EARLY DIAGNOSIS OF PLANT DISEASES CAUSED BY *Ganoderma* spp.

By

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التشـخيـص المبكـر للأمـراض النباتيـة المتسببه عن أنواع فطر *Ganoderma*

رسالة مقدمة من

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ABSTRACT

Labiba Ahmed Reda: Early Diagnosis of Plant Diseases Caused by *Ganoderma* spp. Unpublished Ph.D. Thesis, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, 2013.

Fifteen *Ganoderma* isolates were collected from five naturally diseased host plants i.e. navel orange, morus, casuarina, date palm and fan palm from two governorates in Egypt i.e. Kalyubia and Giza.

Morphological observation, cultural characteristics and chemical test revealed that all fifteen isolates of *Ganoderma* associated with *Ganoderma resinaceum* and *Ganoderma lucidum*.

Phylogenetic analysis of ITS1 sequences revealed that the selective five isolates (1, 4, 7, 10, 13) belong to a single species i.e. *Ganoderma resinaceum.*

Pathogenicity test of five *Ganoderma* isolates, obtaind from naturally diseased navel orange, morus, casuarina, date palm and fan palm plants was carried out under greenhouse conditions. Pathogenicity test revealed that all fungal isolates were pathogenic to their host plants. These isolates caused root-rot symptoms, suddenly wilt without any yellowing symptoms, dieback of twigs and branches on artificially inoculated navel orange grafted on sour orange rootstocks. Visible symptoms were distinguished 12 months after inoculation of young trees.

The same symptoms were observed on navel orange grafted on volkamer lemon rootstocks artificially inoculated with two *Ganoderma* spp. (isolate1 and 13). Meanwhile root-rot symptoms, a slight distinct yellowing and desiccation of the lower leaves were showed on fan palm plants artificially inoculated with *Ganoderma* spp. (isolate1 and 13) 6 months after inoculation.

Polymerase chain reactions (PCR) were used for the confirmation of pathogenicity in inoculated plants using primers Gan1 and Gan2 generated from internal transcribed spacer region of rDNA. Primers Gan1 and Gan2 amplified a DNA fragment of the size of 167 bp when DNA of *Ganoderma* isolates were used for amplification. The amplification of a 167-bp PCR product was achieved with inoculated roots of sour orange rootstocks and volkamer lemon rootstocks 6 months after inoculation with tested *Ganoderma* isolates before disease symptoms had been expressed. Meanwhile the amplification of a 167-bp PCR product was achieved with inoculated roots of fan palm plants 5 months after inoculation with *Ganoderma* isolates before disease symptoms had been expressed. No PCR signal of *Ganoderma* DNA was observed in non-inoculated (healthy) roots. The DNA extract could be diluted up to 1:10 to get a positive reaction.

Physiological studies revealed that the tested fungal isolates produced cellulase, pectin degrading enzymes either in cultural filtrates or of plant tissues.

Data showed an ability of fungal isolates for degradation of lignin on wood chips.

Severe degradation of lignin was detected in young trees of navel orange grafted on sour orange and navel orange grafted on volkamer lemon artificially inoculated with tested fungal isolates. Degradation of lignin in inoculated fan palm seedlings was not significant.

Results showed that no toxins were found in *G. resinaceum* liquid medium.

Results indicated that the temperature of incubation has greeted effect of sour orange root pieces colonization by both *G. resinaceum* isolates. At 15 ºC, rate of colonization of sour orange by both tested isolates was very poor. The fungus attracted to wound area indicated

chemotaxis effect of root pieces on fungal growth. It could be conclude that 20-30 ºC is the optimum for colonization of root pieces by both isolates. Isolate No. 13 tend to be more aggressive at 35 ºC, and isolate No. 1 was more aggressive at 25 ºC.

Scanning electron microscopy revealed that both isolates had great effect on phloem elements and xylem vessels. Fungal hyphae were shown on xylem vessels at different temperature degrees of incubation. Moreover, tylosis was evident in many cases.

Key words: *Ganoderma resinaceum*, pathogenicity, isolation, early diagnosis, PCR, internal transcribed spacer, ITS1, Isolation, navel orange, morus, casuarina, date palm, fan palm, root- rot, taxonomy, Morphological characteristics, cultural characteristics, Laccate fungi, basidiospores, chlamydospores, hyphal system, cutis, Phylogeny, Wood-decay fungi, ligninolytic enzymes, pectintranseliminase, cellulolytic enzyme, cellulase, toxin, Scanning electron microscopy.

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