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Two cyanobacterial culture filtrates as biocides for controlling damping-off and root-rot diseases in fennel (*Foeniculum vulgare* L)

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Abstract

The present study was conducted to evaluate in vitro and in vivo the biological control aptitude of the two cyanobacteria i. e., Anabeana variabilis and Nostoc paludosum, cultural filtrates against Fusarium solani, Macrophomina phaseolina and Pythium aphanidermatum . Culture filtrates of both blue-green algal had antifungal effect against growth of the examined fungi. Under artificial inoculation conditions in a greenhouse experiment, both algae culture filtrates were found to be effective against fennel damping-off and root-rot diseases compared with untreated control. Nostoc paludosum culture filtrate was the more effective than A. variabilis in reducing damping off and root-rot diseases as well as increased activity of hydrolytic enzymes including peroxidase, polyphenoloxidase, chitinase as well as oil content, soluble proteins and total phenols of fennel plants. Under natural field conditions during 2020/ 2021 and 2021/ 2022 seasons, both algal had biocidal effect against the fennel soil-borne diseases and significantly increased growth parameters and plant yield. It was observed that both blue-green algal filtrates increased volatile oil percentage and its frequency compared with untreated seeds.

Keywords: Algae; fennel; soil-borne diseases; Anabaena variabilis; Nostoc paludosum; oil content.

Introduction

Fennel (*Foeniculum vulgare* L) is a member of Umbellifera (Apiaceae). Fennel is considered one of the most popular medicinal crops in the world, which is used against some diseases like cholera, biliousness, dysentery, diarrhea, cough, cold, constipation and as flavouring agent in manufacturing [1, 2]. Fennel attacks by fungal diseases cause considerable yield losses. However, soil-borne pathogens are considered the major factors for the reduction in fennel yield. Among these diseases, seedlings damping-off of seedlings by *Pythium* spp. and *Rhizoctonia solani*, root and crown-rot by *Fusarium* spp., *R. solani*, *Pythium* spp. and *Sclerotium rolfsii*, stem-rot by *Sclerotinia sclerotiorum)* and Fusarium wilt (*Fusarium oxysporum* f.sp. *funiculi*) are the common and most serious diseases of fennel in Egypt and in the world [3, 4, 5]. Damping-off and root rot causes seeds, seedling death at early to late stage resulting in very poor plant stand which ultimately produces very low yield [6, 7, 5]. High crop yield requires extensive use of chemical pesticides for protection against fungal pathogens and these chemical compounds may be phytotoxic to the plant. Seed treatment and soil drenching reduces the risk of environmental pollution, health hazard and not much costly to the growers . The use of biological control represents one of the strategies to control and combat harmful pathogens naturally and represents less harm due to its high nature of sustainability and its outstanding activity as biocides in the required doses [8, 9]. There have been many of successful uses of biocontrol agents of soil-borne fungi causing seedlings damping off and root-rot [10]. Among all the microorganisms, algae are one of the chief biological agents that have been studied for control of plant pathogens, particularly soil-borne fungi [11, 12, 13, 14, 15, 16]. A number of cyanobacteria and eukaryotic algae particularly macroalgae, produce various biologically active compounds, which could be could operate in biological control of plant pathogens includes enzymes, peptides, antibiotics and toxins [17]. *Nostoc* sp. and *Anabaena* sp., are the natural source of biocides against fungi and bacteria [18, 19, 20, 21, 22]. The biofungicidal potential culture filtrate of the blue- green algae has been tested against damping-off and root- rot of faba bean and bean [23]. The aim of this study was to evaluate the effect of two cyanobacteria *i. e., Anabeana variabilis* and *Nostoc paludosum*, cultural filtrates in controlling of fennel soil-borne diseases caused by *Fusarium solani*,

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Macrophomina phaseolina, Pythium aphanidermatum in vitro and *in vivo*. Also, studying their effect on plant yield, the activity of enzymes responsible for disease resistance *i.e.,* peroxidase , polyphenol oxidase and chitinase as well as oil content , soluble proteins and total phenols of the grown plants in soli infested with the tested pathogens.

2. Experimental work

2.1. Materials

Pathogenic fennel fungi: Soil-borne fungi were isolated from diseased root parts of fennel plants grown in fields located at El Qanater El-Khairia location, Qaluobia and Menoufia governorates, Egypt. Root samples were washed, air dried ,cut into small pieces, surface sterilized by dipping in 0.1% mercury chloride solution for two minutes and then washed again with sterile water. The sterilized pieces were put in Pert-dishes contained sterilized Potato Dextrose Agar Plates (PDA) medium and incubated at 28 ± 2 °C for 5 days. The isolated fungi were purified using either a single spore method or a hypha tip technique and then the genera and fungal species were identified according to Booth. [24] and Domsch *et al*., [25].

