International Journal of Plant & Soil Science

Volume 38, Issue 4, Page 61-67, 2026; Article no.IJPSS.155931
ISSN: 2320-7035

Study of Biocontrol Efficacy of SiO_2 Nanoparticles Combined with PGPR (*Pseudomonas* spp. and *Bacillus* spp.) against *Rhizoctonia solani* in Tomato Plants

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i46030

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/155931

Original Research Article

Received: 25/01/2026.
Accepted: 01/04/2026
Published: 04/04/2026

Abstract

Silica nanoparticles have been found to improve rhizosphere microbial diversity while suppressing pathogenic microorganisms by modifying community composition and increasing beneficial taxonomic abundance, hence aiding disease resistance. In the present study, the effect of silicon dioxide Nanoparticles (SiO_2 -NPs) combined with plant growth promoting rhizobacteria (PGPR) on the seedling (Morphological) characters and stomatal function of Tomato plant Variety *(Lycopersicon esculentum* Mill. cv. PKM-1) were studied. The experiment was carried out from the month of October-2025 to November-2025 in Green House at Botany Section, School of Sciences, MANUU. Where healthy and uniform sized-seeds were selected for sowing in

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Cite as: *Robab, M. I., Uddin, W., & Begum, N. (2026). Study of Biocontrol Efficacy of SiO2 Nanoparticles Combined with PGPR (Pseudomonas spp. and Bacillus spp.) against Rhizoctonia solani in Tomato Plants. International Journal of Plant & Soil Science, 38(4), 61–67. https://doi.org/10.9734/ijpss/2026/v38i46030*

pots and they were treated with inoculum and PGPR at 4-leaf stage. The treatments were labelled as control (Untreated), PGPR alone, Combined SiO_2 NPs + PGPR with three replications each. The results indicated that all the growth parameters shown highest mean values for the plants treated with SiO_2 NPs + PGPR, which is followed by PGPR alone followed by the control (Untreated).The ANOVA results indicated that all the treatments were found to be significant. The functioning of stomata was observed to be advantageous to the tomato plants treated with combined SiO_2 NPs + PGPR when compared with PGPR alone treated plants and control (Untreated) plants.

Keywords: Biocontrol; Rhizoctonia solani; damping-off; PGPR, SiO_2-nanoparticles; synergistic effect.

1. Introduction

R.solani is a major soil-borne tomato (*Lycopersicon esculentum* Mill.) disease that causes damping-off and root rot worldwide.One of the earliest approaches to disease control is the use of fungicides.(Mogazy et al., 2024a)The disease results in losses from sowing to maturity in both nursery and transplanted fields. The illness is characterized by the emergence of light brown lesions on the primary root just below the soil surface, with root rot being a significant affliction in tomatoes. The frequency of recurrence is locally determined and primarily contingent upon environmental circumstances. The pathogen is particularly challenging to control in field settings because it persists in soil through resistant structures like sclerotia.(Al-Surhanee et al., 2021)Specific bio-inoculants of *Bacillus* and *Pseudomonas spp.* Plant defense activation can significantly reduce the occurrence or severity of many illnesses on a variety of hosts. Conventional management strategies largely rely on chemical fungicides; however, their extensive use has raised concerns regarding environmental pollution, pathogen resistance, and negative impacts on non-target organisms (Mogazy et al., 2024b).PGPR (*Pseudomonas spp.* and *Bacillus* spp.)inhibit infections by antibiosis, lytic enzymes, siderophore synthesis, competition, and the induction of systemic resistance (ISR).Studies have shown that SiO_2 nanoparticles improve physiological and biochemical parameters in plants, including photosynthetic efficiency, antioxidant activity, and nutrient uptake(Kumari et al., 2024).However, field performance varies depending on environmental conditions and competition with native bacteria. Nanotechnology, specifically silicon dioxide nanoparticles (SiO_2 NPs), can improve biological control by influencing microbial populations and plant immunology. silicon-based compounds have been reported to induce resistance against *R. solani* by regulating gene expression and strengthening plant defense systems(Feng et al., 2024).

