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Cyanobacteria for Sustainable Management of Lupien Root Rot

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CYANOBACTERIA have an essential role in stimulating plant growth and suppressing soil- borne fungi. This study investigated the effect of *Nostoc* sp. and *Anabaena* spp. filtrates on *Fusarium solani* infection of yellow lupine (*Lupinus luteus*) plants, both in *vitro* and in *vivo*. *Nostoc* spp. and *Anabaena* spp. strains were tested for their capacity to suppress pathogenic fungus. A pot experiment in a greenhouse was carried out to apply both cyanobacterial strains individually and in combination against artificial infection by the *F. solani* pathogen. After 45 days of sowing, disease assessment, plant height, fresh weight, dry weight, pigment content, membrane leakage, malonaldehyde, membrane leakage peroxidase, polyphenoloxidase, ascorbic acid, and total phenols were measured in plant leaves. Total bacterial and cyanobacterial counts, dehydrogenase, protease, and chitinase activities were determined in the rhizosphere soil of lupine plants. Survival plants were recorded after 60 days of planting. The results demonstrated that the presence of both cyanobacterial strains was capable of suppressing pathogenic fungus infection as a single treatment; however, the combined application resulted in greater suppression than the single treatment, in single application of cyanobacteria the pre-damping of decreased by 40% and by more than 50% in combined application. Nevertheless, cyanobacteria improved growth measurements, defense enzyme activities, and microbial soil activities, hydrolytic enzymes in soil and plant viability.

Keywords: *Lupinus luteus*, Biocontrol, Cyanobacteria, *Anabaena* sp, *Nostoc* sp.

I. Introduction

The yellow lupine (*Lupinus luteus*) is a promising legume crop in the Fabaceae family (Hama and Strobel, 2020). Lupine is important in agriculture because it can fix nitrogen and provide seeds that are rich in mono- and polyunsaturated fatty acids, fiber, and protein (Knecht et al., 2020). Different lupine species include proteins with high value and potential benefits for human health and ecological production. This genus of plants has an amazing nutritional profile that includes gluten, globulin, protein, dietary fiber, albumin, and polyunsaturated fatty acids. Numerous biological actions were documented, including cytotoxic, anti-inflammatory, antioxidant, and anti-diabetic effects (Ishaq et al., 2022). Among these genera, lupines have the greatest number of species found in North and East Africa, the Mediterranean, and North and South America. Worldwide, there are between 170 and 200 species of lupine, which include both cultivated and wild plants that are often called lupines (Bebeli et al., 2020).

Current sustainable agricultural farming is heavily dependent on high tillage use, overuse of chemical pesticides, irrigation, and fertilizers; the majority of nations' food needs are definitely satisfied, despite many health and environmental issues. Recently, there has been discussion about how to increase agricultural production of crops without compromising the ecology, water supplies, or the fertility of the land and soil (Rehman et al., 2022). Synthetic pesticides made from natural microbial sources and light components have been used in a variety of alternative ways to stop pathogen attack (Ngegba et al., 2022).

Infections by fungi can affect plants grown using any strategy and cause fruit deterioration and severe postharvest losses. Numerous species of *Rhizoctonia*, *Phytophthora*, *Pythium*, *Fusarium*, and *Verticillium* are among the most significant polyphagous soilborne fungi, together with *Sclerotinia sclerotiorum* (Godana et al., 2023). Root rot, collar rot, damping-off, wilting, and yellowing are the results of their impact on the root system, which hinders the absorption of water and nutrients from the soil (Righini and Roberti, 2019).

Algae have long been utilized as soil amendments in agriculture because of their positive effects on plant productivity and health. Algae contain polysaccharides, betaines, micronutrients, and plant growth hormones

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(cytokinins, auxins, abscisic and gibberellic acid). Beginning in the mid-1950s, as a result of studies on algae, their compounds, and how they affect plants, liquid products with extracts of compounds that plants can easily absorb have been produced. Aside from their impact on plant growth, algal extracts have been shown to boost the ability of plants to resist biotic and abiotic stresses. Algae have demonstrated antifungal activity against numerous diseases among biotic stresses. ~~particularly those of horticulture plants (Righini and Roberti, 2019).~~ Algae also contain bioactive chemicals with antibacterial, antiviral, anticancer, antioxidant, and antifungal effects. They have been investigated for use in the manufacturing of pigments, bioactive compounds, medicines, and cosmetics (Sandybayeva et al., 2023). Algae extracts have also shown significant promise as plant disease prevention agents through the production of different defense-related enzymes that help plants fend off fungal, invasion; they can indirectly induce plant resistance in addition to directly combating a range of fungal plant diseases (Husain et al., 2024).

Among the most varied and abundant prokaryotes in the Bacteria super kingdom are cyanobacteria, also referred to as cyanophyta or blue-green algae (Saber et al., 2022, and Khalifa et al., 2021). They originated three billion years ago when photosynthesis caused an oxygenic environment to replace an anoxygenic one (Dadheech, 2024). These environments influenced their evolution by resulting in the formation of free-living, photosynthetic and non-photosynthetic, symbiotic, poisonous, and predatory species (Pathak et al., 2022).

Thus, the goal of this study is to investigate how well double cyanobacterial strains (*Anabaena* sp. and *Nostoc* sp.) inhibit *Fusarium solani* root rot fungus and their effects on the growth parameters of yellow lupine plants.

2. Materials and Methods

2.1 Materials

PDA media (Waksman and Lechevalier, 1961) was used to cultivate the pathogenic fungal strain *Fusarium solani* MT730027, which was graciously provided by the Plant Pathol. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt. Cyanobacterial strains, *Anabaena* OQ207016, *Nostoc* OP730945.1, were grown directly in nitrogen-free BG11 media (Browitzka and Browitzka, 1988) after being provided by the Department of Microbiology, Soils, Water, and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

2.2 Methods

Antifungal activity of cyanobacterial strains

Antifungal activity for both cyanobacterial strains was tested according to Katircioglu et al. (2006), by single cyanobacterial filtrate on Petri dishes and a 2 mm fungal disc on PDA medium. The antifungal properties of both algal culture (at 21days old) and filtrates were tested against pathogenic fungus *F. solani* under sterilized conditions. Filtrates were mixed with sterilized PDA medium before being poured into Petri dishes. There were three replicates for each treatment. Dishes were inoculated with 2 mm discs of tested fungi that were 7 days old. Plates with PDA medium and 2 mm discs were utilized as a control for 7-day-old tested fungus (free algal filtrates). Plates were incubated at 28 degrees Celsius for 7 days. The linear growth (mm) of pathogenic fungus was evaluated when the control treatment's pathogenic fungi covered the entire plate.

Percept reduction of cyanobacterial filtrate was calculated using the following, formula, (Bell *et al*., 1982):

% inhibition = (1 - (Fungal growth / Control growth) x 100.

2.3 Experimental Design

Seeds of yellow lupine (*Lupinus luteus*) variety Giza 2 were obtained from Agriculture Research Center Giza, Egypt, and are known to be susceptible to diseases caused by soil-borne pathogens such as “damping off” by *F. solani*. Soil for pot experiment was provided from the farm of the Agricultural Research Centre in Giza, Egypt. The soil was artificially infested with fungus (at a dosage of 3 % w:w) and well mixed. The soil was transferred to 30 cm diameter pots at a rate of 7 kg each pot. In the greenhouse of the Department of Microbiology, Soils, Water, and Environment Research Institute, ARC, Giza, Egypt, a pot experiment was conducted to study the bio-control activity of two cyanobacterial (*Anabaena* sp., *Nostoc* sp.) strains inoculated with 25ml ($5*10^6$ CFU/ml/pot) against root rot diseases incidence in lupine plants. The physical and chemical characteristics of the experimental farm soil are displayed in Table (1), and the tested pathogenic fungus was *F. solan.* and the experiment included the following treatments, with five replicates in a completely randomized design considered:

a) Control treatment, b) Soil-infested fungus, c) *Anabaena* sp. cyanobacterial strain,

d) *Nostoc* sp. cyanobacterial strain, e) Soil infested fungus treated by *Anabaena* sp. cyanobacterial strain, f) Soil-infested fungus treated by *Nostoc* sp. cyanobacterial strain, g) Soil-infested fungus treated by *Anabaena* sp. and *Nostoc* sp. cyanobacterial strains.

