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Eco-friendly Management of *Rhizoctonia solani,* the Cause of Tomato Root Rot Disease

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ABSTRACT

Background: Controlling soil-borne fungal diseases is challenging due to the fungi's ability to endure in the soil for extended time frames. Tomato plants are one of the crops that suffer from root rot disease caused by *Rhizoctonia solani*. Biocontrol agents, such as rhizobacteria are a new trend to control this pathogen as alternative to chemical fungicides.

Methods: The morphological and physiological traits of four rhizobacterial isolates of *Leclercia adecarboxylata* DKS3, *Bacillus halotolerans* DMC8, *Bacillus subtilis* NAS1 and *Paenibacillus polymyxa* TRS4 were examined *in vitro* and their efficacy in managing tomato root rot disease caused by *R. solani* was assessed under greenhouse conditions, along with their impact on plant growth metrics and enzyme activities.

Result: The isolate DMC8 significantly enhanced the germination percentage of tomato seedlings, whilst the other isolates showed no difference compared to the positive control treatment. The isolates DMC8, NAS1 and TRS4 demonstrated a substantial reduction in disease incidence, ranging from 50% to 55%, in contrast to the positive control, which recorded 85% incidence. All isolates, however, demonstrated a notable reduction in disease severity, ranging from 30% to 49%, in contrast to the positive control, which exhibited a severity rate of 56%. All bacterial isolates exhibited a notable enhancement in growth metrics in the absence of the pathogen, whereas in the presence of the pathogen, isolates DMC8, NAS1 and TRS4 demonstrated a considerable improvement in growth markers. An elevation in the activities of peroxidase and phenylalanine ammonium lyase enzymes was observed, signifying the development of resistance in the plant by the rhizobacterial isolates.

Key words: Biocontrol, Rhizobacterial isolates, *Rhizoctonia solani*, Tomato.

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) holds considerable importance as a food source that is cultivated and exchanged on a global scale, this species belongs to the Solanaceae family, which encompasses approximately 3000 species (Padmanabhan *et al.,* 2016). The tomato crop stands out among all major food crops for experiencing the highest yield losses (Roux *et al.,* 2014). Soil-borne pathogens, especially *R. solani*, are the leading pathogenic agents that cause disease in tomato crops (Hussein, 2023). The decline in tomato production in Iraq can be linked to various factors, such as the contamination of the soil with pathogenic fungi such as *R. solani* (Hussein *et al.,* 2022). *R. solani*, a soil-borne pathogen that causes seedling damping-off and foot rot in tomatoes, was found by Gondal *et al.* (2019). *R. solani*, the main species within the genus *Rhizoctonia*, is a soil-dwelling plant pathogen (Bhamra *et al.,* 2022), it shows considerable differences in cultural morphology, host range and levels of aggressiveness. It has a documented history of inflicting losses on economically important crops worldwide (Ajayi-Oyetunde and Bradley, 2022). *R. solani* affects plants during various growth stages, resulting in considerable reductions in crop yield, the pathogen has the capability to infect seeds present in the soil, seedlings at any stage of emergence, roots and multiple aerial parts of plants such as pods, fruits, leaves and stems (Agrios, 2005). The implementation of biocontrol methods in agricultural production presents a promising solution (Lavanya *et al.,* 2023), as it decreases the reliance on harmful fungicides, thereby lessening pollution within the ecosystem (Madbouly, 2018). Biological control agents function as either outcompeting pests or directly eliminating them (Qaisy *et al.,* 2016; Hussin *et al.,* 2018). In contrast to chemical pesticides, the application of biocontrol agents presents numerous benefits, the outcomes encompass a reduced environmental impact, minimized harm to non-target species and a lower

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likelihood of target pests developing resistance to pesticides (Alwan, 2018; Lahlali *et al.,* 2022). This study focused on assessing rhizobacterial isolates as a sustainable biological alternative to chemical fungicides and fertilizers for the protection of tomato plants.

