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Antifungal Bio-Efficacy Using Cyanobacterial Extracts and Rhizobia Against Root-Rot Disease of Faba Bean (*Vicia faba* L.) Plants

Nahla A. Bouqellah[1], Sahar El-Nahrawy [2*], Ibrahim El-Akhdar [2] and **Nagwa M. M. El-Khateeb[3]**

[(1)]Taibah University, Science College, Biology Department, 42317- 8599, Al Madinah Al Munawwarah, Saudi Arabia

[(2)]Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza 12112, Egypt

[(3)]Agricultural Microbiology, Agricultural Botany Dep., Faculty of Agriculture, Kafrelsheikh Univ., 33516, Kafr El-Sheikh, Egypt

THE SUPPRESSION effect of four biocontrol agents (i.e., cyanobacteria extracts: *Nostoc linckia*, *Anabaena variabilis*, *Oscillatoria agardhii*, and *Spirulina* sp.), in combination with *Rhizobium leguminosarum* biovar *viceae* (TAL–1148), against *Rhizoctonia solani* in faba bean plants was investigated through laboratory and pot experiments. After testing five *R. solani* isolates for pathogenicity, all of the identified fungi were found to be pathogenic and to have induced symptoms of root rot and pre- and post-damping-off. Comparing the results of *in vitro* experiments to the untreated control, algal extracts dramatically suppressed the pathogen's ability to develop mycelially. In comparison to the control (0%), *O. agardhii* showed the highest reduction (59.63%), followed by *N. linckia* (51.85%), *A. variabilis* (48.52%), and *Spirulina* sp. (40.37%). When compared to the other studied cyanobacteria and the control treatment, the combined effect of *Rhizobium* and cyanobacteria extracts (*O. agardhii* and *N. linckia*) produced the highest reduction in pre- and post-emergence damping-off, measuring 13.33 and 6.67%, respectively, in pot studies. Furthermore, in comparison to the control, these treatments enhanced the number and dry weight of nodules, shoot height, dry weight of shoots, N_2%, and N content. Similar results were obtained using the fungicide Rizolex-T50%, which decreased the incidence and severity of the disease but had negative effects on *Rhizobium* and the symbiotic N_2–fixing characteristics. Plants cultivated from treated faba bean seeds showed notable increases in the activity of the oxidative reductive enzymes (i.e., peroxidase and polyphenol oxidase). Thus, it could be concluded that using algal extracts in conjunction with *R. leguminosarum* was thought to be a practical, secure, and economical way to manage this type of soil-borne disease. More further studies are needed to emphasize this sustainable and eco-friendly approach using different phytopathogens.

Keywords: *Rhizoctonia solani*, *Rhizobium leguminosarum* biovar *viceae*, cyanobacteria extracts, Peroxidase, polyphenol oxidase.

1. Introduction

Native to north Africa, southwest Asia, and south Asia, the faba bean (*Vicia faba* L.) is a species of bean (Fabaceae) that is widely cultivated worldwide **(Firdu et al. 2022; AbdEL-Azeiz 2024).** In Egypt, harvested has reached about 25105 ha for that production quantity 1.03 million tons of grains **(FAOSTST 2022).** The proper rhizobia strains (*Rhizobium leguminsarium*) must be added to the roots of faba beans in order to infect the plant and encourage the growth of root nodules **(Li et al. 2023).** The roots of faba beans must be injected with the many soil-borne fungal infections can cause significant root rot and wilt disease that affect a wide range of crops grown in various soil types. Faba bean roots and stem base are known to be attacked by a number of root rot and wilt pathogens, including *Rhizoctonia solani*, *Fusarium oxysporum*, and *Macrophomina phaseolina*. This results in significant losses in seed germination and plant stand, as well as rhizobia strains that infect the plant and encourage the development of root nodules **(Abdel-Kader et al., 2011).** It would be acceptable to tolerate many attempts to manage wilt and root rot infections. Despite their effective outcomes in

*Corresponding author e-mail: saharelnahrawy3@gmail.com
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controlling plant diseases, fungicides are nevertheless regarded as one of the contributing contributors to environmental contamination. Furthermore, it has been extremely difficult to manage disease with fungicides, and nearly all of them are only effective at phytotoxic levels **(McLaughlin et al. 2023; Abdelhady et al. 2024).** Recently, there has been a rise in interest in using alternatives that have a negative environmental impact due to growing concerns about the use of pesticides and their effects on human health and the environment **(Pathak et al. 2022).** Biological control could be an alternative of chemical control. It depends on the potential of beneficial microorganisms. In terms of biological nitrogen fixation, *Rhizobium* sp.'s advantageous effects have received attention recently which its active role in plant pathogen resistance is currently established **(Fahde et al. 2023).** On the one hand, the rhizobia-legumes symbioses' symbiotic nitrogen fixation can lead to higher crop yields, and on the other, a reduction in the need for chemical fertilizers. Additionally, by providing nitrogen (N) to agro-ecosystems and thereby reducing environmental pollution, it may benefit later crops **(Laranjo et al., 2014).**

In recent years, the advantageous effects of *Rhizobium* sp. have been the main focus of biological N_2 fixation. When used as a seed treatment, several strains of *R. leguminosarum* were shown by **Ketta et al. (2021)** to be able to fix nitrogen in an artificial medium and to effectively reduce the occurrence of *Pythium* damping-off in pea. Two *Rhizobium* isolates shielded chickpea plants against *F. oxysporum* infection, as demonstrated by **Arfaoui et al. (2005).** *Rhizobium* spp. have been shown by **Baraka et al. (2009)** to be effective against soil-borne diseases of legume crops, including *M. phaseolina*, *Rhizoctonia* sp., *Fusarium* spp., *Sclerotium rolfsii*, and *Pythium* spp.