Table 1. Properties of the experimental soil chemically and physically.

Distribution of particle sizes			Chemical properties						Textural class
Clay	Silt	Sand	O.M g/kg	EC, dS m^{-1} (1:5)	Available (mg kg^{-1})			pH (1:2.5)	
	%				N	P	K		
40.8	35.4	23.8	18.4	0.99	45.00	12.5	191.90	7.75	Clay loam

2.4 Plant Analysis

2.5 Disease Assessment

Two measurements were taken to determine disease development. After two weeks from planting, pre-emergence damping-off was calculated as the percentage of seeds and seedlings that died before germination compared to the starting seed number. After 45 days, post-emergence damping-off was calculated as the percentage of seedlings that died after germination (post-infected seedlings) compared to the initial seed number. At the end of the 60-day period, the percentage of living plants was recorded as the number of survivors.

The damping-off disease assessment was as follows:

% Pre-emergence = (Number of non-germinated seeds/ Number of seeds sown) X 100

% Post-emergence = (Number of diseased and dead seedlings/ Number of seeds sown germinated seeds) X 100

2.6 Growth parameters and pigments content

Additionally, following a 60-day period, samples of the plants were taken in order to assess some growth features (plant height (cm), fresh and dry weight (g), total chlorophyll and carotenoids (mg/100g FW) (Arnon, 1949).

- Malondialdehyde (MDA) contents

A solution of 0.1% (w/v) trichloroacetic acid was used to homogenize 0.5 g of fresh leaves. One milliliter of the supernatant was combined with four milliliters of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA following ten minutes of centrifugation at 15000 g then, for 30 minutes, the mixture was incubated at 90 °C. After that, the reaction tubes were submerged in an ice water bath to stop the reaction. After five minutes of centrifuging the samples at 10,000 g, the absorbance of the supernatant was measured at 532 nm and at 600 nm; the nonspecific absorption value was eliminated. Using the extinction value of 155 mM-1cm-1, the MDA concentration was calculated *(*Hagege et al., 1990).

- Membrane leakage of electrolytes

Dhindsa et al. (1981) used electrolyte leakage to test the membrane permeability of leaves. Lupine leaves were cut into bits and left for three hours in a beaker filled with distilled water. at room temperature. The solution's conductivity was measured using an Ec-meter (dS/m).

-Defense enzymes

Polyphenol oxidase (PPO)

At 0°C, 100 mg of fresh leaf plant tissues were mixed with sand in 6 ml of 0.1 M phosphate buffer (pH 7.0). Using a clean cloth, the enzyme-containing extract was filtered. Centrifuged for 15 minutes at 3000 rpm, and then kept in an ice bath until needed. Utilizing Sadasivam and Manickam's (2007) methodology, the polyphenol oxidase activity was assessed. Combine two milliliters of enzyme extract with three milliliters of distilled water in a spectrophotometer cell. At ºA490, set the absorbance to zero. To the aforementioned enzyme combination, one milliliter of catechol solution (0.4 mg mL^{-1}) was added, and the reactants were quickly combined. The change in absorbance per minute (ΔA/min) at 490 nm after the addition of catechol solution to start the reaction was used to measure enzyme activity. Likewise, heating at 100 degrees Celsius, which consistently produced zero absorbance, was used to maintain control.

Peroxidase (POD)

According to Beauchamp and Fridovich (1971), a sample of 0.5 g of fresh plant material (leaves) was frozen, then homogenized in 8 ml of 50 mM cold phosphate buffer (pH =7). The homogenate was centrifuged at 4000 rpm at 4 °C for 20 min. The supernatant was used as a raw extract for the enzymatic assays. Mix 1.5 ml of 0.1M phosphate buffer (pH6), 20 µL pyrogallol solution, 500 µL enzyme extract, and 20 µL H_2O_2 solution at zero time. Record the increase in absorbance at 420 nm against the reagent blank. The change in absorbance per 20 seconds

was measured over a linear part of the curve. A unit of peroxidase is defined as the change in absorbance in 20 seconds per millilitre (Maehly and Chance, 1954).

Proline content

Irigoyen et al., (1992) technique was used to determine free proline levels. After homogenizing 50 mg of dry plant leaves in 10 milliliters of 3% aqueous sulphosalicyclic acid, the mixture was centrifuged at 4000 rpm. Add two milliliters of the supernatant, two milliliters of the acidic ninhydrine reagent (1.250 g of ninhydrine was dissolved in 30 ml of glacial acetic acid, 8 ml of orthophosphoric acid, and 12 ml of distilled water. The mixture was heated to be dissolved and kept in the dark), and two milliliters of glacial acetic acid in a test tube. Leave at 100°C for one hour. In an ice bath, the process was terminated. Five milliliters of toluene were used to extract the reaction mixture. Using toluene as a blank, the absorbance of the toluene layer was measured at 520 nm.

Phenol content

To homogenize one gram of fresh plant leaves, ten milliliters of 80% methanol were employed and swirled at 70°C for 15 minutes. Add 5 mL of distilled water, 1 mL of methanol extract, and 250 µL of folin Ciocalteau reagent (1 N). For one hour, incubate one milliliter of saturated Na_2CO_3 solution with one milliliter of distilled water at 25°C. The combination was maintained at 25°C after three minutes. The wavelength at which the blue color absorption was observed was 725 nm. A Folin-reaction with phenol solution was used to create a standard curve that was used to measure the amount of total phenols in fresh tissue. The outcome was given as µg g^{-1} (Zieslin and Ben-Zaken, 1993).

Ascorbic acid

Oser (1979) determined the ascorbic acid (AA), a non-enzymatic antioxidant. After homogenizing 0.1 g of plant leaf tissue in 5 ml of 5% (w/v) sulfosalicylic acid, the sample was centrifuged for 10 minutes at 10,000 rpm. Two milliliters of 2% Na-molybedate, two milliliters of 0.15 N H_2SO_4, one milliliter of 1.5 mM Na_2HPO_4, and one milliliter of tissue extract were all added in the ascorbate reaction mixture. For forty minutes, the mixture was incubated in a water bath at 60°C. The absorbance was measured at 660 nm following cooling and centrifugation for 10 minutes at 3000 rpm. A standard curve created with AA and expressed as mg/g Dw was used to measure the amount of AA.

2.7 Soil Analysis

Total bacterial count was performed on nutrient agar using the spread plate method (APHA, 1992). Total cyanobacterial count was conducted by plating ten-fold serial soil suspension-dilutions in triplicate onto agarized BG11 medium according to (Stanier et al., 1971).

-Dehydrogenase

Dehydrogenase activity in rhizospheric soil was determined according to Casida et al.,(1964) method , 2 g sample was collected and transferred to test tubes. Then mix vigorously after adding 2 ml aliquots of a 0.5% 2,3,5 triphenyl tetrazolium chloride (T.T.C) solution to tris buffer (pH 7.8). It is necessary to soak and briefly immerse soil samples in T.T.C. solution. Rubber silicon stoppers were used to seal the tubes, which were then left in the dark for 24 hours at 30°C. To extract the pink-colored triphenyl formazan (T.P.F.), add 10 ml of pure acetone to each tube and shake constantly for two hours in the dark. After the samples were filtered, the UV spectrophotometer's 485 nm wavelength was used to quantify the intensity of the pink hue that developed. A standard curve was used to determine the formazan concentrations, which were then represented as µg TPF/g dry soil/24 hours. It is necessary to look at and subtract the blank treatment, which did not include soil samples.