Algae: *Anabaena variabilis* and *Nostoc paludosum* were obtained from Dept. of Agric. Microbiol., Nat. Res. Cent., Cairo, Egypt.

2.2. Preparation of the culture filtration

Pure algal cultures of *A. variabilis* and *N. paludosum* were carried out in modified PG11 liquid medium [14] under optimal conditions of continuous white fluorescent light intensity at 950 lux and at 28 °C, for 14 days according to [26, 14, 27]. The biomass of algal was separated from the culture filtrate by centrifugation for 40 mins, speed of 8000 rpm at 10 ^{0}C under sterilize conditions.

2.2.1. Antifungal effect of algal culture filtrate

The antifungal effect of algal culture filtrates was evaluated against growth of the three pathogenic fungi *i. e., Fusarium solani, Macrophomina phaseolina and Pythium aphanidermatum*. Each filtrate was taken under sterilized conditions and added to sterilized PDA medium before pouring in sterilized Petri dishes to give 25 and 50% (v/v). Three replicates were used for each treatment. All dishes were inoculated with 4mm discs, 7-day-old of tested fungi. Plates containing PDA medium with 4mm discs, 7-day-old of tested fungi only were used as control (free algal filtrates). Plates were incubated at 25 ± 2 °C for 5 days. The linear growth (mm) of the three pathogenic tested fungi was measured when the growth of the pathogenic fungi of control treatment completely covered plate.

2.2.2. Total phenols content.

The total content of phenols was evaluated using the Folin–Ciocalteu. Methods described by Lamuela-Raventós [28]. The absorbance was measured at 765 nm. The results were expressed as gallic acid equivalent (GAE)/100 g dry sample.

2.3. Greenhouse experiment

The experiment was carried out in pots (25cm), kept in the greenhouse of Nat. Res. Cent. (NRC). Soil used (clay /sand 2:1) was autoclaved. All the three tested fungi were grown on sterilized corn / sand medium for 15 days at 25° C. Three g of each inoculum per 1kg soil (clay /sand 2:1) were mixed and irrigated for 7days period before sowing. Fennel seeds (local variety obtained from Hort. Res. Inst., Medici. and Arom. Plants Dept. were surface sterilized using 0.1% sodium hypochlorite for three minutes, and washed three times with sterilized water. The seeds were soaked in algae filtrate at the best previous concentration for 10 minutes according to Scott [29]. Ten seeds of fennel were sown per pot (25 cm in diam.) and five pots were used as replicates for each treatment. Control treatment free from algal filtrates was used. Also, both algal filtrates were sprayed on the grown plants 15 days after sown at 30 ml / pot.

2.3.1. Diseases assessment

Pre-and post-emergence damping-off were recorded 15 and 30 days after sowing, whereas the survived plants were assessed 45 days after sowing [16, 5].

2.3.2. Enzymes assay

Determination of polyphenoloxidase (PPO): Polyphenoloxidase PPO activity was assayed by measuring the increase in absorbance at 420 nm using a spectrophotometer. Catechol was used as the substrate following the methods described by Shi *et al*., [30]. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001/minute

Determination of peroxidase (P): Peroxidase (P) activity was assayed by recording 3 ml reaction mixture. The reaction mixture contained 0.1 mM EDTA, 1 ml of 0.2 mol/m3 potassium phosphate buffer with pH = 7.6, 0.1 ml of 2 mM (NADPH), 0.5 ml of 3 mM DTNB, 0.1 ml enzyme extract. Reaction initiated by adding of one unit of P activity. Peroxidase activity was measured at 412 nm using a spectrophotometer. Protein extract was quantified using the methods described by Bradford [31].

Determination of chitinase: Chitinase activity was determined adopting the methods described by Vahed *et al*., [32].

Determination of soluble proteins activity: Total soluble protein was measured following the methods stated by Stocheck [33].

2.4. Field experiment

The experiments were carried out in a field has a back history of high infestation with pathogenic soil-

borne fungi at El Qanater El-Khairia (Bahada location), Qaluobia governorate during two successive winter growing seasons (2020/2021 and 2021/2022). Fennel seeds (local variety obtained from Hort. Res. Inst., Medici. and Arom. Plants Dept.)were soaked for 10 minutes in any of both algal filtrate before sowing [16]. The commercial was used as control. Untreated seeds were used as control treatment. Each plot was 3 x 3.5 m. Twenty five seeds were sown on each ridge and four ridges / plot (100 plant/ plot). Four replicates were used for each treatment in complete randomized plots design.

