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Research Article

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Application of blue-green algae for integrated disease management of barley against foliar pathogens

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ABSTRACT

Barley is one of the most important cereal crops cultivated in the world and Egypt that is extremely drought tolerant, making it an excellent choice for arid and dry areas. However, quality and production is threatened by major barley diseases as net blotch caused by (Pyrenophora teres), powdery mildew caused by (Blumeria graminis f.sp. hordei), leaf rust (Puccinia hodei) and Scald (Rhynchosporium secalis). A field experiment was conducted to evaluate the use of Blue-green Cyanobacteria - O. agardhii for integrated disease management of barley against foliar pathogens and yield related traits in two locations as semi-arid land (Sinai) and normal conditions (Giza) and two varieties (Giza 123 and Giza 133). Seeds and foliar application of Blue-green Cyanobacteria Oscillatoria agardhii showed significant effective net blotch , powdery mildew , rust and spot blotch management. Blue-green Algae applied treatments increased antioxidant enzymes as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), as well as grain yield, kernel weight in most cases and improved barley yield quality parameters and grain protein.

Key words: Algae, antioxidant enzymes, Barley; Blue-green Cyanobacteria–*Oscillatoria agardhii*, integrated disease management, foliar pathogens

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INTRODUCTION

Barley ranks as the world's fourth major cereal crop after maize, wheat and rice. 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. Disease infection is one of the most important biotic constraints, limiting barley productivity. In Egypt, the most common diseases are net blotch, leaf rust and powdery mildew (*Blumeria graminis* f.sp. *hordei*)[1],[2],[3]. *Pyrenophorateres* f. *teres* produces the classic net-type symptoms while *P. teres* f. *maculate* causes spot-type lesions which reduce grain yields and quality parameters required)[1] [4].An integrated disease management is primarily depends on the use of resistant varieties against one or more pathogen (s)[5].

In recent years, the induction of the plant defense response by natural products as well as microorganisms as fungi and bacteria that normally colonize living plants without causing visual damage, has received ample attention[6],[7] . Similarly, Blue-green Algae (cyanobacteria) play a role in soil fertility and system, soil reclamation, bio-controlling of agricultural pests and diseases, change of microbiological. Blue-green Algae (cyanobacteria) are a diverse group of organisms that frequently occur in marine environment and usually produce high biomass yields .Blue-green Algae (cyanobacteria) show a great potential for generation of novel agricultural technologies, attested by the success of already commercialized algae derived benefit products as biofertilizer, biopesticides and

biofungicides[8],[9],[10]. Algae have expressive biotechnological potential as a source of compounds which can stimulate plant growth and protect plants from pathogens[11]. Blue-green Algae (cyanobacteria) show a great potential for suppressive of plant pathologists, *in vitro* by substances produced by various cyanobacteria as *Chaetomium globosum, Cunninghamella blakesleeana, and Aspergillus oryzae, Rhizoctonia solani and Sclerotinia sclerotiorum.*

Biochemically and physiologically, algae are similar in many aspects to other plants. Algae possess the same basic biochemical pathways; all possess chlorophyll-*a* and have carbo-hydrate, protein and products comparable to those of higher plants. Furthermore, algae are the major primary producers of organic compounds; and play a central role as the base of the food chain in aquatic systems. These have been reported to be plant growth regulators (PGRs), vitamins, amino acids, polypeptides, antibacterial or antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity[12]. So, algae are important components of arid and semi-arid ecosystems. Resistance against pests is one of the key factors for plant varieties used in production systems[13].The most prevalent tools to control plant, pests and enhance soil fertilely are the use of intensive agrochemicals irrespective of their high cost and deleterious impacts on health and environments. Some potential applications to consider for cyanobacteriaare the production of antimicrobial compounds for the pharmaceutical industry and the agricultural sector as both bio-fertilizers and biocontrol agents. It is necessary to screen many cyanobacteria before suitable strain can be selected to application.

The objective of this study was thus to evaluate the potentiality of blue-green Algae (cyanobacteria) on controlling of disease severity, yield and yield related traits in two barley cultivars grown in two regions as semi-arid land (Sinai) and normal conditions (Giza).

EXPERIMENTAL SECTION

Algae

Cyanobacteria- *Oscillatoria agardhii* was isolated and characterized by Hoballah *et al*. [14], cultivated on BG- 11 media 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. The extracts were prepared according Karticioglu[15].

