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Phaseolus vulgaris (L.) Growth Promotion by *Cladosporium halotolerans* Inoculation Under Salinity Stress

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Abstract: Salinization induced by salt stress is a critical environmental factor, and limits the expansion of agricultural areas and population distribution in continental regions, including Saudi Arabia. Common beans, a vital source of protein, energy, and dietary fibers, are negatively affected by salt stress. In this research, the endophytic fungus *Cladosporium halotolerans* was utilized to remediate saline soils and enhance common bean growth and resilience. The results of this study demonstrated that soil treatment with *C. halotolerans* enhances the soil properties by decreasing soil pH and increasing soil organic matter content under saline conditions. Inoculation by *C. halotolerans* also significantly improved plant growth parameters, induced systemic resistance to salinity, and increased the levels of chlorophyll b and carotenoids. Fungal inoculation also causes stress relief as indicated by reducing malondialdehyde concentration by 27.4% lower than stressed plants. Microscopic images revealed the active association and colonization of *C. halotolerans* within the roots of the *Phaseolus vulgaris* both under control and saline conditions. Therefore, utilizing endophytic fungi *C. halotolerans* for saline soil remediation appears to be a promising alternative in plant treatments, highlighting their potential as valuable resources for both research and commercial applications under salinity stress.

Keywords: common bean; endophytic fungi; *Cladosporium halotolerans*; salt stress; remediation; plant tolerance

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1. Introduction

Stresses associated with climate change, including drought, salinity, soil compaction, and heat, as well as stresses caused by climate or environmental pollution, restrict global crop yields and their quality. This, in turn, results in significant socioeconomic issues and food insecurity [1]. Salinity stress is a significant environmental abiotic factor that adversely impacts plant yield and the quality of agricultural products [2]. Plant growth and development are hampered by salinity due to water stress, cytotoxicity caused by excessive absorption of ions such as sodium (Na^+) and chloride (Cl), and nutritional imbalance [3]. Furthermore, salinity affects plants' ability to utilize water and absorb nutrients and inhibits nodulation and nitrogen fixation, leading to a decrease in growth rate and alterations in plant metabolic processes. However, numerous breeding approaches and genetic engineering techniques are being employed in research to develop crops with improved resistance to salinity [4]. To address the challenges posed by abiotic stress on plant growth and productivity, using beneficial microbial strains is the most practical, dependable, and sustainable solution. Plant endophytes are essential in supporting and improving plant growth and resilience under various stress conditions [5].

Phaseolus vulgaris (common bean) is a vital grain legume that is grown worldwide and used as a human food and animal feed. It represents a key source of protein, vitamins,

and minerals, accounting for 15% of the global protein supply and 30% of the world's caloric intake as recorded by the United Nations Food and Agricultural Organization (FAO) [6,7]. Common beans and other edible legumes are essential today for addressing major socioeconomic issues and accelerating the shift to plant-based diets as a source of proteins, vitamins, minerals, and fibers, particularly in underdeveloped nations [8,9]. As the bean is mainly grown in poorly irrigated and partly saline conditions throughout the world, it is exposed to great deal of salt stress [10]. Reduction in plant growth and dry-matter accumulation under saline conditions has been reported in several grain legumes, including *P. vulgaris* [11,12]. Growth retardation could be attributed to the inhibition of cell elongation [13].

Plants host a diverse range of microorganisms, including bacteria, fungi, archaea, algae, and protists, both inside and on their surfaces. Over long-term evolution, complex interactions have developed between these species, resulting in symbiotic relationships rather than them existing as separate entities [14]. The biodiversity of endophytic fungi is affected by a number of factors, including biotic and abiotic factors [15]. The hyper-diversity of endophytic fungi is attributed to their ubiquity, as they inhabit approximately 300,000 plant species on earth [16]. The diverse metabolic and genetic strategies used by plant-associated fungi play an important role in mitigating the impact of salt stress and other abiotic stresses associated with extreme environmental conditions. The phrase "Induced Systemic Tolerance" (IST) refers to the activation of abiotic stress responses by microbes [17]. Several endophytic fungi with potential biological control uses have been identified. Notably, *Cladosporium* species have emerged as highly effective agents in protecting plants from a wide range of biotic and abiotic stressors. *Cladosporium* species have proved their practical utility in stimulating plant growth and yielded remarkable bioactive compounds with significant antibacterial and antifungal activity [18]. Moreover, gibberellic acid (GA) and indole-3-acetic acid (IAA) are significant hormones produced by *Cladosporium* species to enhance plant growth and yield [19]. Qiang et al. [20] identified that inoculating plants with the endophytic fungi efficiently relieved water stress.

Some species of *Cladosporium* were used previously to enhance tolerance to biotic stress factors in beans including common bean [21], faba bean [18], and black bean [22]. However, there are only rare studies concerning the effect of *Cladosporium* on alleviating plant growth under abiotic stress factors. The fungus *C. halotolerans* has also been utilized to enhance the growth of various crops [23]. Endophytes isolated from plants that live in extreme environments may help promote plant growth, as these challenging conditions often foster mutualistic relationships between plants and endophytes [24]. *C. halotolerans*, a fungus found in hypersaline environments [25], is assumed to improve plant tolerance to salinity stress. The outcomes provide valuable insights into the interactions between fungal endophytes and common beans, demonstrating the potential of this endophyte to enhance salinity tolerance in the beans. Additionally, in this study, a promising alternative is presented for advancing salinity resistance research in crops.

