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Research article

Innovative Bio-based Products for Management of Biotic and Abiotic Stress in Wheat Plants

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Abstract

Wheat is the most important cereal crop, a staple food for more than one third of world population. Recently, there is increased interest in naturally produced active compounds as alternatives of fungicide and improve plant stress. Blue-green algae (cyanobacteria) secondary metabolites have a diverse antagonistic activity that lead to disintegration of microbial growth and improve plant resistant against stress. So, this study was conducted to evaluate the antifungal activities of *Oscillatoria agardhii* in retarding the growth of wheat pathogenic fungal species and improve resistance to biotic and abiotic stress under stress conditions in compare to the natural environment. Based on zone of inhibition formation and Minimal Inhibitory Concentrations (MIC), it was concluded that the extracts of *O.agardhii* had significant antifungal and antimicrobial efficacy. An experiment was conducted under natural conditions in a farmer's field in middle of Sinai which saline soil and compared in a natural farm with a normal soil to study the effective use of *O. agardhii*, resulted in a significantly greater decrease in the disease incidence of powdery mildew, leaf rust and leaf spots, increased of total soluble protein, proline, soluble carbohydrates, Chlorophyll, carotenoids NPK, K/N ratio, IAA, peroxidase and chitinase in wheat as well as plant growth and yield. The significant increase in grain yield of wheat was observed. Cyanobacteria can be excellent biocontrol sources of plant pathogenic fungi as they can be easily cultured, less expensive compared to synthetic fungicides and ecofriendly, rather they can also promote plant growth.

The investigation, certainly points out the necessity of exploring cyanobacterial strains as potentially outstanding sources of antifungal drugs, Biostimulants and anti-stress that improve plant resistance to abiotic stress.

Key words: Antagonistic properties, Antifungal, Antimicrobial, Biostimulants and anti-stress, Cyanobacteria, Wheat diseases, Stress

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1. Introduction

Arid and semi-arid regions represent about 40% of the world's land area. This also includes the declining acreage of arable land in many parts of the world affected by limited irrigation. Furthermore, altered precipitation pattern onset of climate change will increase the acreage of the dry land. Therefore, this research is essential to ensure world food security; however many agronomic crops lack the genetic ability to tolerate stress demanding the genetic improvement of agronomic crops.

Wheat is the most important grain crop, a staple food for more than one third of the world population. Knowing diseases that may cause injuries and are likely to affect plant health and quality is critical to minimizing the gap between attainable yield and actual yield. It is also one of the world's ancient cereal crops with archaeological remains suggesting that it was first domesticated in the Fertile Crescent around 10,000 years age at about the same time as wheat. Plant diseases are the primary hazards in wheat production. On wheat, the most prevalent diseases were Yellow or stripe rust (*Puccinia striiformis,* f.sp. *tritici*); Leaf or brown rust (*Puccinia recondita,* f.sp. *tritici*); Tan spot (Yellow leaf spot) (*Drecheslera tritici-repentis* (syn. *Helminthosporium tritici-repentis)* as in not final stage; *Pyrenophora tritici-repentis*as a complete stage); - Septoria leaf blotch (*Septoria triici*as in not complete stage); Septoria leaf and glume blotch (*Septoria nodorum*as in not complete stage; *Stagonospora nodorum*as a complete stage); and Powdery mildew (*Blumeria graminis*f. sp. *tritici*)[1,2,3].

Control of fungal pathogens is based on the use of agronomic practices and pesticides, but widespread application of chemicals inundates the agro-eco systems with toxic compounds that affect the balance of the natural food chain. The use of effective and low toxic antimicrobial and antifungal agents are required for the treatment and control of plant pathogens. Biocontrol technologies have gained momentum in disease control of crop plants in recent times as these technologies not only minimize or replace the use of harmful chemical pesticides but also found to be cheaper and efficient in certain disease control programmes.

Salinity not only decreases the agricultural production of most crops, but also, as a result of its effect on soil physicochemical properties, adversely affects the associated ecological balance of the area. Despite the large variety of elicitors, general schemes for cellular elicitor signaling leading to plant resistance can be drawn. The links between the signaling events allow amplification of the signal transduction and ensure specificity to get appropriate plant defence reactions.