According to **EL-sayed and Mousa (2015)** and **Soliman et al. (2018),** algae are thought to be one of the most significant biological agents for combating plant pathogens, particularly soil-borne fungus. Numerous cyanobacteria strains generate intracellular and extracellular compounds, such as antifungal, antibacterial, and antiviral agents, that have varying biological effects on plant pathogens **(Noaman et al. 2004).** Many cyanobacteria may grow in large quantities in mass culture and have antibacterial and antifungal properties; they pose no environmental danger **(Bibi et al. 2024).** Plant bioactive substances, such as total phenolic compounds, total saponins, and alkaloids, have been found to have antifungal activity in algal culture filtrates. These chemicals are used as natural defense mechanisms against pathogenic bacteria, fungus, viruses, and pests **(El-Mahmood and Ameh, 2007).** Extracts of cyanobacterial strains derived from freshwater and marine settings have been discovered to exhibit significant biological activity both *in vivo* and *in vitro*. It seems that much more biological activity has been discovered in extracts of terrestrial cyanobacteria **(Reisser, 2000).** Extracellular compounds from *N. muscorum* are promising as a biological control of soybean seedling damping off, as demonstrated by **Pimentel et al. (2022).** Plant pathogens fungi can be prevented from growing by applying culture filtrates or cell extracts from cyanobacteria and algae to leaves or seeds. These compounds have the ability to produce antibacterial and antifungal compounds, which can be used as protectants against these fungi **(Kulik, 1995). Kiviranta et al. (2006)** identified from crude ethanolic extracts of *Anabaena laxa* Rabenh and called Laxaphycins A, B, C, D, and E. These substances that are physiologically active include poisons and medications. Three cyanobacterial filtrates, *A. subcylindrica*, *N. muscorum*, and *Oscillatoria angusta* have an impact on the proliferation of pathogenic fungi that have been isolated from various faba bean parts. Algal filtrate concentrations and the decrease in fungal dry weight were primarily connected **(Abo-Shady et al., 2007**). According to **Abdel-Monaim et al. (2016**), under greenhouse and field circumstances, four cyanobacteria species, *N. muscorum*, *O. agardhii*, *Spirulina platensis*, and *A. sphaerica* have a protective effect against *R. solani*, *F. solani*, and *M. phaseolina*, which are the causative agents of lupine damping-off and root rot diseases. *Fucus* and *Nostoc* spp. were shown to be the best antifungal agent against *R. solani*, *F. solani*, and *F. oxysporum* by **EL-sayed and Mousa (2015)**, who investigated the inhibitory of algal filtrates on the growth mycelial of *R. solani*, *F. solani*, and *F. oxysporum*. This action could point to algae's capacity to create bioactive secondary chemicals that are released into the environment. These bioactive components have the capacity to produce a wide range of deadly toxins and various antagonistic activities that cause microbial development to disintegrate, which appears to inhibit the growth of the examined fungal isolates *Fucus* and *Nostoc* spp. **(Nandagopal et al. 2021).** The antifungal bioefficacy of Gracilaria confervoides extracts against *R. solani*, *F. solani*, and *M. phaseolina* of the cucumber plant was investigated by **Soliman et al. (2018).** The greatest decrease (100%) on *R. solani* was achieved using chloroform extraction, which was followed by 50% extraction from ethyl acetate. Under greenhouse conditions, the combined inoculation of *Rhizobium* sp. and cyanobacteria improved plant growth, nodule count, and chickpea production. Additionally, these inoculants induced the expression of enzymes linked to pathogenesis and defence **(Prasanna et al. 2005).**

In order to ascertain their effects on certain plant growth parameters, the potential of combined inoculations of both *Rhizobium* and some cyanobacteria extracts was investigated on the

biological control of faba bean root rot disease caused by *R. solani* in both *in vitro* and *in vivo.*

2. Materials and Methods

2.1 Causal organisms' isolation and identification
Samples of faba bean plants exhibiting indications of root rot and damping-off were gathered from various fields situated in the Kafr El-Sheikh Governorate's Sakha Agricultural Research Station. Running tap water was used to thoroughly clean the infected roots, which were then sliced into small fragments and superficially sterilized for 2 min using sodium hypochlorite (5%) before being repeatedly washed with sterile distilled water and dried between sterilized filter papers. In Petri dishes treated with penicillin (20 Iu/ml), the sterilized pieces were placed into potato dextrose agar (PDA) medium and incubated at 25 ± 1 ºC. Every day, the dishes were checked for the growth of fungi. Using single spore or hyphal tip procedures recommended by **Dhingra and Sinclair (1985),** the fungal colonies were purified. As stated by **Burgess et al. (1994),** the pure fungus was identified using their morphological and microscopical characteristics. Experts from the Plant Pathology Institute's Laboratory of Mycology at the Agriculture Research Centre in Giza, Egypt, verified the identification. For additional research, stock cultures were kept at 5 ˚C on PDA slants. A graphical flowchart detailing the different steps undertaken throughout the experiment (Figure 1).

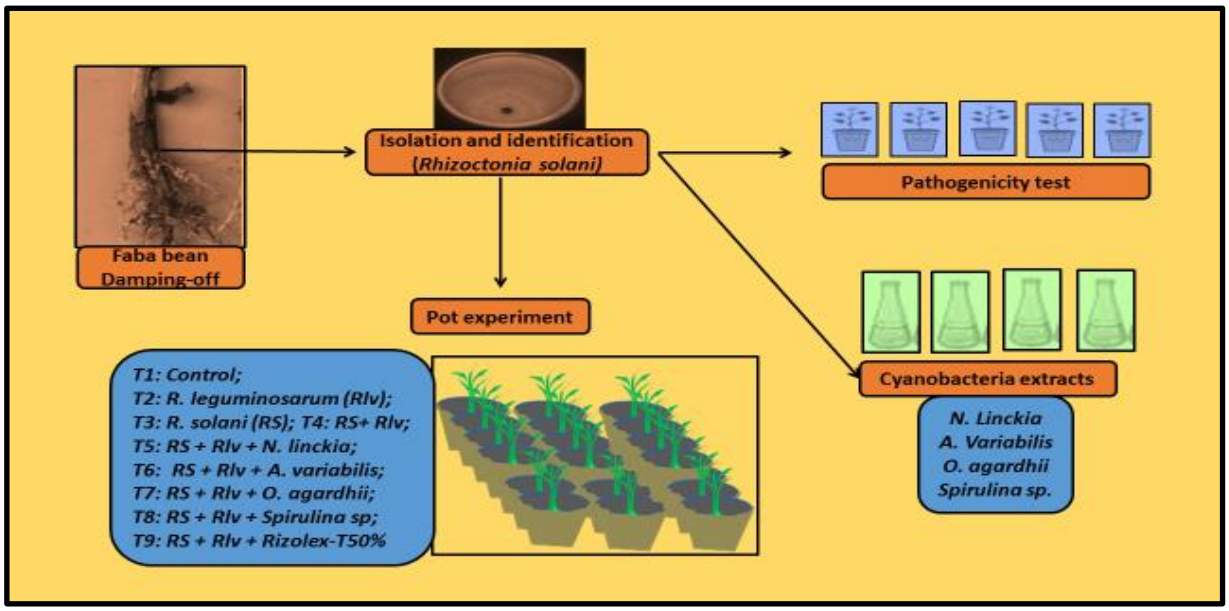

Fig. 1. A graphical flowchart detailing the different steps undertaken throughout the experiment.