MATERIALS AND METHODS

Antagonistic rhizobacterial isolates used

Four rhizobacterial isolates of *L. adecarboxylata* DKS3, *B. halotolerans* DMC8, *B. subtilis* NAS1 and *P. polymyxa* TRS4 were obtained from a prior study (Hussein *et al.,* 2024).

Assessment of antagonistic activity

The fungal pathogen *R. solani* was isolated from infected tomato roots in a previous study (Hussein *et al.,* 2022). The dual culture method was utilized to evaluate the antifungal efficacy of four rhizobacterial isolates *in vitro*, adhering to the protocol established by Hussein *et al.* (2024). The experiment comprised three replicates and was performed twice. The inhibition zone was measured using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Pathogen growth on control plates} - \text{Pathogen growth on dual culture plates}}{\text{Pathogen growth on control plates}} \times 100$$

Characterization of rhizobacteria

The morphological characteristics of the 4 rhizobacterial isolates, such as their colony boundary, color and form, as well as their Gram staining, were examined according to Kloepper *et al.* (1992). The durability of bacterial isolates at several temperatures was assessed in Nutrient Broth injected with 50 µl of bacterial cultures (10^9 cfu ml^{-1}). The cultures were incubated at 15°C, 25°C, 35°C, 45°C and 50°C. The optical density (O.D.) was determined at 420 nm using a UV-Vis spectrophotometer (Shimadzu, Japan) after 48 hours (Hussein, 2019). To assess the growth of bacteria at different pH levels, 50 µl of bacterial cultures (10^9 cfu ml^{-1}) were used to inoculate nutritive broth maintained at different pH values (pH 4, 5, 6, 7, 8, 9) using either HCl or 1N NaOH. O.D. value was measured at 420 nm in a UV-Vis spectrophotometer 48 hours later. The experiment conducted with three replicates and repeated twice (Hussein *et al.,* 2024).

Greenhouse experiment

Four rhizobacterial isolates were evaluated in greenhouse studies for their efficacy in controlling root rot disease in tomato plants caused by *R. solani*, as well as their capacity to enhance plant growth. All bacterial isolates were grown in Nutrient broth for 48 hours at 27°C with 150 rpm shaking. The bacterial cells were pelleted by centrifugation at 6000 rpm for 15 minutes and the cell pellet was resuspended in 2 ml of sterile distilled water. The bacterial suspension was adjusted to 10^9 cfu ml^{-1} in sterile 1% carboxymethyl cellulose (CMC) solution. The fungal inoculum was generated by cultivating *R. solani* on sterilized millet grains for 20 days at 28°C, after which the colonized grains were dried, crushed and combined with sterilized soil at a ratio of 10 g per kg of soil, following the procedure of Etebarian *et al.* (2000). The seeds of tomato (cv. Super Marmande) were subjected to surface sterilization using a 2% sodium hypochlorite solution. The seeds were submerged in a specialized bacterial solution containing 1% CMC and subsequently air-dried, resulting in a bacterial cell population of 10^{-9} to 10^{-11} colonies per seed. Plastic pots (1 Kg) filled with sterile soil and peat moss in a 2:1 volume ratio. Ten grams of fungal inoculum were introduced seven days before seed sowing for the treatments involving pathogenic fungus. Each pot included five coated seeds, except for the negative control treatment, which was exclusively treated with a 1% CMC solution and excluded any bacterial inoculum. The pots were kept in a greenhouse using a fully randomized design (CRD) with four replications. At 0.05% level of significance, means were compared using LSD. The treatments included negative and positive control and rhizobacterial isolates individually and against *R. solani* (Rs).

