

Influence of Antifungal Compound Synthesis by *Anabaena* against Sheathblight and Blast of Rice

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Cyanobacteria are one of the most diverse groups of prokaryotae which are distributed around the world. They are producing a variety of secondary metabolites which are composed of various biological effects. In this study, the cyanobacteria *Anabaena* was separated and purified from Sorkhankol area in the central part of Anzali wetland. Diagnoses were done with valid keys and 16srRNA molecular analysis. Methanol extracts and acetone extracts of *Anabaena* have been examined against *Pyricularia oryzae* and *Rhizoctonia solani* fungus (the factor of Blast and Sheath blight of rice diseases). Antifungal properties of extracts on *Pyricularia oryzae* and *Rhizoctonia solani* have been determined through Park Method. The results indicated that the diameter of inhibition zone for methanol extracts of *Pyricularia oryzae* and *Rhizoctonia solani* were 9 mm and 16 mm respectively, the diameter of inhibition zone for acetone extract of both *Pyricularia oryzae* and *Rhizoctonia solani* were 8 mm. Furthermore, minimum inhibitory concentration of growth (MIC) for methanol extract were 32 µg/ml and 16 µg/ml for *Rhizoctonia solani* and *Pyricularia oryzae* minimum inhibitory concentration of growth (MIC) for acetone extract were 32 µg/ml for both *Rhizoctonia solani* and *Pyricularia oryzae*. phytochemical tests for methanol extracts were conducted in this study.

Keywords: Sorkhankol, cyanobacteria, *Pyricularia oryzae*, *Rhizoctonia solani*.

Cyanobacteria are the most abundant organisms in aquatic ecosystems. These microorganisms are the oldest prokaryotic living creatures on the planet which play an important role in human lives. This alga is a rich source of biologically active metabolites. Recent studies regarding bioactive compounds in blue-green algae in aquatic ecosystems have shown that many compounds have antibacterial, antifungal, antiviral, anticancer, and immunosuppressive properties¹. The bioactive compounds are isolated from cyanobacteria in order to discover new compounds for biological, agricultural and pharmaceutical applications². Given the increasing number of bacteria, fungi, viruses and antibiotic

resistance, the *cyanobacteria* should be considered as a great promise for new drugs, new structures and metabolites with biological activity³. Primary or secondary metabolites produced by these microorganisms demonstrate the potential of bioactive compounds in the industry⁴. In total, only 10 percent of isolated cyanobacteria could be cultivated in the lab and just a small number of these species enjoy commercial uses⁵.

Rice is the main food for about half of the world's population; it is the second greatest consumed nutrition in Iran after the wheat. Rice acreage in Iran is 4.5 tons per hectare. According to the importance of rice in Iran, its diseases also bear considerable significance. One of the most important diseases regarding rice is Blast disease which can reduce the rate of production by 30%. Rice production has caused the largest pesticide business in the world. The share of fungicides in 1988 was 570 Million USD out of total 2.4 billion

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USD which about 92% of that has been used for the control of rice blast disease⁶.

The disease is known as leaf blast, cluster blast and cluster neck. The factor of this disease is a fungus called *Pyricularia oryzae*. Teleomorph of this fungus, that is *Magnoportha grisea*, has not been found in nature. It is the *Ascomycetes* of the *Physosporelleaceae* which produces transparent, spindle-shaped *Ascospores* with a transverse wall in one-wall *Ascs*. It is a Heterotalic fungus which controls the bipolar intercourse while there are additional genes which control the sexual cycle⁷. One another rice disease is the rice sheath blight that is caused by *Rhizoctonia solani* AG1-IA. It originates from *Agonomycetales* which includes sterile hyphae that cannot produce asexual spores. Mycelium of the growth of fungi is colorless when it is young and becomes yellowish-brown when it grows ripe. Hyphae have cross-grid and multi-core wall, and generally have a diameter of 8-12 micrometer; younger hyphae are divided into acute angles (about 45 degrees) and the older hyphae will be divided into perpendicular angles. Hyphae divisions are compressed at the beginning point and a cross wall could be seen near them⁸.

