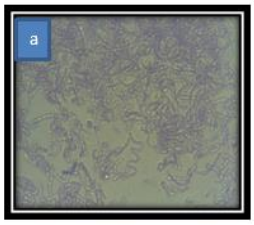
Results in Figure (1) represent light micrographs of cyanobacterial isolates grown on nitrogen free medium (BG11) for 30 days.

Table 2. Cultural and microscopic characterization of cyanobacterial isolates

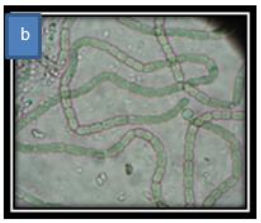
Cultural color	Thallus morphology	Vegetative Cell			Heterocysts			Akinetes				Cyanobacteria Identified Name
		Shape	Width (µm)	Length (µm)	Site	Width (µm)	Length (µm)	Shape	Shape	Width (µm)	Length (µm)	
Dark green	Filaments	oblong	3.2-4.8	4.1-4.5	Terminal and intercalary	3.2-4.8	4.1-4.5	Subspherical	oblong or oval,	4.1-6.3	5-6.3	*Nostoc paludosum*
Green	Filaments	Barrel	4-5	4-5	Terminant ercalary	3-3.5	4-4.5	Subspheric a l conical	3-6 in series, sub-spherical	5-6	6-7	*Anabaena oryzae*
yellowish green later brownish	Filaments	quadrate to short barrel	2.7-3.6	2.7-4.1	Terminal	4.1-4.5	4.1-5	Spherical	-	3.6-7.2	4.1-6.5	*Nostoc entophytum*
Blue-green, or brown; black	Filaments	Subglobose to barrel	2–3	3–4	Intercalary or terminal	5–6	5–7	Globose or ellipsoid	Spherical	10	10	*Nostoc pruniforme*
Oliveceous green, blue green	Filaments	Cylindrica l	3.6-4.1	4.1-5	Terminal	4.2-5	4.2-5.4	Spherical	Ellipsoidal or oblong	5.0-6.3	5-8.1	*Anabaena qelatinicola*

Cont. Table 2. Cultural and microscopic characterization of cyanobacterial isolates

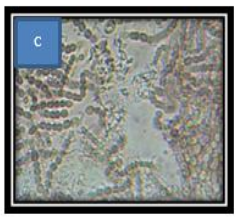
Cultural color	Thallus morphology	Vegetative Cell			Heterocysts			Akinetes			Cyanobacteria Identified Name
		Shape	Width (µm)	Length (µm)	Site	Width (µm)	Length (µm)	Shape	Width (µm)	Lengh (µm)	
Dark green	Filaments	Barrel, granular, yellowish	5-6	5.5-7	Terminal-Intecalary	-	-	-	-	-	*Nostoc muscorum*
Dark green	Filaments	apical cells sometimes slightly larger, oval	1.6-1.8	2.6-3	Terminal	4.6-6.2	5.2-8	Intercalary	6-8.5	6.5-11	*Nostoc viride*
Young cells palegreen, olderones brown	Filaments	quadratic, oblong or barrel	4.1-5.4	3.6-5.4	-	4.5-6.8	5-7.2	terminal or intercalary, cylindrical -	-	-	*Nostoc Rivulare*
blue green	Slimy-gelatinous	Spherical	3-4	3-4	-	-	-	-	-	-	*Chroococcu minor*
Dark green	Solitary and straight	Solitary	4-8.2	2.5-3.1 4-7.1	-	-	-	-	-	-	*Oscillatoria brevis*