2. Materials and Methods

2.1. Preparation of C. halotolerans Inoculum

An endophytic *C. halotolerans* isolated from *Catharanthus roseus* was obtained from the culture collection of the Microbiological Laboratory at the Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. Fungal isolate was grown on PDA medium, cut into small square agar cultures, and transferred into shaker flask with 100 mL of potato dextrose broth (PDB) medium to allow fungal growth for 14 days at 25 °C [25].

2.2. Experimental Design and C. halotolerans Inoculation

The experiment in this study was carried out at the green house of Biological Science department, King Abdulaziz University in Saudi Arabia during the time period from the middle of September 2023 to the middle of October 2023. The weather was mildly windy, with moderate humidity. The average maximum temperature was about 35 °C during

the day while the minimum temperature was 22 °C at night. Ethyl alcohol (70%) and sodium hypochlorite (2.5%) were used to surface sterilize common bean seeds, and then the seeds were washed several times with sterile distilled water [26]. Formerly, the seeds were implanted in sealed plastic pots contained 5 kg of sterilized soil. The plants were grown under natural light conditions in the green house and irrigated conditions each 48 h to keep field capacity level. The pots were arranged in a randomized complete block design in 3 replicates and separated into two groups. One group was irrigated with tap water containing 50 mM NaCl for saline stress conditions, whereas the other was irrigated with tap water (control); both groups were grown for two weeks. Then, each group was divided into two sub-groups, one of them inoculated with *C. halotolerans* suspension and the other not. Fungal inoculation was performed by adding 100 mL/pot of *C. halotolerans* suspension (10^8 cell/mL) to the soil of two-week-old salinized and non-salinized plants that had grown for an extra two weeks. At the end of the experimental period, 30-day-old plants were collected for growth measurements and further plant analysis.

2.3. Soil Analysis

The soil properties including pH, electrical conductivity, and organic matter content were measured at the end of the experimental period.

2.3.1. Soil pH

Soil pH was determined following the method of [27]. Ten ml of distilled water was poured into a flask filled with 10 g of soil. The flask was shaken to mix its contents and allowed to settle for 10 min. The mixture was then filtered using a filter paper to separate the soil from the liquid. A pH meter (Mettler Toledo AG, Columbus, OH, USA) was used to determine the pH level of the solution.

2.3.2. Soil Electrical Conductivity (EC)

The soil EC was determined according to [28]. A total of 10 g of soil was put into 250 cm^2 glass beaker with 50 cm^2 distilled water, left overnight, then filtered through Whatman filter paper. EC meter was used to evaluate the electrical conductive for soil extract using decmins/m as a concentration for soil anions.

2.3.3. Soil Organic Matter

Organic matter percentage of soil and plant samples was determined according to [29]. Five grams of soil was put into an oven and heated at 110 °C for 2 h. The temperature of the soil was allowed to drop by placing the soil in desiccators, after which it was weighed and later placed in a furnace. The soil was heated again at 500 °C for 3 h, and left to cool overnight. Next, the soil was placed in desiccators again and later weighed. Soil organic matter percentage calculated as follows:

$$\frac{\text{Dry soil (g)} - \text{incinerated soil (g)}}{\text{Dry soil (g)}} \times 100$$

2.4. Plant Growth Parameters

After treatment, 30-day-old plants were collected and rinsed with tap water and gently dried with filter paper to eliminate excess moisture. The growth of *P. vulgaris* under varied conditions was assessed by measuring the fresh (FW) and dry weight (DW) of both shoots and roots in grams (g) and the shoot and root length in centimeters (cm). Leaf area in (cm^2) was calculated using the following equation according to the Larche [30] method:

$$\text{Leaf Area} = \text{RLB}$$

where R = coefficient determined by a correlation of L and B of the plant leaf; L = leaf length; and B = maximum leaf breadth.

Dry weights were determined following drying at 70 °C for 48 h until a consistent weight was achieved. Another batch of plants was stored at −80 °C, and then frozen in liquid N_2 for malondialdehyde determination.

2.5. Plant Pigments Determination

Plant pigment including chla, chlb, and carotens was measured according to the method described by Walz et al. [31] using a Lamda 25 UV-Vis spectrophotometer. Briefly, 50 mg of fresh leaves were extracted in 5 mL ethanol (95%) in water bath at 60 °C until the discoloration of the leaves. The volume was then adjusted to 5 mL with ethanol. The absorbance of the developed green color was measured at 664 nm, 649 nm, and 470 nm against 95% ethyl alcohol as a blank.