Percentage of seed germination was calculated after 15 days of seed sowing as follows (Hussein, 2024):

$$\text{Germination (\%)} = \frac{\text{No. of seed germinated}}{\text{Total seed sown}} \times 100$$

The disease incidence (DI) was determined and expressed as a percentage of diseased plants (Hussein and Al Zubidy, 2019) as follows:

$$\text{DI (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

The disease severity index (DSI) was assessed with a six-class scale described by Castro *et al.* (2017) and the disease severity index (%) was calculated (Hussein and Ibrahim, 2019) as follows:

$$\text{DSI (\%)} = \frac{\sum \text{Disease grade} \times \text{Number of plants of this disease grade}}{\text{Total number of plants} \times \text{Maximum disease grade}} \times 100$$

Further, the growth parameters comprising of fresh and dry weight of shoot and root systems were measured and recorded.

Assay of enzyme activity in tomato plants

Peroxidase

For each treatment, 0.5 g of leaf tissues were utilized, which was subsequently macerated in 2 ml of a phosphate buffer solution composed of Na_2HPO_4 and NaH_2PO_4 at a concentration of 0.1 M and a pH of 6, maintained at 4°C. The enzyme extract was acquired by filtering the solution through a linen cloth, facilitating the measurement of the

peroxidase enzyme. The tubes were centrifuged for 15 minutes at 6000 rpm and 4°C. The peroxidase enzyme activity was assessed using a UV-Vis spectrophotometer (Infitek Co., China) by mixing 1 ml of the supernatant with 1 ml of 20% hydrogen peroxide and 1 ml of 1% catechol. The mean of four repetitions was calculated to assess the variation in light absorption at 470 nm (Hussein *et al.,* 2024).

Phenylalanine ammonium lyase (PAL)

To assess the activity of the phenylalanine ammonium lipase (PAL) enzyme, 2.0 g of leaf tissues from each treatment were homogenized and subsequently diluted 1:2 with phosphate buffer at pH 7. The test tubes were filled with the leaf mixture and the phosphate buffer solution. The tubes were centrifuged for fifteen minutes at a speed of 4,000 rpm. The activity of the PAL enzyme was assessed using a UV-Vis spectrophotometer (Infitek Co., China) by combining 0.2 ml of the filtrate, 0.5 ml of a 0.5 M Tris-HCl buffer solution at pH 8 and 0.5 ml of phenylalanine at a concentration of 6 micromoles in test tubes. Following 60 minutes of incubation at 37°C. The hue shift at 290 nm was subsequently recorded by averaging four repeats (Hussein *et al*., 2022; Hussein *et al.,* 2024).

RESULTS AND DISCUSSION

Antagonistic assay

The inhibition rates of four rhizobacterial isolates against *R. solani* varied from 66.67% to 100.00% (Table 1). The isolate *Bacillus halotolerans* DMC8 demonstrated superior performance, exhibiting 100% antagonistic activity (Fig 1). Rhizobacteria are recognized for their ability to produce various secondary metabolites with antifungal properties (Wani *et al.,* 2022). The variation in antifungal efficacy may be attributed to disparities in the ability of rhizobacterial isolates to produce and release these bioactive compounds (Hussein *et al.,* 2024). Isolates like DMC8, TRS4, DKS3 and NAS1 may possess unique metabolic pathways or genetic attributes that enable them to synthesize potent antifungal agents (Wani *et al.,* 2022).

Morphological and physiological evaluation of rhizobacteria

Three of the four rhizobacterial isolates were Gram-positive, except *L. adecarboxylata* DKS3, which was negative (Table 2). Furthermore, the results of the phenotypic examination also revealed that all of the bacterial cells were rod-shaped and the bacterial colonies were all circular, but they differed in terms of elevation and margin, the optimum temperature and pH of all the isolates were 35°C and 7 respectively (Table 2).

