PATHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE TOMATO BACTERIAL SPOT DISEASE

By

Mohamed Ahmed Mohamed Gaber

B.Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 2006

A thesis submitted in partial fulfillment

of

the requirements for the degree of

MASTER OF SCIENCE in Agricultural Science (Plant Pathology)

Under the supervision of:

Dr. Wafaa Mohamed Abd El-Sayed

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

Dr. Nagy Yassin Abd El- Ghafar

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

Department of Plant Pathology Faculty of Agriculture Ain Shams University 2011

Approval Sheet

PATHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE TOMATO BACTERIAL SPOT DISEASE

By

Mohamed Ahmed Mohamed Gaber

B.Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 2006

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

- Dr. Kamal Ahmed Abo-Elyousr Associate Prof. of Plant Pathology, Faculty of Agriculture, Assiut University
 Dr. Ahmed Ahmed Ahmed Mosa Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University
 Dr. Nagy Yassin Abd El- Ghafar
 - Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University
- Dr. Wafaa Mohamed Abd El-Sayed Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University

Date of Examination: 18 / 7 / 2011

PATHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE TOMATO BACTERIAL SPOT DISEASE

By

Mohamed Ahmed Mohamed Gaber

B.Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 2006

Under the supervision of:

Dr. Wafaa Mohamed Abd El-Sayed

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

Dr. Nagy Yassin Abd El- Ghafar

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

ABSTRACT

Mohamed Ahmed Mohamed Gaber : Pathological and Physiological Studies on the Tomato Bacterial Spot Disease. Unpublished M.Sc. Thesis, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, 2011.

Bacterial spot of tomato can affect foliage, fruits, blossoms and stems. This study was conducted to characterize Xanthomonas vesicatoria isolates from different locations in Egypt, and to study isolates diversity. Also, the susceptibility or resistance of some tomato cultivars to infection with bacterial spot was investigated, and to find out some biochemical and physiological changes within infected tomato plants due to infection. Finally the potentiality of some bactericides, bioagents (commercial products) and resistance inducers in controlling bacterial spot disease under naturally and artificially infection conditions was evaluated. The majourty of isolates were obtained from Qalyubia governorate (20 isolates), followed by Beheira (16 isolates) and Sharkia (11 isolates). So the total isolates reached fourty-seven isolates of Xanthomonas vesicatoria which were isolated from leaves and fruits of tomato and pepper, collected from previously mentioned three governorates in Egypt. Xanthomonas vesicatoria isolates were characterized and identified on the basis of morphological, biochemical and physiological characteristics revealing 15 isolates. Isolates TX5 and TX1 were identified as Xanthomonas vesicatoria, 96% and 86%, respectively, using Biolog system. Xanthomonas vesicatoria isolates varried in pathogenicity against tomato plants where isolate TX5 and TX1 were highly pathogenic, while isolates TX3, TX4, PX2 and PX4 were moderatley pathogenic, however TX2, PX1, PX3 and PX5 isolates were less pathogenic. Molecular differences were detected among 10 isolates of Xanthomonas vesicatoria using RAPD-PCR test with one primer, where a specific band was observed in the DNA of the highly virulent isolates only, which can be used as a marker of virulence. Tomato cultivars varried in their

susceptibility to bacterial spot (isolate TX5) where cultivar Castle Rock was the most susceptible cultivar while H 339 was highly resistant to the disease. Five tomato cultivars were screened for defence-related enzymes. Based on the inducible amounts of these enzymes upon pathogen infection, the tomato cultivars were correlated with the disease incidence under greenhouse conditions. Enzyme levels were increased in infected plants compared with control. The present results indicated that, there was no correlation between the degree of host resistance and the enzymes levels. Application of bactericides, bioagents and resistance inducers significantly reduced the disease compared with the control. Under greenhouse conditions, application of bioagents, bactericides and resistance inducers were found to be more effective when spray before inoculation than after inoculation. Efficacy of all treatments were increased with increasing their concentrations. Streptomycin sulphate was more effective against the disease than Galbin copper or Kocide 101. Also, the commercial products Bio-Cure-B or Bio-Cure-F were highly effective against the disease than Symbion N-R or Symbion N-G. Salicylic acid and acetylsalicylic acid as resistance inducers, showed high efficacy in reducing the disease compared with Bion. Under commercial greenhouse conditions, streptomycin sulphate was the most effective treatment against the disease followed by Bio-Cure-B. Application of, commercial bioagents products and resistance inducers, at 3 different intervals gave higher efficacy than one time of application. All tested compounds were more effective under commercial greenhouse conditions than under greenhouse conditions.