2.4.1. Disease assessment

Percentage of pre- and post- damping-off were assessed 15 and 30 days after sowing. Survival plants were recorded after 45 days from sowing.

2.4.2. Crop parameters

Crop parameters i.e., number of branches, plant height (cm), dry weight of inflorescences (g) , dry weight of seeds (g) , weight of 1000 seeds (g) and total yield / plant (g) were determined at the end of each growing season for the tested treatments and the control .

2.4.3. Essential oils assay

Content: Essential oils percentage % of each sample was determined with hydro-distillation for 3 hours at Clevenger-type apparatus using 100 g of each sample according to [34]. The resulted essential oil of each treatment was separately dehydrated with anhydrous sodium sulphate and kept in the deep freezer until GC-MS analyses.

Frequency: The GC-Ms analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Dept. of Medici, and Arom. Plants Res., Nat. Res. Cent. with the following specifications. Instrument: TRACE GC Super Gas Chromatograph (THERMO Scientific Corp., USA), the instrument contains a THERMO mass spectrometer detector. The system was fitted to the GC-MS device by means of a TG-WAX MS column (30 m × 0.25 mm, 0.25 µm film thickness). The analysis was performed using helium gas and the mass spectra of the compounds were obtained by electron ionization (EI) at 70 eV and spectral range of m/z 40-450. The presence and proportions of the compounds were determined using mass spectra (Original Chemicals, Wiley Spectral Library Collection and NSIT Library).

Statistical analysis: Experiments were analyzed with the analysis of variance (ANOVA). The LSD range tests were used for comparisons according to Steel *et al*.,[35]. The means of the treatments were compared using the least significant difference (LSD) test at the 0.05 level. Experimental data were presented as means and standard deviations data were analyzed with SAS, version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Antifungal effect of algal culture filtrate

Data in Table (1) indicate that both tested algal filtrates were effective against the linear growth of tested fungi. *N. paludosum* filtrate was the more effective than *A.variabilis* in reducing linear growth of *F. solani*, *M. phaseolina* and *P. aphanidermatium.* Since, *N. paludosum* filtrate significant reduced linear growth of *M. phaseolina* (2.66 ± 0.20 mm) with 97.00 ± 0.20 % reduction, *F. solani* (4.33 ± 0.88 mm) equal 95.13 ± 0.97% of reduction and *P.aphanidermatium* (5.33 ± 0.88 mm) equal 94.00 ± 0.94% of reduction when compared with untreated control (90.00 ± 0.00 mm).

3.2. Total phenols content

The crude extract of the *A. variabilis* and *N.paludosum* algal filtrates gave positive tests for total phenols content (Figure 1). The estimation of total phenols content in the crude extract revealed that *N. paludosum* contained 59.7 mg GAE/100 g, meanwhile *A. variabilis* contained 32.6 mg GAE/100 g.

3.3. Greenhouse experiment

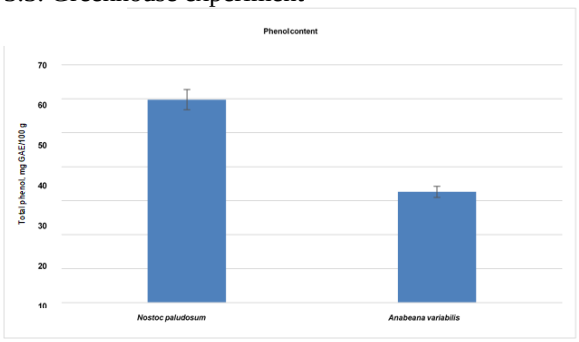

Figure (1) Total phenols content of the crude extract of *A. variabilis* and *N. paludosum* , algal filtrates

Table (1) Evaluation of the tested algal filtrates in reducing the linear growth of tested fungi *in vitro*.