The organic farming production system aims at promoting and enhancing agro-ecosystem health, biodiversity and soil biological activities [4]. Blue-green algae (cyanobacteria) possess a diverse structure and have a wide distribution throughout the globe. Cyanobacteria and eukaryotic algae occur in fresh water, marine and terrestrial soil habitats. A number of cyanobacteria and microalgae produce various biologically active compounds [5]. These include antibiotics which in laboratory tests inhibited

bacteria and fungi that incite diseases of plants antifungal activity [6,7,3]. These microorganisms have been reported to benefit plants by producing growth promoting regulators as abscisic acid, ethylene, jasmonic acid, auxin, cytokinin-like substances and cytokinin isopentenyl adenine [7], vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exo-polysaccharides, that improve plant growth and productivity [5,8,9].

In the present study, we focused on the evaluation of cyanobacteria- *O. agardhii* as the antifungal agent and its ability to control of fungi *P. tritici-repentis* and Septoria complex (*Septoria*s pp.) causing leaf spots of wheat as well as improve resistant to abiotic stress as salinity in new reclaimed region in Sinai and compared with the normal soil (Giza governorate).

2. Materials and Methods

Algae

Cyanobacteria- *O. agardhii* was isolated and characterized by Hoballah *et al* [10]. The isolated strain was then cultivated on BG- 11 media at a light intensity of 200 lux during the 12 hour long light period and temperature was maintained at 26^0C-28^0C. The cultures were kept on a shaker to aid proper aeration and agitation to facilitate growth of the cells. Cyanobacteria- *O. agardhii* extract was prepared from the lysed cells along with methanol solvent that helps in enhancing the activity of secondary metabolites to retard the fungal growth and according to the previous studied [3]. The extracts were prepared according Karticioglu [6]. Cyanobacterial harvested cells were taken in a frozen mortar and pestle. Cells were crushed with acid treated sand for 10-15 minutes in order to aid rupturing of cells. The crushed cells were then placed on ice. Followed by intermitted vortexing and freeze thawing. The freeze-thawed cells were then mixed with the solvent and centrifuged at 12,000 rpm for 30 minutes.

Test Organisms

1. **Gram Positive-** *Bacillus subtilis* (ATCC-6633), *Bacillus pumilus, NCTC 8214 and Staphylococcus aureus* (ATCC 6538).

2. **Gram Negative-** *Escherichia coli* (ATCC-7839) and *Pseudomonas aeruginosa* (ATCC 9027).

Pathogenic fungi

The fungal pathogens were isolated from wheat diseased plants grown in Bohera Governorate. They were identified and characterized on the basis of their morphological properties as *Pyrenophora tritici-repentis*, *Septoria* spp., *Aspergillus niger, and Candida albicans, IMRU* 3669 and were cultivated in Potato Dextrose agar media at 23^0C -25^0C.

Determination of Antagonistic Activity In Vitro Studies

A-Antagonistic study

Antibacterial and Antifungal activity of *O. agardhii* extract was evaluated by disc method using sterilized Whatman filter paper (9mm) and loaded with 10 µml of culture filtrate and allowed to dry and aseptically put on the surface of specific media previously seeded with test organisms then refrigerated for 1-2 hours, for diffusion. Inhibition zones (mm) were checked after incubation period of 48 hours at 30°C for bacteria and 3 days at 72°C for fungi.

B-Minimal Inhibitory Concentrations (MIC)

Oscillatoria agardhii extract added to Sabouraud Dextrose medium to produce the concentrations, i.e., 50,100, 150, 250, 500, 750 and 1000µg/ml, and added distilled water to control dishes. Plats inoculated with 0.1 ml of spore or cell suspension and incubated at (25-28)°C, for three weeks11.

In Vivo Studies

The field trials were performed under field conditions in Sinai and compared with the normal soil (Giza governorate), using bread wheat (*Triticumae stivum*) cv. Gimza 10 and Shaha 93' varieties in 2014 and 2014 seasons. The experimental design was Randomized Complete Block Design with 10 replicate blocks and 1-meter row experimental unit. Ten rows of wheat plants sown with a density of 320 seeds per square meter were grown in each plot. All plots were fertilized immediately after sowing with ammonium nitrate at the rate of 100 kg·N·ha−1.

