PATHOGENICITY OF THE MICROORAGANISMS ASSOCIATED WITH POTATO TUBERS AND THEIR EFFECT ON THE STORABILITY

By

OLA BARAKAT ABD EL-HAFEEZ BARAKAT

B.Sc., Agric.Sc. (Plant Pathology), Ain Shams University, 2007

A thesis submitted in partial fulfillment

of

the requirements for the degree of

MASTER OF SCIENCE

in

Agricultural Science

(Plant Pathology)

Department of Plant pathology

Faculty of Agriculture

Ain Shams University

2014

Approval Sheet

PATHOGENICITY OF THE MICROORAGANISMS ASSOCIATED WITH POTATO TUBERS AND THEIR EFFECT ON THE STORABILITY

By

OLA BARAKAT ABD EL-HAFEEZ BARAKAT

B.Sc., Agric. Sc. (Plant Pathology), Ain Shams University, 2007

This thesis for M.Sc. degree has been approved by:

Dr. Osama Yosof Mohamed Shalaby .....
Prof. Emeritus of Plant Pathology, Faculty of Agriculture, Fayoum University.

Dr. Ahmed Ahmed Ahmed Mosa .....
Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University.

Dr. Magdy Gad El-Rab El-Samman .....
Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University

Dr. Mostafa Helmy Mostafa .....
Prof. Emeritus of Plant Pathology, Faculty of Agriculture, Ain Shams University

Date of Examination: 26 / 8 / 2014

PATHOGENICITY OF THE MICROORAGANISMS ASSOCIATED WITH POTATO TUBERS AND THEIR EFFECT ON THE STORABILITY

By

OLA BARAKAT ABD EL-HAFEEZ BARAKAT

B.Sc., Agric.Sc.(Plant Pathology), Ain Shams University, 2007

Under the supervision of:

Dr. Soad Mohamed Abd Allah

Prof. Emeritus of Plant Pathology, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Mostafa Helmy Mostafa

Prof. Emeritus of Plant Pathology, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University

Dr. Magdy Gad El-Rab El-Samman

Prof. of Plant Pathology, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University

القدرة المرضية للكائنات الحية الدقيقة المصاحبة لدرنات البطاطس وتأثيرها على القابلية للتخزين

رسالة مقدمة من

علا بركات عبد الحفيظ بركات
بكالوريوس العلوم الزراعية (أمراض النبات) ، جامعة عين شمس ، 2007

للحصول على

درجة الماجستير في العلوم الزراعية
(أمراض النبات)

قسم أمراض النبات
كلية الزراعة
جامعة عين شمس

2014

صفحة الموافقة على الرسالة

القدرة المرضية للكائنات الحية الدقيقة المصاحبة لدرنات البطاطس وتأثيرها على القابلية للتخزين

رسالة مقدمة من

علا بركات عبد الحفيظ بركات
بكالوريوس العلوم الزراعية (أمراض النبات)، جامعة عين شمس، 2007

للحصول على
درجة الماجستير في العلوم الزراعية
(أمراض النبات)

وقد تمت مناقشة الرسالة والموافقة عليها

اللجنة:

د. أسامة يوسف محمد شلبي .....
أستاذ أمراض النبات المتفرغ، كلية الزراعة، جامعة الفيوم

د. أحمد أحمد أحمد موسي .....
أستاذ أمراض النبات، كلية الزراعة، جامعة عين شمس

د. مجدي جاد الرب السمان .....
أستاذ أمراض النبات، كلية الزراعة، جامعة عين شمس

د. مصطفي حلمي مصطفي .....
أستاذ أمراض النبات المتفرغ، كلية الزراعة، جامعة عين شمس

تاريخ المناقشة: 26 / 8 / 2014

جامعة عين شمس
كلية الزراعة

رسالة ماجستير

اسم الطالبة : علا بركات عبد الحفيظ بركات

عنوان الرسالة : القدرة المرضية للكائنات الحية الدقيقة المصاحبة لدرنات البطاطس وتأثيرها على القابلية للتخزين

اسم الدرجة : ماجستير في العلوم الزراعية (أمراض النبات)

لجنة الإشراف:

د. سعاد محمد عبد الله

أستاذ أمراض النبات المتفرغ ، قسم أمراض النبات ، كلية الزراعة ، جامعة عين شمس (المشرف الرئيسي)

