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Enhancing sustainable cultivation: The role of plant growth promoting fungi in optimizing Cucumis sativus L. growth

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Abstract

Rhizosphere and root associated fungi, commonly known as plant growth promoting fungi (PGPF), facilitate the growth and productivity of *Cucumis sativus* L. through the formation of mutualistic relationships with the plant roots. These fungi enhance nutrient uptake, promote root development and bolster stress tolerance via the secretion of growth regulating hormones. Despite numerous PGP fungal species have been isolated from the rhizosphere of *C. sativus*, their specific roles in modulating various growth parameters of the *C. sativus* remain insufficiently defined. In the present study, effect of seven PGP fungal species were evaluated such as *Penicillium chrysogenum*, *Paecilomyces variotii*, *Talaromyces purpureogenus*, *Paecilomyces brunneolus*, *Aspergillus flavus*, *Trichoderma viride*, and *Trichoderma atroviride* on the growth parameters of *C. sativus*. Our results revealed that all PGP fungal species positively influenced growth parameters such as, root length, shoot length, fresh and dry biomass, leaf area, specific leaf area, moisture content, flower number, leaf area ratio, leaf weight ratio and chlorophyll content, albeit with varying degrees of efficiency. Notably, *Trichoderma atroviride* exhibited a more pronounced impact on plant growth as compared to other PGP fungal species and the control. Our results suggest that these PGP fungal species may contribute to sustainable cultivation practices by enhancing crop yield and reducing the dependency on chemical fertilizers and pesticides.

1. Introduction

The growing global population and the corresponding rise in demand for agricultural production have resulted in the extensive use of pesticides and chemical fertilizers worldwide (Penuelas et al., 2023). The use of chemical fertilizers enhances crop yields however, substantial amounts of these chemicals accumulate in the soil, posing potential health risks and disrupting the soil's dynamics (Indira et al., 2022; Vanshree et al., 2022; Zhou et al., 2024). Moreover, chemical fertilizers are expensive, contributing significantly to the overall cost of agricultural production (Alexander et al., 2023). Rhizosphere and root associated fungi, commonly referred as plant growth promoting fungi (PGPF), confer numerous benefits to plants. These fungi play a pivotal role in biogeochemical and nutrient cycles, facilitating the utilization of decomposing organic matter through mineral solubilization (Thepbandit and Athinuwat, 2024). Modern agricultural practices emphasize minimizing the reliance on chemical fertilizers and promoting the application of plant growth promoting fungi (PGPF) as alternative strategies to enhance sustainable farming methods (Chaudhary et al., 2022). PGPF have been utilized as biofertilizers and as supplementary agents to chemical fertilizers in the cultivation of field vegetables and forage crops (Kumar et al., 2022). PGPF, are vital constituents of the rhizospheric soil and generally represent a larger proportion of the soil biomass compared to bacteria (Malviya et al., 2011; Adedayo and Babalola, 2023).

PGPF, also produce bioactive compounds, including plant hormones (gibberellins, auxins, cytokinins, and siderophores) and enhance plant resilience to abiotic stresses (Naimuzzaman et al., 2024). Furthermore, rhizospheric fungi can suppress plant pathogens through various antagonistic mechanisms, including the secretion of hydrolytic enzymes, mycoparasitism, competition for saprophytic colonization, and the induction of systemic resistance in host plants (Tyśkiewicz et al., 2022). Previous study has also

demonstrated that soil treated with (PGPF), species significantly enhances plant growth, phosphorus uptake, and yield in various important crops, such as brinjal (Li et al., 2021), wheat (Mohamed et al., 2022), maize (Ain et al., 2024). Several other studies were carried on PGPF genera, including *Gliocladium*, *Penicillium*, *Phoma*, *Phytophthora*, *Rhizoctonia*, *Talaromyces*, and *Trichoderma*, as effective in promoting growth and development in crops like orange (Omomowo et al., 2020), apple (Kuzin et al., 2020), pear (Cantabella et al., 2020), tomato, (Attia et al., 2022) and carrot (Pandit et al., 2022). Additionally, these fungi contribute to enhancing the innate immunity of plants and stimulating the production of essential secondary metabolites.

