
SUMMARY AND CONCLUSION

Root rot caused by *Sclerotium rolfsii* and leaf spot caused by *Alternaria alternata* are the major yield limiting factors in Stevia cultivation. These fungal pathogens spread very rapidly by producing numerous propagules which have different modes of spread. Although fungicides have shown promising results in controlling these diseases, phytotoxicity and fungicide residues are major problems leading to environmental pollution and human health hazards.

Development of fungicide resistance by fungal pathogens further discourages its use for disease control. Sanitation using sterile or clean water supplies, application of organic compost and regulation of watering and temperature during seedling growth contributes to the management of the disease to some extent. Though soil solarization has been successful under hot climatic conditions, it is impractical during the winter season. Thus, existing control measures are not very effective for the control of plant diseases.

Biological control is an alternative approach to the chemical fungicides and is a safe, effective and ecofriendly method for plant disease management. Soil has enormous untapped potential antagonistic microbes like fluorescent pseudomonads and *Bacillus* species which show antagonistic effects against a variety of plant pathogenic organisms. The saprophytic *P. fluorescens* and *B. subtilis* are associated with plant roots. The use of these antagonistic bacteria is gaining importance for plant growth-promotion and biological control.

Hence, the present study entitled “**Bio-management of Root rot and Leaf spot diseases in Stevia rebaudiana using Plant growth Promoting Rhizobacteria**” was aimed to evaluate the efficacy of talc-based powder formulation of bacterial antagonists (*Pseudomonas fluorescens* and *Bacillus subtilis*) in the management of root rot and leaf spot diseases of Stevia. The objectives of the study were to isolate Plant Growth Promoting (PGPR)

rhizobacteria from the rhizosphere soils of Tamil Nadu, characterize them through biochemical and molecular tests, screen the PGPR isolates against the fungal pathogens *S. rolfsii* and *A. alternata*, analyse the secondary metabolites such as hydrogen cyanide (HCN), siderophores, salicylic acid (SA), indole acetic acid (IAA) and chitinase, develop talc-based bioformulations, evaluate their efficacy against *Sclerotium rolfsii* root rot and *Alternaria* leaf spot of *S. rebaudiana* under greenhouse conditions and to study the effect of the rhizobacteria on the induction of defense related compounds in *S. rebaudiana* plants.

In Phase I, ten strains of *Pseudomonas fluorescens*, (AUPF1- AUPF10) and the ten strains of *Bacillus subtilis*, (AUBS1-AUBS10) were isolated from the rhizosphere soils of Tamil Nadu from various crop plants and confirmed by morphological, biochemical and molecular characterization.

The results showed that the *Pseudomonas fluorescens* isolates AUPF5 and the AUPF6 and *Bacillus subtilis* isolates AUBS2 and AUBS5 were found to be the most effective in reducing the mycelial growth of *Sclerotium rolfsii* and *Alternaria alternata* and also recorded the maximum inhibition zone.

In Phase II, the most effective PGPR isolates from Phase I of the study, namely, AUPF6 and AUPF5 of *P. fluorescens* and AUBS2 and AUBS5 of *B. subtilis* were screened for the production of antimicrobial compounds like hydrogen cyanide, siderophores, salicylic acid, indole acetic acid and chitinase. Based on the antimicrobial compounds production, the effective isolates of *P. fluorescens*, AUPF6 and that of *B. subtilis*, AUBS2 were selected for the talc-based formulation study in Phase III.

The *P. fluorescens* isolates AUPF5 and AUPF6 showed moderate HCN production, whereas, the *B. subtilis* isolates AUBS2 and AUBS5 recorded weak HCN production.

Higher production of salicylic acid was recorded in the *P. fluorescens* isolate AUPF6 followed by AUPF5. In the case of *B. subtilis* isolates, AUBS2 recorded significantly higher salicylic acid production than the isolate AUBS5.

The *Pseudomonas fluorescens* isolate AUPF6 significantly produced more amount of siderophore than AUPF5. The *B. subtilis* isolate AUBS2 produced more of siderophore compared to AUBS5. It can thus be inferred that all the isolates showed production of siderophores, with isolate AUPF6 showing maximum production compared to the other isolates.

As regards, indole acetic acid, AUPF6 showed significantly maximum IAA production followed by AUPF5 in the case of *Pseudomonas fluorescens*. However, in the case of *B. subtilis* isolates, the IAA content was significantly higher for AUBS2 than in AUBS5.

AUPF6 showed significantly higher chitinase activity compared to the other isolate AUPF5. The *B. subtilis* isolates AUBS2 and AUBS5 showed chitinase production on par with each other.

Thus, from the results of Phase II, it can be concluded that the *P. fluorescens* isolate AUPF6 and *B. subtilis* isolate AUBS2 produced more amounts of antimicrobial compounds like hydrogen cyanide, siderophores, salicylic acid, indole acetic acid and chitinase than the other isolates.

In Phase III, talc-based formulations were prepared using the effective isolates of *P. fluorescens* (AUPF6) and of *B. subtilis* (AUBS2) and their efficacy on the management of Stevia diseases studied. Application of talc-based formulation of the biocontrol agents AUPF6 and AUBS2 challenged against *Sclerotium rolfsii* and *Alternaria alternata* induced the accumulation of defense related compounds like phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and total phenols in Stevia plants.

Significantly increased activity of phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and total phenols were noticed in plants treated with AUPF6 and AUBS2 which were challenge inoculated with *S. rolfsii* and *A. alternata*.

Native-PAGE analysis showed the presence of two additional peroxidase isoforms PO 1 and PO2 against *S. rolfsii* and three PO isoforms PO1, PO2 and

PO3 against *A. alternata* in Stevia plants treated with AUPF6 and AUBS2, respectively.

Similarly, Native-PAGE analysis of polyphenol oxidase revealed the presence of three additional PPO isoforms like PPO1, PPO2 and PPO3 against *S.rolfsii* and *A. alternata* in Stevia plants treated with AUPF6 and AUBS2.

It can thus be concluded that plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* could be isolated from the rhizosphere soils of different crop plants of Tamil Nadu and confirmed through morphological, biochemical and molecular characterization and the *Pseudomonas fluorescens* isolate AUPF6 and *Bacillus subtilis* isolates AUBS2 could be employed in the management of root rot caused by *Sclerotium rolfsii* and leaf spot disease caused by *Alternaria alternata* effectively.

Recommendations for future studies

- Antimicrobial compounds from the PGPR can be isolated and formulated for the management of Stevia diseases.
- Mixture of PGPR isolates can be incorporated in the formulation and its effectiveness in managing Stevia diseases can be studied.
- Role of individual antimicrobial compounds produced by PGPR against antifungal activity can be estimated quantitatively and qualitatively.
- Transgenic Stevia plants can be produced by incorporating the gene of chitinase, hydrogen cyanide or siderophore etc., which can produce defense against Stevia diseases.