د. مصطفى حلمى مصطفى

أستاذ أمراض النبات المتفرغ ، قسم أمراض النبات ، كلية الزراعة ، جامعة عين شمس

د. مجدى جاد الرب السمان

أستاذ أمراض النبات ، قسم أمراض النبات ، كلية الزراعة ، جامعة عين شمس

تاريخ التسجيل: 14 / 9 /2009

الدراسات العليا

ختم الإجازة **أجيزت الرسالة**
بتاريخ / /2014

موافقة مجلس الكلية **موافقة مجلس الجامعة**
/ / 2014 / / 2014

ACKNOWLEDGEMENT

Firstly, all praises are due to God, who blessed me with kind professors and colleagues, and gave me the support to produce this thesis.

I would like to express my profound gratitude and sincere appreciation to **Prof. Dr. Soad Mohamed Abd-Allah** Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University, for her kind supervision, valuable guidance and her kind encouragements for me during the present investigation.

I wish also to express my deepest thanks to **Prof. Dr. Mostafa Helmy Mostafa**, Prof. of. Plant Pathology, Faculty of Agriculture, Ain Shams University, for his kind attention and efforts through the course of the experiments, useful comments and editing this thesis and help during the preparation and writing this manuscript.

Sincere thanks and deepest gratitude to **Prof. Dr. Magdy Gad El-Rab**, Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University, for his supervision, guidance, help and continuous support in the course of this investigation.

Thanks also extended to all staff members and colleagues at Department of Plant Pathology, Faculty of Agriculture, Ain Shams University.

ABSTRACT

Ola Barakat Abd El- Hafeez: "Pathogencity of the Microorganisms Associated with Potato Tubers and their Effect on the Storability", Unpublished M.sc Thesis, Department of Plant Pathology, Faculty of Agriculture, University of Ain Shams 2014

Dry rot of potato (*Solanum tuberosum* L.) tubers can cause severe economical losses during storage. Pathogenic factors affecting storability of potato tubers. The pathogenic diversity of fungi associated with tuber dry rot was investigated. Three potato cultivars, kept under normal storage conditions, showed varied responses to infection with dry rot, where Annabelle was completely collapsed due to rots in comparison to Desiree cv. and Spunta cv, after two months under normal storage conditions. A total of 21 isolates belong to five fungal Genera, *i.e.*, *Fusarium* (64%), *Penicillium* (14%), *Alternaria* (14%), *Chaetomium* (4%), and *Drechslera* (4%) were recovered from diseased tubers samples of the three potato cultivars. Isolation from tuber adhered soil resulted in one isolate of each of *Fusarium solani*, *Nostoc* sp., and *Bacillus* sp. Identification of isolated fusaria indicate that they belong to three species i.e. *F. solani* (86%), *F. sambucinum* (7%) and *F. oxysporum* (7%). Isolated fusaria greatly varied in their pathogenicity, where, *F. sambucinum* (F_4) showed strong pathogenicity in comparison to the majority of *F. solani* isolates. Freshly isolated *Penicillium* isolates were more vigorous on potato tubers in comparison to isolate of *F. solani*. Subculturing of *Penicillium* sp. isolates caused the losses of their pathogenicity on potato tuber discs. The co-inoculation of potato tubers with isolates of *Penicillium* sp. and *F. solani* resulted in tuber rot by both pathogens.

The chloroform extract of soil led to great reduction of disease caused by *Fusarium solani*, moreover, the extract led to disappearance of tested fungal spores. Treatment of tuber discs with *Bacillus* sp. caused

significant reduction of moderately and weakly pathogenic *F. solani* isolates sporulation specially. Cultural filtrates of *Nostoc* sp. significantly reduced fungal sporulation on rotted tissues.; however, the non-polar fraction of the filtrate had little effect on all figures of rot incidence. The effect of certain agents: warm water shock (42º C for 5 min.), cold water shock (10º C for 5 min.), chitosan (1%), N-acetylcysteine (0.04%), hydrogen peroxide (4%) and calcium chloride (1%) on the infection of potato tuber discs by *Fusarium solani ,* was studied. All tested agents significantly reduced severity of dry rot on potato slices caused by *F. solani*. These findings suggest that these treatments can induce resistance in potato tubers. Peroxidase activity (PO) was determined in the upper 2 mm of the inoculated tuber discs surface. Treatment with warm water shock, N-acetylcysteine, and chitosan increased PO activity over control while, the other three agents decreased it below control. This result supposed that PO activity is not the principle factor in disease resistance of potato tuber discs toward *F. solani* infection.