Rice sheath blight disease is caused by *Rhizoctonia solani* and rice blast disease is resulted from *Pyricularia oryzae* which are the most important limiting factor for the development of the rice crop in most rice-rich countries of the world, including Iran. The above pathogenic fungi can infect potatoes, cotton, beans, lentils, beets, alfalfa as well as eggplant and tomatoes in addition to the rice⁹.

Due to the failure of chemical methods to combat against these diseases, including environmental pollution and high costs, the use of biological methods is highly recommended. The bioactive extracts of cyanobacteria have not been used against diseases of rice sheath blight and rice blast so far in Iran; in fact there are no comprehensive researches done in this field. Cyanobacteria is considered as the best source of biological control which is easily cultivated and is more economical in comparison with anti-fungal and anti-bacterial chemicals compounds, furthermore it is more suitable regarding biological control tools. Based on the importance and necessity of cyanobacteria in biological combat

phenomena and biological control, this group of microorganisms can contribute to the agricultural industry, reducing and preventing the waste of the grains, such as rice. In addition to the isolation and optimization of secondary metabolites of cyanobacteria separated from the central region of Bandar Anzali Wetland, the aim of this study is to evaluate the antifungal activity of these substances and determine the extent of their activity.

MATERIALS AND METHODS

Sampling

Sampling was carried out in Sorkhankol Area, this area is situated in Bandar Anzali Wetland by the geographical coordinates of east longitude E492718- E492404 and north latitude N372620- N372304. Sampling has been done by the use of sterile containers from surface to a depth of 50 cm.

Isolation

In order to isolate sea cyanobacteria, 500 ml of water has been transmitted through a filter with 45.0 micron pores. Then filter-paper was transmitted to the flask containing 500 ml of BG11 cultivation medium (Merck Co, Germany) (BG11 Medium for blue-green algae ATCC medium 616, BG11 Medium)^{10,11}.

Samples were used at 25 ° C as the optimal growth temperature, light intensity of 2500 lux with 16 hours of light and 8 hours of darkness and a pH of 7.5. In order to ventilate the liquid cultivation medium, a shaker with 150 rpm and an air pump were used².

Purification

It is based on a chance or probability of the presence of a particular microorganism under very low concentrations:

Serial dilution

for this purpose, 9 ml of BG11 cultivation medium was added to 10 sterile tubes and then 1 ml of Erlen flask containing cultivated cyanobacteria was added in a tube (1); the content of the first tube was mixed until it was fully diluted up to 10-1. Then 1 ml of it was moved to the next tube in sterile conditions. The former step was repeated in order for the next tubes to obtain a dilution of 10-10.

Pour plate

At first 1.5% agar was added to the liquid

cultivation medium of BG11 in order to have a solid cultivation BG11 medium, and then Nystatin (100 microgram per ml) was added to the cultivation medium in order to eliminate fungal infections and then cycloheximide (100 microgram per ml) was also added to the cultivation medium in order to eliminate the bacterial contamination.

About 1 ml of each of the dilutions were poured into 10 sterile plates, and 15 ml of cultivation BG11 medium was added to the plates (before cooling); then the plates were shaken circularly, put at rest in order to be cooled at 30 ° C and exposed to yellow and white radiation ¹².

Identification of cyanobacteria

Microscopic examination

Morphological or apparent characteristics of cyanobacteria are used for the preliminary identification. The valid identification keys such as Desikhachary, Prescott and algae base website were used in this study ^{13,14}. The grown cyanobacteria were examined with a magnification of 100X (Fig.1).

Molecular Analysis of 16SrRNA

Total DNA was extracted according to modified Marmur method ¹⁵. PCR reaction was carried out using CYAN738F and CAYN1281R special primers for cyanobacteria.

Row	Primer Name	Sequence 5 to 3
1	CYAN738F	ATACCCWGTAGTCCTAGC
2	CYAN1281R	GCAATTACTAGCGATTCTCC

Sequencing details

Applied Biosystems 3730/3730xl DNA Analyzers Sequencing, Bioneer, Korea, Sanger method.

