

## APPLICATION OF CLASSICAL AND MOLECULAR TECHNIQUES IN DETECTION OF *Armillaria mellea* THE CAUSAL AGENT OF ROOT AND CROWN ROT DISEASE FROM SOIL AND WOOD \*

E. YOSEFI HAMEDANI, B. SHARIFNABI\*\* and M. BAHAR<sup>1</sup>

(Received: 19. 7. 2011; Accepted: 31. 1. 2012)

### Abstract

*Armillaria mellea* the causal agent of root and crown rot of trees has a universal distribution and causes extensive economic disease on a broad host range of trees in gardens, forests and urban environments. The pathogen can survive under the bark as inoculum and there is no effective control method against the disease, so pathogen detection from soil and wood is important to predict disease severity and prevent disease dispersal from one tree to another. To achieve a rapid and sensitive detection method for *A. mellea* in soil and wood, sampling was performed from fruiting bodies, soil and wood and after isolation of pathogen on culture media, comparison was done among different detection methods using a semi-selective medium, baiting and molecular methods. Inoculum was prepared for two isolates of *A. mellea* and was inoculated to soil. Pathogen detection was done with soil direct culture method, baiting and nested PCR simultaneously. The culture medium was not effective to detect pathogen in long-term period. In baiting method, *Pelargonium hortorum* was used as a baiting plant that required long time to detect pathogen. The results revealed that nested PCR is an efficient method for detection of *A. mellea*.

**Keywords:** Detection, *Armillaria mellea*, Classical methods, PCR.

See Persian text for figures and tables (Pages ۲۲۳-۲۳۵ ).

---

\*: A Part of MSc. Thesis of the First Author, Submitted to College of Agriculture, Isfahan University of Technology, Isfahan, Iran.

\*\* : Corresponding Author, Email: sharifna@cc.iut.ir

1. Former MSc. Student, Prof. and Assoc. Prof. of Plant Pathol., Respectively, College of Agric., Isfahan University of Technology, Isfahan, Iran.

## References

- ANONYMOUS. 2000. **Armillaria root rot of trees and shrubs**. RPD No. 602. University of Illinois extension.
- ASEF, M. R., MOHAMMADI GOLTAPPEH, E. and ALIZADEH, A. 2003. Identification of *Armillaria* biological species in Iran. **Fungal Divers.** 14: 51-60.
- BAHNWEG, G., SCHULZE, S., MO'LLER, E. M., ROSENBROCK, H., LANGEBARTELS, C. and SANDERMANN, H. 1998. DNA isolation from recalcitrant materials such as tree roots, bark, and forest soil for the detection of fungal pathogens by polymerase chain reaction. **Anal. Biochem.** 262: 79-82.
- BAUMGARTNER, K. 2004. Root collar excavation for post infection control of *Armillaria* root disease of grapevine. **Plant Dis.** 88:1235-1240.
- BAUMGARTNER, K. and A. E. WARNOCK. 2006. A soil inoculant inhibits *Armillaria mellea* *in vitro* and improves productivity of grapevines with root disease. **Plant Dis.** 90:439-444.
- BAUMGARTNER, K., BHAT, R. and FUJIYOSHI, P. 2010. A rapid infection assay for *Armillaria* and real-time PCR quantitation of the fungal biomass in planta. **Fungal Biol.** 114: 107 – 119.
- BEHDAD, E. 1981. **Diseases of Fruit Crops in Iran**. Neda Pub., Tehran. 296 PP (In Persian).
- COETZEE, M. P., WINGFIELD, B. D., BLOOMER, P., RIDLEY, G. S. and WINGFIELD, M. J. 2003. Molecular identification and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. **Mycologia** 95: 285-293.
- DOWNER, J. 2004. *Armillaria* root rot. **Landscape Notes** 17: 1-6.
- GUGLIELMO, F., BERGEMANN, S. E., GONTHIER, P., NICOLOTTI, G. and GARBELOTTO, M. 2007. A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. **J. Appl. Microbiol.** 103: 1490-1507.
- LOCHMAN, J., O. SERY and V. MIKES. 2004. The rapid identification of European *Armillaria* species from soil samples by nested PCR. **FEMS Microbiol. Lett.** 237: 105-110.
- LUSHAJ, B. M., WOODWARD, S., KECA, N. and INTINI, M. 2010. Distribution, ecology and host range of *Armillaria* species in Albania. **Forest Pathol.** 40:485-499.
- NICOLOTTI, G., GONTHIER, P., GUGLIELMO, F. and GARBELOTTO, M. M. 2009. A biomolecular method for the detection of wood decay fungi: a focus on tree stability assessment. **Arboricult. Urban Forest** 35: 14-19.
- ROBINSON-BAX, C. and FOX, R. T. V. 2002. Root rots of herbaceous plants caused by *Armillaria mellea*. **Mycologist** 16: 21-22.
- SCHENA L., IPPOLITO, A. and NIGRO, F. 2002. Identification and detection of *Rosellinia necatrix* by conventional and real-time Scorpion-PCR. **Eur. J. Plant Pathol.** 108: 355-366.
- SCHENA, L. and IPPOLITO, A. 2003. Rapid and sensitive detection of *Rosellinia necatrix* in roots and soils by real time scorpion-PCR. **J. Plant Pathol.** 85: 15-25.
- SCHULZE, S. and BAHNWEG, G. 1998. Critical review of identification techniques for *Armillaria* spp. and *Heterobasidion annosum* root and butt rot diseases. **J. Phytopathol.** 146: 61-72.
- SINGLETON, L. L., MIHAIL, J. D. and RUSH, C. M. (Eds.). 1992. **Methods for Research on Soilborne Phytopathogenic Fungi**. The American Phytopathological Society, APS Press, Minnesota.
- THORN, R. G., REDDY, C. A., HARRIS, D. and PAUL, E. A. 1996. Isolation of saprophytic Basidiomycetes from Soil. **Appl. Environ. Microbiol.** 62: 4288-4292.
- VOLK, T. J. and BURDSALL, H. H. 1995. **A nomenclatural study of *Armillaria* and *Armillariella* species (Basidiomycotina, Tricholomataceae)**. Eko-trykk A/S, Førde, Norway.
- WHITE, T. J., BRUNS, T., LEE, S. and TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322. In: PCR Protocols, **A Guide to Methods and Application**. Academic Press, San Diego, CA.
- WORRALL, J. J. 1991. Media for selective isolation of Hymenomycetes. **Mycologia** 83: 296-302.