Silica nanoparticles have been found to improve rhizosphere microbial diversity while suppressing pathogenic microorganisms by modifying community composition and increasing beneficial taxonomic abundance, hence aiding disease resistance.Recent investigations revealed that silver nanoparticles showed strong antifungal response against R.solani which lead to decreased disease severity and enhanced plant health.(Islam et al., 2024)**.**SiO_2 NPs improved physiological response and defense response activation(Ahamad & Siddiqui, 2021a).When both PGPR and SiO_2 used in combination it reduced oxidative stress and under stress conditions (Sharma et al., 2025). PGPR and SiO_2 NPs have been shown to have synergistic effects, despite most studies focusing on abiotic stressors like drought (El-Abeid et al., n.d.).Combining PGPR and SiO_2-NPs application boosted antioxidant enzyme activity, phytohormone levels, and wheat growth during drought stress, indicating better stress mitigation than either treatment alone.Because of their special and incredibly small size, nanoparticles (NPs) improve crop growth, yield, and nutritional quality(Ahamad & Siddiqui, 2021b).It is crucial to use sustainable, environmentally friendly agricultural methods that can withstand both biotic and abiotic challenges(Syed Nabi et al., 2021).Future P fertilizer demand will rise due to population growth, shifting dietary preferences toward meat, and expanding need for bioenergy crops(Liu & Lal, 2014).In agriculture, nanotechnology is being applied to enhance plant nutrition while preserving soil texture and protecting it from microbial diseases(Gohar et al., 2024).Although the advantages of silicon and its significance for plants are well known, less research has been done on the benefits of silicon nanoparticles over their bulk form(Rastogi et al., 2019).Manufactured nanoparticles (MNPs) might inadvertently enter several environmental compartments, such as fresh water, air, soil, etc., through their handling or indirect routes, such as landfills, sewage sludge, and wastewater during their synthesis(Tripathi et al., 2017).One of the many types of microorganisms associated with plants that might trigger plant defenses is Plant Growth-Promoting Rhizobacteria (PGPR)(Kloepper et al., 2004).Although nanotechnology has made significant strides in pharmacology and medicine, agricultural applications have garnered relatively less attention(Worrall et al., 2018).Challenges like loss of soil fertility, deficiency of water, loss due to weeds and pests, climate change etc can be faced by the use of Nanoagroparticles for the advancements of agricultural sector(Mridha et al., 2025).A number of bacterial and fungal isolates from tomato plant roots and rhizosphere considerably decreased tomato Fusarium wilt(Larkin & Fravel, 1998).Numerous researchers have investigated the special characteristics of nanoparticles (NPs), namely their size-activity interactions, to increase the effectiveness of microbial agents(Mahawar & Prasanna, 2018).Nanomaterials have become important instruments for improving agricultural capacity(Fetyan et al., 2024).

2. Materials & Methods

The tomato seeds (Lycopersicon esculentum Mill. cv. PKM-1) were purchased from the local market of hyderabad.The healthy and uniform sized seeds were surface-sterilized for 10 minutes with 1% sodium hypochlorite solution, then washed several times with double distilled water.The experiment is carried out in the Botany laboratory at Maulana Azad National Urdu University, Hyderabad,Telangana. *Pseudomonas fluorescens* and *Bacillus subtilis (PGPR strains)* were isolated from soil of MANUU campus using serial dilution method and grown in nutrient agar medium. R. solani isolated from infected tomato roots showing damping-off symptoms and it is grown on potato dextrose agar medium.After 7 days medium shows fungal growth. *Rhizoctonia solani* virulent isolated and cultured on PDA medium.

SiO_2 Nanoparticles (SiO_2-NPs) obtained from Nano research laboratory, Jharkhand and the particle size is 30-60nm.Requisite concentration (10ppm, 50ppm, 100ppm) of SiO2-Nanoparticles were dissolved in 10 mL of double distilled water in a 100 mL volumetric flask. Prior to the treatment surfactant (Tween-20) was used. Next,0.01% of Tween -20 was used to wash the roots for 1min and then dipped in SiO_2 Nanoparticles.*Pseudomonas fluorescens* & *Bacillus subtilis* (PGPR Strains), standard inoculum are at 28°C. Cell density was standardized to 10^8 CFU/mL before treatment.

2.1 Greenhouse Studies

A completely randomized design (CRD) with the following treatments was used and three replicates are used.The experiment was setup in combination of Control (no pathogen), *R. solani* + *B. subtilis*+ *Pseudomonas fluorescens* and *R. solani* + *B. subtilis*+ *Pseudomonas fluorescens*+ (SiO_2-NPs).The morphological characters like Shoot length, Root length, Fresh Shoot weight, Fresh root weight, Dry Shoot weight, Dry root weight, Shoot and Root biomass were studied.

2.2 Study of Stomata Characteristics

Treatments:

- **C (Control):** No treatment
- **T1:** PGPR (*Pseudomonas spp.* + *Bacillus spp.*)
- **T2:** SiO_2 nanoparticles + PGPR (moderate concentration)
- **T3:** SiO_2 nanoparticles + PGPR (higher concentration)

2.3 Observation of Stomatal Characteristics

2.3.1 Sample Collection

- Fully expanded leaves were collected at a specific growth stage (e.g., 30–45 days after sowing).