Treatment		Linear Growth (mm) of tested fungi					
		M. phaseolina		*F.solani*		*P.aphanidermatium*	
	Conc.(%)	(mm)	%R**	(mm)	%R	(mm)	%R
A.variabilis	25	24.33 ± 4.25*	72.93 ± 4.73	20.00 ± 2.08	77.73 ± 2.31	19.00 ± 2.64	78.83 ± 2.94
	50	15.60 ± 2.21	82.60 ± 2.46	10.60 ± 0.83	88.16 ± 0.95	9.66 ± 1.20	89.23 ± 1.35
N. paludosum	25	9.60 ± 1.13	89.30± 1.28	10.00 ± 1.15	88.83 ± 1.29	11.33 ± 0.88	87.33 ± 0.97
	50	2.66 ± 0.20	97.00 ± 0.20	4.33 ± 0.88	95.13 ± 0.97	5.33 ± 0.88	94.00 ± 0.94
Control	-----	90.00 ± 0.00	-	90.00 ± 0.00	-	90.00 ± 0.00	-
LSD		2.67	3.36	2.33	3.65	1.96	1.67

*Experimental data were showed as means ± standard deviations

The means of the treatments were compared using the least significant difference (LSD) test at the 0.05 level. **%R= Reduction percent

3.3.1. Data in Table (2) indicate that *A. variabilis* and *N. paludosum* filtrates were effective in suppressing damping-off and root-rot diseases of fennel. Additionally, results Show that *N. paludosum* filtrate was superior than *A. variabilis* filtrate in reducing damping-off and root-rot diseases. Otherwise data indicate that *N. paludosum* decreased damping-off caused by *F. solani* from 3.40±0.44 to 0.80±0.30 %, whereas reduced root-rot from 2.00±0.28 to 0.20±0.03% equal 80.0% reduction and increased the survived plants from 4.45±0.72 to 9.00±0.33% under greenhouse conditions. The results reveal that *M. phaseolina* was less affected by *A. variabilis*, which decreased damping-off from 4.60±0.39 % in control treatment to 1.40±0.26 %, root-rot from 2.40±0.28 to 1.00±0.42 % and increased survived plants from 2.96±0.46% in control treatment to 7.60±0.61 %.

Table (2): Efficacy of the tested algal filtrates in controlling the tested three pathogenic fungi under greenhouse conditions.

Treatments	% Damping-off and root-rot caused by								
	F. solani			*M. phaseolina*			*P. aphanidermatium*		
	Damping off	Root-rot	%Survival plants	Damping off	Root-rot	%Survival plants	Damping off	Root-rot	%Survival plants
A. variabilis	1.00±0.27*	0.60±0.21	8.40±0.32	1.40±0.26	1.00±0.42	7.60±0.61	1.20±0.17	0.60±0.15	8.20±0.14
N. paludosum	0.80±0.30	0.20±0.03	9.00±0.33	1.20±0.37	0.60±0.24	8.20±0.37	1.00±0.31	0.60±0.24	8.40±0.40
+ve control (treated with tested fungi)	3.40±0.44	2.00±0.28	4.45±0.72	4.60±0.39	2.40±0.28	2.96±0.46	4.40±0.68	1.20±0.32	4.40±0.98
-ve control (untreated with tested fungi)	0.00±0.00	0.00±0.00	10.0±0.00	0.00±0.00	0.00±0.00	10.0±0.00	0.00±0.00	0.00±0.00	10.0±0.00
LSD	1.66	1.63	3.33	1.68	3.33	4.69	1.69	3.33	1.78

*Experimental data were showed as means ± standard deviations
The means of the treatments were compared using the least significant difference (LSD) test at the 0.05 level.

3.3.2. Enzymes assay

Results in Figure (2) indicate that both of algal filtrates increased the polyphenol oxidase activity with the three tested fungi .The highest increase was obtained with *N. paludosum*, which increased polyphenol oxidase activity by 0.15, 0.12 and 0.13 mg/min, respectively, followed by *A. variabilis*, being 0.11, 0.09 and 0.08 mg/min compared with untreated control and inoculated with *M. phaseolina, F. solani* and *P. aphanidermatium*, being 0.014 ,0.013 and 0.011 mg/min, respectively. Meanwhile, un-inoculated control recorded 0.017 mg/min.

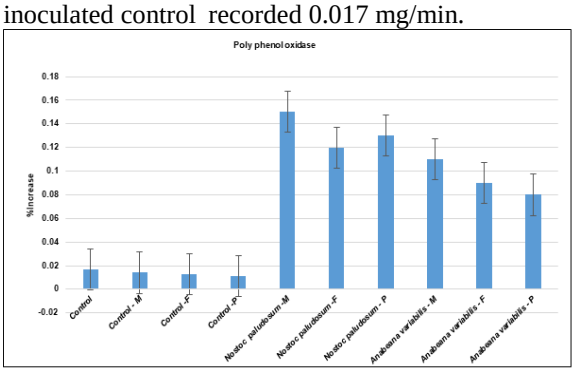

Figure (2) Effect of the tested algal filtrates on poly phenyl oxidase activity of fennel plants grown under artificial with the three tested pathogenic fungi.