Keywords: Potato, Tubers; Dry Rots; *Fusarium* Spp.; *Penicillium* Spp.; Cyanobacteria; *Bacillus* sp.; Co-inoculation; Chitosan; N-acetylcysteine; Hydrogen Peroxide; Induced Resistance; Peroxidase.

CONTENTS

	Page
LIST OF TABLES	V
LIST OF FIGURES	VIII
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	3
2.1. Tuber rot of potato	3
2.2. Causal fungi of dry rot of potato.	4
2.3. Factors affecting potato dry rot incidence and storability of tubers.	7
2.4. Alternative approaches to reduce dry rot disease of potato.	8
2.4.1. Effect of heat and cold shock.	9
2.4.2. Effect of biological control agents.	9
2.4.3. Effect of some chemical agents.	12
2.4.3.1. Effect of chitosan (CH)	12
2.4.3.2. Effect of N-acetylcysteine (NAC)	13
2.4.3.3. Effect of calcium chloride ($CaCl_2$)	15
2.4.3.4. Effect of hydrogen peroxide (H_2O_2)	16
2.4.3.5. Effect of gum arabic (GA)	18
3. MATERIALS AND METHODS	19
3.1. Plant material	19
3.2. Isolation of fungi from rotted tuber tissues.	19
3.3. Culture media.	20
3.4. Identification of the isolated microorganisms.	21
3.4.1. Identification of fungi	21
3.4.2. Identification of bacteria	21
3.5. Inocula preparation	21
3.6. Pathogenicity tests	22
3.7. Disease assessment	22

3.8. Determination of pectinolytic and cellulolytic activity of cultural filtration of *penicillium* spp. 23
3.9. Effect of co-inoculation of the isolated fungi on their pathogenicity 23
3.10. Effect of soil-chloroform extract on the pathogenicity of potato dry rot 24
3.11. Effect of *Bacillus* sp. isolate (B_1) on the pathogenicity of potato dry rot 24
3.12. Effect of cyanobacteria cultural filtrate on the pathogenicity of potato dry rot 24
a. Effect of crude cultural filtrate 24
b. Effect of polar fraction of cultural filtrate. 25
c. Effect of non-polar fraction of cultural filtrates. 25
d. Effect of sterilized culture filtrate of cyanobacteria. 26
3.13. Effect of warm or cold temperature shock on the potato dry rot 26
3.14. Effect of gum arabic on the pathogenicity of potato dry rot 26
3.15. Effect of N-acetylcysteine on the pathogenicity of potato dry rot 27
3.16. Effect of chitosan on the pathogenicity of potato dry rot 27
3.17. Effect of calcium chloride and hydrogen peroxide on the pathogenicity of potato dry rot 27
3.18. Determination of peroxidase enzyme activity 28
3.19. Determination of total proteins 28
4. RESULTS 29
4.1. Pathogenic factors affecting storability of potato tubers 29
4.1.1. Isolation and identification of microorganisms associated with potato tuber rot during storage 30
4.1.2. Isolation and identification of the microorganisms associated with soil-adhered to tubers 32