2.2. Pathogenicity tests
Pathogenicity tests of *Rhizoctonia* isolates were carried out on faba bean cv. Sakha 1 during 2019/2020 season using the homogenized culture technique according to **Muthomi et al. (2007**) in artificially infested sandy soil at the greenhouse of Plant Pathology Department, Faculty of Agriculture, Kafrelsheikh University, Egypt. A week-old culture cultured on 50 ml of potato dextrose broth medium (PDB) was used to prepare the inocula of the acquired isolates. The conical flask (250 ml) was then incubated at 25±1 ºC. In a blender, the contents of the flask were homogenized for one minute. Seven days before to planting, 30cm (5 kg) plastic pots were filled with sterilized soil and combined with 5 fungal inoculums at a rate of 100 ml homogenized culture per pot. For each isolate, three pots were utilized as duplicates. As a control, three other pots were added to the severe with equal volumes of sterile PDB media that had not been inoculated with fungi. Each container had five surface seeds that had been sanitized. Percentage of damping-off was recorded 30 days after planting, respectively according to **Shaban and El-Bramawy (2011)**

Pre-emergence (%) = (Number of non-germinated seeds)/ (Total number of sown seeds) ×100
Post-emergence (%) = (Number of dead seedling)/ (Total number of sown seeds) ×100
Survival plant (%) = (Number of survival plant)/ (Total number of sown seeds) ×100

2.3. Seeds
The Field Crop Research Institute, Agricultural Research Centre, Department of Legume, Sakha Agriculture Research Station, Kafr El-Sheikh, Egypt, provided the beans (*Vicia faba* cv. Sakha 1). After surface-sterilizing the seeds with 70% ethanol, they were cleaned with sterile distilled water and allowed to dry in the open shade.

2.4. Cyanobacteria extracts

Cyanobacteria extracts known as *N. linckia*, *A. variabilis*, *O. agardhii* and *Spirulina* sp., were kindly obtained from Algae Research Unit, Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt. The algal extract was prepared by soaking the algal powder in methanol in a conical flask (50 g dry algae/1L solvent) for two days and the bioactive compounds of the extract were previously analyzed by gas chromatography Spectrophotometry (GC-MS) according to **Deyab et al. (2021) and El-Sheekh et al. (2022).**

2.5 Production of IAA

Using Salkowski's reagent as recommended by **Abdel-Monaim et al. (2016),** colorimetric measurement at 530 nm was used to assess the amount of IAA produced by the Cyanobacteria strains. Cyanobacteria strains were cultivated in BG11 broth medium supplemented with tryptophan (1 mg ml^{-1}) as an IAA precursor under shaking conditions (120 rpm) for two days at 30ºC. Following the incubation period, the cells were centrifuged for 10 minutes at 4°C at 3,000 rpm, and 1 ml of the supernatant was mixed with 2 ml of Salkowski's reagent (250 ml of distilled water, 150 ml of 95-98% H_2SO_4, and 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$). The mixture was then incubated for 30 min at room temperature. A standard curve with established quantities of pure IAA (Sigma-Aldrich, Co.) was used to quantify IAA.

2.6. Estimation of Total Phenolic

The spectrophotometric approach was used to determine the contents of total phenolic (TP) **(Slinkard and Singleton, 1977).** To put it briefly, 0.5 ml of each extract was diluted to 3 ml with distilled water and combined with 0.5 ml of the phenol reagent from Folin-Ciocalteu. 2 ml of a 2% Na_2CO_3 solution were added to the mixture after 5 min, and it was well mixed. After 60 min of dark storage at 30°C, the mixture's absorbance at 650 nm was measured. The calibration curve, which was created using standard amounts of gallic acid solution, was extrapolated to yield the TP. Three separate estimates of phenolic chemicals were made. Gallic acid equivalents (GAE), or mg/g of dried material, were used to express the TP.

2.7. Evaluation of antifungal Activity Assay of Cyanobacteria Extracts

The filter paper disc diffusion method was used to test the antifungal activity **(Thornberry, 1950).** Nine-centimeter petri dishes with 15 ml of PDA medium were split in half evenly. The first half was inoculated with a 0.5-centimeter disc containing individual pathogenic fungi, and the second half was inoculated with a 0.5-centimeter disc saturated with 250 micrograms per millilitre of each crude extract **(Nair et al., 2005).** It was determined what percentage of the pathogen was inhibited. For each treatment, three dishes were utilized as duplicates, along with a control (a disc that was impregnated with sterilized distilled water). Every infected plate was kept in an incubator at 25 °C. After all of the control plates' medium surface was covered in mycelial growth, the pathogens' linear growth was quantified and all of the plates were inspected. Reduction in the growth mycelial of the fungal pathogens was calculated according to **Soliman et al. (2018)** using the following formula:

Reduction in growth (%) = [G1 - G2 / G1] x100

Where: G1: linear growth of pathogenic fungus in control plates, G2: linear growth of pathogenic fungus in dual plates with algae extracts.

2.8. Rhizobial culture

The strain TAL-1148 of *Rhizobium leguminosarum* bv. viceae was procured from the BNF Unit at the SARS located in Kafr El-Sheikh, Egypt. A 500 ml flask containing 250 ml of yeast extract mannitol broth (YM) medium was used to reculture one loopful of the purified strain. For 2 to 3 days, the cultures were incubated at 30 °C and 150 revolutions per minute. Next, using the plate count technique, the number of bacterial cells cultured was calculated as (cell/ml).