Field experiments

The field trial was performed under field conditions in Sinai and compared with the normal soil (Giza governorate), using barley (*Hordeum vulgare* L.), cv. Giza 123 and Giza 133 varieties. Experimental design was Randomized Complete Block Design with 5 replicate blocks and 1-meter row experimental unit. Ten rows of barley plants sown with a density of 300 seeds per square meter were grown in each plot. All plots were fertilized immediately after sowing with ammonium nitrate (NH4 + -N) at the rate of 100 kg· N· ha−1 .

Barley seeds were surface-sterilized, treated with the algae extract. Two months later, 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 h) were determined. Liquid extract were especially sprayed on to the barley leaves at 30 and 60 days after sowing . Each treatment consisted of three pots 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 diseases was assessed as the percentage area of leaves infected during growth periods.

- Disease severity (R) was calculated according to the formula, having added the per cent of the affected leaf area of each leaf and having divided the sum by the number of assessed leaves:

$$R = \frac{\Sigma(n \cdot b)}{N}$$

- Where $\Sigma(n \cdot b)$ – sum of product of the number of leaves with the same percent of severity and value of severity, N – number of assessed leaves.
- Disease incidence, i.e. per cent of disease-affected leaves (P) was calculated according to the following formula**:**

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

- where n – number of affected leaves, N – number of assessed leaves.

Powdery mildew
Leaves colonization by the fungus was quantified by measuring mildew colonies covering the surface of the leaves. Ten days after inoculation, disease severities was recorded according to the following scales: 0 = 0%; 1 ≤ 5%; 3 = 6%– 15%; 5 = 16%–25%; 7 = 26%–50%; and 9 ≥ 50%.

Chemical analysis:
Ten days after spraying, five leaves per plant were separately collected, frozen for 36 h, dried and powdered. Generally, 100 mg dried sample were used for analysis.

Protein determination: The concentration of protein was determined by the method of Bradford [16]using BSA as a standard.

Enzymes extraction and assays
Superoxide dismutase (SOD). SOD activity was estimated by recording the decline in the absorbance of superoxide nitro blue tetrazolium complex by means of the enzyme based on the method of Dhindsa *et al.*[17]. Absorbance was recorded at 560 nm and one unit of enzyme movement was taken as the amount of enzyme.

Catalase (CAT) and peroxidase (POD). Catalase action was precise according to Aebi[18]. As concerning for CAT 3 ml reaction mixture containing 1.5 ml of 100 mmol potassium phosphate buffer (pH = 7.2), 0.5 ml of 75 mmol H2O2, 0.05 ml enzyme extraction. The absorbance recorded in decrease at 240 nm for 60 s. The enzyme action was accounted by calculating the quantity of decomposed H2O2.

Peroxidase (POD) activity was assayed by recording 3 ml reaction mixture. The reaction mixture contained 0.1 mmol EDTA, 1 ml of 0.2 mol/m3 potassium phosphate buffer with pH = 7.6, 0.1 ml of 2 mmol (NADPH), 0.5 ml of 3 mmol DTNB,0.1 ml enzyme extract. Reaction initiated by adding of one unit of POD activity. The raise within absorbance at 412 nm was recorded at 25°C in excess of a period of 5 min on a spectrophotometer. Protein extract was quantified using the technique of Bradford [16]

Determination of growth and yield
All the plants of different treatments were harvested in the same physiological growth state. Data on wheat total dry biomass, grain and yields, harvest index, tiller and spike numbers per plant, seed number per spike, plant and spike height and weight of 1000 kernels were recorded. Spikes were oven-dried at 70 1 °C for 72 h and their dry weights determined. Tiller and spike numbers per plant were recorded from 5 randomly chosen plants. Spike weight per plant was recorded.

Grain protein content (GPC) measurement
Mature grains were ground and passed through a 0.5 mm screen. GPC was measured using the Kjeldahl method [19]with three replication for each sample. Protein content is calculated by duplicating a factor of 6.25 with N content[20].

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.