Sampling of pathogenic fungus of rice

In order to evaluate the rice sheath blight disease, the Rice Research Institute in Gilan province collected the infected leaf samples of rice and transferred them to the laboratory in the summer 2015. *Rhizoctonia solani* (ATCC16118) was used for the examination of rice sheath blight.

Isolation of pathogenic fungus of rice

The pieces by the length of 5 to 10 mm were cut from infected and healthy areas of the rice leaves; and after water rinsing they were sterilized with sodium hypochlorite (NaOCl 5%) for 3 minutes; then some small pieces were separated from infected pieces by sterile scalpel and added

to *potato dextrose agar* (Merck Co, Germany) then they were incubated at 25 °C⁹. Also, *Rhizoctonia solani* (ATCC16118) was used for evaluation of rice sheath blight disease.

Microscopic examination of herbal pathogenic fungi

The isolated funguses were colored by Lacto-phenel-cotonblue and were observed with magnification of X40 (Fig.2).

Extraction of the methanol extracts

For this purpose, at first the grown cyanobacteria were centrifuged in 4000 rpm and added to sediment of methanol solution and incubated at the room temperature and dark conditions for 24 hours; after incubation time, change of the color of solvents were observed and then the colored sediment was removed.

Antibiogram of methanol extract

A 0.5cm piece of rice-plant from the plates containing pathogenic fungi was transferred into center of a plate containing *potato dextrose agar* by the use of sterile scalpel and then Blank paper-discs with a diameter of 6 mm were mixed with 50 macro-liter of the methanol extract (These discs were sterilized in the autoclave for 20 minutes at 121°C and under a pressure of 15 atm) and then they were located in a distance of 1 cm from the plate-wall by the means of sterile forceps and were incubated at 25°C, finally the inhibition growth was evaluated after 5 days ¹⁶.

MIC and MFC of the methanol extract were determined through the fungal suspension ¹⁷.

Chemical Analysis of methanol extract alkaloids

0.5 g of the methanol extract of *Anabaena* cyanobacteria with 15 ml of sulfuric acid solution was solved and then, 0.5 g of sodium chloride was added and stirred after cooling, then it was cleared and the sediment over the strainer alongside with chloridric acid reached the volume of 10 ml, afterwards a few drops of Mayer's reagent were added- yellowish white sediment indicated the presence of alkaloids

Saponins

The purpose of the test was used frothing test, 2ml of methanol extract of *Anabaena* cyanobacteria was added distilled water and shaken it very well, the presence of frothing, indicate saponins.

Tannin

To 2ml of methanol extract of *Anabaena* cyanobacteria, 1% gelatin solution and sodium chloride was added, White sediment indicates tannins.

flavonoids

To the 2ml of methanol extract of *Anabaena* cyanobacteria, few drops of acetate solution were added, yellow colour indicates the flavonoids¹⁸.

Chemical composition (GC-MS analysis)

The Gas Chromatography Mass Spectrometry analysis was carried out. An electron ionization system with Helium gas (as a carrier gas) was used for GC-MS detection. The mass spectra were matched with the library data (NIST/AMIDS).

Extraction of chlorophyll

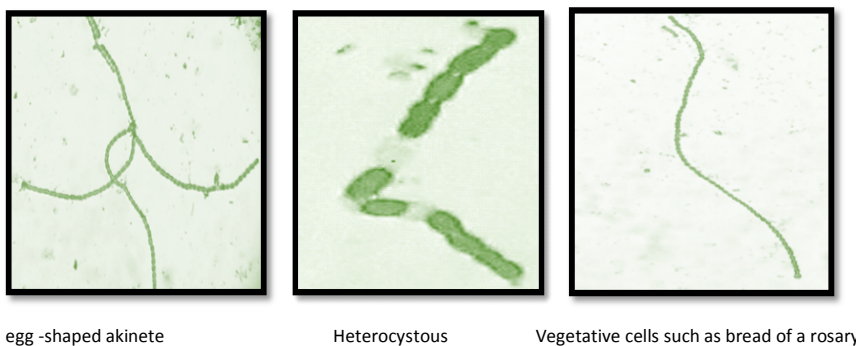
At first, cyanobacterial mass was separated by filtration and then the mass was placed

into the darkness with 2 to 3 ml of 90% acetone for 2 hours at 4 °C, the extracts were centrifuged for 15 minutes at 4000rpm, the supernatant was poured in sterile plates and dry weight was measured (the extracts were diluted by acetone for reusing)¹⁹.

