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Biological Control of Tomato Root Rot Disease by Indigenous Rhizobacteria in Greenhouse Setting

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ABSTRACT

Background: Rhizobacteria are essential for plant health by offering natural antagonism to soil-borne fungi. Rhizobacteria are regarded as an alternative to chemical agents for the integrated control of plant diseases and for enhancing yield in an ecologically sustainable way. Nonetheless, there is a limited comprehension of the precise processes via which rhizobacteria suppress these diseases and the variety of rhizobacterial species implicated.

Methods: Through experiments conducted in 2022-2024, the efficiency of 12 rhizobacterial isolates of *Alloiococcus otitis* BRE6, *Aneurinibacillus thermoaerophilus* SDV1, *Aeribacillus pallidus* ECC4, *A. thermoaerophilus* ECL1, *Bacillus megaterium* SKE2, *Staphylococcus lentus* BZD2, *Enterobacter cloacae* complex BZD3, *B. megaterium* TNK1, *Leclercia adecarboxylata* DKS3, *B. halotolerans* DMC8, *B. subtilis* NAS1 and *Paenibacillus polymyxa* TRS4, in controlling soilborne pathogenic fungi of *Rhizoctonia solani*, *Macrophomina phaseolina* and *Pythium aphanidermatum*, the causal agents of tomato root rot disease was evaluated under greenhouse conditions and plant growth parameters were evaluated.

Result: The results indicated that the combination treatment of 12 rhizobacterial isolates (COR12) achieved the highest germination rate of tomato seeds in the presence of pathogenic fungi, reaching 100% and the lowest rate of disease incidence and severity, reached 5% and 1%, respectively, compared to the positive control treatment, which reached 82% and 53%, respectively. Representing control disease value of 98% compared to the single bacteria treatments. The COR12 treatment achieved a significant increase in the growth parameters represented by plant height and fresh and dry weight of the shoot and root system in the presence of pathogenic fungi compared to the individual bacterial treatments.

Key words: Biological control, Rhizobacteria, Root rot disease, Tomato.

INTRODUCTION

Rhizobacteria are soil-resident bacteria that inhabit plant roots and significantly influence their growth and health (de Andrade *et al*., 2023). Rhizobacteria are considered effective alternatives to chemical pesticides and fertilizers because of their ability to address several issues and constraints linked to these traditional approaches (de Andrade *et al*., 2023). Tomato (*Solanum lycopersicum* L.) playing an essential role in the Iraq's agricultural sector and dietary practices, according to FAOSTAT (2024), its annual production has attained 630,180 tons. Root rot infections are highly significant and devastating plant ailments with a worldwide impact (Williamson-Benavides and Dhingr, 2021). They may result in a reduction in agricultural yield and quality between 10% and 90%, dependent upon the particular crop and pathogen implicated. Moreover, they lead to significant economic detriments for both agricultural producers and consumers (Agrios, 2005). Root rot diseases are caused by several fungal infections, including *R. solani*, *P. aphanidermatum* and *M. phaseolina* (Hussein *et al*., 2022). Bonaterra *et al*. (2022) identified multiple bacterial genera, including *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Stenotrophomonas*, *Streptomyces* and *Xanthomonas*, that demonstrate protective properties against fungal and bacterial pathogens responsible for plant diseases. The mechanisms employed by rhizobacteria include the enhancement of plant resistance, the secretion of potent antimicrobial agents such as antibiotics or cell wall-degrading enzymes to combat pathogens and the occupation of infection sites to outcompete the pathogens (Hussein *et al*., 2024). Rana *et al*. (2024) demonstrated that the soil bacterium *Bacillus halotolerans* NYG5 serves as an effective biocontrol agent against various phytopathogenic fungi, including *M. phaseolina*, *R. solani*, *P. aphanidermatum* and *S. sclerotiorum*, owing to its capacity to produce volatile organic compounds (VOCs). Anak *et al*.

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(2021), revealed that strains of *Bacillus thuringiensis* HV43, *Bacillus subtilis* HV34, *Bacillus cereus* GC and *Bacillus subtilis* HT30 efficiently inhibited root rot disease in bean (*Vigna radiate*) plants induced by *R. solani*, attaining a 100% success rate. The primary hypothesis and aim of this study is to assess the antifungal efficacy of local rhizobacterial isolates against the pathogens *R. solani*, *P. aphanidermatum* and *M. phaseolina*, which cause tomato root rot disease, in a greenhouse environment and to subsequently demonstrate its effectiveness in improving various facets of plant growth.