2.6. Malondialdehyde Determination

The level of membrane damage was evaluated by calculating malondialdehyde (MDA) levels using thiobarbituric acid, following the method outlined by Narwal et al. [32]. In brief, 100 mg of fresh root tissue was homogenized with 1.5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for ten minutes, and 1 mL of the supernatant was combined with 2 mL of 20% TCA containing 0.5% TBA. This mixture was heated at 95 °C in a water bath for 30 min and then rapidly cooled in an ice bath. After cooling, the solution was centrifuged again at 10,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm and 600 nm using a Perkin Elmer Lambda 25 UV/Vis spectrophotometer, with a blank used as the reference.

2.7. Cladosporium halotolerans Colonization in Plant Root

To examine intracellular root arbuscules and vesicles, a single primary root was randomly selected from each plant at the end of the experimental period. Then, the roots were fully submerged in a solution of formalin acid alcohol (FAA) and deionized water (10:5:50:35) for 24 h. Afterward, the roots were removed, rinsed with deionized water, and autoclaved. Root systems containing small amounts of soil were subjected to sonication at 42 kHz for 10 min and rinsed with deionized water. For the soil residues that remained attached to the roots, a soft fine paintbrush was used to remove the debris. The root systems were then submerged in 5% hydrochloric acid for 30 min and incubated at 60 °C in a water bath. After cooling to room temperature, the roots were sectioned into 1 cm pieces, with adjacent sections subjected to different staining methods. For staining, 5 root segments (1 cm in size) were immersed in 0.4% trypan blue solution in phosphate-buffered saline (PBS) for 3 min [33]. The stained samples were then examined under a Vickers compound microscope at 100× magnification. Images were captured using a Bresser HD microscope camera.

2.8. Statistical Analysis

The collected results were statistically analyzed using a two-way analysis of variance (ANOVA) in SPSS software version 21.0 (Chicago, IL, USA) to evaluate the impacts of salinity stress, fungal inoculation, and their interactions on the observed characters. Significant differences between treatments ($p < 0.05$) were identified through the Bonferroni multiple comparison test. The results are shown as mean values with their standard error (SE), derived from 3 replicates.

3. Results

3.1. Soil Characters

Data represented in Table 1 show the effect of *C. halotolerans* inoculation on soil pH, electrical conductivity, and organic matter percentage both under saline and non-saline conditions. As inferred from Table 1, the soil pH value significantly decreased in response to *C. halotolerans* inoculation under saline and non-saline conditions. Salinity treatment did not significantly change the pH value and OM content in *C. halotolerans* untreated soil

(Table 1). On the other hand, salinity stress increased soil EC significantly compared to control samples in the absence of *C. halotolerans* inoculation; meanwhile, *C. halotolerans* inoculation significantly reduced the EC lower than those of uninoculated soil both under non-saline and saline conditions. It is worth mentioning that *C. halotolerans* inoculation significantly increased OM% higher than those of uninoculated soil both under non-saline and saline conditions (Table 1).

Table 1. Effect of *C. halotolerans* inoculation on soil properties under salinity stress.

NaCl Treatment	Fungal Treatment	pH	EC (dS m^{-1})	OM (%)
0 mM	Without *C. halotolerans*	7.43 ± 0.12 ^{a*}	0.79 ± 0.04 ^{d}	30 ± 0.5 ^{c}
	With *C. halotolerans*	7.16 ± 0.05 ^{b}	0.68 ± 0.01 ^{c}	35 ± 0.15 ^{a}
50 mM	Without *C. halotolerans*	7.3 ± 0.15 ^{a}	1.41 ± 0.01 ^{a}	29 ± 0.953 ^{c}
	With *C. halotolerans*	6.6 ± 0.06 ^{c}	1.25 ± 0.03 ^{b}	32 ± 0.75 ^{b}

* Different letters (a, b and c) represent the statistical significance ($p < 0.05$) between various treatments as determined by two-way ANOVA and Bonferroni multiple comparison test.

3.2. Plant Growth Parameters

In this in vivo study, an experiment was carried out to investigate the effect of *C. halotolerans* inoculation on *P. vulgaris* growth parameters including shoot and root fresh weight, dry weight, and length, in addition to leaf area. The plants' growth response to fungal inoculation under salinity stress condition was positive, with no observed adverse effects. Fungal-treated plants showed significant growth enhancement compared to the control plants (which did not receive fungal inoculation). The interaction with *C. halotolerans* offers direct benefits to the plant by improving its salinity resistance (see Supplementary Figure S1).

The results in Figure 1a,b revealed that salinity stress decreased the shoot and root fresh weight (FW) by about 37.9 and 22.2% lower than the non-salinized control in fungal untreated *P. vulgaris* plants. However, inoculation with *C. halotolerans* significantly enhanced the shoot and root FW both under saline and non-saline conditions. Shoot and root FW increased by about 160 and 78.6% higher than the uninoculated plants under saline conditions (Figure 1a,b). In the same context, salinity stress reduced shoot and root dry weight (DW) by about 25 and 40.7% lower than non-salinized plants (Figure 1c,d). Meanwhile, the dry biomass of both shoots and roots in *P. vulgaris* plants showed a substantial increase due to fungal treatment under non-saline and saline conditions. Shoot and root DW increased by about 133.3 and 162.5% higher than uninoculated plants under saline conditions (Figure 1c,d).