Antibiogram of chlorophyll extract

A 0.5cm piece of rice-plant from the plates containing pathogenic fungi was transferred into center of a plate containing *potato dextrose agar* by the use of sterile scalpel and then Blank paper-discs with a diameter of 6 mm were mixed with 50 macro-liter of the methanol extract (These discs were sterilized in the autoclave for 20 minutes at 121°C and under a pressure of 15 atm) and then they were located in a distance of 1 cm from the plate-wall by the means of sterile forceps and were incubated at 25°C, finally the inhibition growth was evaluated after 5 days¹⁶.

MIC and MFC of the methanol extract were determined through the fungal suspension¹⁷.

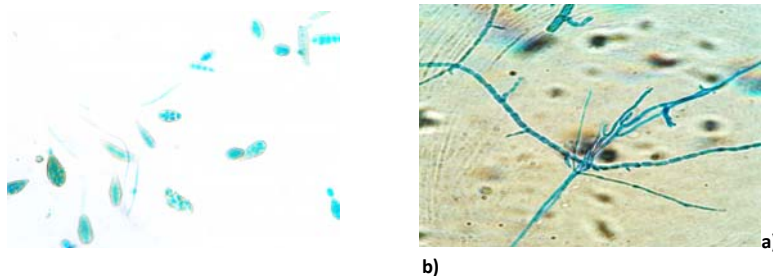
Identification of cyanobacteria

egg-shaped akinete

Heterocystous

Vegetative cells such as bread of a rosary

Fig.1. *Anabaena* Cyanobacteria microscopic features: Vegetative cells are chained such as beads, egg-shaped breads and Heterocystous are placed through the layers of growing cells; trichomes are placed spirally and free of gelatin covers

Identification of rice Fungi (*Pyricularia oryzae* and *Rhizoctonia solani*)

Microscopic image :a (*Pyricularia oryzae* and b (*Rhizoctonia solani*)

Fig. 2. Microscopic characteristics of *Pyricularia oryzae* (blast factor): it produces transparent, streamline-shaped Ascospores with a transverse wall in one-wall Ascs

RESULTS

b) Sequencing of 16SrRNA showed the strain with 534 nucleotide has been 100% phylogenetic relationship with *Anabaena variabilis ATCC29413*^{20,21,22}.

Microscopic characteristics of *Rhizoctonia solani* (sheath blight factor)

it includes hyphae with a cross-grid and multi-core wall, and generally a diameter 12-8 micrometer; young hyphae have acute angles and the older ones are divided in perpendicular angles. Thalloid divisions are compressed at the beginning points, and a transverse wall could be seen in the vicinity of them.

The diameter of inhibition zone (mm)

exhibited against testing fungi by methanol and acetone extracts

MFC level

The amount of MFC is equal to the amount of MIC

Table.1: Results of Phytochemical analysis on *Anabaena* separated from the Sorkhankol

DISCUSSION

Due to the inefficiency of fungicides to control diseases of rice sheath blight and rice blast as well as environmental pollution caused by them in Iran and also the possibility of the emergence of resistant strains of pathogens, the use of other biological controlling methods are of special