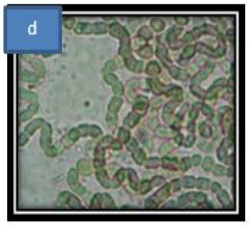
Nostoc muscorum



Anabaena oryzae



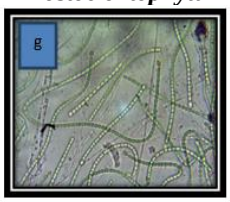
Nostoc paludosum



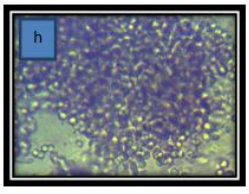
Nostoc pruniforme

Nostoc entophytum

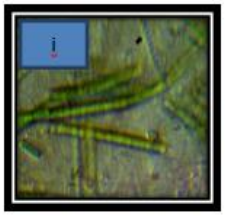
Nostoc rivulare



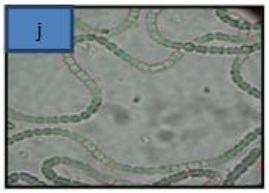
Nostoc viride



Chroococcus minor



Oscillatoria brevis



Anabaena qelatinicola

Fig. 1. Light micrographs of selected cyanobacterial strains.

Determination of dry weight of cyanobacterial isolates and their ability in fixing nitrogen:

The data presented in Table (3) indicate that the biomass dry weight of the cyanobacterial isolates and the fixed nitrogen in their liquid cultures increased gradually with increasing the incubation period. The highest values of dry weight and fixed nitrogen were recorded for all strains after inocubation for 21 days. The highest values of biomass dry weight (98 mg/100ml-culture) and fixed nitrogen (9.81 mg N/100 ml-culture) were recorded for *Anabaena oryzae* among all tested strains after incubation for 21 days. Whereas, the lowest value of biomass dry weight (60mg/100ml-culture) was recorded for *Nostoc verrucosum* and the lowest value of fixed nitrogen (4.27 mg N/100 ml-culture) was recorded for *Nostoc rivulare*. These results are in agreement with those obtained by Abou Elatta (2018) and Zaki *et al.* (2021) who found that the highest dry weight of cyanobacteria increased with increasing the incubation period.

Table 3. Dry weight (mg/100ml-culture) and nitrogen fixation (mg N/100 ml-culture) of the cyanobacteria strains.

cyanobacteria strains	Dry weight (mg/100ml-culture)			nitrogen fixation (mg N/100 ml-culture)		
	Incubation Period					
	7	14	21	7	14	21
Oscillatoria brevis	23	34	67	1.87	2.84	6.46
Chroococcus minor	26	51	65	1.91	2.83	4.18
Nostoc paludosum	31	57	79	2.33	4.14	9.55
Anabaena oryzae	52	74	98	3.39	6.94	9.81
Nostoc pruniforme	24	40	85	2.24	4.16	7.98
Anabaena variabilis	27	44	65	1.35	3.61	7.42
Nostoc verrucosum	28	40	60	2.12	4.58	5.81
Anabaena qelatinicola	30	45	76	2.46	3.89	4.34
Nostoc entophytum	42	55	62	3.11	5.45	8.06
Nostoc rivulare	26	46	66	1.41	2.26	4.27
Nostoc viride	33	62	72	2.41	3.35	4.35
Nostoc muscorum	48	65	91	3.25	5.35	9.81

Molecular identification of the most efficient cyanobacterial isolates:

Based on the results, it can be inferred that the most efficient isolates for nitrogen fixation ability and biomass production belonged to two strains of *Nostoc muscorum* and *Anabaena oryza*e. Molecular identification revealed that these two isolates contained 16S ribosomal RNA (rRNA) genes that were approximately 1500bp in length. The sequences were analyzed using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) and aligned using Align Sequences Nucleotide BLAST (Figure 2). These genes consist of regions of variable DNA sequence that are unique to the species harboring them. Therefore, the identity of an unknown bacterium can be determined from its distinct rRNA gene sequence. To do this, rRNA genes are first amplified using PCR technology. Subsequently, PCR cycle sequencing is conducted, and the rRNA sequence is determined with a capillary sequence analyzer. The resulting sequence is then compared to known rRNA sequences in Gen-Bank® and subjected to a rigorous review process for validation (as shown in Figures 3 and 4).