-Hydrolytic enzymes

Chitinase

A 100 mL Erlenmeyer flask containing 50 mL of chitin and 5 g of soil samples was incubated for 18 hours at 30°C. The samples underwent a 15-minute centrifugation at 5000 rpm, and the chitinase activity test was initiated using the supernatant. For ten minutes, three milliliters of clear supernatant and three milliliters of DNS (di-nitro salicylic acid) were submerged in a boiling water bath. After 5 minutes, 1ml of 40% rochel salt was added and measured at 575 nm, with the absorbance compared to the N-acetyl glucose amine standard curve (Rodriguez-Kabana et al., 1983).

Protease

Soil extracts (5g) were added to 50 mL Erlenmeyer flasks, along with 2.5 mL of Tris buffer and 2.5 mL of sodium caseinate solution. The flasks were then incubated at 30ºC for 18 hours. A soil without sodium caseinate was used as a control. After incubation, samples were centrifuged at 15000 rpm for 10 minutes at 4ºC. The supernatant was used to initiate the protease activity experiment. Mix 3 ml of 1.4M Na_2CO_3 and 1 ml Folin reagent (1N) with 2 ml of supernatant. The absorbance of the blue colour was measured at 700nm and compared to a tyrosine standard curve (Ladd and Bulter, 1972).

2.8 Statistical Analysis

The experimental data obtained were subjected to analysis of variance (ANOVA), according to the procedures outlined by Snedecor and Cochran (1980).

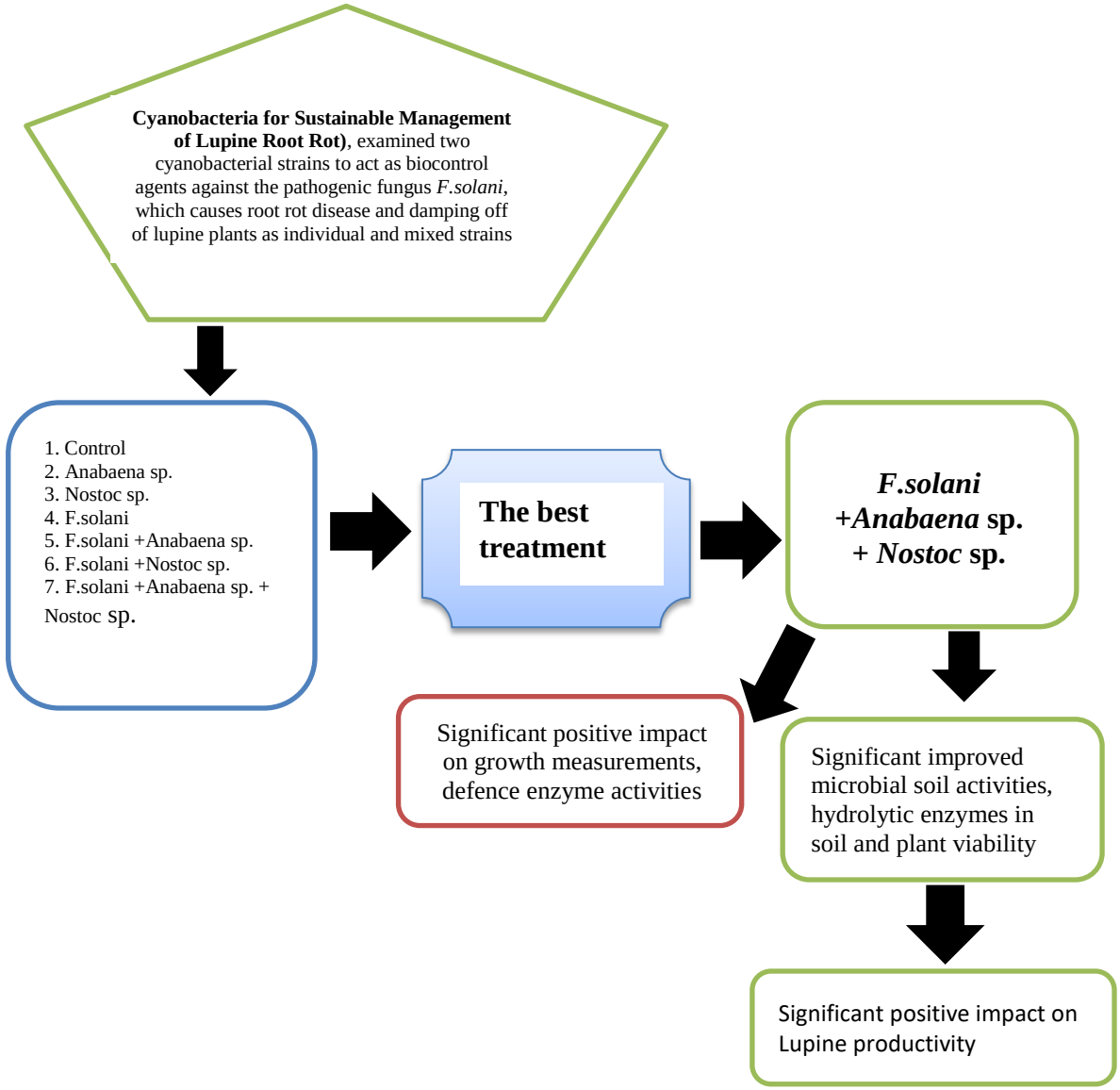

Fig. 1 . Materials and methods of the study.

3. Results

Antifungal activity of cyanobacterial filtrates

The antifungal activity of the filtrate of *Anabaena* sp. and *Nostoc* sp. against *F. solani*, a fungal pathogen that causes root rot, was investigated. The filtrate of both *Anabaena* sp. and *Nostoc* sp. were shown in figure (2) to have antifungal activity against the fungal pathogen *F. solani*. Both strains suppressed the growth of fungal discs

on PAD media in comparison to the control disk. *Anabaena* sp. was redacted the fungus by (45%) and *Nostoc* sp. by (40 %).

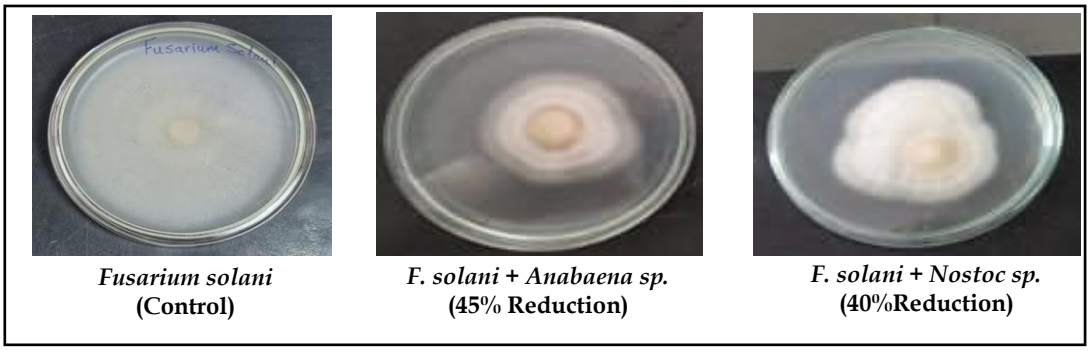

Fig. 2 . Antifungal activity of the filtrate of each of cyanobacteria *Anabeana* sp. and *Nostoc* sp.