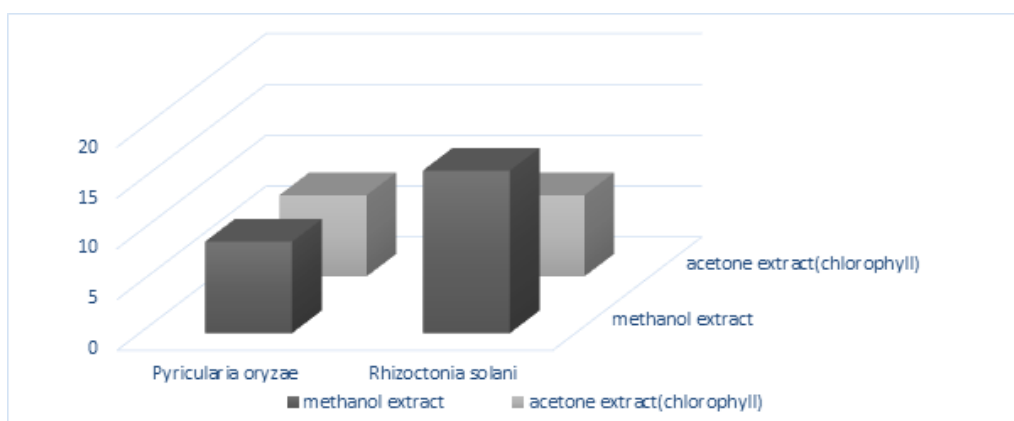


Fig. 3. The diameter of inhibition zone for methanol extracts of *Pyricularia oryzae* and *Rhizoctonia solani*, were 9 mm and 16 mm and the diameter of inhibition zone for both acetone extracts of *Pyricularia oryzae* and *Rhizoctonia solani* were 8 mm

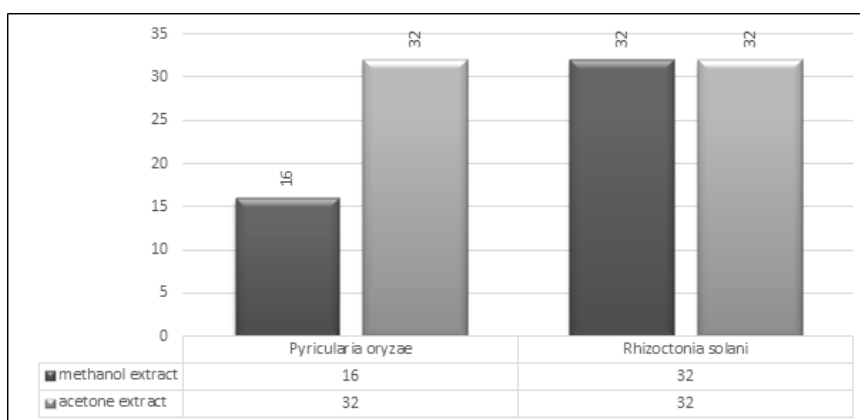


Fig. 4. MIC of methanole extracts for *Rhizoctonia solani* and *Pyricularia oryzae* were 32 µg/ml and 16 µg /ml respectively, and MIC of acetone extracts for both *Rhizoctonia solani* and *Pyricularia oryzae* were 32 µg /ml

importance. Unreasonable use of pesticides increases the cost of production, environmental degradation, loss of natural enemies, secondary pest outbreaks and illegal pesticide residue on agricultural products which as a consequence

jeopardizes the health of the consumers. Cyanobacteria have not been fully studied in terms of antifungal activities but a few studies have been conducted on this issue so far. Given the abundance of cyanobacteria on micro-flours of

Table 1. Results of Phytochemical analysis in *Anabaena variabilis* ATCC29413 isolated from the Sorkhankol

Results	Observations	Effective compound in extract
+	Yellowish – white sediment	Alkaloid
+	Stable lather	Saponin
-	Pink color	Flavonoid
-	Blue or green color	Tannin

Gilan wetlands, *Anabaena* cyanobacteria have been separated from Gilan native wetlands in this research.

Examination of 150 samples of

Table 2. Dry weight of acetone and methanol extract

Dry weight of acetone extract	550 mg/ml
Dry weight of methanol extract	300 mg/ml

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MS Data Review Active Chromatogram Plot - 5/4/2015 2:00 AM