Keywords: Tomato, *Solanum lycopersicum*, bacterial spot, *Xanthomonas vesicatoria*, diversity, defence enzymes, RAPD-PCR.

ACKNOWLEDGEMENT

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

It is an extremely pleasure to thank those who made this thesis possible. I am so grateful to **Prof. Dr. Wafaa M. Abd El-Said**, Professor of Plant Pathology, Faculty of Agriculture, Ain Shams University for her supervision, help, valuable advices and continuous encouragement during this study. I would like to show my gratitude to **Prof. Dr. Nagi Yassin Abd El-Ghafar**, Professor of Plant Pathology, Faculty of Agriculture, Ain Shams University for his kind attention, parental advices and his commitment to complete the work.

I owe my deepest gratitude to **Prof. Dr. Mostafa. H. Mostafa**, Professor of Plant Pathology, Faculty of Agriculture, Ain Shams University and **Dr. E. Gado**, Associate Prof. of Plant Pathology, Faculty of Agriculture, Ain Shams University, who have made available their support in a number of ways, and this thesis would not have been possible unless their help.

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

CONTENTS

| LIST OF TABLES |
|---|
| LIST OF FIGURES |
| LIST OF ABBREVIATIONS |
| I. INTRODUCTION |
| II. REVIEW OF LITERATURE |
| 1. Bacterial spot disease of tomato |
| 1.1. Disease symptoms |
| 1.2. The causal organism |
| 1.3. Detection and identification of the causal bacteria |
| 2. Susceptibility of some tomato cultivars to bacterial spot |
| disease |
| 3. Biochemical effects in tomato plants infected with bacterial |
| spot |
| 4. Diversity within X. campestris pv. vesicatoria isolates |
| 4.1. Pathological diversity |
| 4.2. Molecular diversity |
| 5. Control of bacterial spot disease of tomato |
| 5.1. Chemical control |
| 5.2. Control using resistance inducers |
| 5.3. Biological control |
| III. MATERIALS AND METHODS |
| 1. Pathological studies |
| 1.1. Collection of diseased samples |
| 1.2. Isolation of the causal organism |
| 1.3. Pathogenicity test |
| 1.4. Identification of the pathogen |
| 1.4.1. Morphological and cultural characters |
| 1.4.2. Biochemical and physiological characters |
| 1.4.3. Identification using Biolog system |
| 2. Comparative studies between <i>X. vesicatoria</i> isolates |

| 2.1. Cultural characters | 18 |
|---|----|
| 2.2. Pathogenicity | 18 |
| 2.3. Molecular characterization of isolates | 19 |
| 2.3.1. Isolation of chromosomal DNA from <i>X. vesicatoria</i> | 19 |
| 2.3.2. PCR reaction mixture | 20 |
| 2.3.3. Thermoprofile for PCR | 20 |
| 2.3.4. Separation of amplified products by agarose gel | |
| electrophoresis | 20 |
| 2.3.4.1. Preparation of agarose gel | 20 |
| 2.3.4.2. Gel photographing | 21 |
| 2.3.5. Statistical analysis | 21 |
| 3. Susceptibility of some tomato cultivars to infection with <i>X</i> . | |
| campestris pv. vesicatoria | 21 |
| 4. Assay of some defence related enzymes in different tomato | |
| cultivars inoculated with X. vesicatoria | 22 |
| 4.1. Preparation of samples | 22 |
| 4.2. Changes in peroxidase (PO) enzyme activity | 22 |
| 4.3. Changes in polyphenol oxidase (PPO) enzyme activity | 23 |
| 4.4. Changes in phenylalanine ammonia lyase (PAL) enzyme | |
| activity | 23 |
| 5. Disease control | 23 |
| 5.1. In vitro | 23 |
| 5.1.1. Effect of bactericides on growth of <i>X. campestris</i> pv. | |
| vesicatoria | 23 |
| 5.1.2. Effect of bioagents on growth of <i>X. vesicatoria</i> | 24 |
| 5.1.3. Effect of resistance inducers on growth of <i>X</i> . | |
| vesicatoria | 24 |
| 5.2. <i>In vivo</i> (greenhouse conditions) | 24 |
| 5.2.1. Effect of bactericides on incidence of tomato bacterial | |
| spot disease | 25 |
| 5.2.2. Effect of bioagents on incidence of tomato bacterial | |
| spot disease | 25 |