2.9. Pot experiments

The 2020–2021 pot experiment was conducted at Kafrelsheikh University's Department of Agricultural Botany, Faculty of Agriculture. Cyanobacteria extracts' antifungal efficacy against the pathogen *R. solani* was assessed in pots with simulated infestation circumstances. Bean seeds (3 seeds/pot) were planted in pots (30 cm in diameter) that were filled with 5 kg clay soil (pH, 7.91; EC, 2.86 dS m^{-1}; organic matter 1.12%). For every treatment, there were five replicates. One week prior to seeding, soil in various pots was infected with pathogenic fungus cultivated on barley grain medium at a rate of 5 g/kg. When necessary, pots were irrigated. Before planting, the seeds were soaked in 100 µg/ml of each cyano-bacterium extract for 12 h in previously prepared algal extracts, as previously described. Four times, at two-week intervals, they were sprayed foliarly with 1 L/100 L of extracts after sowing **(Tassara et al., 2008).** Rhizobial isolate liquid cultures (5 ml x 10^8 cell/ml/plant) were generated and added to *Rhizobium*-free liquid media as a control after seedlings were thinned to 2 per pot **(El-Nady and Belal, 2005**). The experiment included the following treatments: T1: Control (uninfested soil); T2: *R. leguminosarum* (Rlv); T3: *R. solani* (RS); T4: RS+ Rlv; T5: RS + Rlv + *N. linckia*; T6: RS + Rlv + *A. variabilis*; T7: RS + Rlv + *O. agardhii*; T8: RS + Rlv + *Spirulina* sp; T9: RS + Rlv + Rizolex-T50%, 3g/kg seeds as seed coating before sowing. Disease incidence of faba bean plants was recorded after 30 days. Plants were collected at 60 and 135 days from

sowing which subjected to the following analysis: disease severity, shoot height, dry weight of shoots, number and dry weight of nodules plant^{-1}, N % and N content **(AOAC, 1990).**

2.10. Estimation of antioxidant enzymes

After 60 days of seeding, new samples were collected from plants developed from both untreated and previously treated bean seeds. Extracts were then utilized to test the biochemical changes linked to the tested algal treatments on the enzyme activities of polyphenol oxidase **(Snell and Snell, 1953)** and peroxidase **(Allam and Hollis, 1972).**

2.11. Statistical analyses

One-way analysis (ANOVA) was used to analyze using the Statistical Package (CO-STATE). Means of the treatments were compared using Duncan's multiple range tests **(Duncan, 1955).**

3. Results

3.1. Isolation and identification of the causal organisms

Five fungi were identified as *R. solani* after they isolated from infected faba bean plants that had obvious signs of root rot and damping-off.

3.2. Pathogenicity tests

Under greenhouse circumstances, faba bean plants were used to evaluate the acquired isolates. Table 1 results show that all of the tested isolates were capable of infecting faba bean roots, resulting in symptoms such as damping-off and root rot, which decreased plant survival. *Rhizoctonia* isolate 5 had the greatest pre- and post-damping-off infection rates (13.33% and 40%, respectively), followed by isolate 3 (26.67 and 20%, respectively), isolates 1 and 2, and finally isolate 5. *Rhizoctonia* isolate 4 had the lowest pre- and post-damping-off values. Conversely, of the examined isolates, the percentage of plants that survived was lower, ranging from 46.67 to 73.33%, in contrast to the control group that had 100% of the plants survive. Based on the data, it can be determined that *R. solani* isolate 5 had the most virulent plants and the lowest percentage of plants that survived (46.67%). *R. solani* was found to be the most virulent isolate 5, causing the highest severity.

3.3. Secondary Metabolite Production by Cyanobacteria Extracts

The production of IAA and total phenol as secondary metabolites in the extracts of *N. linckia, A. variabilis, O. agardhii, and Spirulina* sp is shown in Figs. (2 and 3). The obtained data indicate that *O. agardhii* and *N. linckia* were more production of all secondary metabolites than *A. variabilis* and *Spirulina* sp.

Table 1. Pathogenicity tests of *R. solani* on faba bean plants under greenhouse conditions in 2019/2020.

Isolates	Disease assessment		
	% Pre-emergence damping–off	% Post-emergence damping–off	% Survived plants
Control (un-infested soil)	0.00 d	0.00 d	100.0 a
R1	20.00 b	20.00 b	60.00 c
R2	26.67 a	13.33 c	60.00 c
R3	26.67a	20.00 b	53.33 d
R4	13.33 c	13.33 c	73.33 b
R5	13.33 c	40.00 a	46.67 e

DMRT at the 5% level indicates that there is no significant difference between the numbers in the same column means that are followed by the same letter (s).

3.4. Effect of cyanobacteria extracts on fungal growth reduction (*In vitro*)

Results in Table 2, show that all the tested cyanobacteria extracts reduced the mycelia growth of *R. solani* as compared with the control. The antifungal effects of cyanobacteria extracts against the pathogenic fungus were in the range of 40.37 - 59.63%. The cyanobacteria *O. agardhii* recorded the highest suppressed of the linear growth of R. solani (59.63%) followed by *N. linckia* (51.85%) and *A. variabilis* (48.52%), while the least inhibition effect was recorded in case of *Spirulina* sp (40.37%).

Table 2. The studied cyanobacteria extracts on the mycelial growth of *R. solani*.

Cyanobacteria extracts	Antagonistic effects	
	Mycelial diameter (cm)	Reduction (%)
Control	9.00 a	0.00 e
N. linckia	4.33 d	51.85 b
A. variabilis	4.63 c	48.52 c
O. agardhii	3.63 e	59.63 a
Spirulina sp.	5.37 b	40.37 d

DMRT at the 5% level indicates that there is no significant difference between the numbers in the same column means that are followed by the same letter (s).