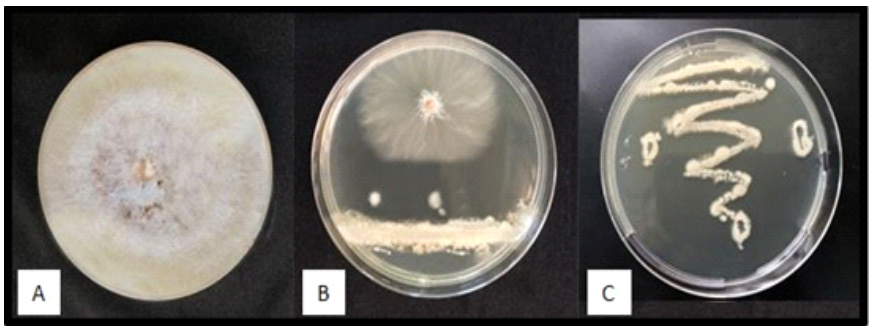
Greenhouse experiment

The result indicateds (Table 3) that the rhizobacterial isolates, in the absence of the pathogen, did not substantially vary from the negative control treatment (plant alone) in attaining a tomato seed germination rate of 100%. In treatments contaminated with the pathogen, the *B. halotolerans* DMC8 treatment significantly increased the seed germination rate to 70%, compared to the positive control treatment (pathogenic fungus alone), which was 60%. The remaining treatments did not exhibit significant differences compared to the positive control treatment (Table 3). The findings align with previous research. Bhatt and Manuel (2014) demonstrated that rhizobacterial isolates greatly enhanced seed germination and seedling

Table 1: Antagonistic activity of rhizobacterial isolates against *R. solani in vitro.*

Isolates code	Inhibition efficacy (%)
Paenibacillus polymyxa TRS4	80.00c
Leclercia adecarboxylata DKS3	66.67d
Bacillus subtilis NAS1	87.46b
Bacillus halotolerans DMC8	100.00a

Different letters within each column represent significant difference (P<0.01) as determined by least significant difference (LSD).



R. solani (A), TRS4 × *R. solani* (B), *P. polymyxa* TRS4 (C)

Fig 1: Interaction of rhizobacterial isolates with *R. solani* in dual culture plate.

vigour in mung bean (*Vigna radiate*) plants subjected to pathogen stress. Research by Olanrewaju *et al.* (2017) has shown that rhizobacteria enhance plant growth and seed germination by counteracting the effects of pathogens through several physiological and biochemical mechanisms.

The data (Table 3) indicated that the treatments with *B. halotolerans* DMC8, *B. subtilis* NAS1 and *P. polymyxa* TRS4 resulted in a significant reduction in disease incidence rates of 50%, 55% and 65%, respectively, compared to the positive control treatment, which recorded an 85% incidence (Fig 2). In contrast, the treatment with *L. adecarboxylata* DKS3 did not demonstrate any significant difference. All therapies, however, resulted in a notable decrease in disease severity, with reductions ranging from 30% to 49%, in contrast to the positive control therapy, which yielded a reduction of 56% (Table 3). The treatments of *B. halotolerans* DMC8 and *B. subtilis* NAS1 demonstrated superiority by attaining a disease severity of 30%. These data illustrated the isolates' have the capacity to function as autonomous biocontrol agents for integrated pest management. These results conform the previous research on the topic. Kloepper *et al.* (2004) revealed that rhizobacteria may successfully protect plants from phytopathogens while enhancing their growth and general health. This is accomplished through many mechanisms, including induced systemic resistance (ISR) and nutritional competition. Additionally, a research by Glick (2012) showed the efficacy of rhizobacteria in controlling soil-borne diseases through antibiotic production and other antagonistic mechanisms. Various biocontrol treatments have demonstrated efficacy in managing *R. solani* across diverse crops. The most efficacious bacterial treatments for managing root rot disease predominantly belong to the *Bacillus* species (Szczech and Shoda, 2004; Hussein *et al.,* 2025). El-Kazzaz *et al.* (2022) demonstrated that *P. polymyxa* reduced both the prevalence and severity of root rot and wilt diseases in pepper plants induced by *R. solani*. The *B. halotolerans* strain effectively mitigated the strawberry gray mold produced by *Botrytis cinerea* (Wang *et al.,* 2021). The results indicated that treatments with rhizobacteria, in the absence of the pathogen, significantly enhanced growth parameters, including plant length and both fresh and dry weight, compared to the negative control treatment, thereby demonstrating the efficacy of these isolates as biofertilizer (Table 4). The treatments contaminated with *R. solani* yielded varied results, the isolates *B. halotolerans* DMC8 and *B. subtilis* NAS1 demonstrated a notable enhancement in the average lengths of the vegetative and root systems (Fig 2), measuring 18.1 cm, 18.5 cm and 9.0 cm, 8.6 cm, respectively, in contrast to the positive control treatment, which measured 12.9 cm and 5.7 cm, respectively (Table 4). The treatments of *B. halotolerans* DMC8, *B. subtilis* NAS1 and *P. polymyxa* TRS4 resulted in a notable enhancement in the average fresh weight of the vegetative and root systems, ranging from 2.226 to 3.450 g and 0.197 to 0.301 g, respectively, in contrast to the positive control treatment, which yielded 2.159 g and 0.175 g, respectively (Table 4). The dry weight for the three isolates DMC8, NAS1 and TRS4 exhibited substantial increases, ranging from 0.445 to 0.690 g and 0.039 to 0.060 g,