The data represented in Figure 2 indicate the negative impact of salinity stress on the shoot and root length in addition to leaf area of fungal untreated *P. vulgaris* plant. Treatment with 50 mM NaCl reduced shoot and root length and leaf area by about 15.9, 15, and 25.7% lower than non-salinized *P. vulgaris* plant in the absence of fungal inoculation, respectively. However, fugal treatment markedly improved the shoot and root length, and leaf area under non-saline and saline conditions, as shown in Figure 2. Under saline conditions, inoculation with *C. halotolerans* enhanced the shoot and root length and leaf area by about 51.5, 79.3, and 103.5% higher than uninfected *P. vulgaris* plants, respectively (Figure 2).

3.3. Plant Pigments Concentration

The results represented in Figure 3 show that the chlorophyll a accumulation was not affected significantly by salinity stress. However, chlorophyll b and carotenoid concentrations reduced significantly in salinized plants by about 34.6 and 25.1% lower than the non-salinized control. On the other hand, the fungal treatment significantly increased the concentration of chlorophyll b and carotenoids under saline conditions. The levels of chlorophyll b and carotenoids increased by 46.1 and 39.58% higher than uninoculated salinized plants, respectively (Figure 3b,c). Fungal inoculation did not show a significant effect on chlorophyll a concentration both under non-saline and saline conditions (Figure 3a).

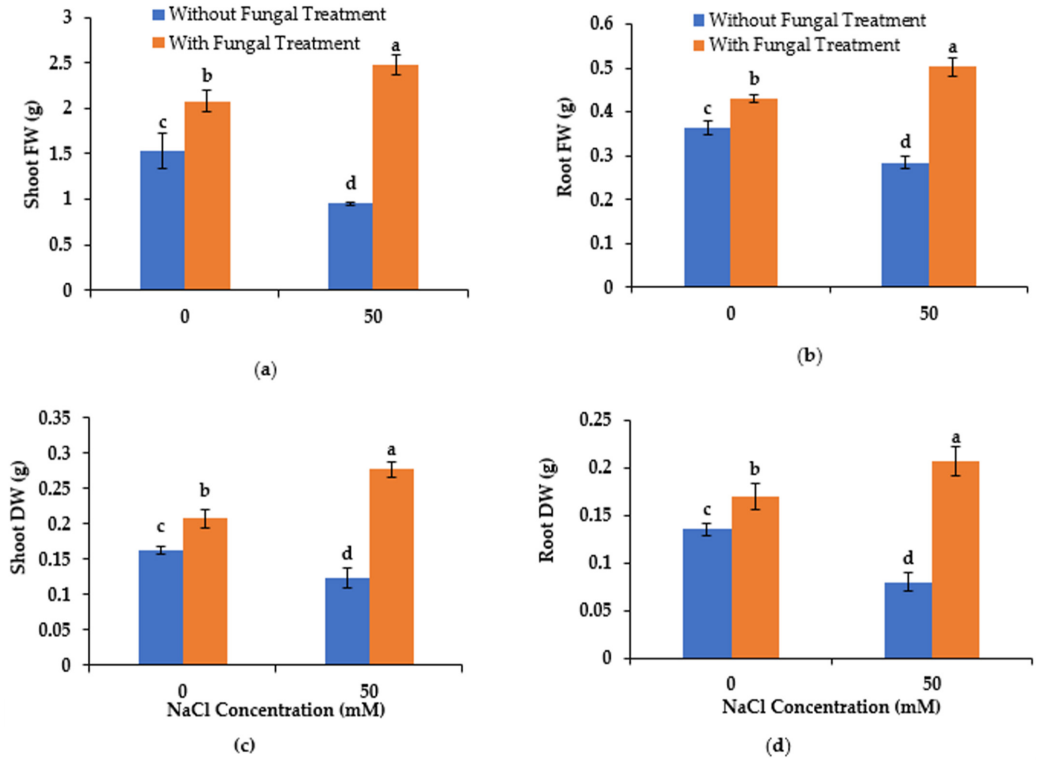

Figure 1. Fresh weight (FW) of (**a**) shoot, (**b**) root and dry weight (DW) of (**c**) shoot, and (**d**) root of non-salinized and salinized *P. vulgaris* plant as affected by *C. halotolerans* inoculation. Each column represents a mean value of three replicates ($n = 3$) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference ($p < 0.05$) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