MATERIALS AND METHODS

Preparing of rhizobacterial isolates inoculum

Twelve rhizobacterial isolates were derived from a prior research (Hussein *et al*., 2024), extracted from the roots of several plant species across Iraqi governorates (Table 1). The bacterial isolates were generated by inoculating 1 ml of fresh individual bacterial cultures, cultivated overnight in 500 ml flasks containing 250 ml of nutrient broth, at 27°C for 48 hours with shaking at 150 rpm. Bacterial cells were pelleted via centrifugation at 6000 rpm for 15 minutes at the room temperature and the supernatant was discarded. The cell pellet was resuspended in sterile distilled water and subjected to centrifugation under identical conditions. The supernatant was discarded and the bacterial cells were washed and resuspended in 2 ml of sterile distilled water. The concentration of cells in the suspension was adjusted to 10^{-9} cfu ml^{-1} in sterile 1% carboxymethyl cellulose (CMC) solution (Hussein, 2024).

Preparing of fungal pathogens inoculum

The fungal pathogens of *R. solani* (AG4) isolate (Rs-12), *P. aphanidermatum* isolate (Pa-m7) and *M. phaseolina* isolate (Mp-03) were previously obtained from the infected roots of diseased tomato plants and have shown a high level of virulence in pathogenicity tests (Hussein *et al*, 2022). Fungal inoculums were prepared individually by cultivating them on millet grains (*Panicum miliaceum*) in 250 ml flasks. The grains were soaked in distilled water and autoclaved twice on two successive days at 121°C for 60 minutes. They were then inoculated with five agar discs (5 mm diameter) from a 5-day-old fungal culture. The flasks were incubated at 28°C in the dark for 20 days, with shaking occurring at least once daily. Once colonized, the grains were transferred into paper pockets, dried and ground. The inoculums were subsequently mixed with sterilized soil at a rate of 10 g per kg of soil (Etebarian *et al*., 2000).

Greenhouse experiment

Twelve rhizobacterial isolates were assessed in greenhouse examinations (Ministry of Agriculture, Department of Plant Protection, Iraq) for their ability to manage prevalent soil-borne pathogenic fungi, specifically *R. solani* (AG4), *M. phaseolina* and *P. aphanidermatum*, while also evaluating their potential for promoting plant growth.

The seeds of tomato (cv. Super Marmande) underwent superficial disinfection by being soaked in a 2% sodium hypochlorite solution for one minute. Following this, they were rinsed three times with sterile distilled water and allowed to dry overnight on sterile filter paper within a sterile laminar flow hood. Subsequently, the seeds were immersed in a formulated bacterial solution in 1% CMC, shaken at 150 rpm at room temperature for three hours and then air dried on sterilized filter paper under the laminar hood overnight. The starting population of bacterial cells on the seed was approximately 10^{-9} to 10^{-11} colonies per seed (Qaisy *et al*., 2016; Alwan, 2018; Hussin, 2018).

Plastic pots measuring 17 cm in diameter and 15 cm in height were filled with a mixture of sterile field soil and peat moss in a 2:1 volume ratio. This mixture underwent sterilization via autoclaving on two consecutive days at 121°C for 60 minutes. For the treatments involving pathogenic fungi, an inoculum of 10 g per kg of pathogens was incorporated into the pots seven days prior to the planting of seeds. Each pot contained five coated seeds, with the exception of the negative control treatment, which was treated solely with a 1% CMC solution and did not include any bacterial inoculum. The pots received watering

Table 1: The origin sources of rhizobacterial isolates.

