STUDIES ON SPIROPLASMA CITRI CAUSING STUBBORN DISEASE IN CITRUS AND WITCHES BROOM INFECTING ALFALFA IN EGYPT

BY

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B.Sc. Agric.Sc. (Agric. Microbiology), Ain Shams University, 2001

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ABSTRACT

RASHA MOHAMED MAHROUS IBRAHIM, Studies On Spiroplasma Citri Causing Stubborn Disease In Citrus And Witches Broom Infecting Alfalfa In Egypt. Unpublished M.Sc.Thesis, Department of Agricultural Microbiology, Faculty of Agricultural, Ain Shams University, 2005.

Spiroplasma citri, the causal agent of stubborn disease, was isolated from leaves and fruits of diseased citrus plants and cultured on solid and liquid C3G medium. On the basis of mode of transmission, symptomatology, shape of colonies on solid medium and examination of stained infected plant tissues with Dienes' stain and by phase contrast microscopy; the isolated agent was identified as *S. citri*. Identification was ensured by ELISA and PCR techniques. A fragment (1053 bp) from the DNA extract of *S. citri* was amplified by PCR using two primers specific to the spiralin gene. The nucleic acid hybridization techniques (Southern and dot blots) was carried out as an alternative sensitive methods for rapid detection of the Egyptian isolate of *S. citri* using a non-radioactive DNA probe specific for spiralin gene region.

Phytoplasma causing Witches'- broom disease in alfalfa was isolated from infected alfalfa and transmitted to alfalfa, tomato and periwinkle by dodder and identified by staining of infected plant tissues with Dienes'stain and DAPI (4¢,6-diamidino-2-phenylindole) stain, PCR (using two universal primers for phytoplasma) and the nucleic acid hybridization technique (Southern and dot blots).

The present study aimed to isolate and identify *S. citri* using some sensitive methods from different infected citrus trees grown at different governorates of Egypt. In addition, isolation and identification of phytoplasma causing witches'-broom in alfalfa plants.

Key words: *Spiroplasma citri*, Phytoplasma, Witches'-broom, Spiralin gene, PCR, DNA hybridization.

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LIST OF ABBREVIATION

B	**Bais pair……**	**bp**
C	**Centimeter…….**	**cm**
	Centigrade…….	**C**
	Complementary DNA……	**c-DNA**
	Hexadecyltrimethyl ammonium bromide…	**CTAB**
D	**Enzyme- linked immunoosorbent assay - Double antibody sandwich……**	**DAS-ELISA**
	Degree……	**°**
	Digoxigenin…….	**Dig-labelled**
	Deoxyribonucleic acid……..	**DNA**
	Deoxyribonuclease…….	**DNase**
	Dideoxy nucleotide triphosphate……	**dNTP**
	Dithiothreitol……..	**DTT**
	4¢, 6-diamidino-2-phenylindole……	**DAPI**
E	**Ethylene diethyl triacetic acid……..**	**EDTA**
	Enzyme linked immunosorbent assay……	**ELISA**
F	**Foetal bovine serum**	**FBS**
G	**Gram(s)……..**	**g**
	Gravity, centrifugal speed…….	**xg**
H	**Hour(s)……..**	**h**
K	**Kilobase……..**	**Kb**
M	**Mycoplasma -like organisms……**	**MLOs**
	Millimeter……. ……	**mm**
	Micro……..	**µ**