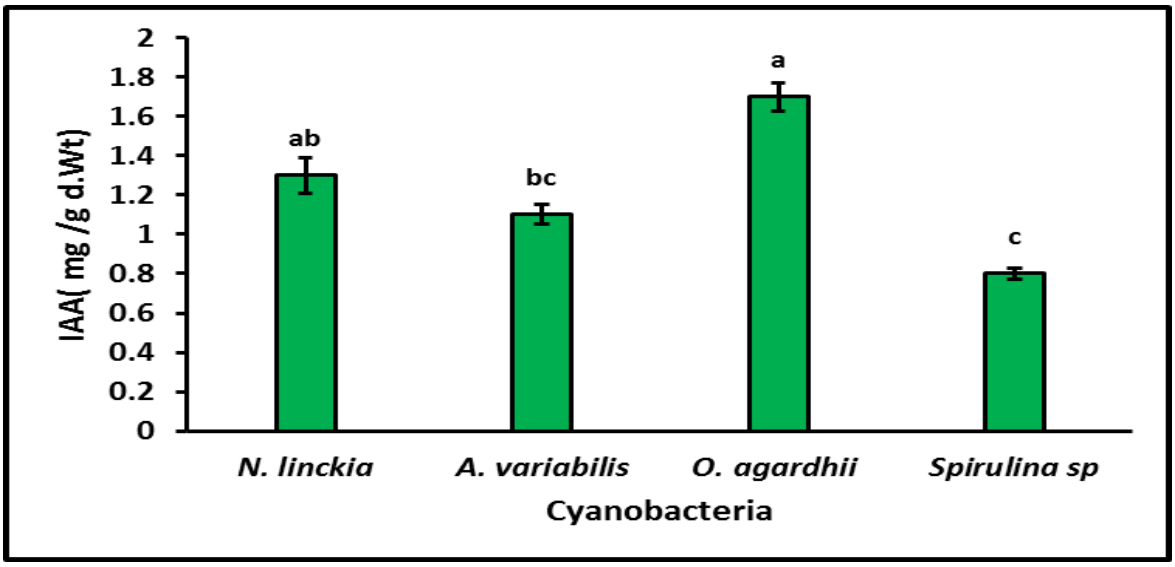

Fig. 2. IAA production in the cyanobacteria culture extracts.

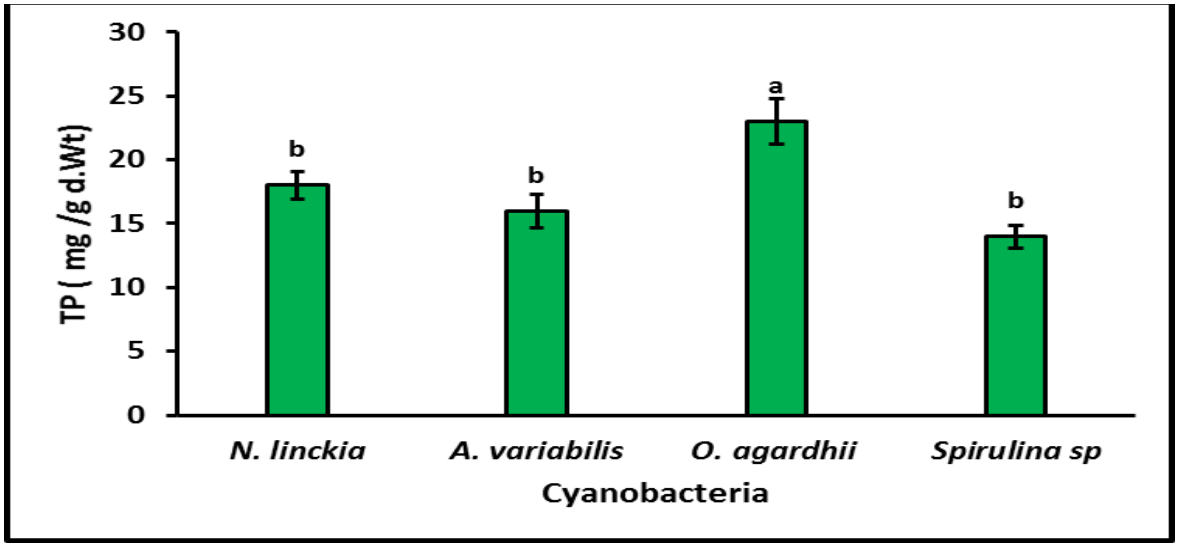

Fig. 3. Total phenols contents in the cyanobacteria culture extracts.

3.5. Pot experiments

3.5.1. Damping-off disease incidence

Cyanobacteria and *R. leguminosarum* shown their effectiveness in managing the damping-off disease on faba bean plants in a greenhouse environment. The data shown in Table 3 unequivocally show that, in greenhouse circumstances, all treatments considerably decreased the pre- and post-emergence damping off caused by an artificial infection with *R. solani*. When faba bean seeds treated *with R. leguminosarum* were planted in soil infested with *R. solani*, the amount of pre- and post-emergence damping-off (20–20%) was significantly lower than in the control group. The species of cyanobacteria extracts that are employed primarily determines how much the occurrence of pathogenic fungus-induced root rot is reduced. When compared to the other studied cyanobacteria, *O. agardhii* and *N. linckia* showed the largest reduction in pre- and post-emergence damping-off, at 13.33 and 6.67, respectively. These were followed by *N. linckia* (13.33 and 13.33), *A. variabilis* (20.00 and 6.67), and *Spirulina* sp. (20.00 and 13.33).

Table 3. Antifungal activity of *Rhizobium* sp. and studied cyanobacteria extracts on damping –off disease incidence of faba bean plants infected with *R. solani* under greenhouse conditions in 2020/2021.

Treatments	Disease assessment		
	Pre-emergence damping–off (%)	Post-emergence damping–off (%)	Survived plants (%)
T1	0.00 d	0.00 e	100.00 a
T2	0.00 d	0.00 e	100.00 a
T3	26.67 a	40.00 a	33.33 g
T4	20.00 b	20.00 b	60.00 f
T5	13.33 c	13.33 c	73.33d
T6	20.00 b	6.67 d	73.33d
T7	13.33 c	6.67 d	80.00c
T8	20.00 b	13.33 c	66.67e
T9	13.33 c	0.00 e	86.67 b

DMRT at the 5% level indicates that there is no significant difference between the numbers in the same column means that are followed by the same letter (s). **T1**: Control (uninfested soil); **T2**: *R. leguminosarum (Rlv);* **T3***: R. solani (RS);* **T4***: RS*+ Rlv; **T5**: *RS* + Rlv + *N. linckia;* **T6***: RS* + Rlv + *A. variabilis;* **T7***: RS* + Rlv + *O. agardhii;* **T8***: RS* + Rlv + *Spirulina sp;* **T9***: RS* + Rlv + Rizolex-T50%.