Pot experiment

A pot experiment was carried out to investigate the ability of two cyanobacterial strains, *Anabaena* sp. and *Nostoc* sp. to suppress pathogenic fungi *F. solani* which causes root rot disease, as individual and mixed treatments for the lupine plant.

Plant Analysis.

Disease Assessment

Forty-five seeds were sown for each treatment. After 15 days, the number of germinated seeds was counted to determine the root rot ratio. After 45 days, the number of damping plants was recorded to calculate the damping off ratio and survival rates were recorded after 60 days. Table (2) showed that *Anabaena* sp. and *Nostoc* sp. filtrates were found to effectively root-rot diseases. The treatment of the fungus F. solani that was infested caused the most significant predamping of ratio (30%); however, when cyanobacteria were present, either as a single strain or in combination, the predamping of decreased by 40% and by more than 50%, respectively. The post-damping off ratio showed a similar pattern, and the plants that survived had the highest number of cyanobacteria Anabaena sp. and Nostoc sp.in the uninfected treatment.

Table 2 . The effectiveness of the cyanobacterial filtrates in suppressing the pathogenic fungus *F. solani* under greenhouse conditions.

Treatments	*F. solani*		
	Pre-damping off %	Post- damping off %	Survival Plants%
Control	7(e)	8(e)	85(ab)
***Anabaena* sp.**	4(d)	3(g)	93(a)
Nostoc sp.	5(d)	4(f)	91(a)
F.solani	30 (a)	25(a)	44(d)
***F.solani +Anabaena* sp .**	16 (b)	12(C)	72(b)
***F.solani +Nostoc* sp.**	17(b)	15 (b)	70(bc)
***F.solani +Anabaena* sp. + *Nostoc* sp.**	12(C)	10 (d)	78(b)

Figure (3 A-C) showed the plant height, fresh weight, dry weight number of buds per plant and pigments content, all growth features increased in the lupine plant with respect to the presence of cyanobacteria in both infected and uninfected plants. The significant plant height was recorded by treatment of *Nostoc* sp. The pigment contents of lupine plant leaves are shown in Figure (3-C) which showed results indicating thatthe presence of cyanobacterial strains increased the content of chlorophyll and carotenoids in both infected and non-infected plants. The treatment of *F.solani + Anabaena* sp. *+ Nostoc* sp. produced the highest levels of chlorophyll and carotenoids (37.01 and 24.20 mg/100g FW), which were followed by treatments of cyanobacterial strains with non-infected plants.

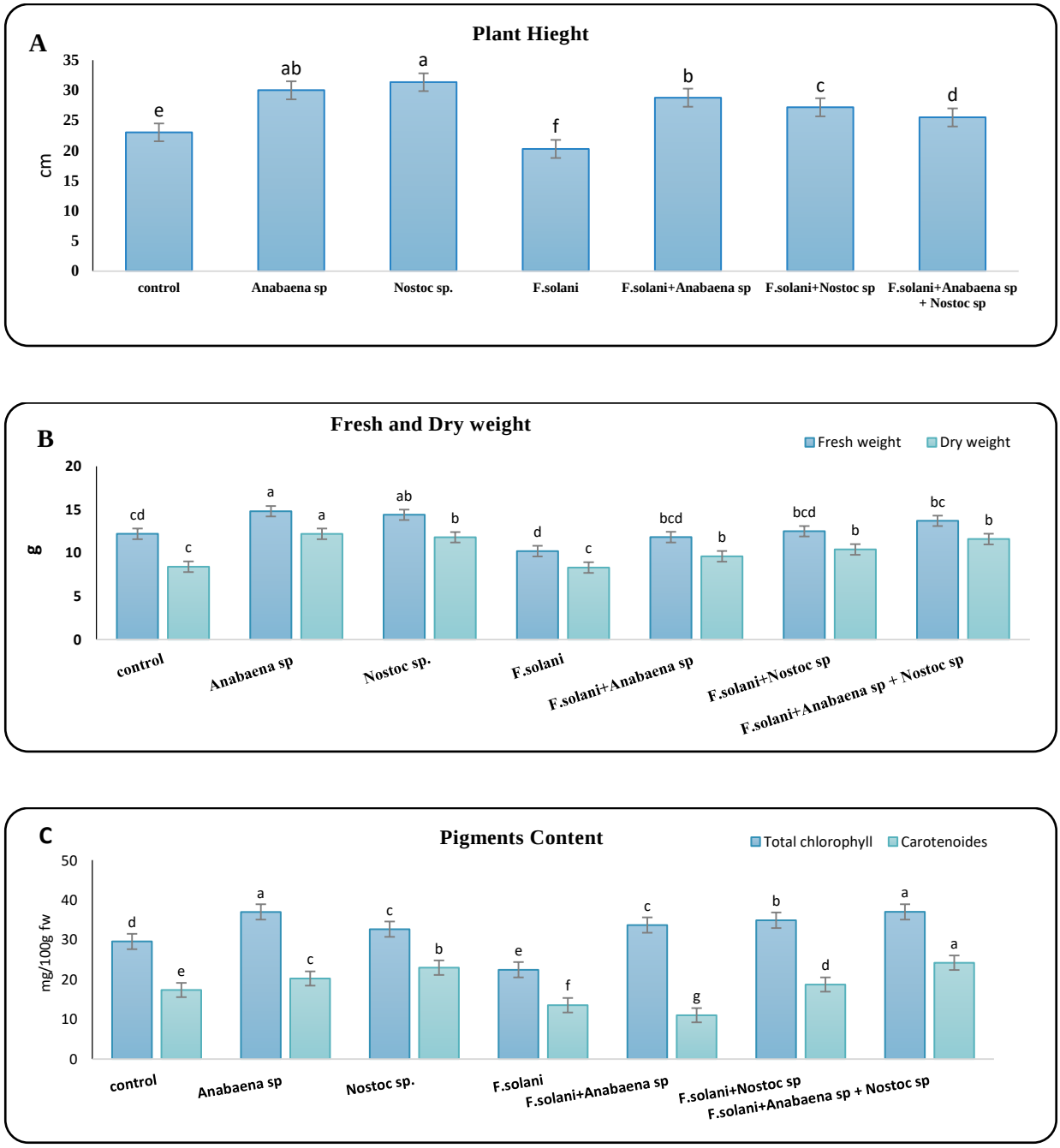

Fig. 3 . Effect of both cyanobacterial strains as individual and mixed inoculation on plant height (A), fresh and dry weight (B) and pigment contents (C) in leaves of lupine.

Malondialdehyde (MDA) contents and Membrane leakage of electrolytes

The presence of a cyanobacterial inoculant reduced MDA accumulation in lupine leaves in the mixed treatment, but there was no significant difference between single strains, while it increased in the presence of the *F. solani* fungal pathogen. The lowest MDA accumulations were (0.60 and 0.63 μmole/g FM) recorded by *Anabeana* sp. and *Nostoc* sp. cyanobacteria treatments, respectively, followed by control treatment and the highest MDA accumulation was (2.64 μmole/g FM) recorded by infected treatment by *F. solani* fungal pathogen (Fig. 4).