Cucumis sativus L. commonly known as cucumber, is one of the most globally consumed vegetable. Its popularity is due to its nutritional benefits, adaptability to diverse climatic conditions, and versatility in culinary applications, making it a valuable crop in the agricultural market. Producing high quality *C. sativus* significantly boosts market sales and enhances farmer income, making it a valuable crop choice for sustainable agriculture. In this study, a greenhouse experiment was carried out to elucidate the roles of various PGP fungal species isolated from the rhizosphere of *C. sativus*. To evaluate the effects of various PGP fungal species on growth parameters of test plant, seven isolated PGP fungal species such as *Penicillium chrysogenum*, *Paecilomyces variotii*, *Talaromyces purpureogenus*, *Paecilomyces brunneolus*, *Aspergillus flavus*, *Trichoderma viride* and *Trichoderma atroviride* were evaluated for their efficacy on growth parameter of test plant such as root length, shoot length, fresh mass, dry mass, leaf area, specific leaf area, moisture content, number of flowers, leaf area ratio, leaf weight ratio and chlorophyll content were evaluated. This avenue of inquiry aims to elucidate the role PGP fungal species within the dynamic interface between plant roots and soil and also helps in developing innovative strategies to improve the resilience and productivity of agricultural systems in the face of increasing challenges.

2. Materials and methods

For the preparation fungal inoculum, seven different fungal species *Penicillium chrysogenum*, *Paecilomyces variotii*, *Talaromyces purpureogenus*, *Paecilomyces brunneolus*, *Aspergillus flavus*, *Trichoderma viride* and *Trichoderma atroviride* were cultured on PDA for 5 days at 28°C. After this incubation period, the actively growing fungal plates were flooded with sterile water separately and gentle agitation was employed to liberate the spores from the mycelia. The liberated spores were then carefully separated from the mycelial debris by passing them through a sieve and were then collected in a beaker. The spore concentration was then adjusted by dilution with sterile water to a final concentration of 10^4 to 10^6 spores/ml. The concentration of fungal spores was selected based on an OD reading of 0.1 of the suspension at 600 nm using a Spectrophotometer. To carry out the Pot house experiment, soil was collected from Kashmir University Botanical Garden and passed through 2mm sieve to remove debris and combined with sand in 3:1 ratio. The soil was packed in sterile polythene bags and autoclaved at 15lb pressure for 20 to 30 min. To ensure the absence of any living microorganisms or contaminants that could interfere with the experiment, the potting mixture was autoclaved thrice.

Following the first autoclave cycle, the material was autoclaved again the following day. This allowed any remaining heat-resistant spores within the material to potentially germinate overnight and subsequently be eradicated. The third autoclave cycle was performed on the third day to ensure thorough eradication of any potential pathogens, including bacteria, fungi, or weed seeds. *C. sativus* seeds were sterilized in (2% NaOCl for 10 minutes followed by streptomycin (100 µg/ml) + cycloheximide (100 µg/ml) for 12 hrs and placed on sterile wet filter paper in 150 mm diameter Petri dishes at 28˚C and 95% humidity. After sterilization, the inoculated seeds were sown into a seedling tray containing sterilized soil at 26˚C and 60% relative humidity. Furthermore, the *C. sativus* seedlings at the three-leaf and heart stage were transplanted from the tray to 15cm clay pots (5 replicates for each treatment in a completely randomized block design) filled with triple autoclaved sand and soil in a ratio of 3:1. The pots were placed in a green house, the pots were irrigated with double distilled water every alternate day and the fungal spore suspension (10 ml in each pot) were applied individually at intervals of 7 days. Plants were grown in the green house for 3 months during which various observations like root length, shoot lengths, fresh mass, dry mass, specific leaf area etc were recorded.