4.1.3. Pathogenicity tests. 32
4.1.4. . Effect of co-inoculation of the isolated fungi on their pathogenicity 37
a. Co-inoculation with *F. solani* and *Penicillium* sp. 37
b. Co-inoculation with *F. solani* and *Alternaria* sp. 39
c. Co-inoculation with *F. solani* and *Drechslera* sp. 40
d. Co-inoculation with *Pencillium* sp. and *Drechslera* sp. 41
4.2.Effect of some factors associated with potato tubers adhered-soil on the potato dry rot severity. 42
4.2.1. Effect of soil-chloroform extract on the pathogenicity of some fungi isolated from potato diseased tubers. 42
4.2.2. Effect of *Bacillus* sp. (B_1) on the potato dry rot. 44
4.2.3. Effect of cyanobacteria cultural filtrate on potato dry rot disease. 46
4.2.3.1. Effect of cyanobacteria crude cultural filtrate. 46
4.2.3.2. Effect of sterilized culture filtrate of Cyanobacteria. 51
4.3.Effect of some physical and chemical agents on the potato dry rot caused by *F. solani* 52
4.3.1.Effect of physical agents (warm and cold temperature shock) on the potato tuber dry rot. 52
4.3.2. Effect of chemical agents on the tuber rot caused by *F. solani.* 55
4.3.2.1. Effect of gum arabic. 55
4.3.2.2. Effect of N-acetylcysteine. 56
4.3.2.3. Effect of chitosan. 56
4.3.2.4. Effect of hydrogen peroxide. 59
4.3.2.5. Effect of calcium chloride. 60
4.3.3.Comparison effect of the best physical and chemical treatments on the infection of potato tuber with dry rot caused by *F. solani.* 62

4.3.4. Effect of the best treatments on peroxidase (PO) activity in potato tuber discs. 64
6. DISCUSSION 66
5. SUMMARY AND CONCLUSION 75
7. REFERENCES 79
ARABIC SUMMARY 1

LIST OF FIGURES

No.	Title	Page
1	Incidence of rot disease on the tubers of two potato cultivars, Desiree, and Annabelle after storage for two months under room temperature	29
2	Frequency of occurrence of the isolated fungi from the potato tuber samples collected in 2012	31
3	Frequency of occurrence of *Fusarium* species isolated from the potato tubers in 2012	31
4	Cultural features of an isolate of cyanobacteria specie isolated from the soil-adhered to potato tuber and grown on Hoagland medium at room temperature for 90 days	32
5	Percentage of the rotted area of inoculated discs by the isolated fungi	34
6	Weight of the rotted area with isolated fungi on the potato tuber discs	34
7	Sporulation capacity of the isolated fungi on the potato tuber discs	34
8	Percentage of rotted area by *Penicillium* isolates and *Fusarium solani* isolate (2) on the potato tuber cv. Spunta	35
9	Pathogenicity of *Penicillium* isolates and *Fusarium solani* isolate (2) on the potato tuber cv. Spunta	36
10	Pectinase and cellulase activity of culture filtrate of *Penicillum* sp. culture filtrate	36
11	Effect of co-inoculation *Fusarium* sp. and *Penicillum* sp. on the percentage of the rotted area of potato tuber discs	38

12	Effect of co-inoculation *Fusarium* sp. and *Penicillum* sp. on the weight of rotted area of potato tuber discs	38
13	Effect of co-inoculation *Fusarium* sp. and *Penicillum* sp. on the sporulation of inoculated potato tuber discs	38
14	Effect of co-inoculation of *Fusarium* sp. and *Alternaria* sp. on the percentage of rotted area of potato tuber discs	39
15	Effect of co-inoculation of *Fusarium* sp. and *Alternaria* sp. on the weight of rotted area of potato tuber discs	39
16	Effect of co-inoculation of *Fusarium* sp. and *Alternaria* sp. on the sporulation of inoculated potato tuber discs	39
17	Effect of co-inoculation of *Fusarium* sp. and *Drechsera* sp. on the percentage of rotted area of potato tuber discs	40
18	Effect of co-inoculaion of *Fusarium* sp. and *Drechsera* sp. on the weight of rotted area of potato tuber discs	40
19	Effect of co-inoculation of *Fusarium* sp. and *Drechsera* sp. on the sporulation of inoculated potato tuber discs	40
20	Effect of co-inoculation of *Penicillium* sp.and *Drchslera* sp. on the percentage of rotted area of potato tuber discs	41
21	Effect of co-inoculation of *Penicillium* sp. and *Drchslera* sp. on the weight of rotted area of potato tuber discs	41
22	Effect of co-inoculation of *Penicillium* sp. and *Drchslera* sp. on the sporulation of inoculated potato tuber discs	41
23	Effect of soil-chloroform extract on the percentage of infection with six fungal isolates on the potato tuber discs	43
24	Effect of soil-chloroform extract on the weight of rotted tissue of tuber slices inoculated with six isolates on potato tuber discs	43