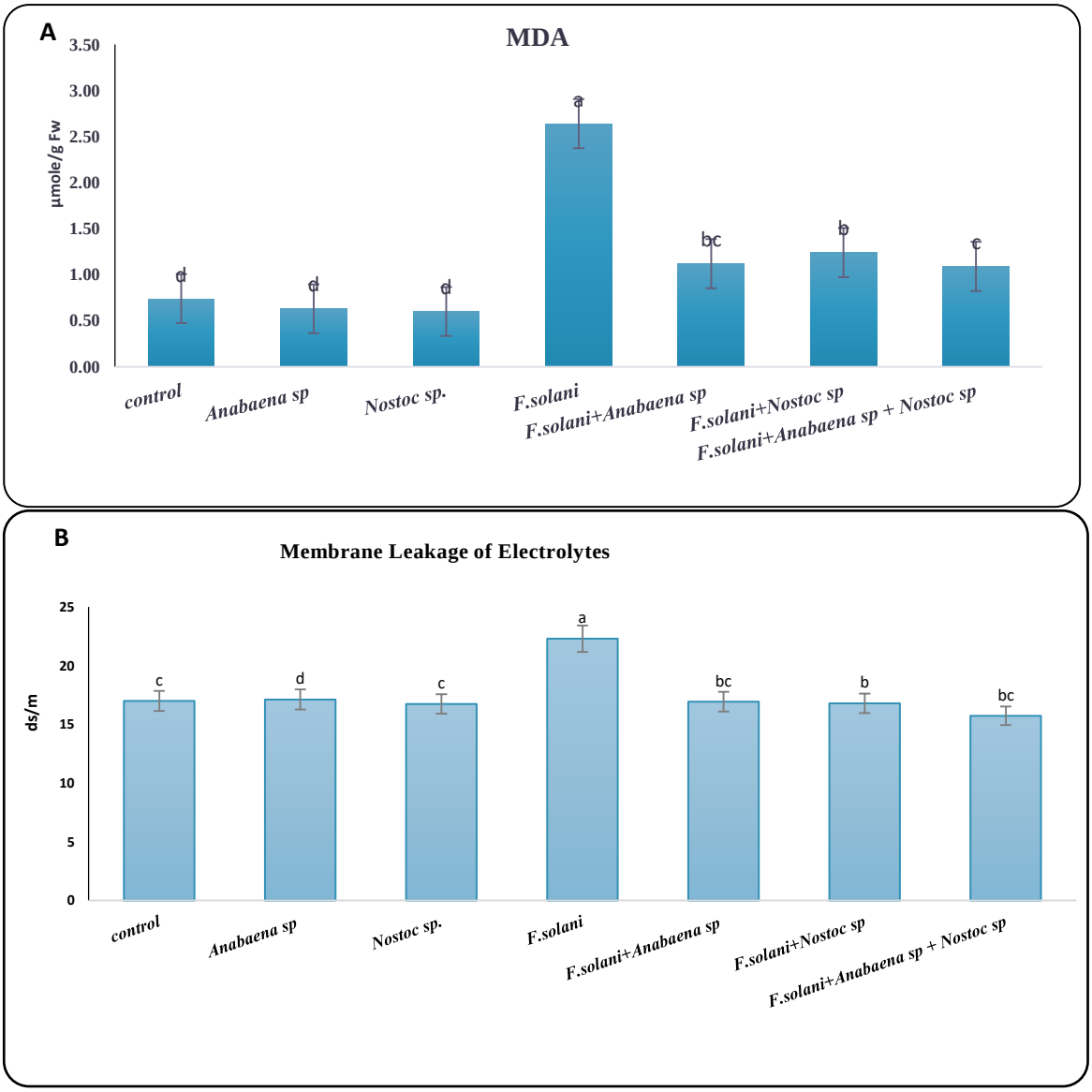

Fig. 4. Effect of both cyanobacterial strains as individual and mixed inoculation on MDA activity (A) and membrane leakage electrolytes (B) in leaves of lupine plant.

Defense Enzyme Activities in plant leaves

Fig. 5. illustrates activities of polyphenol oxidase (PPO) and peroxidase (POD) enzymes, often known as defense enzymes or oxidative enzymes. Cyanobacterial strains *Anabaena* sp. and *Nostoc* sp. showed the highest increase in defense enzymes in the presence of infected with *F. solani* fungus, The presence of both cyanobacterial strains associated with pathogenic fungus resulted in the greatest PPO and POD values (0.45 and 1.9 units per gram of plant tissue, respectively), while the control treatment recorded the lowest PPO and POD activities due to the absence of infection .Single cyanobacterial strain associated with pathogenic fungus (0.42 and 1.83 units per gram of plant) in *F. solani* + *Anabaena* sp. treatment and (0.43 and 1.79 units per gram of plant) *F. solani* + *Nostoc* sp . treatment.

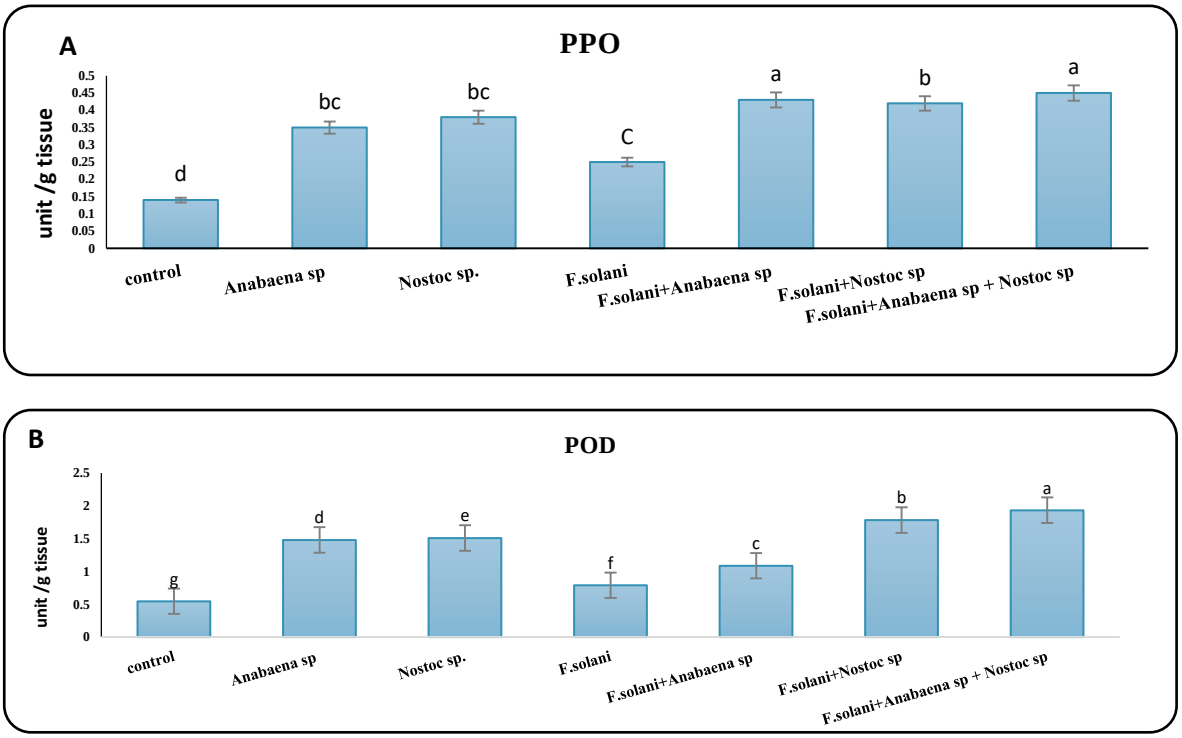

Fig. 5 . Effect of both cyanobacterial strains as individual and mixed inoculation on polyphenol oxidase (A) and peroxidase (B) enzymes activities in leaves of lupine plant.