3. Statistical analysis

Data were analysed as a one-way ANOVA using the IBM SPSS Statistics 25 software package (IBM, USA). Significant differences between treatments were identified using Duncan's new multiple range test at the $p < 0.05$ level of significance.

4. Results

The effect of seven PGP fungal species were evaluated such as *Penicillium chrysogenum*, *Paecilomyces variotii*, *Talaromyces purpureogenus*, *Paecilomyces brunneolus*, *Aspergillus flavus*, *Trichoderma viride*, and *Trichoderma atroviride* on the growth parameters of *C. sativus*. Results revealed (Fig. 1; Table 1) that all the PGP fungal species considerably improved growth parameters such as root length, shoot length, fresh and dry biomass, leaf area, specific leaf area, moisture content, flower number, leaf area ratio, leaf weight ratio and chlorophyll content of *C. sativus*. Root length was highest (12.1 cm) under *Trichoderma atroviride* and lowest (3.36 cm) under control. ANOVA and post-hoc analysis revealed that the increase in root length under the *Trichoderma atroviride* treatment is significantly higher compared to other treatments. Shoot length varied from a highest of 58.66 cm under *Trichoderma atroviride* and lowest of 33 cm under control treatment. Like root and shoot length, shoot fresh mass was also highest (18.22 g/plant) under *Trichoderma atroviride* and lowest (8.78 g/plant) under the control treatment. Shoot dry mass also differed under different treatments and was significantly higher (4.09 g/plant) under *Trichoderma atroviride* and was lowest (1.33 g/plant) under control treatment. The number of flowers ranged from 10.66 under *Trichoderma atroviride* to 2.33 under control. ANOVA and post-hoc analysis revealed that treatments such as *Trichoderma atroviride*, *Trichoderma viride*, *Aspergillus flavus*, *Paecilomyces brunneolus*, *Talaromyces purpureogenus*, *Paecilomyces variotii*, *Penicillium chrysogenum* and control were significantly different from one another. Furthermore, the moisture content percent was highest in control (85.05%) and lowest in *Trichoderma atroviride* (77.92%). ANOVA and post-hoc analysis

revealed that treatments such as *Trichoderma atroviride*, *Trichoderma viride*, *Aspergillus flavus*, *Paecilomyces brunneolus*, *Talaromyces purpureogenus*, *Paecilomyces variotii*, *Penicillium chrysogenum* and control were not significantly different from one another. Leaf area was highest in *Trichoderma atroviride* (112.35 cm^2) followed by the other fungi and least was observed in control (49.3cm^2). Specific leaf area was highest in *Paecilomyces variotii* (166.42 cm^2/g) and lowest in *Trichoderma atroviride* (82.42 cm^2/g). Leaf area ratio was highest in *Talaromyces purpureogenus* (34.56 cm^2/g) and lowest in *Trichoderma atroviride* (21.33 cm^2/g). There were no significant differences between *Paecilomyces variotii* and control. Leaf area ratio was highest in *Talaromyces purpureogenus* (34.56 cm^2/g) and lowest in *Trichoderma atroviride* (21.33 cm^2/g). Leaf weight ratio was highest in *Talaromyces purpureogenus* and *Trichoderma atroviride* (0.25 each). Additionally, chlorophyll content was highest in *Trichoderma atroviride* (7.95 mg/g) and lowest in control (1.95 mg/g). The effects of different treatments *Penicillium chrysogenum, Paecilomyces variotii, Talaromyces purpureogenus*, *Paecilomyces brunneolus*, *Aspergillus flavus*, *Trichoderma viride* and *Trichoderma atroviride* on root length, shoot length, fresh mass, dry mass, leaf area, number of flowers and chlorophyll content revealed significant differences in these growth parameters across the treatments, as compared to control, suggesting that the rhizospheric fungi have a discernible impact on plant growth compared to the control group. Each parameter was measured in five replicates (n = 5), and the results are presented as mean values with their respective standard deviations (SD).