Bacterial isolates code	Cultivar origin	Cultivar location
Alloiococcus otitis BRE6	Hairy willowherb	Baghdad, Rashdiah
Leclercia adecarboxylata DKS3	Tomato	Diyala, Khanaqin
Bacillus halotolerans DMC8	Sweet orange tree	Diyala, Moqdadiah
Aneurinibacillus thermoaerophilus SDV1	Chaste tree	Salah Al Din, Dijeel
Aeribacillus pallidus ECC4	Italian thistle	Erbil, Chomn
Aneurinibacillus thermoaerophilus ECL1	Willowleaf lettuce	Erbil, Chomn
Bacillus megaterium SKE2	Horseweed	Sulimania, Kalar
Staphylococcus lentus BZD2	Florida hopbush	Basra, Zubir
Enterobacter cloacae complex BZD3	Florida hopbush	Basra, Zubir
Bacillus subtilis NAS1	Common sowthistle	Najaf, Abasia
Paenibacillus polymyxa TRS4	Tomato	Thi Qar, Rifai
Bacillus megaterium TNK1	Crown of thorns	Thi Qar, Nasria

every two days using tap water and were maintained in a greenhouse with day/night temperatures of 32/25°C, 40% humidity and a light cycle of 16 hours brightness followed by 8 hours of darkness, without the application of any fertilizer during the experiment. The experiment was carried out with four replicates.

The treatments examined included negative control plants subjected to mock inoculation. Positive control includes *R. solani* (Rs), *M. phaseolina* (Mp) and *P. aphanidermatum* (Pa) individually. Isolates of rhizobacteria free from pathogens, along with treatment of a combination of 12 distinct rhizobacterial isolates (COR12). Isolates of pathogenic fungi *R. solani*, *M. phaseolina* and *P. aphanidermatum* were tested individually against each rhizobacterial isolate individually, as well as against the combination isolates (COR12).

After 15 days of planting, the percentage of seed germination was calculated according to the method of Hussein (2019) as follows:

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

The plants were harvested 60 days after seed planting. 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; where: 0 - Plants with healthy stems and roots, 1 - Plants with minimal stem/ root rot (less than 10%), 2 - Plants with slight stem/ root rot (25%), 3 - Plants with medium stem/ root rot (50%), 4 - Plants with severe stem / root rot (75%) and 5 - Dead Plants (100%) (Castro *et al*., 2017) and the disease severity index (%) was calculated (Hussein and Al Zubidy, 2019) as follows:

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

Control disease value (CDV) was calculated according to the method of Song *et al*. (2004) as follows:

$$\text{CDV (\%)} = \frac{\text{Disease severity index of positive control- Disease severity index of treatment}}{\text{Disease severity index of positive control}} \times 100$$

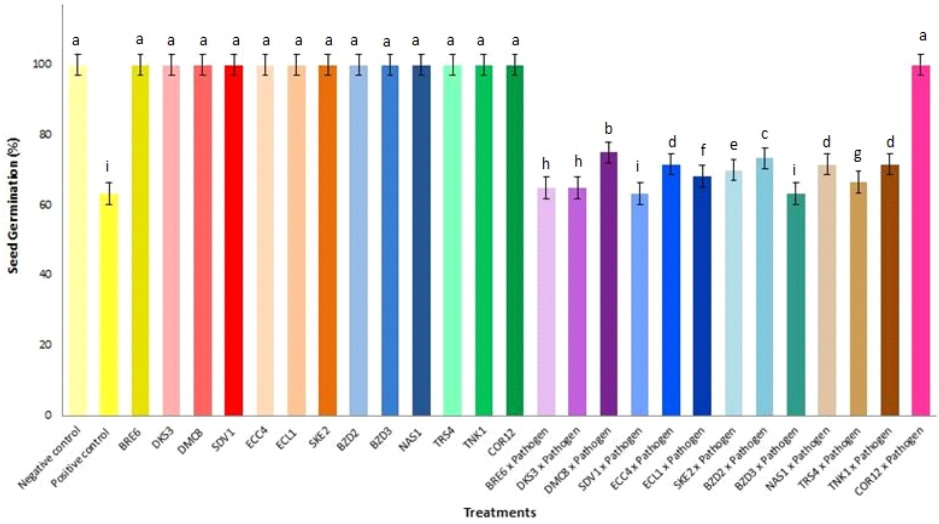
Further, the growth parameters comprising of length, fresh and dry weight of shoot and root systems were measured and recorded, plants were dried in an oven at 70°C until they reached a consistent weight for dry weight (Bhamra *et al*., 2022; Lavanya *et al*., 2023; Bekele *et al*., 2024).