Data in table (3) reported proline content, phenols content and ascorbic acid in leaves of lupine infected and treated plants after 60 days of cultivation, plants accumulate proline under various abiotic and biotic stress situations. Proline build up and antioxidant enzyme induction occur together, consequently, Proline levels increased in infected plants compared to treated and uninfected plants, with the greatest proline reported (3.1 mg/g FW). in *F. solani* treatment and decreasing in treated plants (2.6, 2.4 mg/g FW) in *F. solani + Nostoc* sp. and *F. solani + Anabaena* sp., respectively, while uninfected plants recorded the lowest proline content. Phenolic compounds have antimicrobial and antioxidant characteristics that help plants avoid pathogenic infections and protect tissues from reactive oxygen species. So infected plants give phenolic compounds more than uninfected plants and the highest value of phenolic compound was (115.1 mg/g FW) by treatment of *F. solani+ Anabaena* sp. *+ Nostoc* sp. followed by (107.6 mg/g FW) for treatment of *F. solani + Nostoc* sp., while control and uninfected plants reported less phenolic compounds due to the absence of pathogenic fungi the presence of cyanobacteria increased the plant's health. One of the most prevalent antioxidant compounds in plants is ascorbic acid, it is the first line of defence against harmful reactive oxygen species, shielding plant cells from a variety of environmental variables that cause oxidative stress, such as injury, ozone, excessive salinity, and pathogen attack, Infected plants had higher levels of ascorbic acid than treated or uninfected plants, following the same trend. The highest value of ascorbic acid was (20 mg/g FW) reported by *F. solani* followed by (18 mg/g FW) for *F.solani + Anabeana* sp. treatment and (17 mg/g FW) for *F. solani + Nostoc* sp. treatment.

Table 3. Effect of both cyanobacterial strains as individual and mixed inoculation on proline, phenolic compounds and Ascorbic acid contents in leaves of lupine plant.

Treatments	Proline (mg/g DW)	Phenols (mg/g FW)	Ascorbic acid (mg/g DW)
Control	2.1(d)	89.4(e)	14(c)
***Anabeana* sp.**	1.9(c)	95.4(c)	16(b)
***Nostoc* sp.**	1.8(c)	96.9(d)	15(b)
F. solani	3.1(a)	99.8(b)	20(a)
***F.solani* + *Anabeana* sp.**	2.4(b)	106.9(a)	18(ab)
F. solani* + *Nostoc sp.	2.6(b)	107.6(b)	16(ab)
F. solani* + *Anabeana* sp. *Nostoc sp.	1.9(c)	115.1(a)	15(b)
LSD 0.05	0.1735	2.227	1.48

Soil Analyses

Soil analysis followed the same trend as plant analysis. Figure (6 and 7) demonstrated soil microorganism activity, including total bacterial and cyanobacteria count, dehydrogenase activity, protease and chitinase hydrolytic enzymes. The highest count of bacteria ($58*10^6$ CFU/ml) and cyanobacteria ($18*10^2$CFU/ml) were recorded by mixed treatment in the presence of infected fungus, and the lowest count of bacterial ($45*10^6$, $48*10^6$ CFU/ml) and cyanobacteria ($8*10^{2,}$ $9*10^2$ CFU/ml) recorded by control and *F. solani* treatments. Dehydrogenase enzyme activity (DHA), a measure of energy transfer, is used to assess soil microbial activity. The *F.solani* + *Anabaena* sp. + *Nostoc* sp. treatment had the greatest DHA activity due to an increase in total microbial count, which increased in the presence of cyanobacteria but had non-significant difference from the control

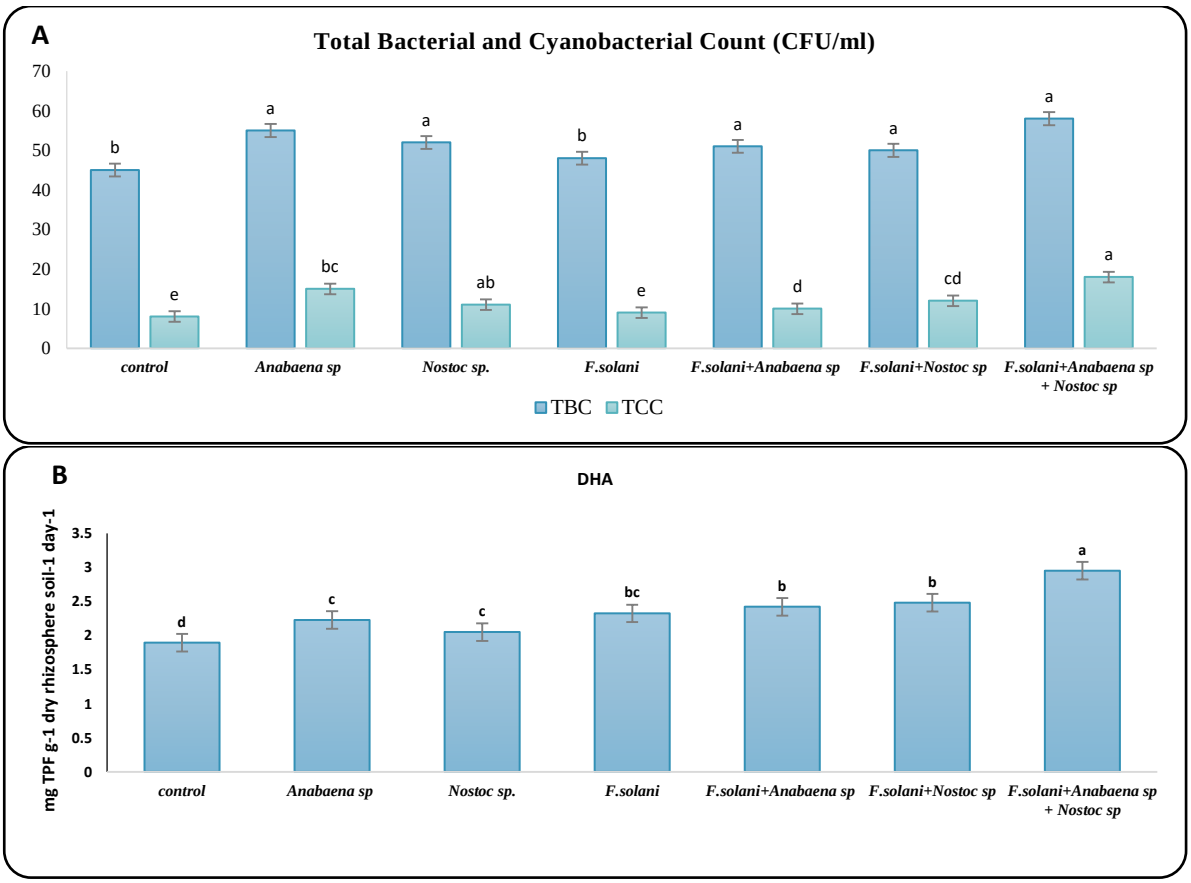

Fig. 6. Effect of both cyanobacterial strains as individual and mixed inoculation on total bacterial and cyanobacterial count (A) and dehydrogenasease enzyme activity (B) in the rhizosphere of lupine plant.

As shown from figure (7), chitinase is a potentially helpful antifungal agent for plant disease resistance and biological control in agriculture. Cyanobacteria produce chitinase for defence against *F. solani*. As a result, chitinase enzyme activity increases in the presence of cyanobacterial strains, either as individual or as mixed strains with the infected pathogen *F. solani* than uninfected and control treatments. *F.solani* + *Anabaena* sp. + *Nostoc* sp. treatment recorded the largest chitinase activity (22.8 Unit/ g soil) followed by *F.solani* + *Anabaena* sp. (19.6 Unit/ g soil) and *F.solani* + *Nostoc* sp. (18.5 Unit/ g soil) and control treatment recorded the lowest chitinase activity (13.4 Unit/ g soil). With the same trend protease enzyme recorded the highest activity in the treatment of *F.solani* + *Anabaena* sp. + *Nostoc* sp. (0.712 Unit/ g soil) , *F.solani* + *Nostoc* sp. (0.602 Unit/ g soil) and *F.solani* + *Anabaena* sp.(0.533 Unit/ g soil), but it reduced in the other treatments due to the absence of the pathogenic fungus.