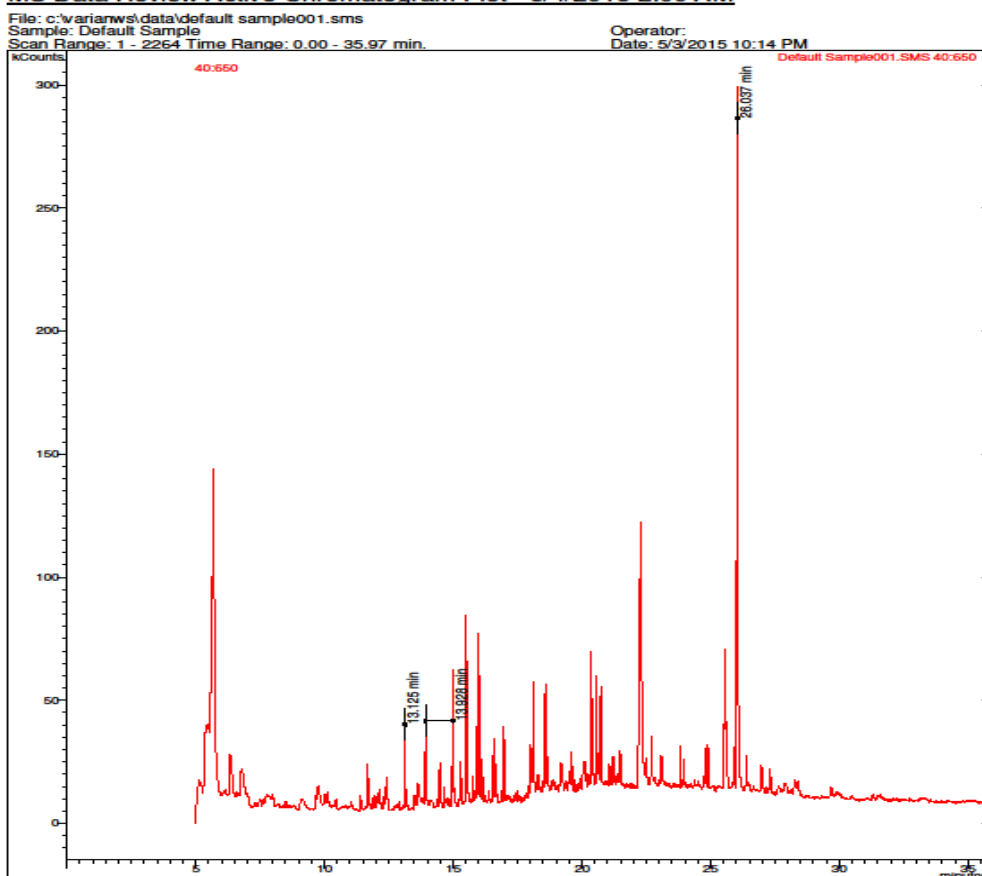


Fig. 5. GC MS Chromatogram of the methanol extract of *Anabena variabilis* ATCC29413

cyanobacteria separated from the Northern provinces by Ghasemi *et al.* showed that 13 samples of cyanobacteria bore antifungal activity. In this study, the antifungal activity of *Anabaena* cyanobacteria was significant¹.

Lampe *et al.* reported that lipids and fatty acids of cyanobacteria would cause damage to the cell membranes and inhibit the growth of some fungal species²³. Katirciooglu *et al.* figured out that antimicrobial properties of cyanobacteria (antibacterial properties, antifungal properties) were related to the ring peptides, alkaloids as well as lipopoly saccharide. Studies suggest that alkaloids and saponins were diagnosed as active and effective ingredients²⁴. Ebana *et al.* reported that alkaloids, as active ingredient in *Anabaena* cyanobacteria, had inhibition effects on pathogen bacteria, and inhibited their growth²⁵. The results of this study showed that the *Anabaena* cyanobacteria has been composed of phytochemical composition with alkaloids and saponins as well as chlorophyll which inhibits the growth of fungi of *Pyricularia oryzae* and *Rhizoctonia solani*.

Ozdemir *et al.* stated diethyl ether and acetone extracts of cyanobacteria of *Spirulina platensis* influenced the herbal pathogenic fungi²⁶. Abedin and Taha showed that the ethanol extract of *Anabaena oryzae* had the highest activity against herbal pathogenic fungi²⁷. Madhumathi and Partners found that the acetone extract of *Phormidium corium*, methanol extract of *Lyngbya martensiana* and ether diethyl extract of *Microcystis aeruginosa* bore the maximum diameter of inhibition zone against herbal pathogenic fungi²⁸.