Soil Analysis

The sample of disturbed soil was taken by a drill and made into composite to have analyses of soil's chemical and physical characteristics. Samples of disturbed soil were air-dried, then sifted with 2 mm sifter for analyses of soil texture.. Samples of dry soil were sifted by 0.5 mm diameter of the sieve hole to analyze chemical characteristics of the soil. The whole soil sample was soaked in water with 2-cm depth to saturate soil's pores for the preparation of soil permeability analysis. Physical

Wheat seeds were surface-sterilized and soaked in cyanobacteria- *O. agardhii* extract for 2 hours. After two months , 10 individual-plant-samples from each experimental unit were carefully harvested an adhering soil removed by washing. Fungal infection of roots was evaluated and fresh weight and dry weight (70°C for 72 hrs) were determined. Liquid formulations were especially sprayed onto the wheat leaves at 30 and 60 days after sowing. Each treatment consisted of three parts and the experiments were conducted three times. In this study, 10^7 cfu mL^{-1} of bacterial cell suspension and 10^5 cfu mL^{-1} of bacterial cell suspension were used. The severity of leaf diseases spots was assessed as the percentage area of leaves infected during growth periods. Disease incidence, i.e. percent of disease-affected leaves (P) was calculated according to the following formula**:**

$$P = \frac{n}{N} 100$$

Where n – number of affected leaves,
N – number of assessed leaves.

Determination of Yield and Its Components

All the plants of different treatments were harvested in the same physiological growth state. Data on wheat total dry biomass, grain and yields, and weight of 100 kernels were recorded. Spikes were oven-dried at 70°C for 72 hrs and their dry weights determined. Tiller and spike numbers per plant were recorded from 5 randomly chosen plants. Spike weight per plant was recorded.

Physiological Analysis

Experiments were performed by plants grown in magenta boxes at 22°C –28°C (depending on the plant species) in a temperature-controlled room with a 12-hrs fluorescent light regime.

Salt: plants were exposed to 300–500mM NaCl in 1ml Hoagland's solution supplemented with 5mM CaCl2 (referred to as 300 or 500mM NaCl solution for 10–14 days by filling the lower chamber of the double decker magenta boxes with 200 ml of one of these salt solutions. After plants started showing symptoms (that is non symbiotic plants dead or severely wilted), they were re-hydrated in sterile water devoid of NaCl for 24–48 hrs, plant health assessed and photographed. All assays were repeated a minimum of three times.

Chemical Analysis

Ten days after inoculation, three leaves per plant were separately collected, frozen for 36 hrs, dried and powdered. Generally, 100 mg dried sample were used for analysis.

Chlorophyll and Protein Content

Soluble protein extraction was carried out according to Bollag and Edelstein [12] and separated according to Laemmli [13]. Leaf Sampling and Chlorophyll determination after the fluorescence recordings, the six leaf samples of each plot were immediately frozen, free-dried, grounded and stored in the dark at room temperature for the determination of their chlorophyll content. The total chlorophyll content of each sample was extracted from 50 mg lyophilized material by 5 ml methanol, which was then filled up to 25 ml. After extraction, the absorbance of the extracts was measured with a UV-VIS spectrophotometer (Perkin-Elmer, Lambda 5, Waltham, MA, USA) and the leaf chlorophyll concentration (LCC) was finally determined.

Determination of total NPK, Photosynthetic Pigments and Solute Accumulation

Plants were harvested at 120 d after planting. Each plant was decapitated and the shoot systems were then weighed. Total NPK, Na, K/Na ration were determined according to Page *et al.* [14]. The variations in their solute accumulations (proline and sugars) and photosynthetic pigment contents (chlorophyll *a* and *b*) were measured. Total water soluble carbohydrates were estimated as described by Thimmaiah [15]. Proline was determined by the method of Bates *et al*. [16] and expressed as µmol g−1 fresh weight (FW) of leaf. The amount of total soluble sugars was estimated in fresh leaf material using the method of Thimmaiah [15]. Chl *a* and *b* concentrations were measured on fresh fully expanded leaves. Fresh tissue (1.0 g) was extracted with 90% acetone, and read using a UV/visible spectrophotometer at 663, 645 and 750 nm wavelengths. Absorbance at 750 nm was subtracted from the absorbance at the other two wavelengths, to correct for any turbidity in the extract, before Chl *a* and *b* concentrations were calculated using the formulae below Strain and Svec [17]:

$$\text{Chl } a(mg\ mL^{-1} = 11.6 \times (A663) - 2.16 \times (A645)$$

$$\text{Chl } b(mg\ mL^{-1} = 20.97 \times (A645) - 2.16 \times (A663)$$

Determination of Phenol Content and Stress Hormones

Free and conjugated phenols were determined in treated leaves, 15 days after plant spraying with chemical elicitors according to A.O.A.C. [18] using the Folin–Danis reagent. Phenols were identified by HPLC using a reverse phase C8 column and compared with a catechol standard (Sigma Chemicals).