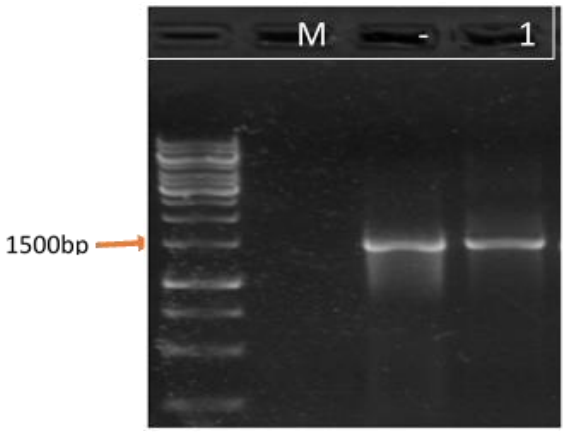


Fig. 2. The sequences nucleotide BLAST program.

Nostoc muscorum

GGAATTGCGATTGCTTACTATGCAGTCGAACGGGCTTTCGGTGATCTGGCGGCTCAGGATGAACGC
TGGCGGTATGCTTAACACATGCAAGTCGAACGGTCTCTTCGGAGATAGTGGCGGACGGGTGAGTAACGCG
TGAGAATCTAGCTTCAGGTCGGGGACAACCACTGGAAACGGTGGCTAATACCGGATGTGCCGAAAGGTG
AAAGATTTATTGCCTGAAGATGAGCTCGCGTCTGATTAGCTAGTTGGTGTGGTAAGAGCGCACCAAGGCG
ACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG
GAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATACCGCGTGAGGGAGGAAG
GCTCTTGGGTTGTAAACCTCTTTTCTCAGGGAATAAAAAAATGAAGGTACCTGAGGAATAAGCATCGGCT
AACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCAAGCGTTATCCGGAATGATTGGGCGTAAAGCG
TCCGCAGGTGGCACTGTAAGTCTGCTGTTAAAGAGCAAGGCTCAACCTTGTAAAGGCAGTGGAAACTACA
GAGCTAGAGTACGTTCGGGGCAGAGGGAATTCCTGGTGTAGCGGTGAAATGCGTAGAGATCAGGAAGAA
CACCGGTGGCGAAAGCGCTCTGCTAGGCCGTAACTGACACTGAGGGACGAAAGCTAGGGGAGCGAATGG
GATTAGATACCCCAGTAGTCCTAGCCGTAAACGATGGATACTAGGCGTGGCTTGTATCGACCCGAGCCGT
GCCGGAGCCAACGCGTTAAGTATCCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAAGACTTG
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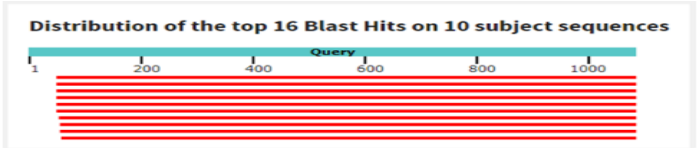

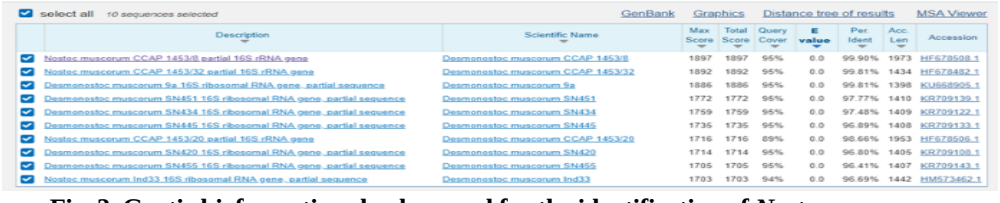

Fig. 3. Gentic bioformations background for the identification of *Nostoc muscorum*.