Results in Figure (3) present that, both of algal filtrates increased the peroxidase activity with the three tested fungi *M. phaseolina, F. solani* and *P. aphanidermatium* .The highest increase was obtained with *N. paludosum*, which increased peroxidase activity by 0.653, 0.304 and 0.2305 mg/min, respectively, followed by *A.variabilis*, being 0.403, 0.225 and 0.198 mg/min, respectively compared with untreated control inoculated with any of the three tested fungi, being 0.193, 0.176 and 0.131 mg/min, respectively. Meanwhile un-inoculated control recorded 0.0854 mg/min.

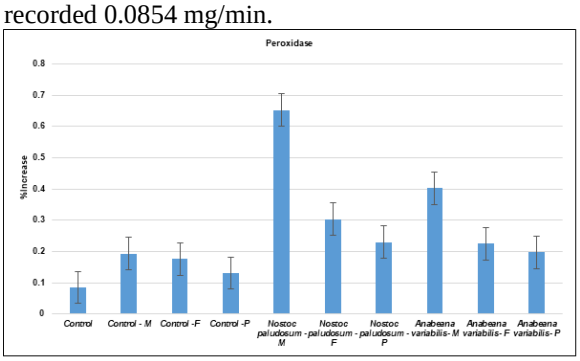

Figure (3) Effect of tested algal filtrates on peroxidase activity of fennel plants grown under artificial with the three tested pathogenic fungi.

Results in Figure (4) show that both of algal filtrates increased chitinase activity with the three tested fungi *M. phaseolina, F. solani* and *P. aphanidermatium*. The highest increase was obtained with *N.paludosum*, which increased chitinase activity by 840.704, 678.3605 and 683.25 ppm , respectively followed by

A. variabilis, being 629.166, 530.122 and 517.704 ppm, respectively compared with untreated control inoculated with any of the three tested fungi, being 592.5, 561.7 and 542.8 ppm as glucose respectively. Meanwhile non inoculated control gave 513.8 ppm as glucose.

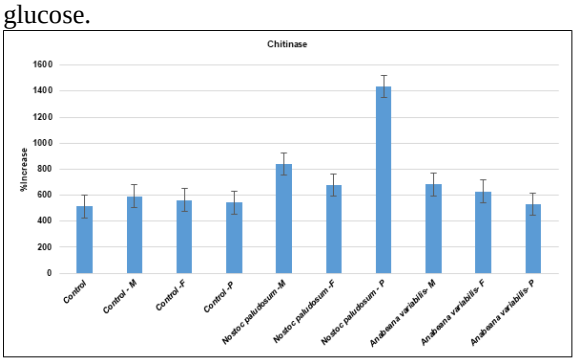

Figure (4) Effect of tested algal filtrates on chitinase activity of fennel plants grown under artificial with the three tested pathogenic fungi.

Results in Figure (5) reveal that, both of algal filtrates increased the soluble proteins activity with the three tested fungi *M. phaseolina*, *F. solani* and *P. a phanidermatium* .The highest increase was obtained with *N.aludosum*, which increased soluble proteins activity by 47.6, 46.0 and 52.5 mg/ml, respectively followed by *A. variabilis*, being 45.6, 42.8 and 39.6 mg/ml, respectively compared with untreated control inoculated with any of the three tested fungi, being 40.7, 40.2 and 39.7 mg/ml, respectively. Meanwhile un-inoculated control signalized 39.8 mg/ml.

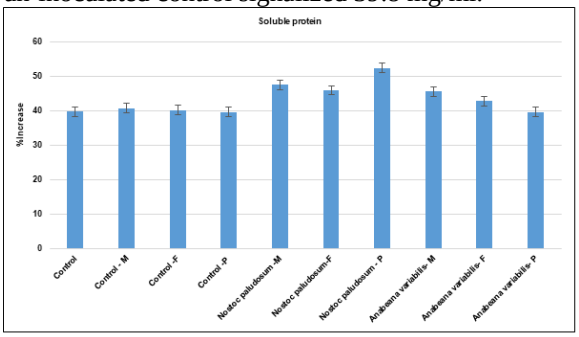

Figure (5) Effect of tested algal filtrates on soluble proteins activity of fennel plants grown under artificial with the three pathogenic tested fungi.