Stress hormonal was tested on plants using the method of Gordon and Weber [19] for the estimation of indole acetic

acid (IAA). Absorbance was read at 530 nm and the amount of IAA produced was expressed µg/mg fresh wet.

Total protein was extracted from wheat leaves and the supernatant prepared according to Tuzun *et al*. [20]. Peroxidase activity was measured according to the methods described by Allam [21]. The chitinase activity was determined by the colorimetric method of Boller and Mauch [22].

Statistical Analysis

Disease assessment results were analyzed using an ANOVA of square- root-transformed data. Data were transformed to acquire the normal distribution necessary for statistical analysis to be carried out. Significant differences were assessed by comparison of sample mean differences with the LSD value.

3. Results

Data reported in (Table1) showed the antimicrobial and antifungal screening of cyanobacteria- *O. agardhii* extract against bacteria as well as pathogenic fungi. *O. agardhii* extract showed inhibitory activity against the tested microorganisms indicator by production of a clear zone around the discs. The highest activity was shown against *Bacillus subtilis* (ATCC-6633) (36.0 ± 0.02 mm) and *Pyrenophora tritici- repentis*(33.0 ± 0.02 mm) flowed by *Septoria* spp.(32.4 ± 0.20 mm) and *Bacillus pumilus,NCTC 8214*(31.0 ± 0.02 mm) and *Staphylococcus aureus* (ATCC 6538) (30.5 ± 0.02 mm) .

Results showed the minimal inhibitory concentration (MIC) for studying cyanobacteria- *O. agardhii* extract was varied according to algae and fungi species, the MIC for *O. agardhii* extract (250) µg/ml for all fungi and bacteria expects *Aspergillus niger* and *Escherichia coli* at 500 µg/ml (Table 2).

Table 1. Antimicrobial and antifungal activities cyanobacteria- *O. agardhii* extract on the agar plate by diffusion assay method

Test microorganisms		Mean values of inhibition zones (mm) ± SD
***Bacillus subtilis* (ATCC-6633)**		36.0 ± 0.02
Bacillus pumilus, NCTC 8214		31.0 ± 0.02
***Staphylococcus aureus* (ATCC 6538)**		30.0 ± 0.02
***Escherichia coli* (ATCC-7839)**		29.5 ± 0.04
***Pseudomonas aeruginosa* (ATCC 9027)**		30.5 ± 0.07
Pyrenophora tritici-repentis		33.0 ± 0.02
***Septoria* spp.**		32.4 ± 0.20
Aspergillus niger		29.0 ± 0.70
Candida albicans		28.2 ± 0.70
L.S.D	at 5 %	1.63

Table 2. Antifungal and antimicrobial activities as MIC of cyanobacteria- *O. agardhii* extract (µg/ml)

Test microorganisms	50	100	150	250	500	750	1000
Bacillus subtilis	+	+	+	-	-	-	-
Bacillus pumilus	+	+	+	-	-	-	-
Staphylococcus aureus	+	+	+	-	-	-	-
Escherichia coli	+	+	+	+	-	-	-
Pseudomonas aeruginosa	+	+	+	-	-	-	-
Pyrenophora tritici-repentis	+	+	+	-	-	-	-
***Septoria*spp.**	+	+	+	-	-	-	-
Aspergillus niger	+	+	+	+	-	-	-
Candida albicans	+	+	+	-	-	-	-

Evaluation of the ability of cyanobacteria- *O. agardhii* extract to induce wheat resistant against biotic and abiotic stress under natural and stress conditions

The effects of cyanobacteria- *O.agardhii* extract on controlling of diseases of wheat were evaluated in saline soil in the Sinai which sandy soil (Table 3) and compared in normal soil in Giza.