RESULTS AND DISCUSSION

Greenhouse experiment

Seed germination

The findings illustrated in (Fig 1) reveal the impact of various rhizobacterial treatments on the germination rates of tomato seeds in the presence of pathogenic fungi that include *R. solani*, *M. phaseolina* and *P. aphanidermatum*. Even with the existence of these pathogens, the findings indicate that rhizobacterial isolates can significantly enhance germination rates. The results indicate that when pathogenic fungi are present, the germination rate of tomato seeds was notably enhanced by all rhizobacterial isolates, with the exception of SDV1 and BZD3. The two outliers



Same letters within each column represent non-significant difference (P<0.05) as determined by least significant difference (LSD).

Fig 1: Effect of antagonistic rhizobacterial isolates on tomato seed germination in greenhouse.

demonstrated germination rates of 63.3%, aligning with the positive control treatment (pathogenic fungi alone), which also showed a seed germination rate of 63.3% (Fig 1). Interestingly, the COR12 combination treatment achieved a germination rate equivalent to that of the negative control treatment (seeds free of pathogens) in the presence of pathogens, resulting in a 100% germination rate. This finding suggests that the COR12 treatment effectively mitigates the detrimental effects of pathogenic fungi, likely due to the advantageous interactions of the bacterial strains that enhance the health and resilience of seeds.

Biological control of root rots disease

The findings shown in Table 2 offer compelling evidence regarding the efficacy of various rhizobacterial isolates in mitigating both the incidence and severity of diseases in tomato plants attributed to the pathogenic fungi *R. solani*, *M. phaseolina* and *P. aphanidermatum*. These findings hold significant value for the development of effective and sustainable biocontrol strategies aimed at managing agricultural diseases. The results indicate a notable reduction in disease incidence caused by pathogenic fungi, attributed to six unique rhizobacterial isolates: DMC8, BZD2, SKE2, NAS1, TRS4 and TNK1. The positive control treatment exhibited a disease incidence of 81.7%, whereas the disease incidence in these treatments ranged from 48.3% to 58.3% (Table 2). The most significant reduction in disease incidence was observed with the combination treatment COR12, which lowered it to 5.0% (Fig 2).

Relative to the positive control treatment, which demonstrated a disease severity of 53.3% (Fig 2), all tested rhizobacterial isolates showed a significant decrease in the disease severity index, with reductions varying from 30.7% to 48.7% (Table 2). This indicates that the intensity of the disease's symptoms can still be mitigated by employing isolates that are less effective in decreasing incidence. The COR12 treatment demonstrated superiority, with the degree of disease lowered to 1.0%, statistically comparable to the negative control (0%).

The research demonstrates considerable heterogeneity in disease control efficacy across the twelve rhizobacterial strains examined. The control disease values in these treatments ranged from 8.7% to 42.4% (Table 2), reflecting

Table 2: Impact of antagonistic rhizobacterial isolates on tomato root rot disease in a greenhouse.

Treatment	Average of DI (%)	Average of DSI (%)	Average of CDV (%)
Negative control (Plant alone)	0.0a	0.0a	-
Positive control (Pathogenic fungi alone)	81.7e	53.3g	-
BRE6 (Alone)	0.0a	0.0a	-
DKS3 (Alone)	0.0a	0.0a	-
DMC8 (Alone)	0.0a	0.0a	-
SDV1 (Alone)	0.0a	0.0a	-
ECC4 (Alone)	0.0a	0.0a	-
ECL1 (Alone)	0.0a	0.0a	-
SKE2 (Alone)	0.0a	0.0a	-
BZD2 (Alone)	0.0a	0.0a	-
BZD3 (Alone)	0.0a	0.0a	-
NAS1 (Alone)	0.0a	0.0a	-
TRS4 (Alone)	0.0a	0.0a	-
TNK1 (Alone)	0.0a	0.0a	-
COR12 (Alone)	0.0a	0.0a	-
BRE6 × Pathogenic fungi	78.3de	48.7f	8.7g
DKS3 × Pathogenic fungi	76.7de	47.0ef	11.8e-g
DMC8 × Pathogenic fungi	48.3b	31.7b	40.4b
SDV1 × Pathogenic fungi	78.3de	47.7f	10.6fg
ECC4 × Pathogenic fungi	73.3c-e	45.7e	14.2ef
ECL1 × Pathogenic fungi	68.3b-e	45.0e	15.5e
SKE2 × Pathogenic fungi	51.7bc	35.7d	33.0d
BZD2 × Pathogenic fungi	50.0b	32.0bc	40.0bc
BZD3 × Pathogenic fungi	73.3c-e	45.7e	14.3ef
NAS1 × Pathogenic fungi	51.7bc	30.7b	42.4b
TRS4 × Pathogenic fungi	58.3b-d	34.3cd	35.6cd
TNK1 × Pathogenic fungi	58.3b-d	33.0bc	38.2bc
COR12 × Pathogenic fungi	5.0a	1.0a	98.1a

