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Effect of Cyanobacteria on Reducing Damping-off and Root Rot Incidence in Lupine Plants, New Valley Governorate, Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to investigate the suppression effect of four cyanobacteria species *viz. Nostoc muscorum*, *Oscillatoria agardhii*, *Spirulina platensis* and *Anabaena sphaerica*, against *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* as the causal agent of lupine damping-off and root rot diseases under greenhouse and field conditions. Cyanobacterial secondary metabolites have a diverse antagonistic activity that lead to disintegration of microbial growth. All cyanobacteria were significantly suppressed of the tested pathogenic fungi *in vitro*. The reduction in the linear growth of the tested fungi was differed depending of type of solvent extract. Acetone extract of *O. agardhii* was recorded the highest growth reduction of all tested pathogenic fungi.
Contents of indole actetic acid (IAA), total phenol and flavonoid compounds and protease enzyme activity showed an increase in the extracts of *N. muscorum*, *O. agardhii* than the extracts of *S. platensis* and *A. sphaerica*. Also, acetone extract of all tested cyanobacteria was recorded the highly secondary metabolites than methanol and water extracts. Treated lupine seeds (cv. Giza 1) with any of the cyanobacteria extracts before sowing results significantly reduction of damping-off and root rot incidence compared with untreated seeds under pot and field conditions. On the other

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hand, treating lupine seeds with cyanobacteria extracts increased plant growth and yield parameters during both growing seasons (2014-2015 and 2015-2016). In general, *O. agardhii* followed by *N. muscorum* highly reduced damping-off and root rot incidence both in pot and field experiments as well as increase of growth (pant height and No. of branches plant^{-1}) and yield parameters (No. of pods plant^{-1}, No. of seed plant^{-1}, seed index (gm) and total yield fed.$^{-1}$ (Kg) in field. Also, acetone extract more effective in this respect than methanol or water extracts.

Keywords: Cyanobacteria; lupine; secondary metabolic; soil borne pathogens; solvent extracts.

1. INTRODUCTION

White lupine (*Lupinus termis* Forsk) is one of the oldest agricultural crops widely used in the world not only as a protein source in fodder production but also for soil improvement [1]. Lupine belongs to the genus *Lupinus* in the Fabaceae family. Lupine seeds contain considerable nutrition due to its high protein (35-45%) and oil content (10-15%). Unfortunately in Egypt and several countries lupine legume plants are infected by several soil-borne fungi causing damping-off and wilt disease which effect both quantity and quality of the yield. Soil-borne fungal diseases are among the most important factors limiting the yield production of lupine resulting in serious economic losses [2]. Many soilborne fungi, including *Rhizoctonia solani* Kühn, *Fusarium solani* (Mart.) Sacc, and *Macrophomina phaseolina* (Tassi) Goid infect lupine plants causing damping-off and root rot diseases. Such diseases cause great decrease in seed yield [3]. The disease can be controlled by certain fungicides [4]. However, disease management using fungicides is not economically practical or environmentally safe. Therefore, the induction of disease resistance in plant may be an alternative approach to diminish the hazardous side effects of chemical fungicides.

Hence, strategies aiming at replacement of chemical pesticides by hazardous free biological agents can be a reasonably good choice. Potential agents for biocontrol activity are rhizosphere competent fungi and bacteria, which in addition to their antagonistic activity are capable of inducing growth responses by either controlling minor pathogens or by producing growth stimulating factors.

Interest in biological control has increased recently, fuelled by public concerns over the use of chemicals in the environment [5]. Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts [6] and which reduce the disease and are perceived as less harmful than conventional fungicides [7]. It has long been recognized as that the biological control became recently an effective strategy for fighting plant pathogens [8]. Algae are one of the chief biological agents that have been studied for the control of plant pathogenic fungi, particularly soil borne disease [9].

Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity [10]. They have received little attention as potential biocontrol agents of plant diseases. Kulik [11] stated that for a number of reasons, cyanobacteria and algae are suitable candidates for exploitation as bio-control agents of plant pathogenic bacteria and fungi: Cyanobacteria produce a large number of antibacterial and antifungal products, many can grow in quantity in mass culture and they are not a threat to the environment [12].

The aim of the present study is focused on detection the ability of some cyanobacteria in suppressing root rot diseases of lupine caused by *R. solani, F. solani* and *M. phaseolina in vitro* and *in vivo* and determine their effects on some growth parameters of Lupine plants.

2. MATERIALS AND METHODS

2.1 Isolation of Pathogenic Organisms

Diseased lupine plants showing root rot were collected from different fields located at New Valley Governorate for isolation. The infected lupine roots were firstly washed with tap water and then the roots were surface sterilized with 2% sodium hypochlorite solution for 2 min. Isolation procedures were carried out according to the method described by Dhingra and Sinclair [13] using the Rose Bengal medium (Dextrose, 10 g; Peptone, 5 g; KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; Rose Bengal 0.05 g; Agar, 15 g in 1 litre of distilled water and pH,

7.2±0.2). The resulted fungi were purified using the hyphal tips and /or single spore technique on Rose Bengal medium and then subculture of each isolated fungus on slant medium for future studies.

Subcultures of the obtained isolates were then kept on PDA slants and stored at 5℃ for further studies. The purified cultures were then tested for their pathogenicity and those proved pathogenic were submitted to identification, and to some pathological studies schemed during the course of this work.