In wheat, powdery mildew, leaf rust and leaf spots are the most important diseases that cause severe losses. Meanwhile, Spots or blotches are the main diseases in wheat in Sinai and powdery mildew in Giza. Wheat i.e. Sakha 93 cv is more resistant to all diseases than Gemmiza 10 (Table 4). In general, the disease incidence was higher in untreated plants and treated with fungicides either for saline or in natural soils. Significant differences were obtained among treatment and untreated control. Results showed that cyanobacteria- *O. agardhii extract* has the potentiality to reduce the disease incidence in wheat cultivars and regions in comparison to the fungicides and untreated. Analysis of data indicated that cyanobacteria- *O. agardhii* extract treatment significantly reduced disease severity under natural and saline conditions in both wheat cultivars regions in comparison to the fungicides and control plants. Cyanobacteria- *O. agardhii* extract was more effective in controlling all the diseases as spot diseases powdery mildew and rust in both wheat cultivars.

Table 3. Physical and chemical properties of the experimental soil and water irrigation analysis in Sinai

Locations	Physical properties			Chemical properties											
	Sand	Silt and clay	Soil texture	EC dS/m	ppm	pH	Cations (meq/L)				Anions (meq/L)				$CaCO_3$ %
							Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}	
	a):Physical and chemical analysis of the soil														
Elqantara	96.57	4.43	Sandy	7.12	716	7.7	7.53	0.82	9.35	0.12	---	1.5	3.38	1.94	
	b): Water irrigation analysis														
Elqantara				8.01	4206	7.5	13.87	4.29	28.35	0.88	---	5.73	36.98	4.68	

Table 4. Diseases incidence (%) of wheat plants cv. Sakha 93 and Gemmiza 10 treated with *O. agardhii* extract and grown under saline soil in Sinai and compared with normal soil in Giza

Location	Wheat variety	Treatment	Powdery mildew	Leaf rust	Spots
Sandy soil (Sinai)	**Sakha**	**Control**	9.7	3.3	20.2
		Fungicide	2.1	2.3	4.5
		Oscillatoria agardhii	0.6	0.5	2.4
	Gemmiza	**Control**	11.3	3.4	23.5
		Fungicide	2.0	1.0	5.4
		Oscillatoria agardhii	1.6	0.9	2.4
Normal soil (Giza)	**Sakha**	**Control**	24.2	9.6	11.3
		Fungicide	3.4	6.0	3.4
		Oscillatoria agardhii	0.3	1.4	0.6
	Gemmiza	**Control**	32.4	11.5	13.4
		Fungicide	7.8	5.6	4.4
		Oscillatoria agardhii	4.7	2.3	0.9
LSD			2.2	2.36	2. 3

Growth and yield of wheat cultivars are highly significantly influenced by salt stress as the result in (Table 5). The data indicated that there was a highly significant difference between control and treated plants with *O. agardhii* extract. Overall, the results suggest that the wheat growth parameter, yield and its compounds were negatively influenced under saline soil that influence growth and yield performance meanwhile its improved under treated conditions. Treated wheat plants with *O. agardhii* extract resulted in a significantly greater increased the growth. *O. agardhii* extract significantly increased yield in comparison with fungicides (Figure 1).

The same results were also obtained that, soluble protein, proline, soluble carbohydrates, Chlorophyll, carotenoids NPK, the K/N ratio in plant leaves of wheat cultivars are highly inter-related and both are significantly influenced by salt stress as the result in (Table 6). Soluble protein, proline and K/N of wheat cultivars are highly significantly influenced by salt stress. In contrast were obtained in clay soil, that soluble protein and carotenoids are highly increased. The data indicated in Table (6) showed that there was a highly significant difference between control, fungicides and treated plants with *O. agardhii* extract. Treated wheat plants with *O. agardhii* extract resulted in a significantly greater increased total phenols, soluble protein, soluble carbohydrates, Chlorophyll, carotenoids NPK, the K/N ratio in plant leaves of wheat cultivars in both regions.

Proline is highly significantly influenced inin plant leaves of wheat cultivars grown in salt soil and treated with *O. agardhii* extract. The same results were also obtained in IAA, total phenols, peroxidase and chitinase in wheat leaves (Table 7). IAA, total phenols, peroxidase and chitinase of wheat cultivars are highly significantly influenced by salt stress. In contrast were obtained in clay soil, that soluble protein and carotenoids are highly increased. Treated wheat plants with *O. agardhii* extract resulted in a significantly greater increased IAA, total phenols, peroxidase and chitinase.