RESULTS

Disease severity
The effects of cyanobacteria- *O. agardhii* extract on controlling of barley diseases were evaluated grown in semi-arid land (Sinai) and compared with the normal conditions in Giza (Table 1). Net blotch, powdery mildew, leaf rust and leaf spots are the most important diseases that causes severe losses of barley varieties. Spots or blotches are the main diseases in barley grown in Sinai and powdery mildew in Giza. In general, the diseases incidence were higher in untreated plants and treated with fungicides grown either in semi-arid land or in normal conditions in Giza. Barley Giza 123 cv is more resistant to all diseases than Giza 133 (Table 1). Significant differences were obtained among treatment and untreated control. Analysis of data indicated that foliar application of cyanobacteria- *O. agardhii* significantly reduced diseases severity in both barley cultivars and regions in compared to the fungicides and control plants. Cyanobacteria- *O. agardhii* was more effective in controlling all the diseases include net blotch , spot diseases, powdery mildew and rust in both barley cultivars.

Table 1: Diseases severity (%) of barley cv. Giza 123 and Giza 133 treaded with *O. agardhii* extract grown in semi-arid land (Sinai) or in normal conditions (Giza)

Location	*Barley variety*	*Treatment*	*Net blotches*	*Powdery mildew*	*Leaf rust*	*Spots*
Semi-arid land (Sinai)	Giza 123	Control	32.7	9.3	12.2	9.3
		Fungicide	6.4	3.1	5.0	6.6
		Oscillatoriaagardhii	0.3	0.0	0.0	1.3
	Giza 133	Control	19.6	6.3	11.3	8.3
		Fungicide	3.5	3.4	4.4	5.6
		Oscillatoriaagardhii	0.6	0.0	0.0	1.4
Normal condation (Giza)	Giza 123	Control	26.3	8.3	36.3	11.3
		Fungicide	12.4	3.4	8.0	11.4
		Oscillatoriaagardhii	2.4	1.3	1.6	0.6
	Giza 133	Control	21.3	7.4	21.5	10.4
		Fungicide	11.2	7.0	8.6	6.4
		Oscillatoriaagardhii	2.3	3.7	2.6	0.8
LSD		-	1.2	2.3	2.16	2. 3

diseases severities were recorded according to the following scales: 0 = 0%; 1 ≤ 5%; 3 = 6%– 15%; 5 = 16%–25%; 7 = 26%–50%; and 9 ≥ 50%.

Enzymes extraction and assays

The effect of cyanobacteria- *O.agardhii* on the activities of antioxidant enzymes is shown in Table 2. The results revealed an increase in SOD and POD activities and CAT activity in leaves of barley. A gradual increase was observed in SOD and POD activities under cyanobacteria-*O. agardhii* in comparison with the control and fungicide. The interaction effect of cyanobacteria- *O.agardhii* X cultivar × drought condition was highly significant ($P < 0.01$) for SOD, POD and CAT .The maximum increase in the enzymes activities was observed in the cultivars of barley cv Giza 123 grown under normal condition in compared to the control.

Growth and grain yield:

Growth and yield of barley cultivars are highly significantly influenced by diseases grown in semi-arid land (Sinai) or in normal conditions (Giza) as the result in Tables (3&4).

The results of data analysis showed that foliar application of barley plants with cyanobacteria *O. agardhii* show significant difference over the control treatment in improving plant growth either grown in semi-arid land (Sinai) or in normal conditions (Giza) (Table 3). In general, the growth were higher in untreated plants and treated with fungicides grown either in semi-arid land or in normal conditions in Giza using Barley Giza 123 cv than Giza 133 (Table 3) .The results of data analysis showed that foliar application has significant effect on some characteristics such as panicle length, tiller number and dry weight.

The same results were obtained in grain yield and yields quality of barley grown in semi-arid land (Sinai) or in normal conditions (Giza). In addition, application of *O. agardhii* extract has significantly contribute to the formation and improved of grain yield and yields quality of barley grown in semi-arid land (Sinai) or in normal conditions (Giza) (Table 4).