3.5.2. Plant growth and N_2- fixing parameters

Table 4 clearly shows that, in comparison to the infested control, all administered treatments considerably enhanced the symbiotic N_2 fixing parameters and development of the faba bean plants. It is evident that rhizobial inoculation of legumes has positive benefits. It was the most effective treatment in increasing all N_2 fixing parameters in the absence of *R. solani*. Rhizobial treatment significantly increased number and dry weights of nodules to, being 89.33 and 0.76 / plant compared to control uninoculated 9.67 and 0.21 / plant, respectively. Also, this treatment increased N_2% and N-content, being 2.26 % and 326.28 mg/plant comparing with uninoculated control with *Rhizobium*, being 1.61% - 153.28 mg /plant, respectively. The fungicide Rizolex-T50% seed dressing greatly decreased the incidence of the disease, however it had extremely notable negative impacts on the N_2 fixing parameters. However, when compared to inoculation with *Rhizobium* alone, the combination inoculation effects of Rhizobia and cyanobacteria extracts demonstrated a substantial increase in the number of nodules, dry weight of nodules, shoot height, dry weight of shoot, N_2%, and N-content of shoots.

Table 4. Antifungal activity of *Rhizobium* sp. and cyanobacteria extracts on symbiotic N_2 fixing parameters of faba bean plants infected with *R. solani* under greenhouse conditions in in 2020/21 at 60 days from sowing.

Treatments	N_2 - Fixation parameters at 60 days from sowing					
	No. of nodules/ plant	Dry weight of nodules (g)/ plant	Shoot length (cm)	Dry weight of shoots (g)/ plant	% N_2 (g)/plant	Total N_2 (mg)/plant
T1	9.67 h	0.21 e	29.27 d	9.23 cd	1.66 d	153.28 d
T2	89.33 a	0.76 a	48.23 a	14.44 a	2.26 a	326.28 a
T3	5.33 i	0.19 e	21.53 e	6.09 e	1.36 e	82.87 e
T4	28.67 f	0.34 d	30.33 d	8.03 d	1.78 d	150.46 d
T5	65.67 c	0.52 bc	35.67 bc	11.17 b	2.01 b	228.26 bc
T6	56.00 d	0.51 bc	35.53 bc	10.60 bc	2.00 bc	204.46 c
T7	68.67 b	0.57 b	38.33 b	11.56 b	2.13 b	245.77 b
T8	52.00 e	0.43 c	34.82 c	10.19 bc	1.97 bc	208.59 c
T9	16.67 g	0.27 e	31.13 d	9.85 bc	1.74 d	171.81 d

DMRT at the 5% level indicates that there is no significant difference between the numbers in the same column means that are followed by the same letter (s). **T1**: Control (uninfested soil); **T2**: *R. leguminosarum (Rlv);* **T3***: R. solani (RS);* **T4***: RS*+ Rlv; **T5**: *RS* + Rlv + *N. linckia;* **T6***: RS* + Rlv + *A. variabilis;* **T7***: RS* + Rlv + *O. agardhii;* **T8***: RS* + Rlv + *Spirulina sp;* **T9***: RS* + Rlv + Rizolex-T50%.

Number and dry weight of nodules per plant were in the range of 52.00 to 68.67 nodule and 0.43 to 0.57 g /plant, respectively compared to 4.67 nodule and 0.17 g /plant in control treatment infested by *R. solani*. Shoot length average in treated faba bean plants was in the range of 34.82 to 38.33 (cm), respectively while in untreated plants it was 21.53 (cm). Dry weight of shoots recorded 10.19 to 11.56 g/plant, compared to 6.09 g /plant in control treatment infested by *R. solani*

(Table 4). Also, results in Table 4 , reveal that the averages of faba bean N_2% and N-content due to application with cyanobacteria extracts and *Rhizobium* sp. were ranged from 1.97 to 2.13 % and 204.46 to 245.77 mg/plant compared to 1.36 % and 82.87 mg/plant in the infested by *R. solani* control plants, respectively. In the presence of *R. leguminosarum* , *O. agardhii* gave the highest increase in the total N-content, being 245.77 mg/plant followed by *N. linckia* 228.26 mg/plant, *Spirulina* sp. 208.59 and *A. variabilis* 204.46 mg/plant, respectively. It is noticeable that application of the fungicide Rizolex negatively affects *Rhizobium* and deprives the legumes from any beneficial effects of Rhizobial inoculation. Although, the total N_2 dropped in treatments with bio-agents but it was still large than in the unprotected plants and protected plants by Rizolex.

3.5.3. Peroxidase and polyphenol oxidase activity

Data presented in Table 5, indicate that bean plants grown from seeds soaked in some cyanobacteria extracts resulted in an increase of peroxidase and polyphenol oxidase activity compared to the untreated control at 60 days from sowing. Data show that the optical density of peroxidase activity was in the range of 2.76 to 3.54 in faba bean plants under application of rhizobia and some cyanobacteria extracts, compared to 1.36 in untreated faba bean plants. The peroxidase enzymatic activity was in the range 102.94 to 160.29% in antifungal treatments application. Mix of rhizobia and *O. agardhii* gave the highest increase in peroxidase activity (195.03%) followed by *N. linckia* (143.38 %), *A. variabilis* (137.50 %) and *Spirulina* sp. (133.08%). Meanwhile treatment with Rhizobia only gave the least activity (132.35%) compared to control (check) treatment and fungicide Rizolex treatment was (87.50%). Results showed that the optical density of polyphenoloxidase were in the range of 1.94 to 2.77 in treated faba bean plants, compared to control plants (1.15). Application of rhizobia and cyanobacteria extracts enhanced the activity of polyphenoloxidase enzyme in faba bean plants from 100.00 to 140.86 %. Furthermore, mix of rhizobia and *O. agardhii* gave the highest increasing in polyphenoloxidase activity (140.86%) followed by *N. linckia* (110.43%), *A. variabilis* and *Spirulina* (106.95 and 102.00%, respectively) followed by inoculation with rhizobia alone (88.69%) compared to control (check) treatment and fungicide Rizolex treatment was (72.17%). The application of cyanobacteria and rhizobia as biocontrol agents resulted in the increase of certain enzymes, including peroxidase and polyphenoloxidase, which are crucial to plants' defense mechanisms against pathogen invasion, according to the findings. The treated bean plants' enzymatic activity rose higher than that of the untreated control, according to the results.

