

## **Abstract:**

Verticillium wilt caused by the *Verticillium dahliae* fungus is one of the most important threatening infections for olives around the world, including olive groves in Iran. Considering the recent development of olive cultivation in Sistan region, this study has been conducted in order to investigate the spread of this disease and contamination of olive trees in Sistan region. For this purpose, sampling was carried out from 20 suspected trees with symptoms such as dieback, and leaf loss from different parts of the olive groves of the Zahak Research Complex, Baqiatoallah Alazam, and Zahak Agricultural Research Center. Of each suspicious tree, five branches from different sectors were investigated. Then DNA was extracted from the collected samples and two methods of spectrophotometry and Agarose gel were used to investigate DNA quantity and quality. After DNA extraction, Real-time PCR analysis was performed using *V. dahliae* ITS specific primers. In this test, the total DNA extracted from the tissues of plants suspected to contamination was used. For this purpose, the extracted DNA samples from 5 samples per tree were mixed in equal proportions and were used. From twenty tested samples (tree) that contained yellow, oily and Cronacae cultivars 12 samples showed *V. dahliae* infection. In the following, in order to determine the pathotypes of the disease agent, the specific primers of *V. dahliae* isolates of type D and ND were used. To this end, we used Nested PCR with total DNA samples extracted from 12 leaf samples of Trees which their infection was previously confirmed with Real-time PCR. The results showed that pathotypes of cause of disease are of ND-type. Finally, for the verification of the results, the Polymerase Chain Reaction Product was transmitted to the South Korean Macrogen Company and sequenced. Then Using the Blasts software on the website (NCBI- Blast Search), the percentage of sequence similarity of the studied samples was determined in *V. dahliae* with sequences recorded at the NCBI database. The majority of the recorded sequences at the level of about 98% had the highest similarity to the gained sequences. These results imply that polymerase chain reaction products have been replicated properly.

**Keywords:** Olive, Verticillium wilt, Defoliating pathotype, Nondefoliating pathotype, Polymerase chain reaction



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**Detection and identification of Verticillium disease in olive orchards of Zahak region by using Real-time and Nested PCR techniques**

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