DI= Disease incidence, DSI= Disease severity index. CDV= Control disease value, Data are the means of four replicates. Same letters within each column represent non-significant difference (P<0.05) as determined by least significant difference (LSD).

the differential efficacy of the isolates in combating pathogenic fungus in tomato plants. The treatment with COR12 demonstrates an outstanding disease control effectiveness of 98.1%. The value far exceeds the results of each treatment, underscoring the possibility of combining several bacterial strains to have a synergistic effect. The remarkable efficacy of disease suppression in COR12 is ascribed to the synergistic metabolic activity, antibiotic production and nutritional competition among the consortium of strains (Glick, 2012).

These results align with previous research on the topic. Research by Kloepper *et al*. (2004) has shown that rhizobacteria may successfully protect plants from phytopathogens and enhance their growth and general health, this is accomplished through many mechanisms, including induced systemic resistance (ISR), nutritional competition. Additionally, synthesize phytohormones, which may enhance plant growth and stress resilience while indirectly mitigating disease impacts. Abdeljalil *et al*. (2016) investigated 25 rhizobacterial isolates sourced from the rhizosphere of healthy tomato plants obtained from various tomato cultivation sites in Tunisia. They assessed the antifungal efficacy of bacterial isolates from *Bacillus* spp., *E. cloacae*, *Chryseobacterium jejuense* and *Klebsiella pneumoniae* against *R. solani*, the pathogen responsible for *Rhizoctonia* root rot in tomato plants. The tomato-associated rhizobacteria demonstrated a substantial reduction in disease severity, ranging from 47% to 100%, compared to the control group infected with the pathogen. Diaz-Diaz *et al*. (2023) discovered that biocontrol agents from *Streptomyces* sp. strains CBQ-EA2 and CBQ-B-8 significantly influenced seed germination and mitigated disease in bean (*Phaseolus vulgaris*) plants afflicted by root rot complex disease induced by *M. phaseolina* and *R. solani*.

Efficacy of rhizobacterial isolates on plant growth metrics
Effect on plant height

The research indicated that all rhizobacterial isolates significantly enhanced the shoot length of tomato plants under pathogen-free conditions. The combined treatment COR12

Table 3: Impact of antagonistic rhizobacterial isolates on the growth characteristics of plants in a greenhouse environment.

