Menoufia University
Faculty of Agriculture
Department of Agricultural Plants



BIOLOGICAL AND MOLECULAR STUDIES ON PHYTOPLASMA AFFECTING SOME ORNAMENTAL PLANTS IN EGYPT.

By

# Shimaa Mohammed Ahmed Mohammed Gad

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#### **Thesis**

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Supervised by

#### **Prof. Dr. Mohammed Ahmed Awad**

Professor of Plant Pathology, Faculty of Agric., Menoufia University

### Dr. Ahmed Abd El-Aziz Kheder

Researcher of Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Centre

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Supervisors: Prof. Dr. Mohammed Ahmed Awad

Dr. Ahmed Abd EL-Aziz Zakaria El-Said Kheder

**Department: Agricultural Botany** 

#### **ABSTRACT**

**Background:** Phytoplasmas known as mycoplasma-like organisms (MLOs) cause diseases in several commercial ornamental plants leads to serious economic losses all over the world. During 2017-2018, phytoplasma disease showing phyllody, yellowing, proliferation, virescence and little leaf symptoms were observed on gazania and other ornamental plants in Giza, Egypt.

**Methods:** Phytoplasma disease was detected and isolated from naturally infected gazania plants during surveys in flower nurseries and open filed in Giza governorate, it was transmitted from naturally infected Gazania to healthy periwinkle and other ornamental plants by dodder (*Cuscuta reflexa*), and insects (*Empoasca decipiens*). Diene's stain was used to detect and differentiate the phloem tissues of leaf sections from infected gazania and periwinkle plants. Transmission Electron Microscopy (TEM) observed the presence of phytoplasma in the sieve tubes and parenchyma cells of leaf midribs in infected plants. DNA extracted from symptomatic samples was used as a template in nested polymerase chain reaction (PCR) using universal primers pairs P1/P7 and R16F2n/R16R2. Sequencing and phylogenetic analysis were performed to identify the detected phytoplasma.

**Results:** Phytoplasma was transmitted successfully from naturally infected Gazania to healthy ornamental plants by dodder, and insect. Light microscopy (LM) and transmission electron microscopy (TEM) revealed that, phytoplasmalike bodies were detected inside phloem, sieve tubes and parenchyma cells of leaf midribs tissues in infected plants and ranging from 200 to 400nm in diameters. The 16srRNA gene from phytoplasma was amplified by nested-PCR assay and direct sequenced using specific primer pairs. Phylogenetic tree was calculated based on obtained sequences data.

**Conclusion:** The phytoplasma associated with Gazania exhibiting phyllody, yellowing, proliferation, virescence and little leaf symptoms was confirmed by the results of LM and TEM observations and Nested-PCR testing. Based on direct sequence date, phylogeny analysis, the associated phytoplasma was classified as related to 16SrII group.

**Key words:** Phytoplasma, Gazania, Light Microscopy, Electron microscopy, Nested-PCR.