Table 2: Morphological and physiological characterization of rhizobacterial isolates.

Isolate	Cell morphology		Colony morphology				Optimum	Optimum
code	Gram stain	Shape	Form	Elevation	Margin	Color	temperature °C	pH
DKS3	-	Rod	Circular	Flat	Entire	Creamy-white	35	7
DMC8	+	Rod	Circular	Flat	Curled	Milky-white	35	7
NAS1	+	Rod	Circular	Convex	Undulate	Creamy-white	35	7
TRS4	+	Rod	Circular	Convex	Curled	Creamy-white	35	7

Table 3: Effects of antagonistic rhizobacterial isolates on tomato root rot disease in a greenhouse.

Treatment	Seed germination (%)	Disease incidence (%)	Disease severity index (%)
Negative control (Plant alone)	100.0a	0.0a	0.0a
Positive control (Pathogen alone)	60.0c	85.0c	56.0e
DKS3 (Alone)	100.0a	0.0a	0.0a
DMC8 (Alone)	100.0a	0.0a	0.0a
NAS1 (Alone)	100.0a	0.0a	0.0a
TRS4 (Alone)	100.0a	0.0a	0.0a
DKS3 × Rs	65.0c	80.0c	49.0d
DMC8 × Rs	70.0b	50.0b	30.0b
NAS1 × Rs	65.0c	55.0b	30.0b
TRS4 × Rs	60.0c	65.0b	34.0c

Rs= *R. solani*, Data are the means of four replicates. Significant differences (P<0.05) are shown by different letters in each column using least significant difference (LSD).

respectively, in comparison to the positive control treatment, which recorded 0.432 g and 0.035 g, respectively (Table 4). The treatment with *L. adecarboxylata* DKS3 bacterium did not result in any notable enhancement under biotic stress conditions. The results shown in Table 4 conform the previous research findings demonstrating the beneficial effects of rhizobacteria on plant growth. Abdeljalil *et al.* (2016) discovered that rhizobacterial isolates of *B. thuringiensis* and *B. subtilis* significantly enhanced the growth of tomato plants infected with *R. solani*, resulting in a 62-76% increase in plant height, a 53-86% increase in root fresh weight and a 34-67% increase in the fresh weight of aerial parts. Kang *et al.* (2021) found that isolates of *L. adecarboxylata* displayed significant differences in plant growth traits when cucumber (*Cucumis sativus* L) seeds were infected, resulting in a marked increase in shoot length, root length and shoot fresh weight of the plants. Zhang *et al.* (2015) discovered that *P. polymyxa* and *B. subtilis* significantly enhanced the growth of tomato plants in greenhouse pot experiments, resulting in increased plant height, root length and total fresh and dry biomass compared to an untreated control group.