2.4 Stomatal Analysis Method

- The method used was nail polish impression:
  - A thin layer of transparent nail polish was applied on the abaxial (lower) leaf surface.
  - After drying, the film was peeled off using transparent tape.
  - The peel was mounted on a glass slide.
- Slides were observed under a light microscope (40× objective).
- The following parameters were recorded: 1)Stomatal type 2)Stomatal density (number/mm²) and 3)Stomatal aperture size (µm).

2.5 Statistical Analysis

- one-way Analysis of Variance (ANOVA) was performed.
- The treatments were tested for Significance of differences at $p \leq 0.05$ and $p \leq 0.01$ levels.
- Statistical software such as OPSTAT was used for analysis.

3. Results and Discussion

The present study evaluated the effect of PGPR (*Pseudomonas spp.* and *Bacillus spp.*) alone and in combination with SiO_2 nanoparticles (SiO_2-NPs) on plant growth parameters. The results revealed a consistent enhancement in all measured parameters under treated conditions compared to the control. A gradual increase in shoot length was observed across treatments, with mean values of 5.10 cm (Control), 5.76 cm (PGPR), and 6.80 cm (SiO_2 + PGPR) was observed.Root length showed a significant increase from 6.00 cm in control to 8.00 cm in PGPR and 9.67 cm in SiO_2 + PGPR. The fresh shoot weight showed 6.00 g (control), 7.00 g (PGPR) and 8.67 g (SiO_2 + PGPR). Fresh root weight increased from 3.43 g (control), 4.97 g (PGPR) and 5.40 g (SiO_2 + PGPR). A pronounced increase in dry root weight was recorded, with values of 1.33 g (control), 1.87 g (PGPR), and 3.28 g (SiO_2 + PGPR). Dry shoot weight was showed as 1.65 g (control), 2.17 g (PGPR) and 2.73 g (SiO_2 + PGPR).

Table 1. Showing mean values for the characters of tomato Plant PKM-1 variety

Tomato Plant PKM-1 Variety	Plant Characters					
	Mean Shoot Length (in cm)	Mean Root Length (in cm)	Fresh Shoot Weight (in g)	Fresh Root Weight (in g)	Mean Dry Root Weight (in g)	Mean Dry Shoot weight (in g)
Control	5.10	6.00	6.00	3.43	1.33	1.65
PGPR	5.77	8.00	7.00	4.97	1.87	2.17
SiO_2 NPs+ PGPR	6.80	9.67	8.67	5.40	3.28	2.73

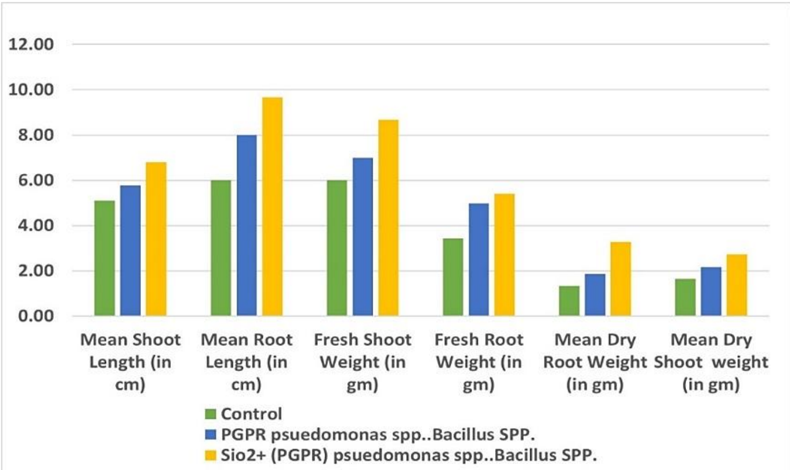

Fig. 1. Showing mean values of growth plant parameters-control, PGPR-Treated and SiO_2-NPs PGPR combined Treated

Table 2. Showing One-way ANOVA analysis of Growth Parameters of Tomato Plants PKM-1 Variety

Parameters	MS (T)	MS (E)	F	Sig.	SE(m)	SE(d)	CD (5%)	CV (%)
Shoot Length	0.810	0.003	243.11	**	0.033	0.047	0.134	1.01
Root Length	3.845	0.132	29.14	**	0.210	0.297	0.846	16.98
Fresh Shoot Weight	3.754	0.321	11.69	*	0.327	0.463	1.319	8.19
Fresh Root Weight	8.770	0.088	100.23	**	0.171	0.242	0.689	9.86
Dry Root Weight	6.351	0.864	7.35	*	0.537	0.759	2.164	11.10
Dry Shoot Weight	5.947	0.053	112.68	**	0.133	0.188	0.535	6.04

** = Significant at* ***$p \leq 0.05$*** *and ** = Significant at* ***$p \leq 0.01$***

One-way ANOVA analysis indicated that all the treatments had a highly significant effect