Kollimalai *et al.* reported that *Rhizoctonia solani* are sensitive against methanol and chloroform extracts of cyanobacteria as the *Synechocystis salina*, the *Spirulina subsalsa*, the *Oscillatoria cortiana*, the *Oscillatoria salina*, the *Oscillatoria willei*, the *Phormidium fragile* and *Phormidium tenue*²⁹. Shrivastava showed that the extract of cyanobacteria as *Phormidium fragile* and *Oscillatoria boryana* have had effects on the growth of herbal pathogenic fungi such as *Rhizoctonia solani*, and have prevented their growing³⁰. The results of this study represented that the methanol extract and the acetone extract of *Anabaena* cyanobacteria have had inhibitory

effect on the growth of *Rhizoctonia solani* and *Pyriculariaoryzae*. The minimum inhibitory concentration of growth (MIC) of the methanole extracts for *Rhizoctonia solani* and *Pyricularia oryzae* were 32 µg/ml and 16 µg /ml respectively, and the minimum inhibitory concentration of growth (MIC) of acetone extracts for both *Rhizoctonia solani* and *Pyricularia oryzae* were 32 µg/ml.

The diameters for inhibition zone of growth regarding methanol extracts for *Rhizoctonia solani* and *Pyricularia oryzae* were 9 mm and 16 mm respectively. The diameters for inhibition zone of growth regarding acetone extracts for both *Rhizoctonia solani* and *Pyricularia oryzae* were 8 mm. The results also showed that the *Anabaena* cyanobacteria separated from Sorkhankol Area has been composed of phytochemical compounds possessing traits of alkaloids and saponins that could prevent the growth of pathogens such as *Pyriculariaoryzae* and *Rhizoctonia solani*. According to the results, it seems that the blast and Sheath blight diseases could be controlled by using cyanobacteria as a means of biological control while the risks of using chemical poisons could be reduced.

REFERENCES

1. Ghasemi, Y., Tabatabaei., Yazdi, M., Shokravi, S., Soltani, N., Zarrini, G. Antifungal and antibacterial activity of Paddy-filds Cyanobacteria from the north of Iran. *Journal of Sciences, Islamic Republic of Iran.*, 2003; **14**: 203-209.
2. Hanan, M., El-Kassas, Y. Active substance from some bluegreen algal species used as antimicrobial agents. *African Journal of Biotechnology.*, 2010; **9**(19): 2789-2800.
3. Singh, S., Bhushan, K., Banerjee, U. Bioactive Compounds from Cyanobacteria and Microalgae. An Overview. *Critical Reviews in Biotechnology.*, 2005; **25** (3): 73-95.
4. Tuney, I., Cadirci, B.H., Nal, D., Sukatar, A. Antimicrobial activiters of the extracts of marine algae from the coast of Urla . *Turk. J.Biology.*, 2006; **30**: 171-175.
5. Hajimahmoodi, M., faramarzi, M., Mohammadi, N., Soltani, N., Oveisi, M., Nafissi-Varche, N. Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. *Journal of Applied Phycology.*, 2009; **22**: 43-50.

