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Short Report

FIRST REPORT OF *Diplodia malorum* FUCKEL THE CAUSAL AGENT OF CANKER DISEASE OF APPLE TREES IN IRAN*

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Abstract

In summer of 2009, a disease with symptoms such as stem and trunk cankers were seen in apple Golden Delicious cultivars trees in west Azerbaijan orchards. The disease was in progress due to environmental stresses such as drought and frostbite. Bark and vascular system of the tree was darkened at the site of infection and cankers were scaling on main trunk and branches. To isolation of the fungi, infected tissues were surface sterilized for 3 min in 70% ethanol and were washed 3 times with sterile distilled water. Pieces were taken from sterilized tissues and were transferred to PDA, and then were kept at 25 °C, in the dark. The growing fungi were purified by hyphal tip method. To induce the formation of asexual fruiting bodies, the method of using pine needles were used. Inoculated Petri plates were kept for 4 weeks at 25 °C under near-ultraviolet light (near-UV) with 12:12h photoperiod to produce the pycnidia (Pavlic *et al*. 2008). Different macroscopic and microscopic characters such as color and growth characteristics of colony, color, shape and dimensions of conidium, conidiogenous cell and pycnidium were studied. Also, the ITS region of one selected isolate was amplified and sequenced. Based on morphological and ITS sequence analysis, *Diplodia malorum* was identified. Descriptive characters of the species are as: Colony at first white, after two weeks turned to olive buff to olive greenish and finally after three weeks became dark. Conidiomata pycnidial, 600 × 500 mm in diameter, solitry or aggregated, spherical to oblong, dark brown to black, immersed, partially erumpent when mature. Conidiogenous cells hyaline, smooth, cylindrical, swollen at the base, 7 - 16 × 2.5 - 5 µm, proliferating at the same level to produce periclinal thickening, or proliferating percurrently, giving rise to 2-3 annelations. Conidia aseptate, thick-walled, smooth outer surface and verruculose inner surface, oblong to cylindrical with broadly rounded ends, hyaline, becoming dark brown and 1-septate after release from the pycnidium (rarely two septum), 17.5-34 × 11-16 µm. The results of Blast search showed high similarity (100 percent) of the studied isolate with the isolates belong to *Diplodia malorum* deposited in Gene bank(Phillips *et al*. 2012). The pathogenicity tests were carried out on potted 2 years old seedlings and fruits of apple Golden cultivar based on completely randomized design. After eight weeks post inoculation, symptoms of bark darkening and canker were seen in inoculated areas. In fruits, six days after inoculation the brown rot symptoms were seen in the inoculated area which was progressed into the fruit flesh. In the controls, no symptoms were developed. Reisolation and identification of the inoculated fungi were done from the newly infected tissues and fulfilled Koch's postulates. To our knowledge, this is the first report of isolation and pathogenicity confirmation of *Diplodia malorum* from apples in Iran.

References

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