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Enhancing Tomato Defense Mechanisms Through Endophytic Colonization by *Metarhizium anisopliae* and *Beauveria bassiana*: A Novel Approach to Biological Control Against *Tuta absoluta*^{*}

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Abstract

When plants interact with microbes, they use complex defense systems to maintain their safety. A plant may efficiently control the risks posed by phytopathogens and herbivores by triggering a cascade of effective reactions across its tissues when it recognizes them. In this study, the systematic growth of two entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, as endophytes in different tomato plant tissues with spraying method was evaluated using quantitative real-time PCR (qPCR). Moreover, the response of tomato plant immune responses in the presence and absence of this endophyte to *Tuta absoluta* herbivory was assessed using Real-time PCR. In the first experiment, both fungi demonstrated a notable increase in the colonization of various tomato tissues between 14 and 28 days post-inoculation, with this increase in concentration appearing initially in the leaves, followed by the stems and roots of the host plant. In addition, the highest fungal concentration was observed in tomato tissues colonized by *M. anisopliae* as an endophyte. In this study, we discovered for the first time that pre-inoculation with *M. anisopliae* or *B. bassiana* significantly activated *TGA*, *ERF*, and *PR-10*, gene expression pattern in the phytohormone pathways of tomatoes after a tomato leaf miner herbivory, compared to infested plants grown in the absence of the fungus. Additionally, after herbivory, tomato-colonized plants exhibited a strong induction of *P450* in the phenylpropanoid pathway, along with other defense-related genes, such as *WIP*, *nsLTP*, and *PRODH*, compared to tomatoes without endophytes. These findings suggest that integrating *M. anisopliae* and *B. bassiana* into tomato pest management strategies can reduce reliance on chemical pesticides, promoting a more sustainable and environmentally friendly approach to agriculture. This study provides a precedent for further exploration of endophytic fungi in biological control, potentially extending these benefits to other crops and pest challenges in agricultural systems.

Keywords: Insect pathology, Microbial control, Entomopathogenic fungi, Immune response, Real-time PCR, Tomato leaf miner.