| 5.2.3. Effect of inducers on incidence of tomato bacterial |
|--|
| spot disease |
| 5.3. Under commercial greenhouse conditions |
| 5.3.1. Effect of bactericides on severity of tomato bacterial |
| spot disease |
| 5.3.2. Effect of bioagents on severity of tomato bacterial spo |
| disease |
| 5.3.3. Effect of inducers on severity of tomato bacterial spot |
| disease |
| 5.3.4. Disease assesment |
| 6. Statistical analysis |
| 7. The media and buffers used in this investigation |
| 7.1. Nutrient agar (NA) medium |
| 7.2. Tween-B medium |
| 7.3. King's B (KB) medium |
| 7.4. TBE buffer (5 X) |
| 7.5. Ethidium bromide |
| 7.6. Sample loading dye (5x) |
| IV. RESULTS |
| 1. Pathological studies |
| 1.1. Isolation of the causal organism |
| 1.2. Identification of the causal organism |
| 1.2.1. Morphological and cultural characters |
| 1.2.2. Biochemical and physiological characters |
| 1.2.3. Identification using Biolog system |
| 2. Comparative studies between <i>X. vesicatoria</i> isolates |
| 2.1. Cultural characteristics |
| 2.2. Pathogenicity test |
| 2.3. Molecular diversity |
| 3. Susceptibility of some tomato cultivars to X. vesicatori |
| infection |

| 4. Activity of some enzymes in different tomato cultivars treated | |
|---|----|
| with X. vesicatoria | 44 |
| 4.1. Peroxidase (PO) enzyme activity | 44 |
| 4.2. Polyphenol oxidase (PPO) enzyme activity | 46 |
| 4.3. Phenylalanine ammonia lyase (PAL) enzyme activity | 46 |
| 5. Disease control | 48 |
| 5.1. In vitro | 48 |
| 5.1.1. Effect of bactericides on growth of <i>X. vesicatoria</i> | 48 |
| 5.1.2. Effect of bioagents on growth of <i>X. vesicatoria</i> | 50 |
| 5.1.3. Effect of resistance inducers on growth of X . | |
| vesicatoria | 50 |
| 5.2. In vivo (greenhouse experiments) | 52 |
| 5.2.1. Effect of bactericides on bacterial spot disease | 52 |
| 5.2.2. Effect of bioagents bacterial spot disease | 58 |
| 5.2.3. Effect of resistance inducers | 63 |
| 5.3. Under commercial greenhouse conditions | 67 |
| 5.3.1. Effect of bactericides on severity of tomato bacterial | |
| spot disease | 67 |
| 5.3.2. Effect of bioagents on severity of tomato bacterial spot | |
| disease | 68 |
| 5.3.3. Effect of inducers on severity of tomato bacterial spot | |
| disease | 70 |
| V. DISCUSSION | 72 |
| VI. SUMMARY | 77 |
| VII. REFERENCES | 82 |
| VIII. ARABIC SUMMARY | |