2.2 Pathogenicity Tests

The purified fungal isolates secured from diseased lupine plant organs were tested for their pathogenicity on healthy lupine plants cv. Giza 1 grown in pots (25 cm in diameter) containing sterilized soil and sown with disinfested seeds. Soil sterilization was carried out using formalin solution 5%. The disinfested soil before sowing the seed was left to aeration for 3 weeks to get rid of the chemical remains. The obtained isolates (19 isolates) were grown separately on barley grain medium in conical flasks for 7-10 days to be used as a source of inoculum. Inocula of these tested fungi were applied separately at the rate of 5% of the soil weight [4], mixed thoroughly with the soil then irrigated and left 7 days for establishment. Disinfested lupine seeds were sown in the infested pots at the rate of 5 seeds/pot. Four pots were used for each isolate, (which were considered as replicates). Pots containing sterile soil mixed with barley grains free of any fungi were sown similarly with disinfested lupine seeds at the same rate to be used as control treatment. Pots were kept under observation and irrigated as needed.

2.3 Disease Assessments

Results were recorded after 30 days of planting for damping- off and after 90 days for rot root and wilt. The percentage of pre and post emergence damped-off seedlings was estimated per each replicate. The root rot plants of each replicate were removed from the soil after the inoculation period, washed thoroughly to remove soil debris then disease severity was estimated according to the percentage of root discoloration as follows: 0 = roots without discoloration (no infection), 1= 1-20%, 2= 21- 40%, 3= 41-75%, 4= 75-100% and 5= completely dead plants. Disease severity index (DSI) for each replicate was calculated by the formula suggested by Liu et al. [14] and calculated as follows:

$$DSI = \sum d \times 100 / d\ max \times n$$

Whereas: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate. Reisolation was carried out from some of the experimentally diseased plants to fulfill Koch's postulations and the developing fungi were compared with the original isolates. Identification of the obtained pathogenic fungal isolates was carried out according to Dhingra and Sinclair [13]. Representative of these isolates were sent to Assuit University Mycological Center (AUMC) for verification.

2.4 Blue Green Algae (Cyanobacteria)

Nostoc muscorum, *Oscillatoria agardhii*, *Spirulina platensis* and *Anabaena sphaerica* were obtained from Microbiology Department, Soil, Water and Environment Research Institute, Agric. Res. Centre.

2.5 Cyanobacteria Growth and Different Extracts Preparation

Pure algal culture of the cyanobacteria, *Nostoc muscorum*, *Oscillatoria agardhii*, *Spirulina platensis* and *Anabaena sphaerica* were selected to evaluate their antifungal activity against *R. solani*, *F. solani*, *M. phaseolina*. BG11 medium was used for maintenance of cyanobacteria [15]. The cultured media were incubated at 30±2℃ without aeration and under continuous illumination of fluorescent lamps with intensity 2500 Lux. The cultures were shaken every day to prevent algal cell clumping and adherence of algal cells to the containers.

2.6 Extracts Preparation

The algal pellets (37 gm) were extracted using serial Exhaustive Extraction Method [16] with methanol, acetone, and water. All extracts were dried and weighed to estimate the concentration in 1 ml. Dried extracts were either reconstituted in deionized water.

2.7 Antifungal Activity Assay of Cyanobacteria Extracts

The antifungal activity was tested by using the filter paper disc diffusion method [17] employing

24 hours cultures of the above mentioned organisms. Petri dishes (9 cm in diameter) contains 15 mL of PDA medium were divided into two equal halves, the first half was inoculated with a disk (0.5 cm in diameter) of pathogenic fungi, individually and the second half was inoculated with a disk (0.5 cm in diameter) saturated with 250 µg/ml of each crude extract [18]. The percentage of inhibition of the pathogen was calculated. Each treatment was replicated three times with five plates per replication. The percentage reduction (Mr) of colony diameter by each extract was computed as following

$$\text{Inhibition (\%)} = (M1-M2)/M1 \times 100$$

Where: M1=Colony diameter in the untreated medium (control) and M2=Colony diameter in the treated medium.

2.8 Production of IAA

The production of IAA by the Cyanobacteria strains was determined by colorimetric measurement at 530 nm using Salkowski's reagent. Cyanobacteria strains were grown under shaking (120 rpm) for 2 days at 30ºC in BG11 broth medium supplemented with tryptophan (1 mg ml^{-1}) as IAA precursor. After incubation, the cells were centrifuged (3,000 rpm for 10 min at 4℃) and 1 ml of supernatant was combined with 2 ml of Salkowski's reagent (150 ml of 95-98% H_2SO_4, 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$, and 250 ml distilled water) and incubated for 30 min at room temperature. The quantification of IAA was carried out using a standard curve with known concentrations of pure IAA (Sigma–Aldrich, Co.).