6. Padasht, F. chemical control of rice blast disease and pathogen resistance against Antifungal.Rice. Research Institute., 1379.
7. Izadpanah, k., Ashkan, M., BaniHashemi, Z., Rahmaniyan, H., Minasian, V. *Plant Pathology.*, 1389; (2):P677.
8. Jones, R.K., Belmar, S.B. Characterization and Pathogenicity of Rhizoctonia spp. Isolated from rice, soybean and other crops grown in rotation with rice in Texas. *Plant Dis.*, 1989; **73**:1004-1010.
9. Torabi, M., Binesh, H. Rice sheath blight disease transmission and susceptibility of some varieties. *Plant diseases.*, 1364; Cover. **21**: 15-25.
10. Stanier, R. Y., Kunisawa, R., Mandel, M., Cohen –Blazire, G. Purification properties of unicellular blue – green algae (order Chlorococcales) *Bacteriol Rev.*, 1971; **35**(2):171-205.
11. Summers, M.L., Wallis, J.G., Campbell, E. L., Meeks, J. C. Genetic evidence of a major role for glucose-6-phosphate dehydrogenase in nitrogen fixation and dark growth of the cyanobacterium Nostoc sp. Strain ATCC 29133. *J Bacteriol.*, 1995; **177**(21):6184-6194.
12. Andersen, R.A. Algal culturing techniques. Elsevier Academic Press., 2005; 589 p.
13. Desikachary, T.V. Cyanophyta. Indian Council of Agricultural Research, New Delhi., 1959; 684 pp.
14. Prescott, G.W. Algae of the Western Great Lake Areas. William C. Brown Company Publisher, Dubuque. Iowa., 1962 ;Jun:977-985.
15. Marmur, J. A procedure for the isolation of deoxyribonucleic acid from microorganisms, *J. Mol. Biol.*, 1961; **3**: 208–218.
16. Park, c.s. Identification of some bacteria from paddy antagonistic to several rice fungal pathogens. *J Phytopathology.*, 1989; **138**(3):189-208 pp.
17. Wayne, P. Clinical Laboratory Standard Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved standard., 2008; M27-A3. Clinical Laboratory Standard Institute.
18. Tiwari, P., Kumar, B., Kaur, M, Kaur, G., Kaur, H. Phytochemical Scening& Extraction. *International Pharmaceutica Scientia.*, 2011: **1**(1).
19. Huang, T.L., Cong, H.B. A New Method for Determination of Chlorophylls in Freshwater Algae [J]. *Environmental Monitoring and Assessment (SCI journals source).*, 2007; **129**: 1-7.
20. Altsch, S.F., Madden, T.L., Schaeffer, A.A., Zhang, Z., Miller, W., Lipman, D.J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acid .*, 1997; **25**: 3389-3402.
21. Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S., Chun, J. Introducing EZTaxon-e: a prokaryotic 16SrRNA Gene sequence database with phylotypes that represent uncultured Species. *Int J Syst Evol Microbiol.*, 2012; **62**: 716-721.
22. Myers, E.W., Miller, W. Optimal alignments in linear space. *Comput Appl Biosci.*, 1988; **4**: 11-17.
23. Lampe, M.F., Ballweber, L.M, Isaacs, C.E., Patton, D.L., Stamm, W.E. Killing of Chlamydia trachomatis by novel antimicrobial lipids adapted from compounds in human breast milk. *Antimicro. Agents Chemo.*, 1998; **45**: 1239-1244.
24. Katircioglu, H., Beyatli, Y., Aslim, B., Yüksesdag, Z., Atici, T. Screening for antimicrobial agent production in fresh water. *Internet J. Microbiol.*, 2006; **2**: 2.
25. Ebana, R., Madunagu, B., Ekpe, E., Otung, I. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria cymoides*, *Kola nitida* and *Citrus aurantifolius*. *J. Appl. Biotechnol.*, 1991; **71**: 398 – 401.
26. Ozdemir, G., Karabay, N., Dolay, M., Pazarbasi, B. Antibacterial activity of volatile extracts of *Spirulina plantensis*. *Phytother. Res.*, 2004; **18**: 754-757.
27. Abedin, R.M.A., Taha, H.M. Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman Design for antimicrobial activity of *Spirulina platensis*. *Global J. Biotech. Biochem.*, 2008; **3**: 22-31.
28. Madhumathi, V., Deepa, P., Jeyachandran, S., Manoharan, C., Vijayakumar, S. Antimicrobial activity of cyanobacteria isolated from freshwater lake. *Internat. J. Microbiol. Res.*, 2011; **2**: 213-216.
29. Kollimalai, S., Kandasamy, K. Antimicrobial activities of marine cyanobacteria isolated from mangrove environment of south east coast of India- *Journal of Natural Products.*, 2012; **5**: 147-156.
30. Shrivastava, D.K. Toxicity of cyanobacteria against pathogenic bacteria and fungi. *Indian J.Sci.*, 2014; **4**(1):01-07.