Anabaena oryzae

GAGATCGTGGCGGACGGGTGAGTAACGCGTGAGAATCTAGCTTCAGGTCGGGGACAACCACTGGA
AACGGTGGCTAATACCGGATGTGCCGAAAGGTGAAAGATTTATTGCCTGAAGATGAGCTCGCGTCTGATT
AGCTAGTTGGTGTGGTAAGAGCGCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGAAA
GCCTGACGGAGCAATACCGCGTGAGGGAGGAAGGCTCTTGGGTTGTAAACCTCTTTTCTCAGGGAATAAA
AAAATGAAGGTACCTGAGGAATAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATG
CAAGCGTTATCCGGAATGATTGGGCGTAAAGCGTCCGCAGGTGGCACTGTAAGTCTGCTGTTAAAGAGCA
AGGCTCAACCTTGTAAAGGCAGTGGAAACTACAGAGCTAGAGTACGTTCGGGGCAGAGGGAATTCCTGG
TGTAGCGGTGAAATGCGTAGAGATCAGGAAGAACACCGGTGGCGAAAGCGCTCTGCTAGGCCGTAACTG
ACACTGAGGGACGAAAGCTAGGGGAGCGAATGGGATTAGATACCCCAGTAGTCCTAGCCGTAAACGATG
GATACTAGGCGTGGCTTGTATCGACCCGAGCCGTGCCGGAGCCAACGCGTTAAGTATCCCGCCTGGGGAG
TACGCACGCAAGTGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAA
TTCGATGCAACGCGAAGAACCTTACCAAGACTTGACATGTCGCGAATCTTCTTGAAAGGGAAGAGTGCCT
TAGGGAGCGCGAACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTCGTTTTTAGTTGCCAGCATTAAGTTGGGCACTCTAGAGAGACTGCCGGTGACAA
ACCGGAGGAAGGTGGGGATGACGTCAAGTCAGCATGCCCCTTACGTCTTGGGCTACACACGTACTACAAT
GCTACGGACAGAGGGCAGCAAGCTAGTGATAG

Fig. 4. Gentic bioformations background for the identification of *Anabaena oryzae.*

CONCLUSION

Generally, on the basis of the obtained results it can be concluded that cyanobacteria were found in soil polluted with insecticides. The detected cyanobacterial genera in such soil were *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp. and *Chroococcus* sp. *Nostoc* genus was found to be the most dominant genus among the detected genera in the insecticides polluted soil. Moreover, in liquid cultures of cyanobacteria the biomass dry weight and the fixed nitrogen increased with increasing the incubation period.

REFERENCES

Abdel-Razek, M. A.; Abozeid, A.M.; Eltholth, M.; Abouelenien, F.A.; El-Midany, S.A.; Moustafa, N.Y. and Mohamed, R.A. (2019). Bioremediation of a pesticide and selected heavy metals in wastewater from various sources using a consortium of microalgae and cyanobacteria. Slov Vet., 56: 61-73.

Abou Elatta, A.E.A. (2018). Microbiological Studies on Cyanobacteria in Rice Plant. M.Sc. Thesis, Fac.of Agric. Mansoura Univ., Egypt.

Abou Elatta, A.E.A.; El-Zawawy, H.A.H.; Afify, Aida H. and Hauka, F.I.A. (2023). Degradation of chlorpyrifos by the cyanobacteria strains in rice fields. J. Agric. Chem. and Biotech., Mansoura Univ., Vol. 14(5): 43-49.

Afify, Aida H. and Ashour A.Z.A. (2018). Use of cyanobacteria for controlling flax seedling blight. J. Agric. Chem. and Biotechn., Mansoura Univ. Vol. 9(11): 259-261.

Bergey's Manual of Systematic Bacteriology 2nd (2001). Rippka R., R.W. Castentholtz, I. Iteman and M. Herdman (2001). Form-genus I. Anabaena Bory. In: Boone, D.R. and W.R. Castenholz, (Eds). Springer, Berlin, pp. 566-568.

Bicker, W.; Lammerhofer, M.; Genser, D.; Kiss, H. and Lindner, W. (2005). A case study of acute human chlorpyrifos poisoning: novel aspects on metabolism and toxicokinetics derived from liquid chromatography–tandem mass spectrometry analysis of urine samples. Toxicol. Lett., 159: 235–51.

Black, C.A.; Evans, D.D. and White, J.L. (1965). Methods of soil analysis: chemical and microbiological properties. ASA.

Chen, Z.; Juneau, P. and Qiu, B. (2007). Effect of three pesticides on the growth, photosynthesis and photo inhibition of the edible Environ. Sci. Pollut. Res. (2011) 18:1351–1359 1357. cyanobacterium Ge-Xian-Mi (Nostoc) Aquat Toxicol 81:256– 265.