Assay enzyme activity in tomato plants

The research revealed peroxidase enzyme activity significantly changed in tomato plants subjected to several treatments, including rhizobacterial isolates both independently and in conjunction with *R. solani*. The peroxidase enzyme is crucial for the plant's defense mechanisms, particularly by facilitating the neutralization of reactive oxygen species and fortifying cell walls to inhibit pathogen invasion (Hiraga *et al.,* 2001). The results (Table 5) demonstrated that the rhizobacterial isolates, when exposed to the pathogen, led to a significant enhancement in peroxidase enzyme activity, in contrast to treatments utilizing rhizobacteria only. The enzyme activity in pathogen-contaminated treatments varied between 18.11 and 20.21 $\Delta 470.min^{-1}.g^{-1}.F.wt^{-1}$ (Table 5), significantly exceeding the enzyme activities observed in the positive and negative control treatments, which were 7.10 $\Delta 470.min^{-1}.g^{-1}.F.wt^{-1}$ and 4.82 $\Delta 470.min^{-1}.g^{-1}.F.wt^{-1}$, respectively. The elevated peroxidase activity seen in the presence of pathogens signifies that the rhizobacterial isolates effectively induce systemic resistance in tomato plants, hence enhancing their defensive responses. The findings align with previous studies indicating that some beneficial bacteria may

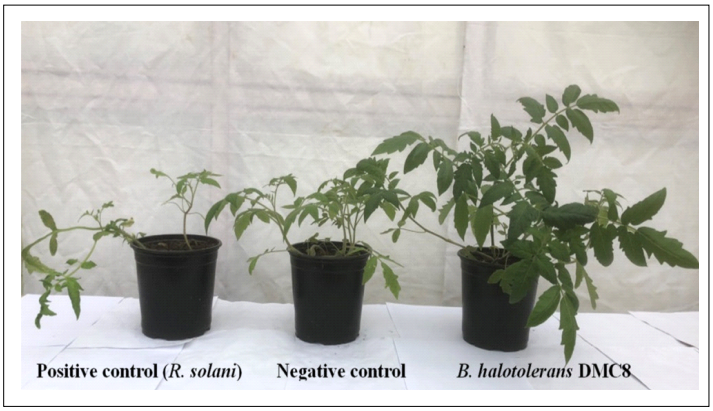


Fig 2: Biological control of tomato root rot disease using rhizobacterial isolates.

Table 4: Impact of antagonistic rhizobacterial isolates on the growth characteristics of plants.

Treatment	Plant length (cm)		Plant fresh weight (g)		Plant dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
Negative control	25.1c	15.6c	5.910c	0.585c	0.886dc	0.117b
Positive control	12.9f	5.7e	2.159g	0.175f	0.432hg	0.035d
DKS3	26.4b	16.1c	6.263b	0.641b	1.256cb	0.128ab
DMC8	26.8a	17.1b	6.713a	0.683a	1.343ba	0.137a
NAS1	28.3a	17.2a	6.716a	0.689a	1.343ba	0.138a
TRS4	27.0b	17.1b	6.260b	0.643b	1.251cb	0.129a
DKS3 × Rs	13.0f	6.3e	2.226g	0.197f	0.445hg	0.039d
DMC8 × Rs	18.5d	9.0d	3.151e	0.287d	0.631fe	0.058c
NAS1 × Rs	18.1d	8.6d	3.450d	0.301d	0.690ed	0.060c
TRS4 × Rs	15.4e	7.0e	2.768f	0.264e	0.553gf	0.053c

Rs= *R. solani*, Data are the means of four replicates. Significant differences (P<0.05) are shown by different letters in each column using least significant difference (LSD).

Table 5: Effect of antagonistic rhizobacterial isolates on enzymes catalyzed defense (PO, PAL) of tomato plants in greenhouse.