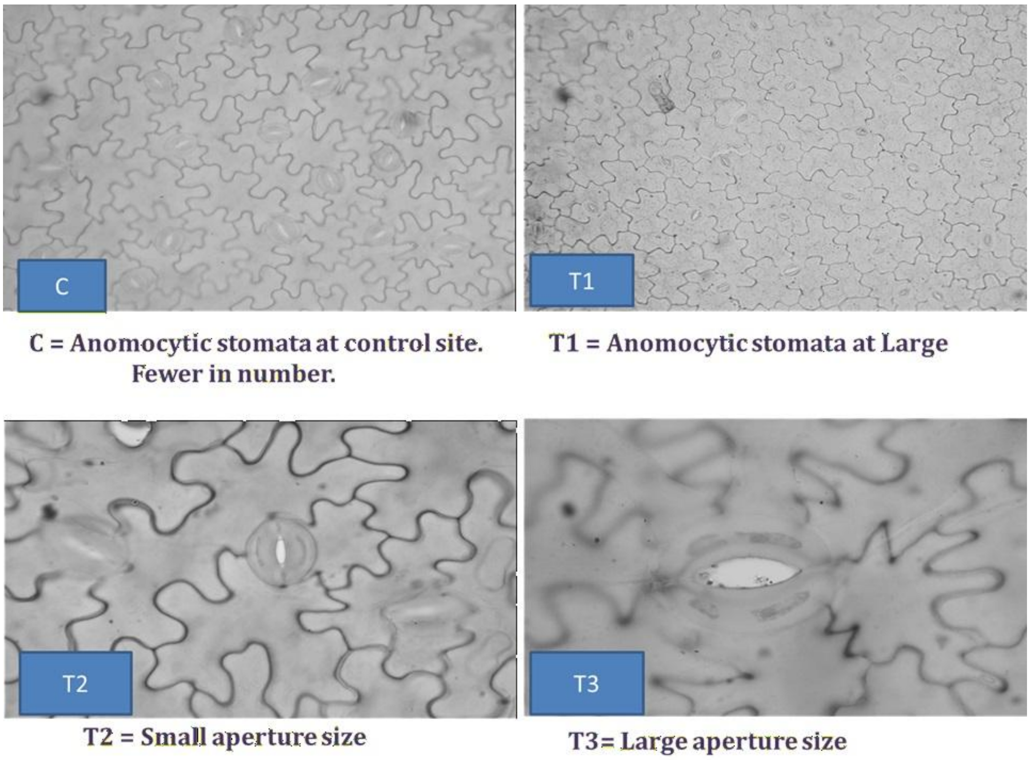

Fig. 2. Showing stomatal characteristics in control (untreated), T1(PGPR Alone), T2 (SiO_2 NPs + PGPR moderate concentration) and T3 (SiO_2 NPs + PGPR higher concentration).

Microscopic analysis revealed that all treatments, including control (C), T1 (PGPR), T2 (SiO_2 + PGPR – moderate effect), and T3 (SiO_2 + PGPR – enhanced effect), exhibited anomocytic stomata, indicating that stomatal type remained unaffected by microbial and nanoparticle treatments.

The treatments led to variations in stomatal density and stomatal aperture.The control(Untreated) plants showed little aperture opening and fewer stomata leading to low rate of gas exchange and reduced physiological activity. PGPR treated plants showed more stomata compared with control(Untreated), improved nutritional uptake and better stomatal function.

Combined treatment (SiO_2 + PGPR) decreased stomatal aperture and lead to partial stomatal closure.Due to this, there will be effective conservation of water by the plant.

The treatment (SiO_2 + PGPR higher efficacy) showed larger stomatal apertures on the leaf of plants with higher CO2uptake and increased photosynthesis.

These findings are consistent with previous studies reporting that PGPR improve plant physiological traits(Vacheron et al., 2013), while silicon enhances stress tolerance and regulates transpiration dynamics(Abdelrhim et al., 2021). The combined application appears to create a balance between growth promotion and stress adaptation, as evidenced by variations in stomatal behavior.

4. Conclusion

The SiO_2-NPs combined with PGPR showed more growth enhancing effect on tomato plants compared to control(Untreated) and PGPR alone treated plants.It supports that NPs facilitate the microbial efficiency which makes them suitable as biocontrol agents.It may involve rhizosphere dynamics, enhanced plant defence responses which leads to decreased disease severity and enhanced plant growth.Field validation and optimizing bioformulations of NPs for disease management of tomato plants are needed for sustainable agriculture. The

ANOVA results revealed that all the treatments are significant.The stomatal study indicates that combined treatment of SiO_2 NPs + PGPR has influenced significantly on the functioning of stomata.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Acknowledgement

This present research work is UGC funded minor research project. I sincerely thank Botany Section, School of Sciences, Maulana Azad National Urdu University, Hyderabad for providing me this opportunity to undertake this research work.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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