Desikachary, T. V. (1959). "Cyanophyta". New Delhi: Indian Council of Agricultural Research. 686 pp.

El- Ayouty, E.Y. and Ayyad, M.A. (1972). "Studies on blue-green algae of the Nile Delta 1-Description of some species in a wheat field". Egypt. J. Bot., 15: 283-321.

El-Gamal, A.D.; Ghanem, N.A.E.; El-Ayouty, E.Y. and Shehata, E.F. (2008). Studies on soil algal flora in Kafr El- Sheikh Governorate, Egypt. Egyptian J. Phycol., 9: 1-23.

El-Saadny, A.Y. (2013). A study on therelations between different nitrogen fixing consortia and their effects on growth and yied of rice. Ph.D. Thesis, Fac. of Agric. Mansoura Univ., Egypt.

El-Zawawy, H. A. H. (2016). Microbiological and Ecological Studies on The Activity of Cyanobacteria in Different Types of Soil. Ph.D. Thesis, Fac.of Agric. Azhar Univ. Egypt.

Gavrilescu, M. (2005). Fate of pesticides in the environment and its bioremediation. Eng. Life Sci.5: 497-526.

Jackson, M.L. (1973). "Soil chemical Analysis, Constable and CO_2". Agric. Exp. Mad. Wisconsin. P: 183-187.

Jha, M.N. and Mishra, S.K. (2005). Biological responses of cyanobacteria to insecticides and their insecticide degrading potential. Bull. Environ. Contam. Toxicol. 75: 374–381.

Komarek, J.; Kastovsky, J.; Mares, J. and Johansen, J.R. (2014). Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) using a polyphasicapproach. Preslia 86: 295-335.

Lakshmi, C.V.; Kumar, M. and Khanna, S. (2008). Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int. Biodeterior. Biodegrad., 62: 204–209.

Manual, Oxoid (1990): Culture media ingredients and other laboratory services 6th ed. Published by Unipath Limited, Wade Road. Basingstoke Hampshire, R. G. 24 OPW. England

Mohapatra, P.K.; Patra, S.; Samantaray, R.K. and Mohanty, R.C. (2003). Effect of the pyrethroid insecticide cypermethrin on photosynthetic pigments of the cyanobacterium *Anabaena doliolum* Bhar. Pol. J. Environ. Stud. 2: 207–212.

Nawaz, K.; Hussain, Choudary, N.; Majeed, A.; Ilyas, U.; Ghani, A.; Lin, F.; Ali, K.; Afghan, S. and Raza, M.I. (2011). Eco-friendly role of biodegradation against agricultural pesticides hazards. Afr. J. Microbial. Res., 5: 177–183.

Naz, S.; Ul-Hasan, M. and Shameel, M. (2004). Taxonomic study of *Anabaena* Bory (Nostocophyceae,Cyanophyta) from northern areas of Pakistan. Pak. J. Bot., 36 (2): 283-295.

Parte, S.G.; Mohekar, A.D. and Kharat, A.S. (2017). Microbial degradation of pesticide: a review. Afr. J. Microbiol. Res. 11: 992–1012.

Pinevich, A. V. (2008). Paradoxes of Biodiversity, Phylogeny, and Taxonomy of cyanobacteria. Moscow University Biological Sciences Bulletin, 63 (1): 21–24.

Piper, C. S. (1950). "Soil and Plant Analysis". Inter. Sci. Publisher, Inc. New York, USA.

Prasad, S.M.; Kumar, D. and Zeeshan, M. (2005). Growth, photosynthesis, active oxygen species and antioxidant responses of paddy field cyanobacterium Plectonema boryanum to endosulfan stress. J. Gen. Appl. Microbiol. 51: 115–123.

Rani, K. and Dhania, G. (2014). Bioremediation and biodegradation of pesticide from contaminated soil and water - a noval approach. Int. J. Curr. Microbiol. Appl. Sci. 3: 23–33.