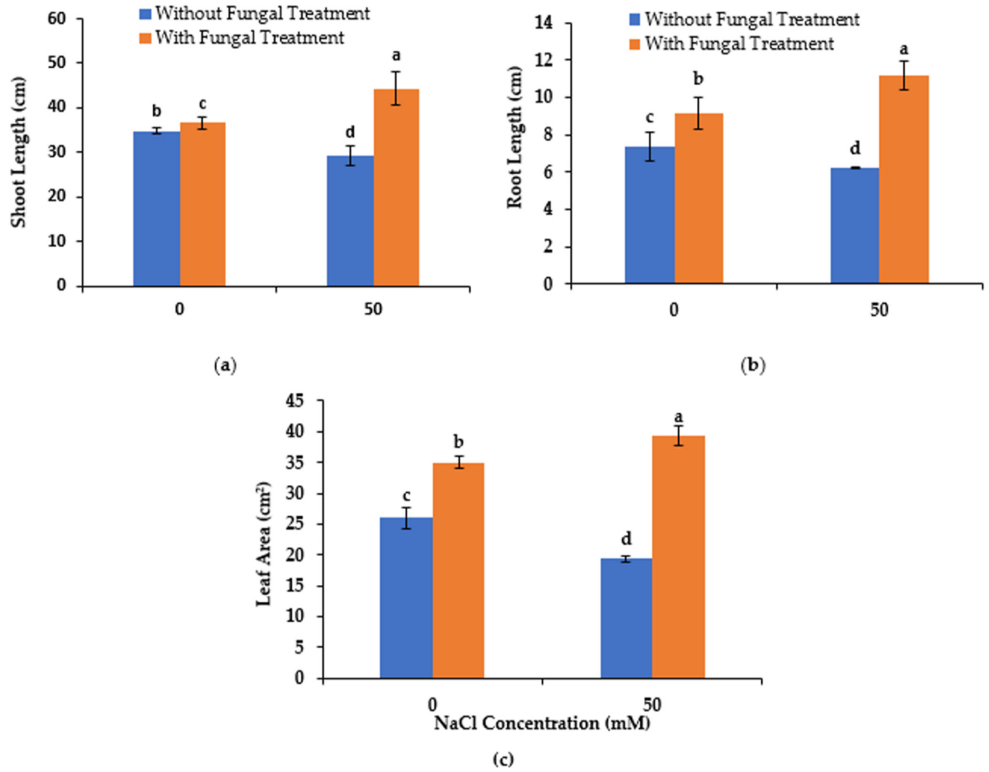

Figure 2. (**a**) Shoot length, (**b**) root length, and (**c**) leaf area of non-salinized and salinized *P. vulgaris* plant as affected by *C. halotolerans* inoculation. Each column represents a mean value of three replicates

(n = 3) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference ($p < 0.05$) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