3.4. Field experiment

3.4.1. Data presented in Table (3) reveal that both of algal filtrates reduced reducing (Pre- and Post-emergency) damping-off. Also, data show that, *A.variabilis* and *N. paludosum* , filtrates were found to reduce the disease incidence compared with control during the two successive growing seasons of 2020/2021 and 2021/2022. *N. paludosum* showed the highest effect in reducing disease incidence 1.75±0.47 and 2.50±0.26% followed by *A. variabilis*, being 2.25±0.62 and 2.71±0.28% during the two successive growing seasons (2021 and 2022), respectively in comparison with the control.

3.4.2. Data presented in table (4) that applying any of the tested algal filtrates for treating fennel seeds increased yield growth parameters in both seasons of 2020/2021 and 2021/2022 growing seasons . *N. paludosum* was the most effective than *A.variabilis* which showed the highest increased in plant height, number of branches, dry weight of inflorescences ,dry weight of seeds ,dry weight of 1000 seeds, and plant yield during the two successive growing seasons being 197.0±17.5 cm, 9.3±1.1 , 219.0±20.8g, 192.0±18.4g, 29.3±3.4g, 510.9±23.4 g, respectively in the 2020/2021growing season and gave 214.0±24.1cm, 27.0±3.5g, 310.0±36.0g, 196.4±17.0g, 31.2±2.0g, 649.5±64.0g, respectively in 2021/ 2022 growing season in comparison with the control.

3.4.3. Effect of the tested algal filtrates on volatile oil percent of fennel seeds and its components: Data shown in Figure (6) show that both *A. variabilis* and *N. paludosum* filtrates increased oil percentage in fennel plants compared with treated commercial biocide and untreated control . The highest oil percentage was observed in plants treated with *N. paludosum*, which increased from 0.18 to 1.55%. Meanwhile, *A. variabilis* increased oil percentage from 0.18 in control treatment to 1.5 % and the commercial biocide gave moderate effect (0.95%).

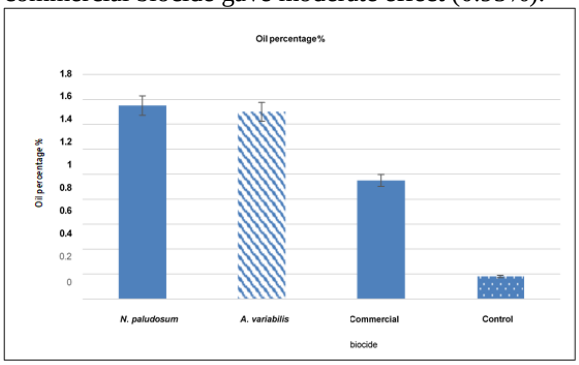

Figure (6) Effect of the tested algal filtrates on volatile oil percent of fennel seeds.

3.4.4. Effect of the tested algal filtrates on volatile oil composition: A total of 14 components were identified by GC-Ms from the essential oil of fennel plants. The composition of essential oil is shown in Table (5). *N. paludosum* was superior in increasing all the compounds of the essential oils than *A. variabilis* filtrate. *N. paludosum* increased the main compounds of the essential oils (estragole) from 77.17% in control treatment, 86.76% in commercial biocide to 87.17%. Meanwhile, *A. variabilis* increased the percentage of

estragole from 77.17 to 86.84%. L-fenchone and L-fenchone were also found as major components, which increased in fennel plants treated with *N. paludosum* the same results were recorded with *N. paludosum.*

Table (3): Effect of tested algal filtrates in controlling fennel soil borne disease under field conditions during 2020/ 2021 and 2021/2022 growing seasons**.**

Treatment	2020/2021 season				2021/2022 season			
	Damping off	Root-rot	% incidence	%Survival Plants	Damping off	Root-rot	% incidence	%Survival Plants
A. variabilis	1.50±0.50*	0.75±0.25	2.25±0.62	22.75±0.62	2.25±0.09	1.00±0.00	2.71±0.28	18.53±3.77
N. paludosum	1.25±0.25	0.50±0.28	1.75±0.47	23.25±0.47	1.75±0.02	0.75±0.25	2.50±0.26	15.00±4.48
Commercial biocide	1.50±0.50	1.25±0.25	2.75±0.62	22.25±0.62	1.75±0.25	1.25±0.25	3.00±0.40	22.00±0.40
Control	7.25±1.88	2.50±0.64	9.75±1.25	15.25±1.25	7.50±0.64	3.00±0.91	10.50±0.86	14.50±0.86
LSD	0.6	0.65	2.3	2.65	2.41	0.66	2.23	2.63

*Experimental data were showed as means ± standard deviations
The means of the treatments were compared using the least significant difference (LSD) test at the 0.05 level.

