

University of Zabol Graduate School Faculty of Agriculture Department of plant protection

The Thesis Submitted for the Degree of (M.Sc)

Title

Quantitative measurement of Verticillium dahliae in infected olives and evaluation of resistance to the disease using Real – time PCR technique

Supervisors

Dr. M. Salari

Dr. H. Jafary

Advisors

Dr. N. Panjehkeh

H. M. Javadi

By

S. Khanmohammadi

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Abstract

Verticillium Wilt is very important disease that causes destruction of olive vascular system and ultimately redounded to plant death. Use of resistant cultivars is the most effective methods to disease control. In this study, first infected olives from Tarom region were sampled. After surface disinfection, sample parts has taken on the general medium PDA and proprietary medium Czapec dox agar then incubated in Ancubator in 24°C and dark conditions. After the isolated fungi using standard single-spore method, purification was performed in water agar medium. DNA extraction from Verticillium grown on PDB liquid medium was performed and kind of type pathotype of pathogenic fungi using Nested-PCR technique and specific primers D type was determinated. Following, 5 months plants of cultivars include Zard (Tarom native), Arbequin, Oblonga and Koroneiky in Tarom Olive Research Station for fungi quantitative assessment and determined their sensitivity or resistant to disease were inoculated with 10⁷ suspension of fungi agent of disease with Root Dip methods. Number of plants to the control were considered. DNA extraction performed from inoculated plant roots and control samples at intervals time after sampling. Gradually increased the amount of fungal DNA in the plant using Real-time PCR technique and utilizing Sybr Green system with specific primers were quantitated. After the pathogenic fungi isolated from infected trees, results of this study showed that the Zard, Arbequin and Oblonga varieties, respectively had the high concentration of fungal DNA in total DNA extraction at certain intervals time after inoculation, therefore are respectively more sensitivity than the Koroneiky cultivar to the disease and Koroneiky cultivar somdeal is resistant to the disease. The results show that Real-time PCR techniques suitable for evaluating genotype resistance to olive Verticillium Wilt Disease which can be common used in the method for screening disease resistant varieties.

Key words: *Verticillium dahliae-* Resistant - Real – time PCR- Olive