2.9 Estimation of Total Phenolic

Content Total phenolic (TP) contents were determined by the spectrophotometric method [19]. In brief, a 0.5 ml of each extract was made up to 3 ml with distilled water, and then mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 2 ml of a 2% Na_2CO_3 solution were added to the mixture and thoroughly mixed. The mixture was kept at 30℃ for 60 min in dark place, and then the absorbance was recorded at 650 nm. The TP was determined from extrapolation of calibration curve that was constructed by standard concentrations of gallic acid solution. Estimation of phenolic compounds was carried out in triplicate. The TP was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

2.10 Determination of Total Flavonoid Content

Total flavonoid (TF) was determined by a colorimetric method as described by Zhishen et al. [20]. A 0.5 ml of each extract was made up to 1 ml with methanol. Afterwards 0.4 ml of distilled water was added followed by 0.3 ml of 5% $NaNO_2$ solution and the mixture was left for 5 min. Thereafter, 0.3 ml of (10%) $AlCl_3$ solution was added and allowed to stand for 6 min. Two ml of (1 M) NaOH solution was added to the mixture and the final volume was adjusted to 10 ml with distilled water. The mixture was thoroughly shaken and allowed to stand for 15 min. Absorbance of the reaction mixture was read at 510 nm. The concentrations of total flavonoids were determined as quercetin equivalents (mg/g of dry weight).

2.11 Protease Activity

Protease activity was determined by a modified procedure 15, 16 using 2% casein in 0.2 M carbonate buffer (pH 10) as a substrate. Casein solution (0.5 ml) with an equal volume of suitable diluted enzyme solution was incubated at 40ºC. After 10 min the reaction was terminated by addition of 1 ml of 10% trichloroacetic acid. The mixture was centrifuged, supernatant was taken to 5 ml of 0.44 M Na_2 CO_3 and 1 ml of two-fold diluted Folin-Ciocalteau reagent was added. After 30 min the color developed was read at 660 nm against reagent blank prepared in the same manner. Tyrosine served as the reference standard. The optical density of these solutions was measured.

2.12 Greenhouse Experiment

Pathogenic isolates of *R. solani*, *F. solani*, *M. phaseolina* inoculum was prepared as described before for use in pathogenicity test. Plastic pots (30 cm diameter) were packed with sterilized sandy clay soil infested with fungal inocula at the rate 3% (w/w), seven days before planting. Lupine disinfested seeds were soaked in the solution of each extract (methanol, acetone and water) for 12 h, then sown in infested pots at rate 5 seeds pot^{-1}. Also, in control treatment, lupine seeds soaked in water for the same time and seeding in infested soil with the pathogen at the same rate. Five pots were used per treatment as a replicates. Percentages of damping-off and root rot severity were recorded after 30 and 90 days from planting, respectively.

2.13 Under Field Conditions

The experiments were carried out in a field naturally infested with the casual organisms of damping-off and root rot of lupine located at the experimental farm of El-Kharga Agriculture Station, New Valley governorate during two successive winter growing seasons (2014/15 and 2015/16). Selected seed samples of lupine cv. Giza 1 were treated following dipping method. The seeds were dipped in each algal extracts with the same concentration (250 µg/ml) for 12 hour in algae previously prepared extracts (Acetone, methanol and water). All treatments were arranged in a complete randomized block design with three plots as replicates. A plot was 3 ×3.5 m with five rows; seeds were sown in hills (2 seeds hill-1 and 20 cm apart). In the control treatment, lupine seeds were soaked in water for the same time and sown with the same method. Percentages of damping-off and root rot disease incidance were calculated as well as some vegetative growth and yield parameters, i.e. plant height (cm), number of branches, pods and seeds per plant, weight of 100 seeds (seed index) and total yield per feddan were recorded. Also, protein percentage content in seeds was recorded using the method of Jackson [21].

2.14 Statistical Analysis

All experiments were performed twice. Analyses of variance were carried out using MSTAT-C program version 2.10 [22]. Least significant difference (LSD) was employed to test for significant difference between treatments at P≤0.05 [23].

3. RESULTS

3.1 Isolation and Identification of the Causal Organisms

Isolation trails resulted 19 different fungal isolates obtained isolates were screened microscopically for *R. solani* (8 isolates), *F. solani* (6 isolates), and *M. phaseolina* (3 isolates) as well as 2 isolates not identified.

3.2 Pathogenicity Tests

Results presented in Fig. 1 show that all tested isolates able to infect roots of lupine causing damping-off and root rot symptoms thus reduce the survived plants. *Rhizoctonia solani* isolate LR3 recorded the highest presentage of damping-off disease being 85% followed *R. solani* by isolate LR2 (65%) then *F. solani* isolate LR10 (50%), while *R. solani* isolte LR4 gave the lowest damping-off percent (10%). On the other hand, all tested isolates caused root rot/wilt ranging between 10.25-55.36%. *Fusarium solani* isolates LR11 and LR9 followed by *M. phaseolina* isolate LR16 and *R. solani* isolate LR8 caused the highest root rot severity, whereas recorded 55.36, 55.00, 52.25 and 50.36%, respectively. While, isolate LR18 causing the lowest percentage of root rot disease.

In general, *R. solani* isolates LR3 and LR2, *F. solani* isolate LR9 and LR11, and *M. phaseolina* isolate LR16 were the more virulent ones.

3.3 Antifungal Activity

Data present in Table 1 show that all solvents extract of the four cyanobacteria resulted in significant inhibitory effect of the growth of the tested pathogenic fungi. The cyanobacteria *O. agardhii* recorded the highest suppressed of the linear growth of all tested pathogenic followed by *N. muscorum*, while the least inhibition effect of all the tested fungi were recorded in case of *A. sphaerica*. The reduction in the linear growth of the tested fungi was differed depending of type of solvent extract. In general, acetone extract of all tested cyanobacteria suppressed linear growth of the tested pathogenic fungi more than methanol and water extracts. Acetone extract of *O. agardhii* recorded the highest suppressed of all tested fungi, while methanol extract showed moderate inhibited effect of all fungal species and water extract was the lowest ones. On the other hand, *M. phaseolina* was more sensitive to cyanobacteria extracts than *R. solani* and *F. solani*.