Table (4). Efficacy of tested algal filtrates on growth parameters and yield / plant under field conditions during 2020/ 2021 and 2021/2022 growing seasons**.**

Treatment	2020/2021 season						2021/2022 season					
	Plant height (cm)	No. of branches / plant	Dry Weight of Inflorescences (g)	Dry Weight of Seeds (g)	Weight of 1000 seeds (g)	yield / plant (g)	Plant height (cm)	No. of branches / plant	Dry Weight of Inflorescences (g)	Dry Weight of Seeds (g)	Weight of 1000 seeds (g)	yield / plant (g)
A. variabilis	170.0±26.4*	9.0±1.5	147.0±6.5	165.0±16.0	29.9±1.8	256.3±26.9	185.3±3.78	24.3±5.4	215.0±11.5	133.6±8.7	28.0±4.1	219.0±14.7
N. paludosum	197.0±17.5	9.3±1.1	219.0±20.8	192.0±18.4	29.3±3.4	510.9±23.4	214.0±24.1	27.0±3.5	310.0±36.0	196.4±17.0	31.0±2.0	649.5±64.0
Commercial biocide	183.0±15.6	6.3±1.1	170.3±10.8	140.0±14.6	23.6±5.6	248.9±34.4	180.0±28.9	23.8±2.8	201.7±19.7	126.6±9.6	26.7±1.8	288.5±36.2
Control	150.0±25.1	3.6±0.8	156.0±28.0	96.0±7.8	21.7±1.4	75.0±11.8	171.0±20.5	8.0±1.5	156.2±26.7	108.9±6.0	22.0±1.5	110.0±3.7
LSD	3.65	0.3	5.36	4.63	0.33	8.53	4.53	1.66	6.63	4.85	1.3	8.76

*Experimental data were showed as means ± standard deviations
The means of the treatments were compared using the least significant difference (LSD) test at the 0.05 level.

Table (5) Effect of the tested algal filtrates on volatile oil composition of fennel seeds**.**

Components	%, Area			
	A. variabilis	***N. paludosum***	**Commercial biocide**	**Control**
α-Pinene	0.37	0.59	0.31	0.28
à-Phellandrene	0.19	0.21	0.17	0.15
D-Limonene	5.41	9.78	4.81	4.59
Eucalyptol	0.32	0.71	0.123	0.11
trans- à –Ocimene	0.30	0.50	0.26	0.15
ç-Terpinene	0.24	0.50	0.19	0.11
L-Fenchone	6.77	6.91	6.41	6.12
Limonene oxide	0.05	0.10	0.05	0.05
Trans-Limonene oxide	0.02	0.02	-	-
Camphor	0.02	0.05	-	-
Estragole	86.84	87.17	86.76	77.17
Fenchyl acetate	0.11	0.24	0.09	0.07
d-Carvone	-	0.06	-	-
Anethole	0.54	1.35	0.53	0.02

4.Discussion

Fennel plants suffer from the infection by many fungal soil-borne fungi such as *F. solani, M. phaseolina* and *P. aphanidermatium* that attack the plants from seedling to maturing and caused damping-off and root-rot diseases. Acquired resistance by beneficial microorganisms appear to be effective and alternative to chemical fungicides in controlling soil-borne diseases [36, 2]. Culture filtrates of two cyanobacteria (blue-green algal) *i.e.*, *Anabeana variabilis* and *Nostoc paludosum* were effective *in vitro* and *in vivo*. *In vitro*, the growth of the three pathogenic fungi were significantly decreased by the culture filtrate of *A. variabilis* and *N. paludosum* at 25 and 50% concentrations. In this concern, the filtrate of *N. paludosum* was more efficient in inhibiting the growth of the three pathogenic fungi. This inhibition may be due to that the filtrate of *Nostoc* contains a wide variety of compounds with biological activities such as phenols, antibiotics, enzymes and toxins [37,38,

20]. Salem *et al.* [38] showed that methanol extract of *Nostoc* sp. have antifungal activity against *A. niger, R. solani* and *F. oxysporum.* Cyanobacteria produce various secondary metabolites having hormonal, toxic, antimicrobial and antineoplastic effects . Kshetrimayum and Surya [20] reported that all the extracts of *Nostoc* inhibited the growth of *F. culmorum* due to a lipopeptide compound. In general, chitosanase homologs and microcystin show fungicidal activity and have also been present in *Anabaena laxa, A. iyengarii,* and *A. fertilissima*. Phenolic chemicals substances are good electron donors because their hydroxyl groups directly increase antioxidant effects, reduce free radicals and scavenge oxygen in biological systems, preventing oxidative stress of wild vegetables [39].