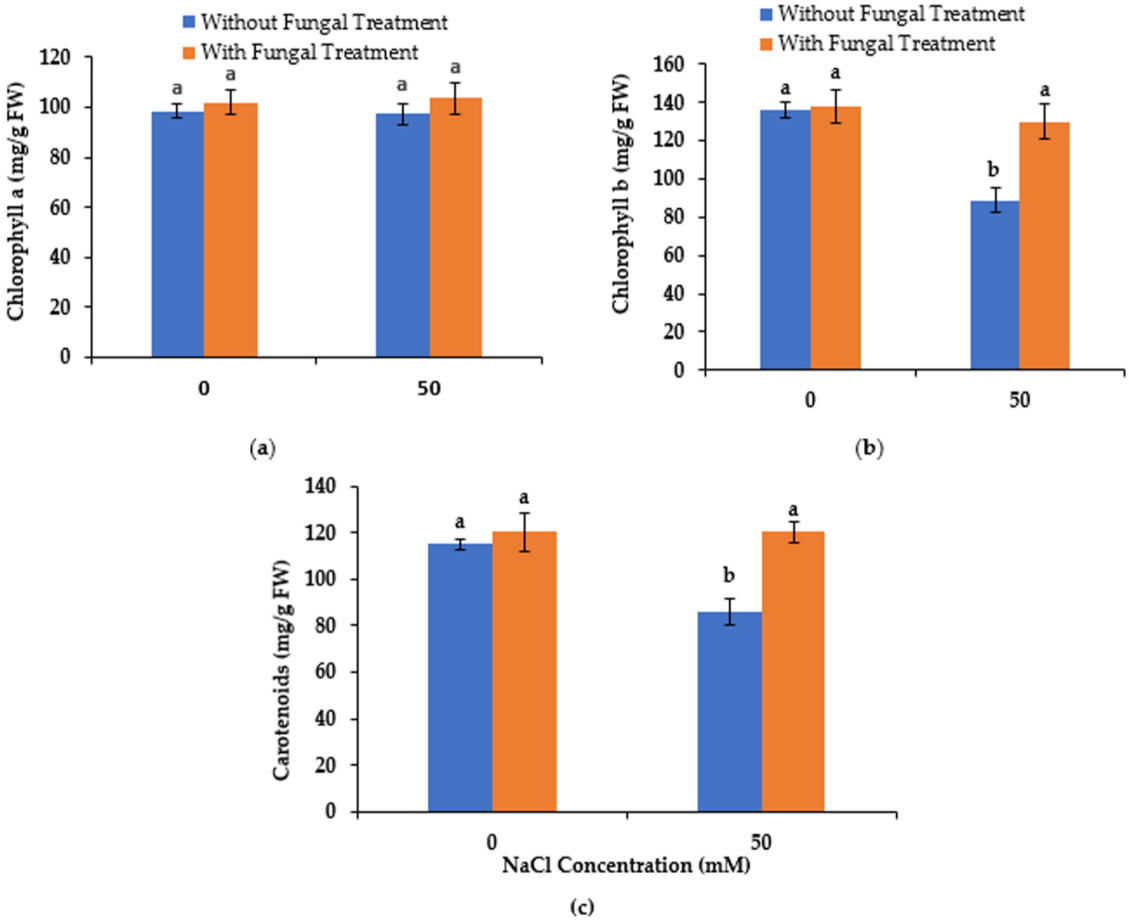

Figure 3. Concentrations of (**a**) chlorophyll a, (**b**) chlorophyll b, and (**c**) carotenoids in leaves of non-salinized and salinized *P. vulgaris* plant as affected by *C. halotolerans* inoculation. Each column represents a mean value of three replicates (n = 3) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference ($p < 0.05$) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

3.4. Malondialdehyde Concentration

The data represented in Figure 4 clearly indicate that salinity stress markedly increased the MDA concentration in *P. vulgaris* roots by about 64.8% higher than the non-salinized control, which indicated a high degree of lipid peroxidation. However, fungal inoculation of the soil notably mitigated salt stress-induced lipid peroxidation in *P. vulgaris* roots by reducing MDA levels by about 27.43% lower than uncolonized stressed plants (Figure 4).

3.5. Root Colonization with C. halotolerans

In this experiment, we initially used a Vickers compound microscope to examine the colonization patterns of endophytic fungi in stressed plant roots from the following two samples: one from the treated plant and the other from the control plant. Microscopic images revealed the active association and colonization of *C. halotolerans* within the roots of the *P. vulgaris* plant. Endophyte–plant interactions were regarded as a mutualistic symbiosis, where both organisms benefit from their association. However, the specific advantages may vary significantly based on environmental factors and the particular endophyte and host

species. Results from the common bean–endophyte relationship demonstrated mutualism, with enhanced plant growth and no disease symptoms. Additionally, the cross-section of the common bean root in Figure 5 revealed that fungal colonization did not damage the root tissue or cell walls. Hyphae were observed colonizing root hairs, with hyphal swellings resembling appressoria also present on root cells. The hyphae keep growing, leading to the formation of dense concentrations of branched structures (Figure 5).

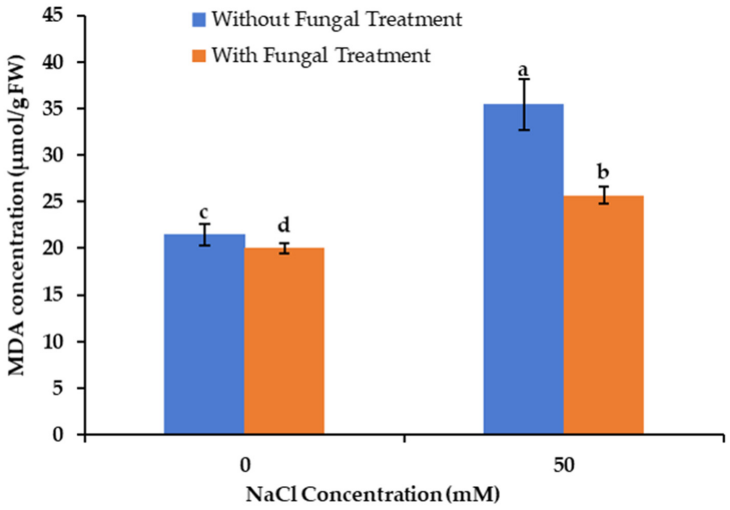

Figure 4. Malondialdehyde (MDA) concentration in roots of non-salinized and salinized *P. vulgaris* plant as affected by *C. halotolerans* inoculation. Each column represents a mean value of three replicates ($n = 3$) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference ($p < 0.05$) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