5. Discussion

The study presents a comprehensive evaluation of the effects of plant growth promoting fungi (PGPF) on the growth parameters of *C. sativus*. Previous studies have also demonstrated that specific fungal species can stimulate key growth parameters, including seed germination and other developmental stages. *Trichoderma* species, which are well-documented for their biostimulators effects, likely due to their ability to produce phytohormones and solubilize essential nutrients (Asghar et al., 2024) have also been shown to elicit systemic resistance in plants, aiding them in coping with abiotic and biotic stresses (Gupta and Bar, 2020). The use of these fungal inoculants provides insight into reducing chemical inputs while maintaining high crop yield. Chemical fertilizers and pesticides, while effective, are increasingly scrutinized due to their impact on human and environmental health, as well as the development of pathogen resistance (Indira et al., 2022; Vanshree et al., 2022).

In the context of plant growth enhancement, different fungal treatments were used to evaluate how these fungal species, which include *Penicillium chrysogenum*, *Paecilomyces variotii*, *Talaromyces purpureogenus*, *Paecilomyces brunneolus*, *Aspergillus flavus*, *Trichoderma viride*, and *Trichoderma atroviride*, impact various growth parameters of *C. sativus*. The results highlight that C. *sativus* seedlings treated with diverse rhizospheric plant growth promoting fungal species, particularly *Trichoderma atroviride*, significantly improves growth parameters like root and shoot length, fresh and dry weight, and leaf chlorophyll content. For instance, *Trichoderma atroviride* -treated plants exhibited the highest root length (12.1 cm) and shoot length (58.66 cm), indicating a robust root system and enhanced vegetative

growth. Interestingly, treatments also affected moisture retention, with control showing the highest moisture content, contrasting with *Trichoderma atroviride*, which exhibited the lowest. This variation in moisture content could reflect the different capacities of fungal species to enhance water use efficiency and dry mass accumulation or adapt to fluctuating water availability in the soil matrix. Moreover, *Paecilomyces variotii*, and *Talaromyces purpureogenus*, which showed significant improvements in specific leaf area and leaf area ratio, could be contributing to an optimized plant architecture that maximizes light capture, a feature particularly advantageous in competitive plant environments. This investigation aligns with current research trends that emphasize sustainable agricultural practices and the reduced use of chemical fertilizers and pesticides. Given the context of global food security and the associated challenges posed by environmental stressors on crop productivity, this study offers a promising approach to addressing the yield demands of *C. sativus* which is widely cultivated and consumed in world.

6. Conclusion

This study highlights that plant growth promoting fungi (PGPF), can serve as eco-friendly bio stimulants, enhancing crop resilience and productivity while minimizing dependence on pesticides and chemical fertilizers. The study also demonstrates that PGP fungal species significantly improved growth parameters, including root and shoot length, biomass accumulation and chlorophyll content which indicates strengthened plant structure and photosynthetic efficiency. The observed bio-enhancement suggests that such fungi could play a vital role in advancing food security and eco-friendly crop management practices.

Declarations

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AUTHORS CONTRIBUTIONS

All authors listed in this submission have made significant contributions to the conceptualization and design of the study, as well as the analysis and interpretation of the data. Each author has actively participated in drafting the manuscript, critically revising it for key intellectual content, and approving the final version for publication

Data availability statement

No datasets were generated or analysed during the current study

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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Ethics, Consent to participate, Consent to publish

