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Exploitation of Root Gall Associated Fungal Endophytes for the Biological Control of *Meloidogyne graminicola* in Rice

Suvasri Dutta

Abstract

Root-knot nematodes, particularly *Meloidogyne graminicola*, represent a major biotic constraint in rice cultivation, especially under direct-seeded and water-limited conditions. The limitations of chemical nematicides, including environmental persistence and the emergence of resistance, necessitate the development of sustainable alternatives. This study investigated the potential of root gall-associated fungal endophytes as biocontrol agents against *M. graminicola*, with a specific focus on the isolate *Aspergillus niger* F4.

The fungal endophytic community associated with infected rice roots was characterised using both high-throughput sequencing and culture-based methods. Root gall tissues harboured stress-tolerant and potentially antagonistic taxa, in contrast to the mutualistic communities of healthy roots. Among the 32 isolates screened, *A. niger* F4 demonstrated strong nematicidal activity, inducing nearly 100% juvenile mortality in vitro and significantly reducing galling and nematode reproduction factor in rice plants. Chemotaxis assays revealed strong repellence, while microscopy suggested methuosis-like cell death in treated nematodes.

Further experiments established that F4 culture filtrate is effective against the *Meloidogyne incognita* in tomato. Root dipping reduced gall formation and reproduction by over 85%, matching the performance of the commercial nematicide Velum® Prime. Biochemical and transcript analyses indicated the induction of host defence through jasmonate- and ethylene-mediated signalling pathways alongside direct toxicity.

Metabolomic analyses identified sphinganine as the principal active compound. Sphinganine exhibited potent, dose-dependent nematicidal activity with minimal cytotoxicity to mammalian cells. Treated nematodes displayed cuticular disruption, oxidative stress, and differential expression of genes associated with metabolism, neuromuscular activity, and stress response.

To enable field deployment, a water-dispersible granule (WDG) formulation of the culture filtrate was developed. The optimised formulation (B6) retained bioefficacy under storage and significantly suppressed *M. graminicola* populations under both greenhouse and field conditions, with performance comparable to commercial nematicides.

This research presents a framework for harnessing gall-associated endophytes in nematode management. The findings position *A. niger* F4 and its metabolites as promising candidates for the development of next-generation, ecologically benign nematode management solutions for sustainable rice cultivation.

(Abhishek Mukherjee, Supervisor)

Abhishek Mukherjee, Ph.D.
Associate Professor
Agricultural and Ecological Research Unit
INDIAN STATISTICAL INSTITUTE
New Barganda, Giridih - 815 301
Jharkhand, India

Suvasri Dutta.

(Suvasri Dutta)