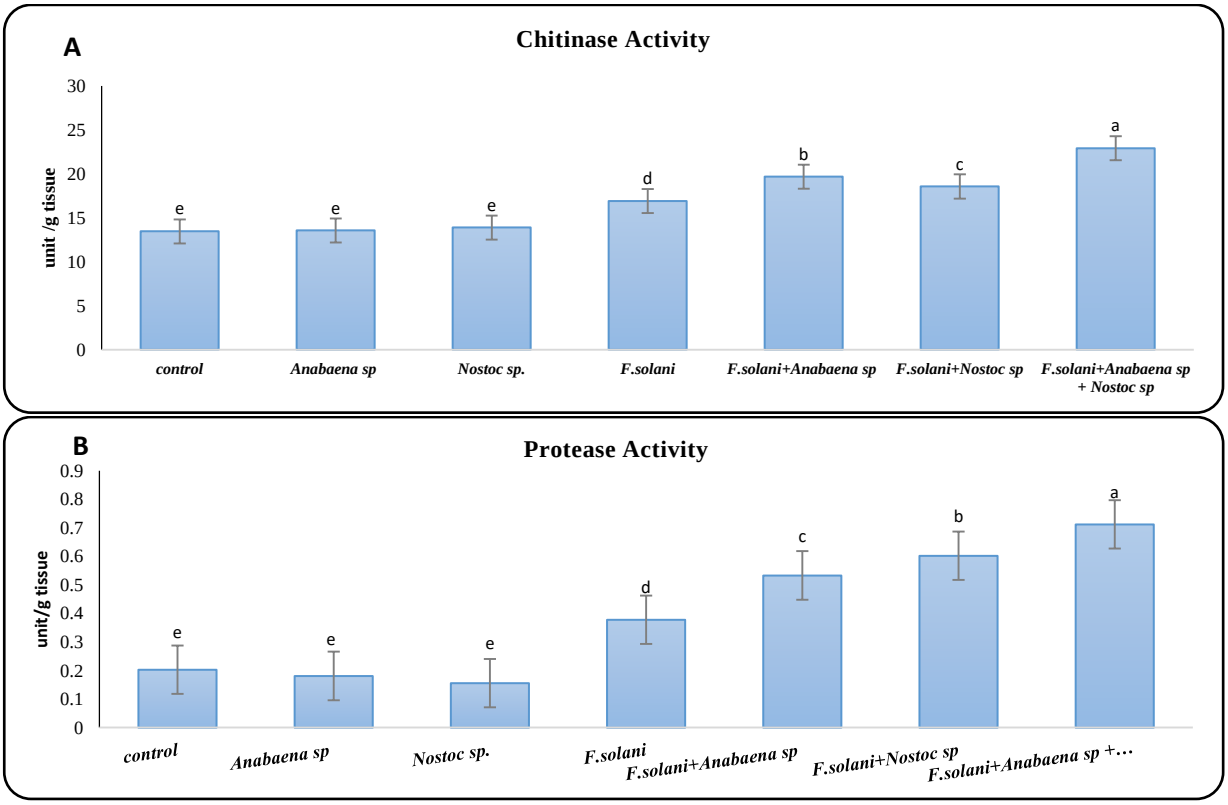

Fig. 7. Effect of both cyanobacterial strains as individual and mixed inoculation on chitinase (A) and protease (B) enzymes activities in rhizosphere of lupine plant.

4. Discussion

Fungal pathogens remain a significant challenge in agricultural systems, causing fruit damage, postharvest losses, and widespread crop infections. Prominent soil-borne fungi such as *Rhizoctonia*, *Verticillium*, *Pythium*, and *Phytophthora*, along with species of *Fusarium* and *S. sclerotiorum*, are among the most destructive pathogens in farmed cropping systems (Godana et al., 2023). Their ability to infect multiple host plants (polyphagy) and thrive under diverse environmental conditions exacerbates their impact, leading to substantial economic losses. Lupine crops in Egypt are severely impacted by soil-borne diseases like damping-off, root rot, and wilt, leading to significant yield losses (Abou El Nour et al., 2020). These diseases, caused by fungi such as *S. sclerotiorum*, *Fusarium* and *Rhizoctonia*, are challenging to manage due to their wide host range and adaptability (Pastrana et al., 2016). Biological control, particularly using cyanobacteria like *Nostoc* and *Anabaena*, offers a sustainable alternative to chemical pesticides. These microorganisms produce bioactive compounds with proven effectiveness against pathogens causing root rot and damping-off (Yadav et al., 2022; El-Sheekh et al., 2022). Cyanobacteria-based biocides also provide environmental advantages by degrading rapidly and reducing non-target effects, making them suitable for organic and sustainable farming systems (Shah et al., 2021). Expanding research into these biocontrol agents could enhance their application for managing soil-borne diseases.

The results of this study demonstrate that filtrate of *Nostoc* and *Anabena* cyanobacterial strains have effective biocontrol agents against *F. solani* fungi, primarily due to their ability to produce diverse bioactive chemical compounds. These compounds include enzymes capable of breaking down fungal cell walls, offering a promising solution for controlling fungal pathogens in both biotechnological and agricultural applications. Enzymes like chitinase, which hydrolyzes β-1,4-glycosidic linkages in chitin, selectively degrade the structural components of fungal cell walls and inhibiting fungal growth (Rkhaila et al., 2021). This enzymatic activity not only limits fungal infection but also highlights cyanobacteria's role as a source of potent natural antifungals. The study further confirms that algal extracts inhibit fungal infections through two primary mechanisms: direct antifungal activity and the induction of plant resistance. Results showed that cyanobacteria inoculated plants have significant increase in all measured parameters in plant or in soil compared to control ones, and the most significant treatment was the mixture *Nostoc* and *Anabena* treated plants. And this funding similar to (Shukla et al., 2021), who demonstrated that algal extracts inhibit fungal infections through both direct antifungal action and

plant resistance. The antifungal efficacy of cyanobacteria is attributed to their synthesis of specific chemical compounds, including chitinase homologues, endoglucanase, benzoic acid, and phenolic compounds (Rkhaila et al., 2021; Nowruzi and Nemati, 2023). These compounds act synergistically, with chitinase breaking down fungal cell wall components, endoglucanase degrading cellulose, and phenolic compounds interfering with fungal metabolism and growth. The ability to produce such a diverse array of bioactive agents underlines the versatility and potential of cyanobacteria in integrated pest management strategies.

Cyanobacteria play a vital role in enhancing crop growth, nutrition, yield, and soil fertility. Their presence has been shown to stimulate chlorophyll production and overall plant development, underscoring their significance in sustainable agricultural practices. Cyanobacteria promote plant growth through several mechanisms, including nitrogen fixation (diazotrophy), auxin production, and the secretion of nitrogenous and carbon-based compounds, as well as secondary metabolites. These activities contribute to improved nutrient availability and plant health, ultimately supporting higher productivity (Bharti et al., 2021). Membrane leakage and MDA concentration are commonly associated indicators of oxidative stress, reflecting the destabilization of cell membranes under adverse conditions (Garcia-Caparros et al., 2021). Electrolyte leakage assays are widely utilized to assess the extent of cell damage in plant tissues caused by both biotic and abiotic stressors. These stressors include pathogen attacks, insect herbivory, physical wounding, UV radiation, oxidative stress, and environmental challenges such as salinity, drought, cold, and heat stress (Mearaji et al., 2021). This method effectively measures membrane stability by determining the proportion of cells compromised under stress conditions. Malondialdehyde (MDA), a key product of lipid peroxidation, serves as a reliable biomarker for oxidative stress in plants. Elevated MDA levels in infected plants indicate extensive membrane damage, as corroborated by Pan et al. (2021), who observed heightened MDA accumulation in stressed plants.