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Clinical trial

Not applicable

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

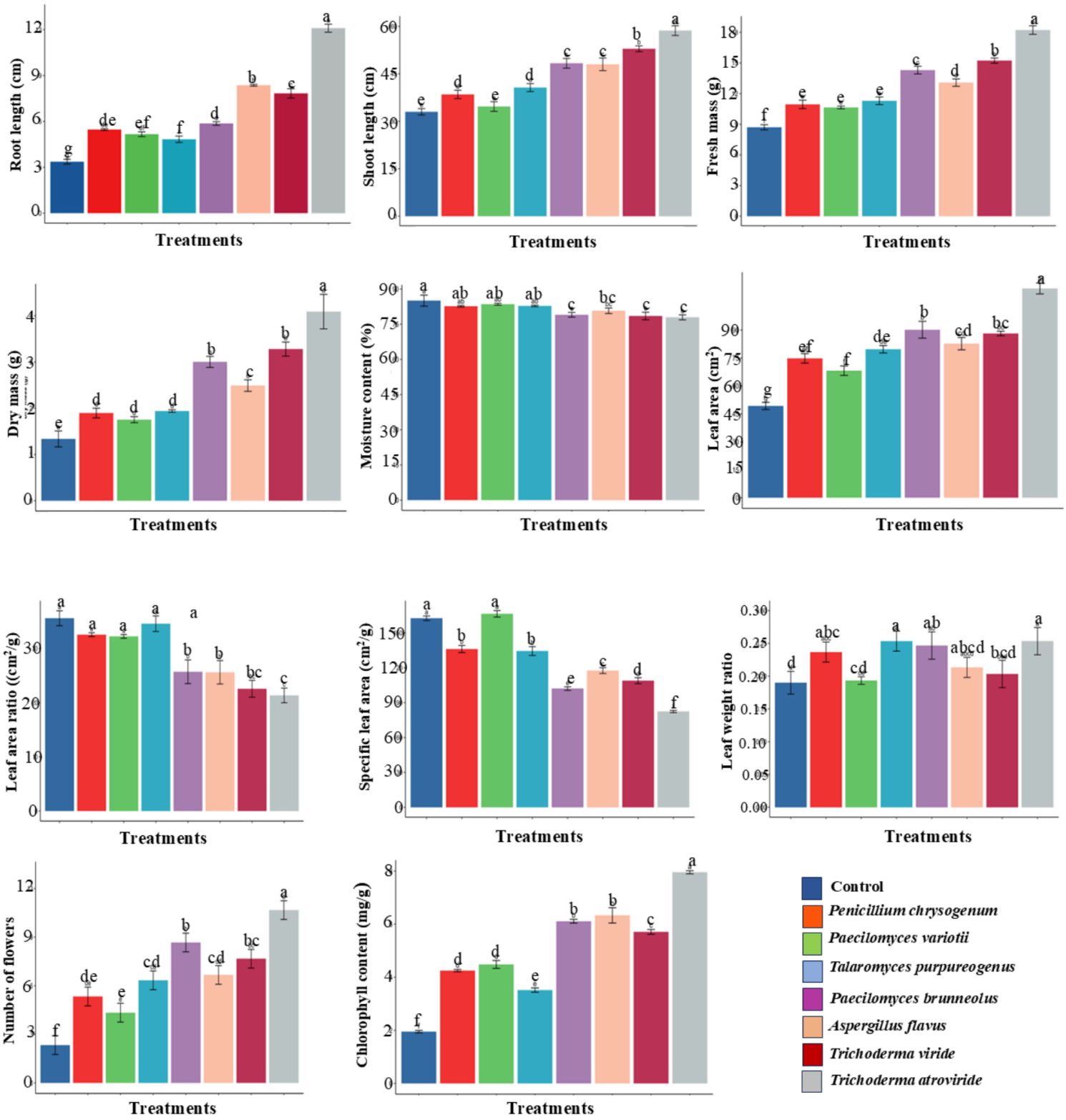

Figure 1

Effect of plant growth promoting fungi on growth parameters of *C. sativus*. Letters represent the significant differences $p < 0.05$



Figure 2

Effect of plant growth promoting fungal species on growth parameters of *C. sativus* Where T0: Control, T1:*Penicillium chrysogenum,* T2: *Paecilomyces variotii,* T3: *Talaromyces purpureogenus*, T4: *Paecilomyces brunneolus*, T5: *Aspergillus flavus*, T6: *Trichoderma viride* T7: *Trichoderma atroviride*

Supplementary Files

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- Table1.docx