3.4 Pot Experiment

Data present in Table 2 show that the four tested cyanobacteria able to reduced significantly damping of and root rot incidence caused by *R. solani*, *F. solani* and *M. phaseolina* compared with check treatment. The reduction of damping-off and root rot caused by pathogenic fungi were depends mainly on the type of the cyanobacteria species, type of solvent used and the tested fungal species. *Oscillatoria agardhii* was gave the highest reduction of damping-off and root rot compared with the other tested cyanobacteria in

all pathogenic tested fungi followed by *N. muscorum*. Also, acetone extract of all cyanobacteria tested was more effective for controlling damping –off and root rot diseases in lupine plants and methanol extracts were moderate, while water extracts were the least ones in this respect. In general, acetone extract of *O. agardhii* recorded the highest reduction of damping-off and root rot caused by any pathogenic tested fungi.

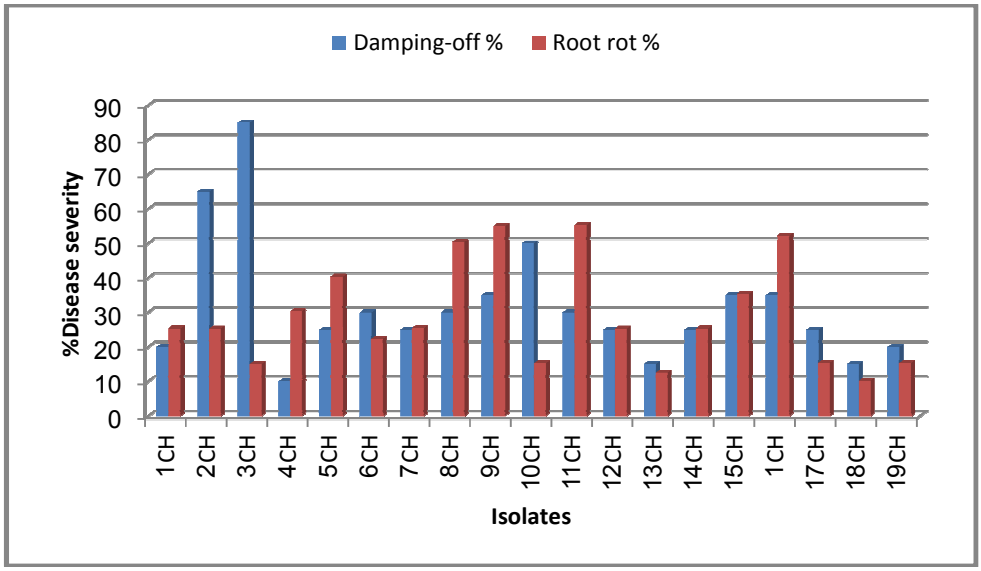

Fig. 1. Pathogenicity tests of soil borne fungi isolated from lupine roots under the greenhouse conditions. Different letters indicate significant differences among treatments within the same color column according to least significant difference test (P ≤0.05). Percentages of damping-off were recorded 30 days after planting, while root rot disease index was determined 90 days after planting

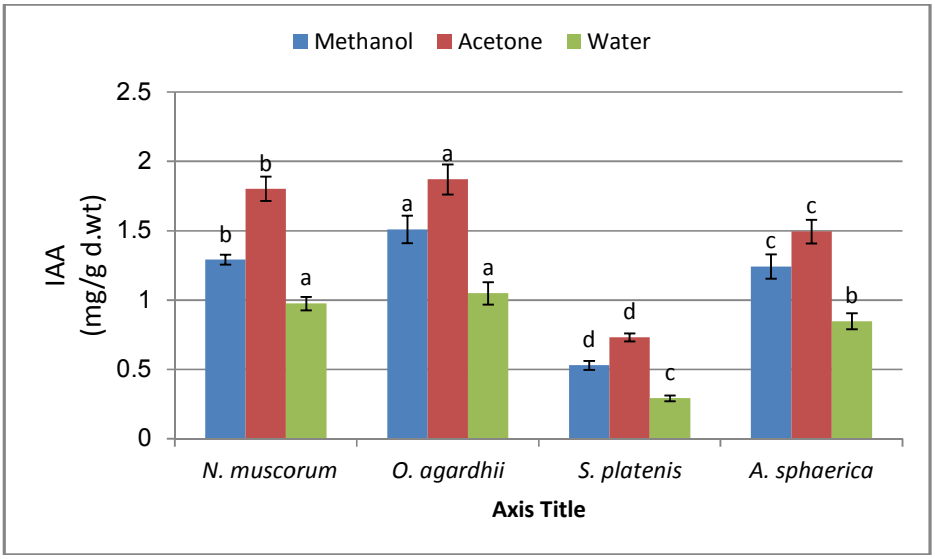

Fig. 2. IAA production in algal culture filtrates of cyanobacteria strains. Values in the column followed by different letters indicate significant differences among treatments according to LSD test at 0.05. Bars indicate the standard deviation

Table 1. Antifungal activity of cyanobacteria extracts against pathogenic fungi isolated from lupine plants