The findings of this study revealed that membrane leakage of electrolytes increased significantly in infected plants compared to treated and uninfected plants, following a pattern similar to that observed with malondialdehyde (MDA) accumulation. Plants infected with *F. solani* exhibited the highest levels of electrolyte leakage, indicating severe membrane damage and oxidative stress. Conversely, plants treated with both cyanobacterial strains displayed the lowest electrolyte leakage, demonstrating the protective effects of cyanobacteria against oxidative stress. Plants treated with a single cyanobacterial strain also showed reduced membrane leakage, though to a lesser extent than the dual treatment, highlighting the enhanced efficacy of combined cyanobacterial applications. These results align with the observations of Ngou (2020), who reported a similar trend in treated plants compared to infected controls. The reduced leakage and MDA levels in cyanobacteria-treated plants suggest that these treatments effectively mitigate oxidative stress, likely through the modulation of antioxidant defenses and the stabilization of cellular structures.

The application of *Anabaena* sp. has been shown to enhance plant defense by increasing the activity of several key defense enzymes. According to Righini et al. (2022), enzymes such as endochitinase, N-acetylhexosaminidase, chitin 1,4-chitobiosidase, β-1,3-glucanase, and peroxidases were significantly activated in plants treated with *Anabaena* sp. Furthermore, biofilm-forming *Anabaena* sp. was found to stimulate the activity of peroxidases, phenylalanine ammonia lyase (PAL), and polyphenol oxidases (PPO) in both roots and shoots, demonstrating its potential to boost plant systemic resistance (Kapoore et al., 2021). The ability of cyanobacteria to produce bioactive metabolites, including polyphenols and flavonoids with strong antifungal properties, positions them as a sustainable alternative to synthetic fungicides in managing phytopathogenic fungi (Senousy et al., 2022). Additionally, their capacity to enhance soil nutrient bioavailability and plant physiological immunity highlights their broader role in sustainable agriculture (Bhardwaj et al., 2024). The increased expression of PPO and peroxidase (POD) enzymes in mixture treated plants underscores cyanobacteria's role in fortifying plant defense mechanisms. Polyphenol oxidases, which are typically located in chloroplasts, play a crucial role in plant defense. Under stress conditions such as senescence, wounding, pest or pathogen interactions, and postharvest handling, the compartmentalization of PPO and vacuolar phenolic substrates is disrupted. This interaction triggers defense-related oxidative reactions, highlighting the role of PPO in protecting plants during stress events (Upadhyay, 2020).

Proline plays a critical role in plant responses to biotic and abiotic stress. Since the 1960s, numerous studies have demonstrated that plants rapidly accumulate substantial amounts of free proline under various stress conditions. This accumulation is thought to contribute to stress tolerance by stabilizing proteins, protecting cellular structures, and balancing osmotic pressure (Singh et al., 2022). The ability of proline to mitigate stress effects highlights its significance as a biochemical marker and functional molecule in stress physiology. Phenolic compounds, another crucial class of secondary metabolites, are synthesized via the phenylpropanoid pathway and

are integral to plant defense mechanisms. These aromatic compounds help plants combat a wide range of abiotic stresses, such as drought, salinity, and UV radiation, as well as biotic stresses caused by pathogens like bacteria, fungi, and viruses (Kumar et al., 2020). Phenolic compounds provide protection through both antioxidant and non-antioxidant mechanisms. Acting as ROS (reactive oxygen species) detergents, they stabilize cell membranes and prevent lipid peroxidation, thus maintaining cellular integrity under stress conditions (Mounir et al., 2022). Ascorbic acid (vitamin C) also plays a multifaceted role in plant defense. Beyond its well-known antioxidant properties, it functions in the detection of reactive oxygen species and the activation of defense-related signaling pathways. Several studies have reported an increase in ascorbic acid levels following pathogen attacks, suggesting its involvement in enhancing plant immune responses (Dumanović et al., 2021). Elevated ascorbic acid levels promote the biosynthesis of oxalic acid, which leads to the accumulation of hydrogen peroxide (H_2O_2), a vital signaling molecule in plant defense (Wu et al., 2024). This process not only limits pathogen spread but also strengthens the plant's overall defense system.

These findings align with the work of Raymaekers et al. (2020), who highlighted the potential of leveraging plant immune-defense mechanisms to reduce reliance on chemical pesticides. By inducing disease resistance through natural or synthetic elicitors, this approach aims to offer sustainable solutions for controlling plant diseases. Such strategies underscore the growing focus on eco-friendly methods to enhance crop protection while minimizing environmental impact. Soil enzyme activities serve as valuable indicators for assessing the economic and sustainability impacts of agricultural practices and for diagnosing soil types (Techen et al., 2020). Among these, dehydrogenase enzyme activity (DHA) is particularly significant as it reflects soil microbial activity and energy transfer processes. In addition to DHA, soil microbes, particularly plant growth-promoting rhizobacteria (PGPR), produce a variety of hydrolytic enzymes, including chitinase, glucanase, protease, and cellulase, which play critical roles in biocontrol by lysing phytopathogens through hyperparasitism. The antagonistic properties of these enzymes against phytopathogens are essential for effective biocontrol (Wang et al., 2021; Singh et al., 2022).

Cyanobacteria have shown significant potential as biocontrol agents due to their ability to produce hydrolytic enzymes and secondary metabolites with antifungal properties. For instance, *Anabaena* sp. generates chitinase enzymes that inhibit fungi such as *Fusarium solani*, *Fusarium oxysporum*, and *Rhizoctonia solani* (Bhardwaj et al., 2024). In addition, cyanobacterial extracts enhance the efficacy of other bioagents against bacterial, fungal, and nematodal pathogens (Afify and Ashour, 2023). Strains like *Nostoc spp.* act as bioagents against phytopathogenic fungi, with phenols and polysaccharides contributing to their antifungal activity. Chitinase, in particular, enhances plant defense by breaking down chitin in fungal cell walls, insect peritrophic membranes, and other structures. It activates defense pathways, offering protection against pests and diseases (Nayak et al., 2020). Similarly, proteases degrade peptide bonds in proteins, contributing to pathogen suppression by modifying their structure and function (Shankar et al., 2021). These enzymes collectively bolster plant resistance and reduce reliance on chemical inputs.

Cyanobacteria also produce antagonistic compounds, such as benzoic acid and majuscule amide C, which have been identified in *Anabaena laxa* and *Calothrix elenkinii*, respectively, further underscoring their biocontrol potential (Shah et al., 2021). *Anabaena iyengarii* and *Nostoc sp.* have been reported to produce chitinase homologs and microcystins, which exhibit strong fungicidal properties (Khder et al., 2022). Applications of cyanobacteria in soil and on plant surfaces have demonstrated significant effectiveness in managing damping-off diseases. For example, treatments with *Nostoc muscorum* and *Nostoc entophytum* on soils infected with *R. solani* improved seedling survival rates and enhanced plant growth, including root and shoot biomass (Kalyanasundaram et al., 2020). Similarly, *Nostoc linckia* has been effective in reducing wilt disease caused by *F. solani* in tomato plants (El-Sheekh et al., 2022).

5. Conclusion

Cyanobacteria play a vital role in integrated pest and crop management by suppressing soil-borne pathogens, improving plant resilience, and enhancing soil health. The findings support their broader application in sustainable agriculture, particularly for managing destructive diseases in lupine crops. Future research should focus on optimizing cyanobacterial formulations for field application and understanding their long-term ecological impacts.

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