Roger, P.A. and Ardales, S. (1991). "Blue-Green algae collection". Internat. Rice Res. Institute, IRRI, Pub. Manila, Philippines.

Sammauria, R.; Kumawat, S.; Kumawat, P.; Singh, J. and Jatwa, T. K. (2020). Microbial inoculants: potential tool for sustainability of agricultural production systems. Archiv. of Microbiol., 202(4): 677-693.

Seckbach, J. (2007). Algae and cyanobacteria in extreme environments, in Cellular Origin, Life in Extreme Habitats and Astrobiology, Seckbach, J., Ed., New York: Springer Sci., Business Media, Vol. 11: pp. 1–811.

Shariatmadari, Z. and Riahi, H. (2010). New records of heterocystous cyanophyta from paddy fields of Iran. Boil. Fert. Soil., 8:229-234.

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

Staub, R. (1961). "Ernahrungsphysiologisch aurakologische untersuchengen an der planktischen blualg *Oscillatoria rubescence* D. C". Scheweiz. Zeitschr Hydrobiologie, 23: 82-198.

Tariq, M.; Afzal, S.; Hussian, L. and Sultana, N. (2007). Pesticides exposure in Pakistan Rev., Environ. fnt. 33: 1107-1122.

Venkataraman, G.S. (1981). "The cultivation of algae". Indian Council Agric. Res., New Delhi, India.

Verma, J.P.; Jaiswal, D.K. and Sagar, R. (2014). Pesticide relevance and their microbial degradation: a-state-of-art. Rev. Environ. Sci. Biotechnol. 13: 429–466.

Watanabe, A.; Nishigaki, S. and Konishi, C. (1951). "Effect of nitrogen-fixing blue-green algae on the growth of rice plants". Nature, 168: 748-749.

Zaki, Randa M.; Mehesen, Ahlam A.M.; Ashour, Eman H. and Afify, Aida H. (2021). Characterization of soil-indigenous cyanobacterial strains and bioactivity assessment. J.Agric. Chem. and Biotechn., Mansoura Univ. Vol.12(11): 195-199.

Zulpa, G.; Zaccaro, M. C.; Boccazzi, F.; Parada, J. L. and Storni, M. (2003). Bioactivity of intra and extracellular substances from cyanobacteria and lactic acid bacteria on —wood blue stain fungi. Biological control, 27(3): 345-348.

خصائص سلالات السيانوبكتيريا المعزوله من عينات تربه ملوثه بمبيدات الآفات الحشريه

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الملخص

تهدف هذه الدراسه إلى عزل السيانوبكتيريا من عينات تربه ملوثه بمبيدات حشريه من أراضى مزروعه بالأرز فى محافظة كفر الشيخ وقد تم الحصول على إثنى عشرة عزله نقيه . تم تنقية عزلات السيانوبكتيريا بالطرق المختلفه وتم تعريف عزلات السيانوبكتيريا طبقا لطرق التعريف القياسيه المزرعيه (اللون) وكذلك المورفولوجية (شكل ولون الثالوس وحجم الهيتروسست بالإضافه إلى الخلايا الخضريه و التكاثريه) وإتضح عند تنمية أجناس السيانوبكتيريا أن كل من أنابينا ونوستوك وأوسيلاتوريا وكذلك كرووكوكس لها القدره على تكوين الهيتروسست . وقد سجل جنس النوستوك أعلى نسبة فى الإنتشار بينما سجل جنسى الأوسيلاتوريا والكرووكوكس أقل إنتشار وذلك عند العزل من الأراضى الملوثه بهذه المبيدات الحشريه . وقد أثبتت جميع العزلات كفاءة فى الوزن الجاف وكمية النتروجين المثبت وكان أفضلهما سلالتى النوستوك والأنابينا ومن خلال التعريف الجزيئي ثبت أنهما متماثلين بنسبة ٩٩و٩ % لكل من نوستوك مسكورم و أنابينا اوريزا . وبهذه النتائج يمكن أن نوصى بإستخدام تلك السلالات من السيانوبكتيريا فى التسميد الحيوى وخصوصا فى الأراضى الملوثه بمبيدات الآفات .