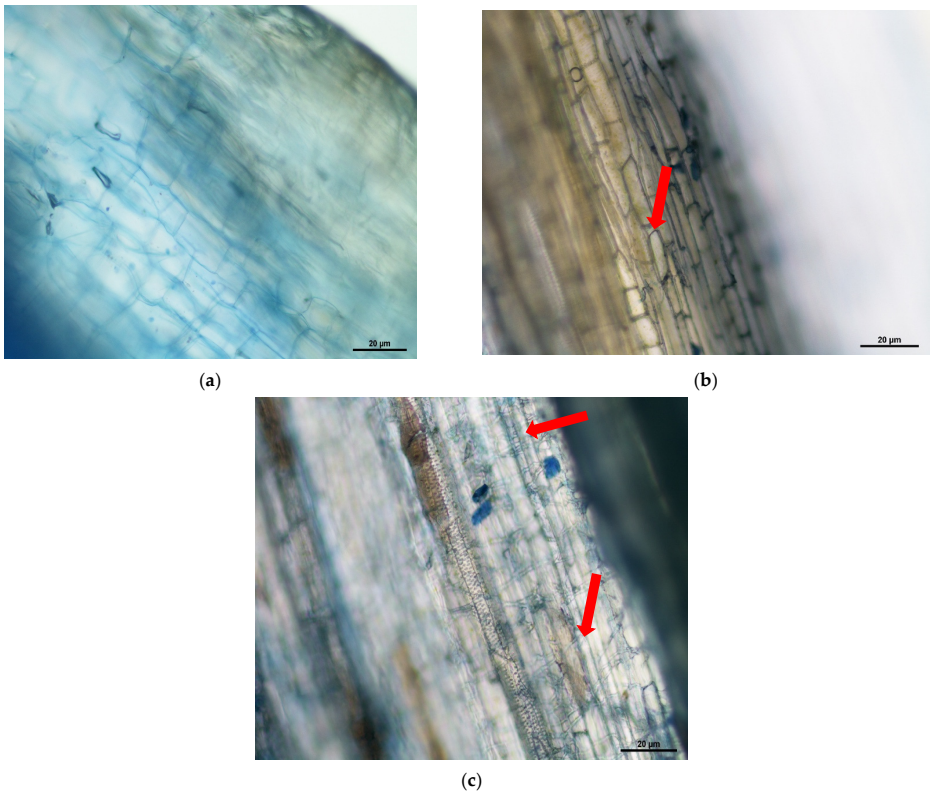

Figure 5. (**a**) Root sectioning of uninoculated plants. (**b**,**c**) Intracellular colonization by endophytic *C. halotolerans*, dark septate hyphae within a healthy root tissue of the treated plant under salinity. The

red-colored arrow head indicates the presence of fungal mycelia as observed under a Vickers compound microscope stained with trypan blue staining.

4. Discussion

Abiotic stresses, such as salinity, are estimated to cause a 50% decrease in crop yields, posing a significant risk to the world's food supply [34]. In general, when plants are subjected to salt stress, their initial reaction is osmotic shock, which is followed by the closing of stomata. This stomatal closure reduces photosynthetic capacity by restricting the supply of CO_2. However, studies have shown that raising external CO_2 levels during salt stress often does not result in increased photosynthesis rates. This indicates that non-stomatal factors may play a role in reducing photosynthesis under saline conditions, such as the overproduction of reactive oxygen species (ROS) and the loss of K^+ in plant cells due to the buildup of Na^+ [35]. The common bean plant is unfortunately sensitive to salt, leading to a considerable reduction in growth when cultivated in saline soils [36]. Research efforts in sustainable common bean agriculture have historically centered on the two primary strategies of enhancing soil quality to reduce salinity levels and developing bean varieties that are more resilient to salt stress [37].

Endophytic fungi are a diverse group of microorganisms that can be found in almost all plants, from the coldest to the hottest environments. These fungi live inside plant tissues without causing harm and can be found in various habitats, including polar regions, deserts, oceans, and tropical forests [38]. Thus, plants can benefit from the diverse metabolic and genetic strategies employed by the endophytic fungi to mitigate the adverse effects of salt stress and other environmental challenges caused by extreme conditions [17]. In this study, soil inoculation with the endophyte *C. halotolerans* significantly enhanced soil characteristics by decreasing soil pH and EC and increasing soil organic matter content. Similar results were observed by [39], where fungal inoculation over time greatly improved soil chemical properties when compared to the control treatment, increasing soil organic matter, soil organic carbon, total nitrogen, phosphorus, calcium, potassium, magnesium, sodium, sulfur, and iron in soils. The induced accumulation of organic matter content in soil samples that were inoculated with *C. halotolerans* could be related to the symbiotic relationship between the common bean plant and this endophytic fungus that directly inhibits the activity of the soil's microorganisms that can decay plant debris and on the other side, increase the abundance of microbiota that improve soil properties, as concluded by Cheng et al. [40] and Ortas [41]. The wealth of soil organic matter might represent a reservoir for most of the macro-elements that are needed for plant growth and crop production including N, P, K, Ca, and Fe [42]. In accordance with the results of this investigation, previous studies have demonstrated that soil amendments with aerial parts of mucuna and cowpea as organic fertilizers increased the soil organic matter content after harvesting due to the decomposition of this green manure with soil microorganisms [40,42].

Plant growth parameters, including shoot and root length, biomass, and leaf area, were measured in response to fungal inoculation under saline and non-saline conditions. Additionally, physiological parameters such as chlorophyll a, b, carotenoids, and MDA concentration were measured. The results of this study demonstrate the beneficial effects of *C. halotolerans* inoculation on all measured growth parameters of common bean under salinity stress. Furthermore, the fungal treatment resulted in a notable increase in Chl b and Car content compared to the controls, under both saline and normal conditions. The data also showed that the MDA concentration was markedly reduced under saline conditions in response to fungal inoculation. These outcomes are consistent with other findings by Lu [43], who reported that inoculating *C. halotolerans* enhanced stem growth in *Dendrobium officinale* under heat stress. This improvement was attributed to the enhancement of activity of several protective enzymes, higher proteins and soluble sugars, chlorophyll accumulation, enhanced photosynthetic rate, and reduced intercellular CO_2 and MDA content. Similarly, *C. cladosporioides* has been shown to promote the growth of barley and wheat across various salt concentrations [44].