Table 5. Growth and yield of wheat plants cv. Sakha 93 and Gemmiza 10 treated with cyanobacteria- *O. agardhii* extract and grown under saline soil in Sinai and compared with normal soil in Giza.

Location	Wheat variety	Treatment	*Hundred grain weight (g)*	*Number kernels in five spikes*	*Weight kernels in five spikes (g)*	*Grain Yield of sample (g)*	*Grain Yield for total sample (g)*
Sandy soil (Sinai)	Sakha	**Control**	4.2	50	5.2	11.7	19.9
		Fungicide	5.6	104	7.5	15.5	23
		Oscillatoria agardhii	5.3	190	10.2	21.6	33.8
	Gemmiza	**Control**	5.3	88	8.2	16.4	24.6
		Fungicide	7.0	129	10.3	27.0	28.2
		Oscillatoria agardhii	6.6	182	14.1	34.7	43.8
Normal soil (Giza)	Sakha	**Control**	8.3	131	10.7	39.4	50.1
		Fungicide	7.6	231	10.8	37.4	48.2
		Oscillatoria agardhii	7.3	310	13.2	36.8	50
	Gemmiza	**Control**	5.4	218	10.5	31.0	41.5
		Fungicide	5.8	226	11.5	49.3	50.8
		Oscillatoria agardhii	4.5	372	28.7	63.2	71.9
LSD			1.2	6.6	1.5	3.5	4.5

Table 6. Chemical components and physiological characteristics of wheat plants cv. Sakha 93 and Gemmiza 10 treated with cyanobacteria- *O. agardhii* extract and grown under saline soil in Sinai and compared with normal soil in Giza.

Characters	Sandy soil (Sinai)						Normal soil (Giza)					
	Wheat Sakha			Wheat Gemmiza			Wheat Sakha			Wheat Gemmiza		
	Control	*Fungicide*	*Oscillatoriaa gardhii*	*Control*	*Fungicide*	*Oscillatoriaa gardhii*	*Control*	*Fungicide*	*Oscillatoria agardhii*	*Control*	*Fungicide*	*Oscillatoriaa gardhii*
Proline (umol/g fresh weight)	**4.90**	**5.95**	**6.99**	**4.43**	**5.52**	**6.52**	**4.76**	**5.09**	**5.54**	**4.09**	**4.23**	**5.12**
Soluble carbohydrates %	**10.7**	**11.32**	**12.02**	**10.8**	**11.30**	**11.28**	**10.9**	**10.02**	**10.32**	**10.0**	**10.20**	**11.01**
Chl. a (mg/g fresh weight)	**2.10**	**2.02**	**2.55**	**0.76**	**1.84**	**2.53**	**2.02**	**2.05**	**2.21**	**0.80**	**0.85**	**1.05**
Chl. b (mg/g fresh weight)	**1.15**	**1.40**	**1.70**	**1.02**	**1.29**	**1.71**	**1.10**	**1.10**	**1.21**	**1.01**	**1.12**	**1.28**
Chl. a+b (mg/g fresh weight)	**3.03**	**3.42**	**3.85**	**3.06**	**3.09**	**3.13**	**3.02**	**3.05**	**3.32**	**3.01**	**3.02**	**3.14**
Chl. a/Chl. b	**1.23**	**1.44**	**1.65**	**1.12**	**1.35**	**1.92**	**1.14**	**1.15**	**1.15**	**1.10**	**1.25**	**1.98**
Carotenoids (mg/g fresh weight)	**0.54**	**0.66**	**0.93**	**0.54**	**0.81**	**0.91**	**0.36**	**0.33**	**0.76**	**0.50**	**0.67**	**1.21**
Crude protein%	**10.7**	**11.6**	**12.36**	**9.87**	**13.50**	**12.84**	**10.6**	**10.36**	**12.87**	**9.78**	**10.31**	**12.02**
N% (grains)	**1.76**	**2.21**	**2.55**	**1.45**	**2.32**	**2.26**	**2.20**	**2.25**	**2.32**	**1.40**	**1.47**	**2.01**
P%	**0.15**	**0.25**	**0.32**	**0.13**	**0.21**	**0.28**	**0.22**	**0.22**	**0.23**	**0.16**	**0.20**	**0.26**
K%	**0.32**	**0.42**	**0.51**	**0.21**	**0.34**	**0.46**	**0.40**	**0.41**	**0.45**	**0.28**	**0.29**	**0.34**
Na%	**0.31**	**0.31**	**0.41**	**0.30**	**0.31**	**0.35**	**0.30**	**0.31**	**0.35**	**0.13**	**0.21**	**0.25**
K/Na ratio	**1.13**	**1.35**	**1.42**	**1.25**	**1.21**	**1.53**	**1.15**	**1.12**	**1.23**	**1.20**	**1.21**	**1.46**

