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Enhancing the pathogenicity of *Arthrobotrys conoides* and *A. oligospora* against *Meloidogyne javanica* J₂ by transferring of protease (AcI) gene and evaluation of antagonistic capability of transgenic isolates

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Title: Enhancing the pathogenicity of *Arthrobotrys conoides* and *A. oligospora* against *Meloidogyne javanica* J₂ by transferring of protease (*AcI*) gene and evaluation of antagonistic capability of transgenic isolates

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Abstract Antagonistic fungi are well-known as viable alternatives to chemical control of root-knot nematodes. In this paper, serine protease *AcI* as an important pathogenicity factor was used to enhance the antagonistic activity of *Arthrobotrys conoides* and *A. oligospora* against *Meloidogyne javanica* J₂ (second stage juveniles). *AcI* gene was extracted from *A. conoides* and cloned in pCambia1304 vector. The recombinant plasmid was then transferred to these fungi using two strains of *Agrobacterium tumefaciens* (LBA4404 and AGL1). Transgenic isolates were confirmed by PCR amplification of Hygromycin B resistance gene (*hph*) as selectable marker, protease assay using casein substrate and *in vitro* bioassay. Transferring of *AcI* gene by homologous recombination to *A. conoides*, increased protease activity. Our results showed that production of protease in both transgenic species was increased compared to wild type. Bioassay results indicated that pathogenicity rates of transformants, in both the number of traps formed and the number of trapped J₂, increased compared to the wild type. The results showed that inhibition of root-knot nematode, *M. javanica*, was markedly increased by transgenic isolates of *Arthrobotrys* spp. compared to the wild types.

Keywords Antagonistic activity, *Arthrobotrys conoides*, *A. oligospora*, *Meloidogyne javanica* J₂, *AcI* serine protease, *Agrobacterium tumefaciens*-mediated transformation.

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