Table 2: Antioxidant enzymes in barley, Giza 123 and Giza 133 varieties treaded with *O. agardhii* extract grown in semi-arid land

Characters	***Semi-arid land (Sinai)***						***Normal condition (Giza)***					
	Giza 123			**Giza 133**			**Giza 123**			**Giza 133**		
	Control	*Fungicide*	*Oscillatoria agardhii*	*Control*	*Fungicide*	*Oscillatoria agardhii*	*Control*	*Fungicide*	*Oscillatoria agardhii*	*Control*	*Fungicide*	*Oscillatoria agardhii*
Superoxide dismutase (SOD) unit/mg protein	3.3	4.6	12.3	3.0	4.5	11.3	3.0	3.9	11.4	2.6	3.9	10.9
Catalase (CAT) unit/mg protein	2.9	4.5	11.3	2.6	3.3	10.6	2.3	4.3	10.5	2.0	3.5	9.8
Peroxidase (unit)	19.8	18.7	23.9	18.6	19.9	26.9	17.5	18.5	23.8	16.7	17.8	22.9

(Sinai) or in normal conditions (Giza)

Table 3: Growth parameters of barley cv. Giza 123 and Giza 133 treaded with *O. agardhii* extract grown in semi-arid land (Sinai) or in normal conditions (Giza)

Location	***Wheat variety***	***Treatment***	**Hundred grain weight (g)**	**Number kernels in five spikes**	**Weight kernels in five spikes (g)**
Semi-arid land (Sinai)	Giza 123	Control	6.0	159	7.00
		Fungicide	9.8	235	13.6
		Oscillatoriaagardhii	11.3	208	11.6
	Giza 133	Control	5.4	151	9.4
		Fungicide	6.2	216	10.3
		Oscillatoriaagardhii	7.5	192	9.8
Normal condation (Giza)	Giza 123	Control	7.9	195	10.7
		Fungicide	6.3	193	13.2
		Oscillatoriaagardhii	13.9	220	15.3
	Giza 133	Control	6.3	189	9.45
		Fungicide	6.3	191	10.1
		Oscillatoriaagardhii	6.8	199	12.6
LSD			0.6	2.4	0.9

Table 4: Barley yield cv. Giza 123 and Giza 133 treaded with *O. agardhii* extract grown in semi-arid land (Sinai) or in normal conditions (Giza)

Location	*Wheat variety*	*Treatment*	Grain Yield of sample (g)	Grain Yield for total sample (g)	Grain Protein %dw
Semi-arid land (Sinai)	Giza 123	Control	26.4	37.4	9.3
		Fungicide	53.7	64.1	10.6
		Oscillatoriaagardhii	58.7	68.1	12.8
	Giza 133	Control	19.1	28.5	8.9
		Fungicide	49.2	61.5	9.8
		Oscillatoriaagardhii	54.2	664	12.3
Normal condation (Giza)	Giza 123	Control	23.1	33.7	9.8
		Fungicide	66.0	75.3	11.5
		Oscillatoriaagardhii	69.4	81.8	13.3
	Giza 133	Control	21.2	30.2	10.9
		Fungicide	60.0	70.8	11.3
		Oscillatoriaagardhii	63.4	73.3	12.4
LSD			1.3	1.3	0.8

DISCUSSION

Barley varieties were found to be susceptible to foliar pathogens in semi-arid areas where shoot fly infected is severe[1],[2]. Application of fungicides in shoot fly cannot be advisable as this pest results in poor crop stand and marginal yield advantages and increase the problems associated with environmental pollution and cost.

Algae have been proven to play an important role in crop production and protection [9],[11]. Furthermore, algae are the major primary producers of organic compounds; and play a central role as the base of the food chain in aquatic systems. In the last decades, different researchers have studied the replacement of chemical pesticides by natural components of different plant and microalgal sources of fungicidal agents[21],[21]. These natural materials in addition to their lethal activities on pathogens, preserves the environment of pollution and maintain the equal distribution of fauna.

Based on the results of this study, foliar application of cyanobacteria- *O. agardhii* on barley was found to be very effective on decreasing foliar diseases and increasing plants yield and its quality. Cyanobacteria - *O. agardhii* provided an important role in integrated disease management, accelerate biochemical reactions as antioxidant enzymes i.e. Catalase (CAT) and peroxidase (POD) and superoxide dismutase (SOD) as well as stimulate growth in general, which will lead for better quality and higher yield. Cyanobacteria have received little attention as potential biocontrol agents of plant diseases [22]. Besides forming the basic food source for these food chains, they also produce the oxygen necessary for the metabolism of the consumer organisms [23].

In this case, barley with a high level of antioxidant enzyme activities which are related to its capacity for better protection mechanisms against oxidative damage could be introduced to farmers as diseases and drought tolerant cultivar of plant in arid regions.

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