Tested cyanobacteria	Solvents extract	Inhibition (%)			
		R. solani	*F. solani*	*M. phaseolina*	Mean
Nostoc muscorum	Methanol	41.25	45.47	50.14	45.62
	Acetone	48.67	52.25	58.36	53.09
	Water	31.42	35.26	42.15	36.28
	Mean	40.45	44.33	50.22	-
Oscillatoria agardhii	Methanol	45.09	45.09	45.09	45.09
	Acetone	50.23	50.23	50.23	50.23
	Water	35.90	35.90	35.90	35.90
	Mean	43.74	43.74	43.74	-
Spirulina platensis	Methanol	26.14	30.24	35.45	30.61
	Acetone	33.12	35.36	42.15	36.88
	Water	16.59	17.36	19.09	17.68
	Mean	25.28	27.65	32.23	-
Anabaena sphaerica	Methanol	30.14	37.25	39.36	35.58
	Acetone	38.36	43.56	48.76	43.56
	Water	28.57	32.14	37.59	32.77
	Mean	32.36	37.65	41.90	-
LSD at 0.05 for:					
Tested cyanobacteria (A)	=	2.96			
Solvents extract (B)	=	3.02			
Fungi (C)	=	2.85			
Interaction (A×B×C)	=	6.58			

3.5 Secondary Metabolite Production by Cyanobacteria Extracts

The production of indole acetic acid (IAA), total phenol and flavonoid contents and protease enzyme as secondary metabolites in the extracts of *N. muscorum*, *O. agardhii, S. platensis* and *A. sphaerica* is shown in Figs. (2-5). The obtained data indicate that *O. agardhii* and *N. muscorum* were more production of all secondary metabolites than *A. sphaerica* and *S. platensis*. Acetone extract of all cyanobacteria was increased production of IAA, total phenols and flavonoid contents and protease enzyme than the other solvent extracts.

3.6 Field Experiment

3.6.1 Effect of cyanobacteria on damping-off and root rot incidence under field conditions

Damping-off and root rot severity were significantly reduced due to soaking the lupine seeds in all the tested cyanobacteria extracts before sowing compared with check treatment in both growing seasons (Table 3). In this regard, the extract of *O. agardhii* was the highest efficient in reduction of damping-off and root rot severity in both growing seasons while the extract of *S. platensis* was the lowest one for controlling damping –off and root rot. On the other hand, acetone extract of any cyanobacteria types was more effective for controlling damping-off and root rot diseases in lupine plant than methanol or water extracts. Acetone extract of *O. agardhii* recorded the highest reduction of damping-off and root rot whereas reduced damping-off from 29.5 and 32.5% in check treatment to 4.19 and 5.51% in both growing seasons respectively.

3.7 On Growth and Yield Parameters

Data present in Table 4 indicate that lupine seeds treated with cyanobacteria extracts significantly improved all growth and yield parameters *viz.* plant height (cm), number of branches plant^{-1} and yield components *viz* . No. of pods plant^{-1}, No. of seed plant^{-1}, seed index (gm), total yield fed.$^{-1}$ (kg), compared with untreated seeds in both growing seasons (2014-15 and 2015-16). The enhancement in growth and yield parameters were varied depending with cyanobacteria type and type of solvent extract. *Cyanobacteria O. agardhii* recorded highest the average growth and yield parameters in both growing seasons followed by *N. muscorum* and *A. sphaerica*. While, *S. platensis* extracts gave the lowest increased in plant growth and yield parameters. Acetone extract of all cyanobacteria improved plant growth and increased yield

parameters more than methanol and water extracts. In general, acetone extract of *O. agardhii* recoded the highest plant height (120.25 and 128.36 cm) and No. of branches (7.25 and 7.29 branch plant^{-1}) in both growing seasons, respectively. Also, this treatment recorded the highest yield parameters *viz.* No. pods plant^{-1} (22.14 and 24.33 pod plant^{-1}), No. seeds (66.96 and 69.63 pod plant^{-1}), seed index (35.26 and 35.29 gm 100 seeds^{-1}), and total yield fed.$^{-1}$ (899.36 and 910.23 Kg fed.$^{-1}$) in both growing seasons respectively. On the other hand, all cyanobacteria extracts increased protein contents in seeds but were slight increase compared with untreated seeds (control).

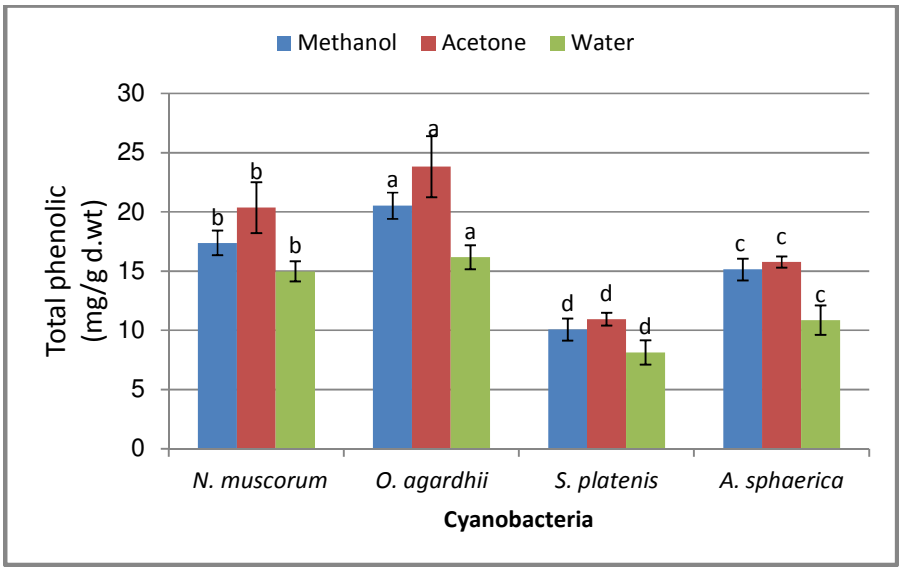

Fig. 3. Total phenols contents in the cyanobacteria culture extracts of cyanobacteria strains. Values in the column followed by different letters indicate significant differences among treatments according to LSD test at 0.05. Bars indicate the standard deviation

