

**TOLERANCE OF SOME PHYTOPATHOGENIC
FUNGI TO FUNGICIDES**

By

NADIA ALY FOUAD

A thesis submitted in partial fulfillment

of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Science

(Plant Pathology)

31388 ✓

632.952

N.A

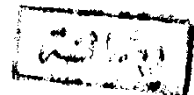
Department of Agricultural Botany

and Plant Pathology

Faculty of Agriculture

Ain Shams University

1990



Approval Sheet

TOLERANCE OF SOME PHYTOPATHOGENIC FUNGI TO FUNGICIDES
By

NADIA ALY FOUAD

B. Sc. in Plant Pathology, Fac. of Agr.
Ain Shams University. 1967.

M. Sc. in Plant Pathology, Fac. of Agr.
Ain Shams University. 1980.

This thesis for Ph. D. degree has been approved by:

Prof. Dr. : M. M. EL - Zayat
Prof. of : Plant Pathology
Fac. of Agric., Ain Shams Univ.

M. M. El Zayat

Prof. Dr. : A. A. Abdel - Razik
Prof. of : Plant Pathology
Fac. of Agric., Assiut Univ.

A. Abd. El Razik

Prof. Dr. : M. Z. EL - Shanawany
Prof. of : Plant Pathology
Fac. of Agric., Monofya Univ.

M. Z. El Shanawany

Date of examination : 14 / 6 / 1990



TOLERANCE OF SOME PHYTOPATHOGENIC
FUNGI TO FUNGICIDES

By

NADIA ALY FOUAD

B.Sc. in Plant Pathology Fac. of Agric.
Ain Shams Univ. 1967

M.Sc. in Plant Pathology Fac. of Agric.
Ain Shams Univ. 1980

Under the Supervision of Prof.Dr. Mohammed El-Zaiaat
Prof.Dr. Wallie-El-Deen Ashor
Professors of Plant Pathology, Agric. Bot. & Plant
Pathology Dept. Ain Shams Univ.

Abstract

Two pathogens, associated with damping - off and root rot disease of soybean seedlings, were isolated and identified as *Rhizoctonia solani* and *Sclerotium rolfsii*.

The *in vitro* sensitivity test towards different systemic and non systemic fungicides, showed that Vitavax at 0.1 and 0.5 $\mu\text{g/ml}$ inhibited the mycelial growth of *R.solani* and *S. rolfsii*, respectively. Benlate was more potent than Tecto, Derosal and Topsin - M against the two pathogens. *S. rolfsii* was more sensitive to PCNB and Captan than *R.solani*.

Tolerance of the two pathogens to the tested fungicides was performed by subculturing the wild types on medium containing gradually increasing concentrations of the fungicides. *S. rolfsii* was adapted more easily than *R. solani* to the fungicides tested except Vitavax where the reverse was obtained.

Tolerance did not influence the pathogenic activities of the tolerant isolated, as compared with the wild types. The morphological characters of the tolerant isolates differed in their mycelium colour, number and weight of the sclerotia formed. The tolerance to systemic fungicides was stable until 9 generation, while after 5 generation the tolerance of both pathogens to non - systemic fungicides was reversible.

The enzymatic activities of polyphenol oxidase and peroxidase were not affected greatly by tolerance to the fungicides. Adaptation to Benlate stimulated the enzymatic activities of both enzymes in the culture filtrates and in the mycelium matrix. In addition the adaptation process did not affect the polygalacturonase (PG) pectic methylestrase (PME) and Cellulase (Cx) activities. Thus the tolerant isolates were pathogenic to soybean seedlings similar to the wild type isolates.

Adaptation of the two pathogens affects markedly the metabolic pathways of free amino acids content within the fungal cells. The total amino acids increased in the tolerant isolates as compared with the wild type, indicating that these amino acids may play an important role in the mechanism of tolerance.

Total RNA and DNA were increased by the fungicide tolerance. Their ratios were also increased. This increasing may play a role in detoxification of the fungicides in the tolerant cells.

