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The Effect of Rhizospheric Fungal Species of Tomato (*Solanum lycopersicum* L.), on In-Vitro Growth of the Tomato Early Blight Pathogen

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Abstract

Tomato (*Solanum lycopersicum* L.) is a globally consumed crop, susceptible to various biotic and abiotic stresses. Among biotic stresses, plant diseases, particularly Early Blight caused by *Alternaria* spp. leads to heavy losses in the field and post-harvest stages. The conventional use of synthetic agrochemicals to control these diseases poses environmental risks, underscoring the need for sustainable alternatives. Hence, this study aimed to assess the potential of rhizospheric fungi in controlling *Alternaria* spp. Infected tomato leaves, stem parts, and fruits were collected from commercial cultivations in four agro-ecological regions in Sri Lanka (two from mid-country in the wet zone, and two from the intermediate zone). All samples were washed using running tap water, and 1cm² sections, including the edges of lesions, were cut, surface sterilized, placed on potato dextrose agar (PDA), and incubated at room temperature until colonies appeared. Fungal colonies that emerged from lesions, were sub-cultured onto fresh PDA, and pure cultures were obtained using the hyphal-tip purification method. *Alternaria* isolates were identified morphologically and confirmed the pathogenicity by Koch's postulate method. Species-level identification involved genomic DNA extraction and sequencing ITS regions. The soil dilution plate technique was used to isolate rhizospheric fungi from two agroecological regions. Composite samples (10 g) of soil attached to the roots of 5 uprooted healthy plants, from each field were used. Soil suspensions were inoculated onto PDA using the spread-plate method and incubated at room temperature for 5 days. A total of 27 isolates were obtained and morphologically identified up to the genus level after preparing pure cultures. The dual-culture plate technique was used to assay percentage inhibition, and according to Tukey's pairwise comparison, *Trichoderma* sp. and *Purpureocillium lilacinum* exhibited significantly high ($p \leq 0.05$) inhibition of *Alternaria* colony growth, (68.25±1.18% and 64.35±3.31% respectively). Microscopic observations of inhibition zones between *Alternaria* and antagonistic rhizospheric fungal colonies, including *Trichoderma* sp. and *P. lilacinum* showed the presence of mycoparasitism-related structures, such as haustoria, coils, loops, and knobs. Qualitative assay for fungal chitinase enzyme production demonstrated high activities in *P. lilacinum*, *Trichoderma* sp. and *Acremonium* sp., whereas in a quantitative assay for fungal glucanase enzyme, testing the ability of fungal culture filtrates to release 1µg of glucose per minute, from Beta-glucanase solution, *P. lilacinum*, *Trichoderma* sp., and *Paecilomyces* sp. showed significantly high ($p \leq 0.05$) activities (105.14± 3.62, 100.11±4.09 and 99.83 ±6.44 µg glucose/L respectively). In conclusion, this study highlights the potential of rhizospheric fungi like *P. lilacinum* and *Trichoderma* sp. as effective biocontrol agents against *Alternaria* spp. and these findings lay the groundwork for future applications in enhancing tomato crop resilience against pathogens and promoting sustainable and eco-friendly agricultural practices.

Keywords: Rhizospheric fungi, Mycoparasitism, ITS region, Chitinase enzyme, Glucanase enzyme