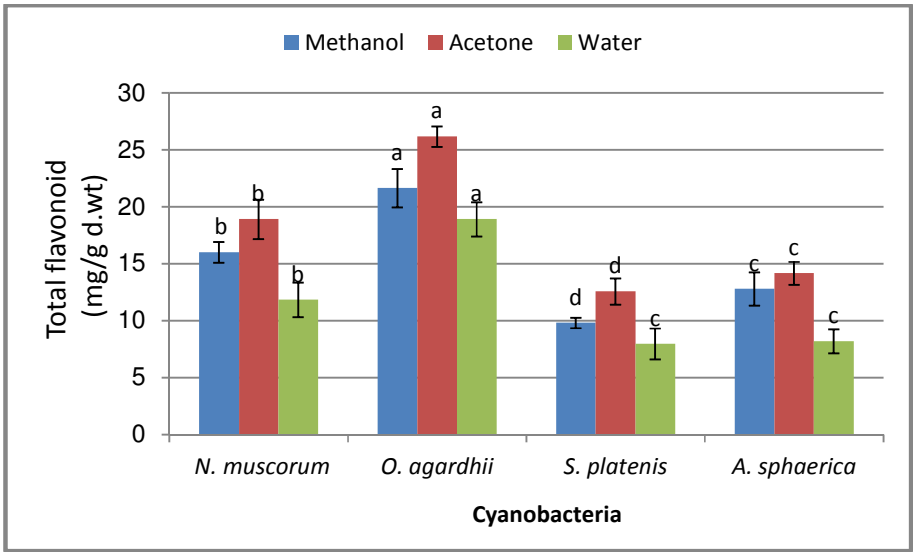

Fig. 4. Total phenols contents in the cyanobacteria culture extracts of cyanobacteria strains. Values in the column followed by different letters indicate significant differences among treatments according to LSD test at 0.05. Bars indicate the standard deviation

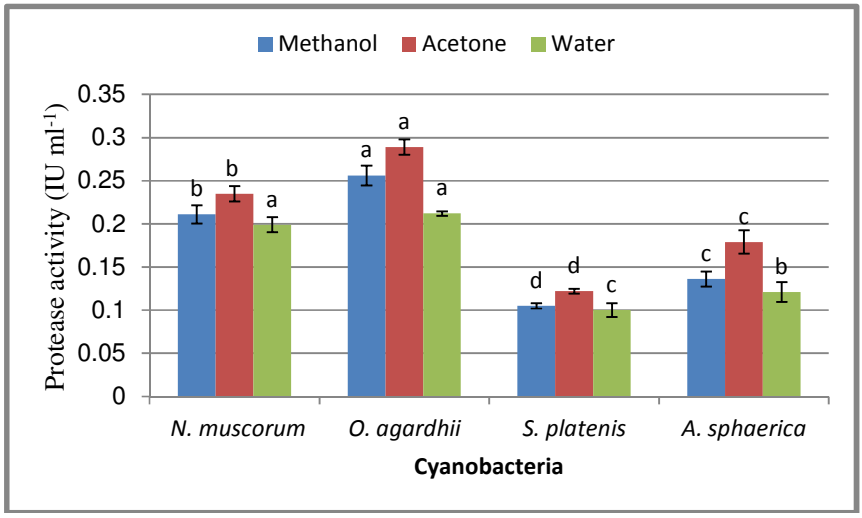

Fig. 5. The activity of protease enzymes in the algal culture extracts of cyanobacteria strains (IU ml^{-1}). Values in the column followed by different letters indicate significant differences among treatments according to LSD test at 0.05. Bars indicate the standard deviation

Table 2. Effect of soaking lupine seeds in cyanobacteria extracts on damping-off and root rot diseases under artificial infection with *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* in pots

Tested cyanobacteria	Solvents extract	*R. solani*		*F. solani*		*M. phaseolina*	
		% Damping-off	% Root rot	% Damping-off	% Root rot	% Damping-off	% Root rot
Nostoc muscorum	Methanol	30.00	14.90	10.0	18.30	15.0	20.33
	Acetone	25.00	11.47	5.0	12.14	10.0	15.02
	Water	45.00	18.25	10.0	25.30	20.0	27.36
	Mean	33.33	14.87	8.33	18.58	15.00	20.90
Oscillatoria agardhii	Methanol	25.00	10.30	5.0	10.47	5.0	12.14
	Acetone	15.00	7.25	0.0	7.56	0.0	8.47
	Water	40.00	14.58	5.0	18.47	15.0	18.75
	Mean	26.67	10.71	3.33	12.17	6.67	13.12
Spirulina platensis	Methanol	30.00	19.36	20.0	20.36	15.0	28.5
	Acetone	35.00	15.47	15.0	17.45	15.0	21.14
	Water	50.00	22.47	25.0	28.36	25.0	32.47
	Mean	38.33	19.10	20.00	22.06	18.33	27.37
Anabaena sphaerica	Methanol	25.0	15.47	10.0	17.24	10.0	18.25
	Acetone	20.0	13.25	5.0	15.47	10.0	15.47
	Water	45.0	20.14	15.0	25.32	20.0	22.3
	Mean	30.00	16.29	10.00	19.34	13.33	18.67
Control		70.0	30.0	35.0	50.42	35.0	45.09
LSD at 0.05 for:		Damping-off			Root rot		
Tested cyanobacteria (A)	=	3.96			1.96		
Solvents extract (B)	=	3.24			2.32		
Fungi (C)	=	2.85			2.09		
Interaction (A×B×C)	=	7.89			5.48		