ACKNOWLEDGMENT

The author wishes to express her appreciation and gratitude to Prof. Dr. Mohamed M. EL_Bayat and Prof. Dr. W. E. Ashour, Professors of Plant Pathology, Department of Agric. Botany and Plant Pathology, Faculty of Agriculture, Ain Shams University for their continuous supervision, guidance, valuable criticism and encouragement throughout this investigation and preparation of this thesis.

Deep thanks are due to Prof. Dr. Z. M. EL_Tobshy, Head of Post Harvest Dis. Dept.; and to Dr. M. M. EL_Shiekh Aly, Researcher at the Post Harvest Dis. Dept., Pl. Path. Inst., ARC for their great help in the determinations of the chemical analyses.

The author would like^{to} express her sincere thanks to Dr. A. M. Amr, Asst. Researcher and Head of Cotton & Fiber Diseases Dept.; and to Dr. E. M. EL_Sherif, Asst. Researcher and Head of Mycology Res. & Plant Dis. Survey Dept., Pl. Pathology Institute, Agricultural Research Center for their gracious help during this investigation.

Sincere thanks and gratitude are extended to Dr. M. B. Mahmoud, Lecturer of Pesticides, Fac. of Agric., Al Azhar Univ.; to Dr. M. Abd El_Monem Mohamed and Dr. S. M. Hanafy, Researchers of Pl. Path., Pl. Path. Inst., ARC for their gracious help and the preparation of this thesis.

The author is indebted to all members of the Fungicides Res. Department, Pl. Path. Inst., ARC for their continuous help and encouragements.

Special thanks and graceful appreciations are due my husband Prof. Dr. Moustafa A. Amer, Professor of Plant Physiology, Dept. of Agric. Botany and Plant Pathology, Fac. of Agric., Ain Shams Univ. for his continuous help and encouragement and for the great sacrifices he made during the period of this investigation and the preparation of this thesis.

CONTENTS

Page

Introduction.	
Review of Literature.	
1 - Pathogens	1
2 - Fungicides	1
2 - A - Mode of action.	2
B - Effect of certain fungicides on the growth of pathogenic fungi in <u>vitro</u> .	2
3 - Resistance.	6
4 - Behaviour of adapted fungi	10
4 - A - Morphological and cultural characteristics.	18
B - Physiological studies :	18
B - 1 - Pectolytic and cellulolytic enzymes.	19
2 - Oxidative enzymes	19
5 - Behaviour of pathogenicity.	23
6 - Stability of resistant isolates.	26
7 - Mechanism of resistance.	27
Materials and Methods.	28
1 - Isolation and Identification of causal fungi.	31
2 - Pathogenicity test.	31
	32

3 - Fungicides used	33
4 - Effect of fungicides on the growth of pathogenic fungi <u>in vitro</u> .	39
5 - Adaptation of <i>R. solani</i> and <i>S. rolfsii</i> to fungitoxicans.	40
6 - Behavior of adapted isolates.	41
6 - 1 - Morphological and cultural characteristics.	41
2 - Physiological studies.	42
2 - I - Enzymes	42
I - A - In <u>vitro</u> .	42
B - In <u>vivo</u> .	42
II - Biochemical changes in resistant strains.	45
3 - Pathogenicity of adapted isolates.	48
7 - Stability of resistant isolates to fungicides.	53
Results :	54
1 - Etiology of soybean damping - off and root rot diseases.	55
2 - In <u>vitro</u> sensitivity testing of the two pathogens to different systemic and non - systemic fungicides.	57
3 - Acquired resistance of the two pathogens to the tested fungicides.	61

4 - The morphological characteristics of acquired isolates.	67
5 - Stability of acquired resistance for tolerant isolates of the two pathogens.	72
6 - Pathogenicity of the tolerant isolates.	77
7 - Physiological and biochemical studies on isolates of the two pathogens in <u>vitro</u> and in <u>vivo</u> .	79
7 - A - In vitro studies	80
A - 1 - Enzymes activities.	80
1 - a - Polyphenol oxidase and peroxidase.	80
b - Pectolytic and cellulolytic enzymes.	94
2 - Phenols content.	101
3 - soluble sugars content.	105
4 - Amino acids content.	109
5 - Nucleic acids content.	115
B - In <u>vivo</u> studies.	116
B - 1 - Phenol oxidase and peroxidase enzymes.	116
2 - Pectolytic and cellulolytic enzymes.	122
Discussion	128
Summary.	148
References.	155
Arabic Summary.	