Treatment	PO (Δ470/min/g/ F.wt.)	PAL (μg Cinnamic acid/h/ g/F.wt.)
Negative control	4.82j	7.82i
Positive control	7.10i	13.95h
DKS3 (Alone)	13.55h	16.91g
DMC8 (Alone)	17.35e	19.21e
NAS1 (Alone)	16.87f	19.67d
TRS4 (Alone)	14.63g	17.89f
DKS3 × Rs	18.11d	19.20e
DMC8 × Rs	20.21a	20.70a
NAS1 × Rs	19.95b	20.33b
TRS4 × Rs	18.98c	19.72c

Rs= *R. solani*, Data are the means of four replicates. Different letters within each column represent significant difference (P<0.05) as determined by least significant difference (LSD).

activate induced systemic resistance (ISR) in plants, hence enhancing the synthesis of defense-related enzymes like peroxidase (Van Loon *et al.,* 1998).

Phenylalanine ammonium lyase (PAL) is a crucial enzyme in plant defense mechanisms. It activates the phenylpropanoid pathway, which generates defense-related compounds such as phytoalexins and lignin (Gho *et al.,* 2020). The findings (Table 5) indicateds that all rhizobacterial treatments, irrespective of pathogen presence, significantly increased PAL activity compared to the control group. The treatment of rhizobacterial isolates against *R. solani* resulted in an increase in PAL activity ranging from 19.20 to 20.70 μg Cinnamic Acid.h^{-1}.g^{-1}.F.wt. The increases were substantial relative to the positive control (13.96 μg Cinnamic Acid.h^{-1}.g^{-1}.F.wt.) and the negative control (7.82 μg Cinnamic Acid.h^{-1}.g^{-1}.F.wt.). This indicateds that the rhizobacterial isolates significantly enhanced PAL activity and maintained elevated enzyme levels even in the absence of pathogens, suggesting a strong priming effect. This corresponds with the principle of priming, wherein plants pre-conditioned by advantageous microbes exhibit a more robust and rapid defensive response to subsequent pathogen assaults (Goellner *et al.,* 2008). The results demonstrated the effectiveness of rhizobacterial treatments in promoting the production of essential defense enzymes in tomato plants. The notable increase in peroxidase and phenylalanine ammonia lyase activity in pathogen-contaminated treatments suggests that these bacteria may induce a heightened state of readiness in plants, allowing for a more effective response to pathogen attacks. Yasmin *et al.* (2016) identified a robust correlation between the activity of the antioxidant enzymes peroxidase (PO), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) in plants and disease suppression, proposing that these enzymes may function as elicitors of induced systemic resistance (ISR). Al-Himiry (2013) conducted an experiment demonstrating that the application of a mixture of rhizobacterial isolates of *P. putida* and *E. cloacae* as an inoculum significantly improved the resistance of tomato plants against *F. oxysporum* f. sp. *lycopersici*, correlating with increased levels of peroxidase, phenylalanine ammonia lyase and phenolic compounds. Jayaraj *et al.* (2004) demonstrated that the application of *B. subtilis* strain AUBS1 significantly reduced the occurrence of sheath blight disease in rice during greenhouse trials.

CONCLUSION

Three isolates of *B. halotolerans* DMC8, *B. subtilis* NAS1 and *P. polymyxa* TRS4, reduced the root rot disease incidence caused by *R. solani* in tomato plants. All of the four isolates, however, resulted in a considerable decrease in the disease severity. The isolates showed significant abilities in enhancing plant growth, including height and both fresh and dry biomass of the plants. The results demonstrate the effectiveness of rhizobacterial treatments in augmenting the production of essential defensive enzymes, namely peroxidase (PO) and phenylalanine ammonia lyase (PAL), in tomato plants. This study shows that rhizobacterial isolates may promote plant development in sustainable agriculture as biocontrol agents and biofertilizer. These results provide the groundwork for future field studies on crop productivity and soil health in different environments.

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Conflict of interest

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