Table 5. Determination of peroxidase (PO), and polyphenoloxidase activity (PPO) in faba bean plants treated with *Rhizobium* sp. and the studied cyanobacteria extracts under greenhouse conditions.

Treatments	Enzymatic activities			
	PO (mM H_2O_2 g^{-1} FW min^{-1})		PPO (µM tetra-guaiacol g^{-1} FW min^{-1})	
	Activity	To control (%)	Activity	To control (%)
T1	2.82 d	107.37	2.06 e	79.13
T2	3.16 c	132.35	2.17 d	88.69
T3	1.36 f	-	1.15 g	-
T4	2.76 d	102.94	1.94 f	68.69
T5	3.31 b	143.38	2.42 b	110.43
T6	3.23c	137.50	2.38 b	106.95
T7	3.54 a	160.29	2.77 a	140.86
T8	3.17 c	133.08	2.33 c	102.00
T9	2.55 e	87.50	1.98 f	72.17

DMRT at the 5% level indicates that there is no significant difference between the numbers in the same column means that are followed by the same letter (s). **T1**: Control (uninfested soil); **T2**: *R. leguminosarum (Rlv);* **T3***: R. solani (RS);* **T4***: RS*+ Rlv; **T5**: *RS* + Rlv + *N. linckia;* **T6***: RS* + Rlv + *A. variabilis;* **T7***: RS* + Rlv + *O. agardhii;* **T8***: RS* + Rlv + *Spirulina sp;* **T9***: RS* + Rlv + Rizolex-T50%.

3.5.4. Plant growth and yield parameters

When compared to the uninoculated control, the data in Table 6 demonstrate a highly significant increase in the symbiotic N_2 fixing and yield components of faba bean following inoculation with *R. leguminosarum*. Results in Table 6 show that the combinations of cyanobacteria extract with *R. leguminosarum*, were more effective in comparison with using *Rhizobium* with Rizolex-T50%, in the presence of *R. solani* under greenhouse conditions. Combined inoculation enhanced number and dry weight of pods /plant, number and dry weight of seeds /plant, 100-seed weight (g) and N_2% and total N-content of seeds mg/plant. Data also showed that number and dry weight of pods ranged from 4.00 to 4.67 pods /plant and from 15.02 to 17.24 g/plant, compared to 1.51 pods and 6.48 g/plant in the control plants infested by *R. solani*, respectively. No significant differences

were recorded between inoculated treatments in number of seeds /plant. Differences in dry weight of seeds were in the range of 11.52 to 13.06 g/plant, compared to 3.97 g/plant in the control infested by *R. solani*.

Results in Table 6, clearly show that the average of faba bean N_2% and N-content with cyanobacteria extracts and *Rhizobium* applications ranged from 3.06 to 3.25 % and from 2545.09 to 2765.79 mg/plant) compared to 1.86 % and 1234.00 mg/plant in the control plants infested by *R. solani*. In the presence of *R. leguminosarum* , *O. agardhii* gave the highest value in the total N-content, being 2765.79 mg/plant, followed by *N. linckia* 2700.93 mg/plant, *A. variabilis* 2583.94 and *Spirulina* sp 2545.09 mg/plant , respectively.

Table 6. Antifungal activity of *Rhizobium* sp. and the studied cyanobacteria extracts on symbiotic N_2 fixing parameters of bean plants infected with *R. solani* under greenhouse conditions in 2020/21 at 135 days from sowing.

Treatments	Yield parameters at 135 days from sowing						
	No .of Pods/ plant	Dry weight of pods (g)/ plant	No. of Seeds/ plant	Dry weight of seeds (g)/plant	weight of 100seeds / plant	% N_2 /plant	Total N_2 (mg)/plant
T1	2.67 ab	8.98 f	9.50 d	7.75 d	76.54 c	2.71 de	2075.05 d
T2	5.00 a	18.54 a	14.93 a	12.88 a	88.72 a	3.50 a	3105.10 a
T3	1.51 c	6.48 g	7.67 e	3.97 e	66.26 d	1.86 f	1234.00 e
T4	3.33 b	13.56 e	10.78 cd	8.90 c	78.61 bc	2.80 cde	2201.18 d
T5	4.43 ab	17.24 b	14.42 a	11.83 b	84.95 ab	3.18 abc	2700.93 b
T6	4.00 ab	15.98 c	13.95 a	11.52 b	82.64 abc	3.13 abc	2583.94 c
T7	4.67 a	17.03 b	14.64 a	13.06 a	85.11 ab	3.25 ab	2765.79 b
T8	4.00 ab	15.02 cd	13.18 ab	11.83 b	83.27 abc	3.06 bcd	2545.09 c
T9	3.73 b	14.42 de	11.97 bc	8.93 c	79.75 bc	2.59 e	2068.51 d

DMRT at the 5% level indicates that there is no significant difference between the numbers in the same column means that are followed by the same letter (s). **T1**: Control (uninfested soil); **T2**: *R. leguminosarum (Rlv);* **T3***: R. solani (RS);* **T4***: RS*+ Rlv; **T5**: *RS* + Rlv + *N. linckia;* **T6***: RS* + Rlv + *A. variabilis;* **T7***: RS* + Rlv + *O. agardhii;* **T8***: RS* + Rlv + *Spirulina sp;* **T9***: RS* + Rlv + Rizolex-T50%.