Table 7. Hormonal stress of wheat plants cv. Sakha 93 and Gemmiza 10 treated with cyanobacteria- *O. agardhii* extract and grown under saline soil in Sinai and compared with normal soil in Giza

Characters	Sandy soil (Sinai)						Normal soil (Giza)					
	Wheat Sakha			Wheat Gemmiza			Wheat Sakha			Wheat Gemmiza		
	Control	*Fungicide*	*Oscillatoriaagardhii*	*Control*	*Fungicide*	*Oscillatoriaagardhii*	*Control*	*Fungicide*	*Oscillatoriaagardhii*	*Control*	*Fungicide*	*Oscillatoriaagardhii*
Total phenols (mg/g fresh weight)	0.19	0.23	0.36	0.21	0.23	0.34	0.18	0.26	0.31	0.23	0.27	0.42
IAA (mg/g fresh weight)	99.7	101.4	132.3	98.8	109.9	121.3	87.8	91.9	117.7	88.8	122.3	128.8
Peroxidase (unit)	15.6	15.9	19.9	18.8	18.7	20.9	14.5	15.5	17.8	14.7	16.8	19.9
Chitinase (unit)	5.6	5.7	7.4	5.8	5.8	8.7	5.2	5.5	7.7	5.4	5.5	7.7

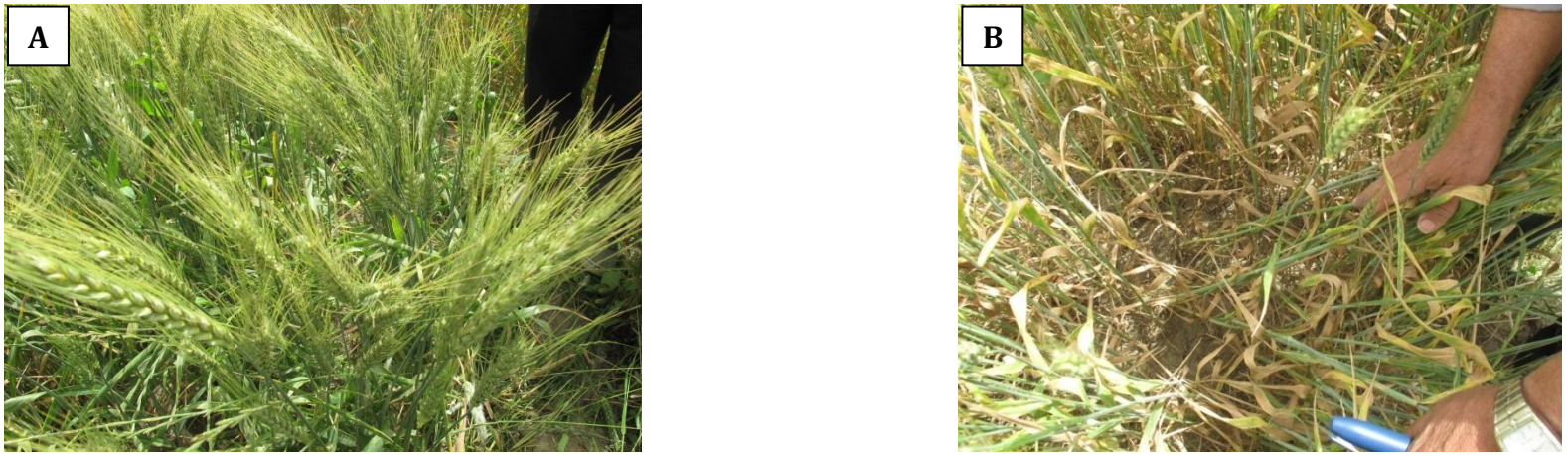


Figure 1. Diseases and growth of wheat plants treaded with Cyanobacterial extracts (A) and grown under saline soil in Sinai in compared with untreated control (B).