Endophytic fungi enhances plant growth through various mechanisms. *Cladosporium* species produce a diverse groups of secondary metabolites that support plant adaptation to novel environments and improve plant fitness and performance, making them effective agents for promoting plant growth [21]. Of these metabolites, gibberellins play a pivotal role as growth-stimulating hormones, particularly in seed germination, stem elongation, and leaf expansion [45,46]. Additionally, Pandey [47] highlighted the remarkable capability of *C. cladosporioides* for producing ammonia and solubilizing potassium, both of which are critical plant growth-promoting qualities that enhance nutrient availability during early seedling development. Studies have also shown that endophytic fungi could solubilize phosphate and zinc which significantly adds to their effectiveness as PGP [48]. Moreover, these fungi produce indole-3-acetic acid, the primary auxin in plants, which regulates organ development and various growth processes. They also secrete other beneficial secondary metabolites, such as exopolysaccharides and phenols, which endorse plant growth through their antioxidant properties [49].

In contrast to the treated plants, the control plants experienced a decline in growth due to salinity stress. This was evident in reduced plant height, the fresh weight (FW) of shoots and roots, and the dry weight (DW) of shoots and roots of the studied common bean plants. There are several species of *P. vulgaris* that are recorded as extremely salt sensitive species by earlier studies [50–52]. A total of 50 mM NaCl is enough to develop salinity stress in *P. vulgaris*, as proved by recent studies that used 50 to 60 mM NaCl as salinity stress concentrations [53–56]. Excessive salts in the soil lower water availability, inhibit metabolic processes, and affect nutrient composition, osmotic balance, and hydraulic conductivity resulting in stunted growth and productivity of plants [57]. Endophytic fungi are well-known as organisms that inhabit the internal tissues of plants without causing any damage to their host [58]. They penetrate plant cell walls by altering their rigidity, allowing the plasma membrane to invaginate and internalize the fungi. The fungi remain enclosed in membrane-bound compartments, avoiding direct contact with the plant's cytoplasm [59]. Endophytic fungi associated with host plants can boost pigments accumulation and contribute to the overall growth of the plants. In this research, the endophytic fungus increased pigment levels and improved all growth parameters under salinity stress conditions. Furthermore, fungal endophytes may influence gene expression associated with salt stress tolerance, enabling improved adaptation to saline conditions. Therefore, boosting the salt tolerance of crops remains a key objective for plant breeders to meet the future food demands of the next generations [60]. In this study, the endophytic fungus *C. halotolerans* exhibited a positive effect on the common bean plant, completing its growth and producing spores within the plant's roots without causing any disease symptoms or affecting plant structures or the cell wall. On the contrary, the fungus enhanced the plant's performance, increased its salt tolerance, and improved the photosynthesis process, which in turn led to greater plant size and improved growth.

Overall, the application of *C. halotolerans* represents a promising biological strategy for enhancing the resilience of common beans to salinity stress, potentially leading to improved crop yields in marginal soils. This fungus can not only help mitigate the adverse effects of salinity on plant growth but may also contribute to the overall health of the soil ecosystem. Future studies should delve deeper into the specific metabolic pathways modulated by *C. halotolerans*, as understanding these mechanisms will be crucial for optimizing its application in agricultural settings.

Additionally, investigating the long-term effects of this fungal symbiont on plant health will provide insights into its sustainability and effectiveness as a biocontrol agent. It is also essential to explore how *C. halotolerans* interacts with the soil microbiome, as these relationships can significantly influence nutrient availability, disease resistance, and overall plant vigor. By conducting comprehensive research that integrates these aspects, we can develop more effective strategies for managing salinity stress in crops and enhancing agricultural productivity in challenging environments.

5. Conclusions

The global extent of agricultural land impacted by salinity is projected to rise as a result of climate change. The increasing demand for food production and the depletion of common bean resources have generated interest in alternative methods for bean improvement. Endophytes such as *C. halotolerans*, which thrive in highly saline environments, have proven effective in various agricultural practices to mitigate the impact of salinity stress on crop productivity. The key findings of this research demonstrated the fungal treatment's ability to enhance the soil properties and plant growth under saline conditions. Shoot and root FW and DW increased by about 160, 78.6, 133.3, and 162.5% higher than uninoculated plants under saline conditions, respectively. Furthermore, fungal inoculation alleviates membrane damage, as indicated by reducing malondialdehyde concentration by 27.4% lower than stressed plants. In this study, it is also highlighted that endophytic fungi offer numerous direct and indirect benefits to crop plants. Further studies to investigate how plants and endophytes respond to various combinations of stressors is crucial for developing long-term strategies for sustainable agriculture in challenging conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems8040135/s1, Figure S1: fungal untreated and fungal-treated *P. vulgaris* seedlings grown under salinity stress condition (50 mM NaCl): (A) one week post-inoculation with *C. halotolerans*; (B) two weeks post-inoculation with *C. halotolerans*.

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