4. DISCUSSION

Several soil-borne fungi attack lupine plants during its various growth stages from seedling till maturity causing damping-off and root rot diseases. The present results indicated that damping-off and root rot diseases are incited by the soil borne fungi, *Rhizoctonia solani,*

Fusarium solani and *Macrophomina phaseolina*. These results are in agreement with these obtained by Abdel-Monaim et al. [3].

The management of soil-borne plant pathogens is particularly complex because these organisms live in or near the dynamic environment of the rhizosphere and can frequently survive a long period in soil through the formation of resistant survival structures. Several commercially available products have shown significant disease reduction through various mechanisms to reduce pathogen development and disease. Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. However, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture.

Acquired resistance by using biotic agents as biological control seems to be one of alternatives to substitute for, or at least to decrease the use of fungicides in plant disease control. Excessive and improper use of pesticides including fungicides presents a menace to the health of human, animal and environment [24]. The present study was planned mainly to investigate the possibility of minimizing the infection by damping-off and root rot diseases of lupine using the extracts of four cyanobacteria as a biological control agents.

In this study, solvents extract of the four cyanobacteria *viz. Nostoc muscorum*, *Oscillatoria agardhii*, *Spirulina platensis* and *Anabaena sphaerica* resulted in significant inhibitory effect of the growth of the tested pathogenic fungi *in vitro.* The acetone extract of *O. agardhii* was more effective for suppressed of all tested pathogenic fungi than the others.

On the other hand, the obtained data showed that lupine seeds treated with cyanobacteria extracts recorded the highly protection against infection with soil borne pathogens more than untreated seeds under greenhouse and field conditions and significantly improved plant growth and yield parameters under field conditions. The solvent extracts of all cyanobacteria more active for reducing damping-

Table 3. Effect of soaking lupine seeds in cyanobacteria extracts on damping-off and root rot diseases under field condition during 2014-15 and 2015-16 growing seasons

Tested cyanobacteria	Solvents extract	Season 2014-15		Season 2015-16	
		% damping-off	% root rot	% damping-off	% root rot
Nostoc muscorum	Methanol	8.69	6.75	11.03	7.94
	Acetone	6.55	5.19	9.19	6.12
	Water	12.58	8.26	15.54	9.73
	Mean	9.27	6.73	11.92	7.93
Oscillatoria agardhii	Methanol	6.99	4.66	9.19	5.49
	Acetone	4.19	3.28	5.51	3.87
	Water	11.18	6.6	14.7	7.77
	Mean	7.45	4.85	9.80	5.71
Spirulina platensis	Methanol	12.36	8.76	14.03	10.32
	Acetone	9.78	7.00	9.86	8.25
	Water	14.23	10.17	18.38	11.98
	Mean	12.12	8.64	14.09	10.18
Anabaena sphaerica	Methanol	10.23	12.03	16.19	14.02
	Acetone	8.25	9.12	13	12.05
	Water	17.02	14.36	19.54	16.23
	Mean	11.83	11.84	16.24	14.10
Control		29.5	22.9	32.5	21.03
LSD at 0.05 for:		Damping-off		Root rot	
Tested cyanobacteria (A) =		1.96		1.02	
Solvents extract (B) =		2.12		1.32	
Seasons (C) =		NS		NS	
Interaction (A×B×C) =		5.21		3.26	

Table 4. Effect of soaking lupine seeds (cv. Giza 1) in cyanobacteria extracts on plant growth and yield parameters under field conditions during 2014-15 and 2015-16 growing seasons