LIST OF TABLES

Table (1)	: The average percentages of germination, post - emergence damping - off and healthy survivals of Soybean grown in potted soil artificially infested with <i>Rhizoctonia solani</i> or <i>Sclerotium rolfsii</i> in comparison with the control after 50 days from planting.	Page 56
Table (2)	: Mycelial growth (mm) of <i>R. solani</i> on PDA medium amended with different concentrations of some systemic and nonsystemic fungicides after 5 days at 25°C	53
Table (3)	: Mycelial growth (mm) of <i>S. rolfsii</i> on PDA medium amended with different concentrations of some systemic and nonsystemic fungicides after 5 days at 25°C	59
Table (4)	: ED ₅₀ values of the fungicides dosage - linear growth response curves for <i>R. solani</i> and <i>S. rolfsii</i> in fungicide - amended PDA medium after 5 days at 25°C	60
Table (5)	: Effect of extended exposure of <i>R. solani</i> to subsequent increasing concentrations of the fungicides in PDA amended medium on the build up of fungicide - tolerant isolates	62
Table (6)	: Effect of extended exposure of <i>S. rolfsii</i> to subsequent increasing concentrations of the fungicides in PDA amended medium on the build - up of fungicide - tolerant isolates	64

	Page
Table (7) : ED ₅₀ values of the fungicides dosage - linear growth response curves for Fungicide - tolerant isolates of <i>R. solani</i> and <i>S. rolfsii</i> in fungicide - amended PDA medium after 5 days at 25°C	66
Table (8) : Morphological characters of <i>Rhizoctonia solani</i> isolates adapted to certain fungicides after 8 and 30 days of growth at 25°C on PDA.	68
Table (9) : Morphological characters of <i>Sclerotium rolfsii</i> isolates adapted to certain fungicides after 8 and 30 days of growth at 27°C on PDA.	72
Table (10) : Effect of Subcultivation of the induced tolerant isolates of <i>R. solani</i> on PDA fungicide free medium on maintaining of tolerance expressed as mean mycelial growth (mm)	75
Table (11) : Effect of successive subcultivation of induced tolerant isolates of <i>S. rolfsii</i> on fungicide free - medium on maintaining of tolerance expressed as mean mycelial growth (mm)	76
Table (12) : Average percentages of germination post - emergence damping - off and health survivals of soybean plants grown in sterilized soil inoculated with adapted isolates of <i>R. solani</i> or <i>S. rolfsii</i> and parent wild type isolates after 50 days from planting under greenhouse conditions	78
Table (13-a): Activity of polyphenol oxidase enzyme (Activity/minute as optical density and % from wild type) in the filtrate and mycelial matrix of tolerant isolates of <i>R. solani</i> grown on Czapek's that allowed their growth, after 7 and 15 days of incubation at 25°C / 27°C	83

Table (13-b): Activity of peroxidase enzyme (Activity/minute as optical density and % from wild type) in the filtrate and mycelial matrix of tolerant isolates of <i>R. solani</i> grown on Czapek's that allowed their growth, after 7 and 15 days of incubation at 25°C / 27°C	83
Table (14-a): Activity of polyphenol oxidase enzyme (Activity/minute as optical density and % from wild type) in the filtrate and mycelial matrix of tolerant isolates of <i>S. rolfsii</i> grown on Czapek's that allowed their growth, after 7 and 15 days of incubation at 25°C / 27°C	87
Table (14-b): Activity of peroxidase enzyme (Activity/minute as optical density and % from wild type) in the filtrate and mycelial matrix of tolerant isolates of <i>S. rolfsii</i> grown on Czapek's that allowed their growth, after 7 and 15 days of incubation at 25°C / 27°C	91
Table (15) : Pectin - methylestrase (PME), Polygalacturonase (PG) and Cellulase (Cx) activities in culture filtrates of <i>R. solani</i> tolerant isolates grown on Tolboys & Busch liquid medium, amended with the highest concentrations of certain fungicides that allowed growth, after 7 and 15 days of incubation at 25°C	94
Table (16) : Pectin - methylestrase (PME), Polygalacturonase (PG) and Cellulase (Cx) activities in culture filtrates of <i>S. rolfsii</i> tolerant isolates grown on Tolboys & Busch liquid medium, amended with the highest concentrations of certain fungicides that allowed growth, after 7 and 15 days of incubation at 25°C	98