Treatment	Average of plant length (cm)		Average of plant fresh weight (g)		Average of plant dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
Negative control	25.1i	15.6e	5.910f	0.585h	0.886f	0.117d
Positive control	12.7o	5.8j	2.144k	0.174l	0.429k	0.035g
BRE6 (Alone)	26.4gh	15.8de	6.045ef	0.603gh	1.209e	0.122cd
DKS3 (Alone)	26.8fg	16.1c-e	6.263cd	0.641f	1.256c	0.128b-d
DMC8 (Alone)	28.5c	17.1b-d	6.713b	0.683bc	1.343b	0.137b
SDV1 (Alone)	26.0h	16.3c-e	6.157c-e	0.633f	1.232c-e	0.127b-d
ECC4 (Alone)	27.5ef	16.4c-e	6.557b	0.653d-f	1.312b	0.131bc
ECL1 (Alone)	26.9fg	16.6b-e	6.338c	0.647ef	1.268c	0.130bc
SKE2 (Alone)	27.8de	16.4c-e	6.669b	0.666c-e	1.335b	0.133bc
BZD2 (Alone)	27.9de	17.2a-d	6.694b	0.673b-d	1.339b	0.135b
BZD3 (Alone)	26.5f-h	16.1c-e	6.079d-f	0.610g	1.217de	0.122cd
NAS1 (Alone)	28.3cd	17.2a-d	6.716b	0.689b	1.343b	0.138b
TRS4 (Alone)	27.0f	17.1b-d	6.260cd	0.643f	1.251cd	0.129bc
TNK1 (Alone)	28.3cd	17.3a-c	6.719b	0.690b	1.343b	0.138b
COR12 (Alone)	33.5a	18.6a	8.296a	0.791a	1.659a	0.158a
BRE6 × Pathogenic fungi	13.0o	5.9ij	2.316jk	0.178l	0.463jk	0.035g
DKS3 × Pathogenic fungi	13.1o	6.4h-j	2.365j	0.221k	0.473j	0.044e-g
DMC8 × Pathogenic fungi	17.0j	7.5f	2.815gh	0.267ij	0.564gh	0.054e
SDV1 × Pathogenic fungi	13.2o	5.8j	2.315jk	0.178l	0.464jk	0.035g
ECC4 × Pathogenic fungi	12.9o	5.8j	2.343jk	0.177l	0.469jk	0.040fg
ECL1 × Pathogenic fungi	14.0n	6.8g-j	2.363j	0.226k	0.473j	0.046e-g
SKE2 × Pathogenic fungi	15.8lm	7.8f-h	2.715h	0.256ij	0.543hi	0.051ef
BZD2 × Pathogenic fungi	16.1lm	7.8f-h	2.816gh	0.265ij	0.564gh	0.053e
BZD3 × Pathogenic fungi	12.8o	5.8j	2.318jk	0.177l	0.464jk	0.035g
NAS1 × Pathogenic fungi	16.8jk	8.2fg	3.010g	0.275i	0.602g	0.055e
TRS4 × Pathogenic fungi	15.6m	7.3f-i	2.589i	0.249j	0.518i	0.050ef
TNK1 × Pathogenic fungi	16.3kl	7.6f-h	2.778h	0.262ij	0.556hi	0.052e
COR12 × Pathogenic fungi	32.2b	18.0ab	8.153a	0.766a	1.631a	0.153a

Rs= *R. solani*, Mp= *M. phaseolina*, Pa= *P. aphanidermatum*, Data are the means of four replicates. The same letters within each column represent non-significant difference (P<0.05) as determined by the least significant difference (LSD).

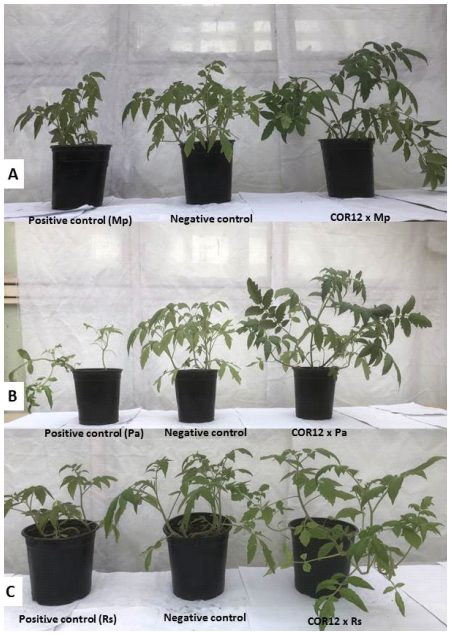
exhibited the most significant development, with a shoot length of 33.5 cm (Table 3). COR12 surpassed all opponents, achieving a remarkable shot length of 32.2 cm (Table 3).

The rhizobacterial isolates significantly augmented the root length of tomato plants, with the treatments of COR12, TNK1, BZD2 and NAS1 exhibiting the most substantial increases in root length (Table 3). Upon exposure to detrimental fungi, six particular strains exhibited enhanced root lengths, illustrating COR12's robustness and ability to promote root growth.

Effect on plant fresh weight

The findings indicated that rhizobacterial isolates, especially COR12, notably enhanced the shoot fresh weight of tomato plants in pathogen-free environments, achieving a peak shoot fresh weight of 8.296 g (Table 3). In the context of disease, the isolates led to an increase in shoot weight, varying from 2.363 g to 3.010 g, in contrast to the positive control's weight of 2.144 g (Table 3). COR12 showed significant effectiveness in promoting plant growth under stress conditions, highlighted by its impressive shoot fresh weight of 8.153 g.