4. Discussion

Nowadays, there has been a rise interest in using alternatives to pesticides to avoid the negative environmental impact on human health and the environment. The goal of the current study is to identify substances that are safe for the environment and for people. Biological control could be an alternative to the chemical control. It depends on the potential of beneficial microorganisms. The efficiency of the cyanobacteria strains was positively correlated with the content of IAA and 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 the growth and yield compared to the other cyanobacteria (**Abdel-Monaim et al. 2016)**. The evidence from **Pimentel et al. (2022)**, who found that extracellular components from *N. muscorum* are promising as a biocontrol of soybean seedling damping-off, is consistent with our results. According to **Kulik (1995),** seedlings were treated with filtrates or cell extracts from cyanobacteria to ward off damping-off fungus.

Ayaz et al. (2023), who discovered that, when compared to a control, all of the tested algal filtrates reduced *R. solani* mycelial growth. It can be applied to induce plant defenses, which offer defense against a wide range of pathogenic organisms. This behaviour could suggest that algae have the capacity to secrete bioactive secondary metabolites into their surroundings. Given that the investigated fungi, *Nostoc* sp. and *Anabaena* sp., can create a range of deadly toxins, these bioactive compounds appear to prevent their proliferation **(Surakka et al., 2005).** According to **Abdel-Monaim et al. (2016),** of all the pathogenic fungi examined, *O. agardhii* cyanobacteria showed the most suppression of linear growth, followed by *R. solani* and *N. muscorum*, while *A. sphaerica* showed the least amount of inhibition.

The results of **Fahde et al. (2023)** corroborate the outcomes of the current investigation. Indeed, some researchers have linked the excretion of antibiotic substances to *R. leguminosarum* antagonistic properties against *F. oxysporium* f. sp. *lentis*. Additionally, *R. leguminosarum* antimicrobial activity is attributed to its protein nature, which has fungicidal action on *F. oxysporum* conidia **(Essalmani and Lahlou, 2002)**. Furthermore, it has been reported by **Arfaoui et al. (2005)** that *Rhizobium* may suppress fungal diseases biologically through a variety of ways. These processes include the host's induced or improved resistance, the creation of antibiotics, the competition for nutrients or iron, and the stimulation

of plant growth. When compared to the control, the combined effect of cyanobacteria and rhizobia extracts shown the greatest effects for regulating pre- and post-emergence damping-off **(Abdel-Kader and El-Mougy, 2013 and Prasanna et al., 2017).**

The outcomes that reported by **Tassara et al. (2008**), which found that *N. muscorum*, a member of the Cyanobacteria genus, exhibited antifungal activity against soil fungus. Furthermore, according to **Biondi et al. (2004)**, *Nostoc* ATCC 53789, a recognized producer of cryptophycin, is a source of naturally occurring insecticides with cytotoxic effects that fight fungi like *R. solani* as well as insects and worms. According to **EL-sayed and Mousa (2015**), the reason why soaking seeds in algal filtrates works so well could be because the active ingredients are absorbed and stop infections and diseases from spreading. According to **Biondi et al. (2004),** the effectiveness of irrigating soil with culture filtrates may be attributable to the potential of chemicals that resemble antifungals to infiltrate the fungal cell and subsequently induce modifications in the fungal metabolism. Also, **Saleh et al. (2000**) found similar outcomes, indicating that inoculating faba bean cv. Giza 674 with *R. leguminosarum* led to a considerable increase in the number of nodules, dry weight, and N-content of the shoots. The same results were observed by **Abdel-Monaim et al. (2016)** showed that lupine seeds treated with cyanobacteria extracts recorded the highly protection against infection with soil borne pathogens and significantly improved plant growth and yield parameters under field conditions. Furthermore, these findings concur with earlier studies that show red algae extracts to be effective antiprotozoal and anti-mycobacterial agents in addition to enhancing plant development **indices (Jimenez, et al. 2011 and Sultana, et al. 2011).**

According to **Nawar and Kuti (2003),** peroxidase and the emergence of plant resistance have good correlations. Additionally, **Caruso et al. (2001)** provided experimental evidence in favour of the theory that peroxidase functions as a defence against invasive infections. According to **EL-Sayed and Mousa (2015),** cyanobacteria extract treatments significantly decreased the occurrence of faba bean root rot**.** Furthermore, it was evident that plants developed from treated seed had higher levels of peroxidase and polyphenol oxidase enzyme activity than plants grown from untreated seed. Bean seedlings were significantly protected against the white rot disease by treatments with extracts of cyanobacteria and *Rhizobium*. It might have to do with the cyanobacteria's capacity to activate the enzymes in bean plants that boost disease resistance.

Attia et al. (2019) found that applying *Rhizobium* strains to faba bean seeds significantly affected the green pod's length and width, quantity of green pods, weight of the green pods, overall seed weight, healthy-looking seed, and discolored seed. These results were approved with **Ghazi (2006)** who indicated that the highest values of number as well as nodules dry weight, shoot dry weight, seeds dry weight, pods dry weight plant^{-1} and nitrogen content (mg/plant) of broad bean seeds were obtained from the treatments which were inoculated with *R. leguminosarum* (strain 317). Also, inoculation with *R. leguminosarum* in the presence of *R. solani* showed significantly increase in symbiotic N_2 fixing and yield parameters of faba bean compared to the infested by *R. solani* control. These results are in agreement with those recorded by **Arfaoui et al. (2005)** and **Baraka et al. (2009)**, **Abdel-Monaim et al. (2016)** and **Fahde et al. (2023).** These results are in harmony with the findings of **(Abdel-Monaim et al., 2016 and Prasanna et al., 2017; Alsalamah et al. 2024)**. In this regard, **Khalequzzaman and Hossain (2007)** discovered that treating faba bean seeds with *Rhizobium* strains had a substantial impact on the length and width of the green pod, the number of the pods, the weight of the pods, weight of the seeds, the healthy-looking seeds, and the discolored seeds.

5. Conclusion

In conclusion, results indicated that the efficiency of the combined effect of *R. leguminosarum* and cyanobacterial extracts to protect faba bean plants against root rot disease caused by *R. solani* without affecting the symbiotic N_2 fixation and enhancing growth parameters has been clearly proved throughout the present study. We may draw the conclusion that using algae extracts is a practical, secure, and economical way to manage this type of soil-borne disease. More research is required to highlight this environmentally beneficial and sustainable strategy that makes use of several phytopathogens.

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