4. Discussion

Diseases are primary hazards in wheat production as tan spot (*Pyrenophora tritici-repentis*) and Septoria complex (*Septoria* spp.), powdery mildew (*Blumeria graminis* f. sp. *Tritici*) and (*Puccinia striiformis,* f.sp. *tritici*); Leaf rust (*Puccinia recondita,* f.sp. *tritici*), an economically important disease in different regions, which can considerably reduce the yield of susceptible cultivars up to about 40-60% [3,23].

Salinity is one of the major physiological stresses that lead to crop reduction productivity worldwide especially in arid and semiarid regions. It reduces seed germination and crop yield of most crops and its effect on soil physic-chemical properties. Salt effects are the combined result of the complex interaction among different morphological, physiological, and biochemical processes. Morphological symptoms are indications of the injurious effects of salt stress.

Cyanobacterial extract has significant antifungal activity. The degree of efficiency is subjected to the kind of biological treatment being imparted. This kind of investigation, although, creates a very general view of cyanobacterial possibility to produce biologically active compounds but certainly points out the necessity of exploring cyanobacteria as potentially excellent sources of these substances and reveals the most prospective strains for further investigations.

The minimal inhibitory concentrations (MIC) is defined as a lesser concentration of contrary which inhibits the fungal growth under optimum test condition; from the other hand, the fungi are eukaryotic organisms and similar to it's eukaryotic hosts in structure and metabolism. So, the antifungal agents work on inhibit (or kill) the pathogenic fungi and in same time may be effects on host tissues, therefore, the study includes the MIC tests to detect the lesser concentrations inhibit the growth fungi *In vitro*.

The results of MIC tests showed there is a somewhat variation in sensitivity of fungi against algae extracts, that's may be due to the differing nature and to the variation of metabolism in the extract. Cyanobacteria have received little attention as potential biocontrol agents of plant diseases [9]. Cyanobacteria are known to produce antibiotic and antifungal compounds [5,7,9]. Various strains of cyanobacteria and green algae are known to produce intracellular andextra cellular metabolites with diverse biological activities such as antibacterial, antifungal and antiviral activity. Kim [8] reported that cyanobacterial strain - *Oscillatoria,* exhibited antifungal activity against seven phytopathogenic fungi causing diseases in hot pepper. These antifungal activities are very interesting in the perspective of cyanobacterial research. In this respect, some reported that extract of *C. vulgaris* is suppressive to some microorganisms, of which *Fusarium oxysporum* and *Tetrany chusurtica* Koch due to antifungal compounds that have inhibitor properties (secondary metabolites) retarding the growth of other microorganisms as peptides, alkaloids and phenols [24].

Cyanobacteria produce extracellular polymers of diverse chemical

composition, especially exopoly saccharides that enhance microbial growth and as a consequence, improve soil structure and exoenzyme activity [25]. Algae play an important role in agriculture where they are used as biofertilizer and soil stabilizers.

Field experiments confirmed the effectiveness of cyanobacterial strain - *Oscillatoria* in reducing the infection of diseases in addition increased of total soluble protein, Chlorophyll, carotenoids NPK, the K/N ratio in wheat as well as plant growth and yield. Plants treated with cyanobacterial strain - *Oscillatoria* generally develop resistance to host, because application of elicitors on plant surface activates multiple signaling pathways of intracellular defense [26]. In a broad sense, "elicitors", for a plant refer to chemicals from various sources that can trigger physiological and morphological responses and phytoalexin accumulation. Their variability is less than the rest pathogens, which have been "chosen" by plants and animals as "telltale signs" of different groups of pathogens [27,28]. Elicitor needs to be recognized on plant by a receptor (protein), which activates the expression of defence genes. The selected bacterial species might be formulated as a biofungicide seed treatment in compared with the neutral products which use as an inducer. These biofungicides may be readily integrated within a disease management program for the control of the biotic disease and abiotic stress as salinity.

The investigation, indicated that cyanobacterial strain - *Oscillatoria,* have potentially as antifungal, biostimulants and anti-stress that improve plant resistance to abiotic stress.

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