Under artificial inoculation conditions in a greenhouse, results showed that culture filtrates of cyanobacteria (blue-green algal) *i. e., A. variabilis* and *N. paludosum* applied to seeds and sprayed on the grown plants protected fennel plants against damping-off and root-rot caused by *F. solani, M. phaseolina* and *P. aphanidermatium* in compared with the commercial biocide and untreated control. Algal filtrate can be used as one of the important elements for controlling a large number of soil-borne pathogenic fungi. The fungicidal activity with the use of the culture filtrates of the *A. variabilis* and *N. paludosum* strains is strongly suggested to be due to the presence of bioactive compounds as well as enhanced natural defense mechanisms of oxidative enzymes *i.e.,* peroxidase, polyphenol oxidase, chitinase and soluble protein against the three pathogenic fungi. Results of the study showed an increase in the defense enzymes such as peroxidase and polyphenyl oxidase that are critical biochemical markers for a host's defense mechanism against pathogens. Similar results were obtained by Ingle and Deshmukh [40] stated that peroxidase and polyphenyl oxidase were concerned in oxidation of phenols and polyphenols respectively as well as plant cell wall lignification to prevent pathogen infestation. Kumar *et al.* [41] showed under controlled conditions a low disease severity was observed, along with an increase in polyphenol oxidases and peroxidases, were also triggered in pathogen-unchallenged coriander, cumin, and fennel plants by applied *Anabaena species, A. laxa*, and by *Calothrix elenkinii* as biomass culture. Thapa *et al.* [42] obtained that the isolates of *Nostoc spp., Anabaena spp*., and *C. elenkinii* have the same habitat of the pathogen *M. oryzae* due to increase in the leaf antioxidant enzymes polyphenol oxidase, peroxidases activities involved in plant defense induction.

Plants manufacture chitinase enzymes as a part of their defense system against invasive fungal infection as assist in the breakdown and assimilation of fungal cell walls and degrade hyphae [43]. Proteins are rich macromolecules that represent the main functions of living cells. Sabatini *et al*., [44] reported that total soluble proteins are studied as an index of metabolic changes under stress conditions.

Under natural field conditions, seeds and plants treated with culture filtrate produced by the two tested cyanobacteria performed better efficiency against damping-off and root-rot diseases. This indicates that the treatment with the cyanobacterial culture filtrate successfully expressed antagonistic action against pathogens and enhanced plant resistance. This inhibitory effect is mostly due to the presence of antifungal compounds that inhibited the fungal spore growth. According to Devappa *et al*.,[9] the aqueous extracellular product from cyanobacterium *Nostoc muscorum* was efficient to control the plant pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum* and reduced the spreading of *Erysiphe polygoni* causing powdery mildew on turnips, damping-off disease in tomato seedlings, and *Botrytis cinerea* on strawberries and also reduced the growth of some saprophytes and pathogenic fungi as *Aspergillus oryzae*, and *Chaetomium globosum Sclerotinia sclerotiorum* and *Rhizoctonia solani*. At the same time, it promote plant growth, yield, oil contents and its components. Algae contain many phytochemical compounds that stimulate and help plant and crop growth and increase its tolerance to various stresses. Alwathnani and Perveen [45] noticed the effect of *Nostoc calcicola* and *N. linckia,* on the root, shoot length, fruit number, and weight of tomato in two stage (after 50 and 100 days of the plantation) due to the different components of the extracts **.**

Cyanobacteria and green bacteria have also been known to be effective in plant growth and as a biofertilizer. In this regard [46,47] used organic fertilizer enriched with *Anabaena variabilis* and *A. laxa* which helped to enhance and increase soil organic carbon, nitrogen fixation, and significantly increase plant growth, quantity and quality of tomato yield and its content of mineral elements.

Anabaena variabilis and a *Nostoc* sp. increased germination of seeds, plant resistant, growth and yield when utilized as biofertilizer and biofungicides [48,49,50,51].

5. Conclusion

The present work indicated that *A.variabili* and *N. paludosum* had antifungal activities against the causal agent of fennel soil-borne diseases *in vitro* and *in vivo* experiments. Our study indicated that *N. paludosum* had a high content of phenolic compounds. *In vivo* experiments, *N. paludosum* filtrate was more effective than *A.variabilis* in reducing soil-borne diseases, enhancing plant resistance, showing an increase in the defense enzymes like peroxidase (P), polyphenyl oxidase (PPO), chitinase and soluble proteins and promoting plant growth, yield and oil content .

6. Conflicts of interest

The authors declare no conflict of interest.

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