Tested cyanobacteria	Filtrate extracts	Plant height (cm)		No. of branches plant^{-1}		No. of pods plant^{-1}		No. of seed plant^{-1}		Seed index (gm)		Total yield fed.$^{-1}$ (Kg)		% Protein	
		Season 2014-15	Season 2015-16	Season 2014-15	Season 2015-16	Season 2014-15	Season 2015-16	Season 2014-15	Season 2015-16	Season 2014-15	Season 2015-16	Season 2014-15	Season 2015-16	Season 2014-15	Season 2015-16
Nostoc muscorum	Methanol	90.35	95.23	6.42	6.52	16.35	18.23	48.23	52.36	31.45	31.25	715.36	726.36	37.23	37.33
	Acetone	105.36	109.32	6.96	7.02	18.25	20.12	55.42	58.36	32.14	32.25	755.36	766.21	37.36	37.25
	Water	88.41	92.04	5.56	6.01	14.36	15.23	43.56	45.02	31.25	31.36	686.25	692.35	36.96	36.89
	Mean	94.71	98.86	6.31	6.52	16.32	17.86	49.07	51.91	31.61	31.62	718.99	728.31	37.18	37.16
Oscillatoria agardhii	Methanol	102.36	105.36	7.02	7.25	18.69	19.99	56.38	59.03	33.36	33.35	825.36	836.35	38.23	38.36
	Acetone	120.25	128.36	7.25	7.29	22.14	24.23	66.96	69.63	35.26	35.29	899.36	910.23	38.35	38.42
	Water	95.36	104.32	6.09	6.28	16.58	18.23	50.25	53.27	31.02	31.22	755.25	766.32	37.58	37.29
	Mean	105.99	112.68	6.79	6.94	19.14	20.82	57.86	60.64	33.21	33.29	826.66	837.63	38.05	38.02
Spirulina platensis	Methanol	82.36	88.02	4.96	5.03	14.25	15.26	43.26	46.69	30.25	30.7	523.36	539.36	36.56	36.59
	Acetone	88.36	92.54	5.23	5.29	16.35	17.36	50.02	54.06	30	30.25	556.23	569.08	37.02	36.09
	Water	75	78.23	4.56	4.86	14.02	15.02	44.26	48.36	29.89	30	505.36	515	36.05	36.09
	Mean	81.91	86.26	4.92	5.06	14.87	15.88	45.85	49.70	30.05	30.32	528.32	541.15	36.54	36.26
Anabaena sphaerica	Methanol	96.09	99.02	6.02	6.22	16.35	17.36	50.02	54.36	31.12	31.25	582.23	596.36	36.59	36.65
	Acetone	102	108.26	6.53	6.85	18.29	19.86	56.23	60.14	32.15	32.02	612.32	625.35	36.99	36.92
	Water	84.23	89.25	5.32	5.83	15	16.02	46.03	48.23	30.25	30.42	555.02	566.36	35.63	35.41
	Mean	94.11	98.84	5.96	6.30	16.55	17.75	50.76	54.24	31.17	31.23	583.19	596.02	36.40	36.33
Control		65.23	67.25	4.25	4.55	10.42	11	30.16	32.23	28.01	28.06	445.23	452.36	34.21	33.36
LSD at 0.05 for:															
Tested Cyanobacteria (A)	=	5.26		0.95		1.52		2.63		1.52		11.25		2.01	
Filtrate extracts (B)	=	4.59		0.84		1.66		3.02		NS		10.19		1.69	
Seasons (C)	=	NS		NS		NS		NS		NS		NS		NS	
Interaction (A×B×C)	=	9.26		NS		2.26		5.26		NS		25.06		NS	

off and root rot more than water extract. Acetone extract of all cyanobacteria more effective than the others either under greenhouse or field conditions especially in case of *O. agardhii*. In addition, the efficiency of the cyanobacteria strains was positively correlated with the content of indole acetic acid (IAA), total phenols and flavonoids compounds and protease enzyme as secondary metabolites in the extracts of *N. muscorum*, *O. agardhii, S. platensis* and *A. sphaerica*, where the treated seeds with the acetone extract of *O. agardhii* and/or *N. muscorum* were more efficient in decreasing the infection by soil borne pathogens in greenhouse and in fields as well as increasing plant growth and yield components compared with the other cyanobacteria. These results are in conformity with the obtained data by De Caire et al. [25] who reported that extracellular products from *N. muscorum* are promising as a biological control of soybean seedling damping-off. Kulik [11] mentioned that filtrates or cell extracts from cyanobacteria were applied to seeds as protectants against damping-off fungi such as *Fusarium* sp., *Pythium* sp. and *R. solani*.

On the other hand, the potential activity of cyanobacteria to inhibit certain soil borne diseases could be attributed to produce a wide range of plant growth regulators such as abscisic acid, ethylene, jasmonic acid, auxin and cytokinin- like substances as well as the cytokinin isopentenyl adenine [26]. The antifungal activity has been due to the presence of plant bioactive compounds, i.e total phenolic compounds, total saponins and alkaloids in the algal culture filtrates, employed as natural defense mechanisms against pathogenic bacteria, fungi, viruses and pests [27]. The capacity of indole acetic acid biosynthesis has been found in free-living and symbiotic cyanobacteria of the genera *Nostoc, Chlorogloeopsis, Calothrix, Plectonema, Gloeothece, Anabaena, Cylindrospermum* and *Anabaenopsis* [28]. The activity of protease enzymes in the cyanobacteria filtrates revealed that hydrolytic enzymes may contribute to be the fungicidal activity of the cyanobacterial strains, besides other bioactive compounds, including indole acetic acid [9]. Many cyanobacteria are known to release various kinds of biologically active substances like proteins, vitamins, carbohydrates, amino acids, polysaccharides and phytohormones that function as elicitor molecules to promote plant growth and help them to fight against biotic and abiotic stress. These metabolites produced by the cyanobacteria affect the gene expression of the host plants and thereby bring about qualitative and quantitative changes in the phytochemical composition of the plants [29].

5. CONCLUSION

Cyanobacteria known as biocontrol agents bring about induced systemic resistance (ISR) fortifying the physical and mechanical strength of the cell wall and changing physiological and biochemical reaction of the host leading to synthesis of defense chemicals against the pathogens. Such changes can lead to enhanced plant growth, improved yields and quality of produce. Although induction of systemic resistance has been studied by different investigators, little is known about the mechanisms of nitrogen fixing cyanobacteria in helping plants to overcome biotic stress induced by the pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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