Table (17) :	Total, Free and Conjugated phenol contents (as mg/g fresh weight in mycelial extracts of tolerant isolates of <i>R. solani</i> and <i>S. rolfsii</i> grown for 15 days on Czapek's liquid medium, amended with the highest concentrations of certain fungicides that allowed their growth.	Page 102
Table (18) :	Total, reducing and non - reducing sugar contents (as mg/g fresh weight) in mycelial extracts of <i>R. solani</i> and <i>S. rolfsii</i> grown for 15 days on Czapek's liquid medium, amended with the highest concentrations of certain fungicides that allowed their growth.	106
Table (19) :	Free amino acids content (mg/1g fresh weight) in mycelial growth of <i>R. solani</i> tolerant isolates grown for 15 days in Czapek's liquid medium, amended with the concentrations of certain fungicides that allowed their growth.	110
Table (20) :	Free amino acids content (mg/1g fresh weight) in mycelial growth of <i>S. rolfsii</i> tolerant isolates grown for 15 days in Czapek's liquid medium, amended with the concentrations of certain fungicides that allowed their growth.	113
Table (21) :	The nucleic acids DNA and RNA contents (μ g/0.1g dry weight) in mycelial growth of the two pathogens <i>R. solani</i> and <i>S. rolfsii</i> in Czapek's liquid medium, amended with the highest concentrations of certain fungicides that allowed growth, after 15 days of incubation.	116
Table (22) :	Polyphenoloxidase and Peroxides enzymes activities (Activity / minute as optical density and % from wild type in Soybean Plants infected with wild type and adaptive isolates of <i>R. Solani</i>	120

Table (23) :	Polyphenoloxidase and Peroxides enzymes activities (Activity / minute as optical density and % from wild type in Soybean Plants infected with wild type and adaptive isolates of <i>S. rolfsii</i> after 21 and 28 days from planting.	120
Table (24) :	Pecticmethylestrase (PME), Polygalacturonase (PG) and cellulase (Cx) enzymes activities in soybean seedlings infected with wild type and adaptive isolates of <i>R. solani</i> after 21 and 28 days from planting	124
Table (25) :	Pecticmethylestrase (PME), Polygalacturonase (PG) and cellulase (Cx) enzymes activities in soybean seedlings infected with wild type and adaptive isolates of <i>S. rolfsii</i> after 21 and 28 days from planting	127

LIST OF FIGURES

	Page
Fig (1) Inhibition of mycelial growth of <i>R.solani</i> by the different fungicides in PDA medium.	59
Fig (2) Inhibition of mycelial growth of <i>S. rolfsii</i> by different concentrations of Derosal, Tecto and Topsin_M in PDA medium.	59
Fig (3) Inhibition of mycelial growth of <i>S. rolfsii</i> by different concentrations of Benlate, PCNB and Captan in PDA medium.	59
Fig (4) Inhibition of mycelial growth of <i>R.solani</i> and <i>S. rolfsii</i> by different concentrations of Vitavax in PDA medium.	59
Fig (5) Mycelial linear growth of <i>R.solani</i> isolates adapted to certain fungicides after 8 days of growth on PDA (% of wild type).	69
Fig (6) Sclerotial numbers, size and weight of <i>R.solani</i> isolates adapted to certain fungicides after 30 days of growth on PDA (% of W. T.).	70
Fig (7) Mycelial linear growth of <i>S. rolfsii</i> isolates adapted to certain fungicides after 8 days of growth on PDA (% of W. T.).	73
Fig (8) Sclerotial number, size and weight of <i>S. rolfsii</i> isolates adapted to certain fungicides after 30 days of growth on PDA (% of W. T.).	74