The root system's weight displayed a similar trend, as all isolates showed a significant rise in root fresh weight when grown in pathogen-free conditions. COR12 consistently exhibited the greatest root fresh weight, measuring 0.791 g (Table 3). Upon exposure to pathogens, eight isolates showed a significant enhancement in root fresh weight, suggesting that COR12 facilitates root growth, thereby improving the uptake of nutrients and water, essential for plant development and stress resilience.



Tratments of tomato inoculated with *M. phaseolina* (A), Tratments of tomato inoculated with *P. aphanidermatum* (B), Tratments of tomato inculated with *R. solani* (C).

Fig 2: Biocontrol of tomato root rot diseases caused by soil-borne fungi in greenhouse conditions.

Effect on plant dry weight

All rhizobacterial isolates notably enhanced the dry weight of the plant's shoot system in treatments free of pathogenic fungi. The COR12 combination treatment achieved the highest dry weight of 1.659 g (Table 3), demonstrating the effectiveness of the bacterial mixture in enhancing shoot development more effectively than the individual isolates. The effectiveness of the rhizobacterial isolates varied when pathogenic fungi were present. Eight isolates demonstrated a significant increase in the dry weight of the shoot system, with values ranging from 0.473 g to 0.602 g, in comparison to the positive control treatment. The COR12 combination treatment demonstrated significant protective and growth-promoting effects, even under conditions of pathogen-induced stress.

Isolates from rhizobacteria notably increased the dry weight of the root systems of tomato plants when pathogenic fungi were not present, with the COR12 combination treatment achieving the highest dry weight of 0.158 g (Table 3). The COR12 treatment exhibited remarkable results, achieving an average root dry weight of 0.153 g, which is significantly higher than that of both the negative and positive controls.

Prior investigations have demonstrated the positive effects of rhizobacteria on the growth of plants. Abdeljalil *et al.* (2016) demonstrated that rhizobacterial isolates of *B. thuringiensis* B2, *B. subtilis* B10 and *E. cloacae* B16 notably enhanced the growth of tomato plants affected by the pathogenic fungus *R. solani*, leading to a 62-76% increase in plant height, a 53-86% increase in the fresh weight of roots and a 34-67% increase in the fresh weight of aerial parts. Abdel-Monaim *et al*. (2012) demonstrated that employing PGPR strains of *Azotobacter* sp., *B. cereus* and *B. megaterium* enhanced several growth parameters of tomato plants. Kang *et al*. (2021) observed notable differences in plant development traits when cucumber seeds were infected with isolates of *L. adecarboxylata* MO1. Zhang *et al*. (2015) demonstrated that six distinct bacterial strains significantly enhanced the growth of tomato plants in greenhouse pot experiments. Suprapta *et al*. (2014) demonstrated that five isolates of *E. cloacae* derived from cogongrass plants positively influenced plant growth and enhanced rice seedling yield. Patel *et al*. (2023) noted remarkable characteristics that promote plant growth when fenugreek seeds were treated with the *B. subtilis* ER-08 strain in greenhouse conditions.

CONCLUSION

The greenhouse trials showed that rhizobacterial isolates can enhance tomato seed germination, reduce disease incidence and severity and promote plant development under pathogenic stress conditions. The COR12 combination

treatment, consisting of twelve bacterial strains, consistently outperformed individual isolates, achieving a 100% germination rate and significantly reducing disease incidence and severity. The collaborative function of these bacteria highlights the potential for employing bacterial groups in comprehensive pest management strategies. The COR12 treatment also significantly enhanced plant development metrics, including shoot and root length and the weight of fresh and dried plant material. The effectiveness of disease control varied among the rhizobacterial isolates, with COR12 achieving the highest efficacy at 98.1%. Specific strains like *B. subtilis* NAS1, *B. halotolerans* DMC8, *S. lentus* BZD2 and *B. megaterium* TNK1 exhibit significant potential as biocontrol agents, positioning them as viable options for sustainable agriculture.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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