LIST OF TABLES

Page

| Table (1): | Number of tomato and pepper samples collected from naturally infected tomato plants from three governorates, during growing seasons 2007 – 2008. | 31 |
|------------|---|----|
| Table (2): | <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> isolates which isolated from diseased tomato samples which collected from three governorates, during growing season 2007 – 2008 | 33 |
| Table (3): | Morphological and cultural characters of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> isolates | 34 |
| Table (4): | Biochemical and physiological characters of isolated bacteria from infected tomato plants | 35 |
| Table (5): | Variation in pathogenicity for 10 isolates of X. vesicatoria on tomato plants (Castle Rock, cv.) under artificial inoculation conditions | 40 |
| Table (6): | Susceptibility of different tomato cultivars to infection with <i>X. vesicatoria</i> | 44 |
| Table (7): | Activity of peroxidase enzyme (PO) in five tomato cultivars inoculated with the pathogen <i>X. vesicatoria</i> | 46 |
| Table (8): | Activity of Polyphenol oxidase enzyme (PPO) in five tomato cultivars inoculated with the pathogen <i>X. vesicatoria</i> | 48 |
| Table (9): | Activity of Phenylalanine ammonia lyase enzyme (PAL) in five tomato cultivars inoculated with the pathogen <i>X. vesicatoria</i> | 50 |

| Table (10): | Effect of different bactericides on growth of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> as optical density and inhibition zone (mm), at different concentrations, <i>in vitro</i> | 52 |
|-------------|--|----|
| Table (11): | Effect of some bioagents on growth of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> (inhibition zone (mm) and percentage of efficiency), <i>in vitro</i> | 54 |
| Table (12): | Effect of different bioagents as foliar treatment on incidence of tomato bacterial spot disease, 24 hours before inoculation with the pathogen, at different concentrations, under artificial inoculation conditions | 55 |
| Table (13): | Effect of different bioagents as foliar treatment on incidence of tomato bacterial spot disease, 24 hours after inoculation with the pathogen, at different concentrations, under artificial inoculation conditions | 57 |
| Table (14): | Effect of different bioagents as foliar treatment on disease incidence of tomato bacterial spot disease, 24 hours before inoculation by the pathogen, at different concentrations, under artificial inoculation conditions | 59 |
| Table (15): | Effect of different bioagents as foliar treatment on incidence of tomato bacterial spot disease, 24 hours after inoculation by the pathogen, at different concentrations, under artificial | |
| | inoculation conditions | 61 |

VI

| Table (16): | Effect of different resistance inducers as foliar treatment on incidence of tomato bacterial spot | |
|-------------|---|----|
| | disease, 24 hours before inoculation by the | |
| | pathogen, at different concentrations, under | |
| | artificial inoculation conditions | 63 |
| Table (17): | Effect of different resistance inducers as foliar | |
| | treatment on incidence of tomato bacterial spot | |
| | disease, 24 hours after inoculation by the | |
| | pathogen, at different concentrations, under | |
| | artificial inoculation conditions | 65 |
| Table (18): | Effect of number of application for some | |
| | bactericides as foliar treatment on incidence of | |
| | tomato bacterial spot disease, under commercial | |
| | greenhouse conditions | 67 |
| Table (19): | Effect of number of applications of some | |
| | bioagents on incidence of tomato bacterial spot | |
| | disease under commercial greenhouse conditions | 69 |
| Table (20): | Effect of number of application times of some | |
| | resistance inducers on incidence of tomato | |
| | bacterial spot disease under commercial | |
| | greenhouse conditions | 70 |
| | | |

LIST OF FIGURES

| Fig. (1): | Naturally infected tomato plants with <i>X. vesicatoria</i> the causal of bacterial spot | 30 |
|-----------|--|----|
| Fig. (2): | Number of tomato and pepper samples which collected from different locations, through growing seasons 2007 – 2008. | 31 |
| Fig. (3): | Typical symptoms of bacterial spot disease on tomato plants as resulted in pathogenicity test | 32 |
| Fig. (4): | No. of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> isolates which isolated from tomato samples collected from different locations through growing seasons 2007-2008 | 33 |
| Fig. (5): | Colonies shape of different <i>X. vesicatoria</i> isolates on Tween B medium | 36 |
| Fig. (6): | Computer sheet of identifying isolate no. TX1 of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> using biolog system. | 37 |
| Fig. (7): | Computer sheet of identifying isolate no. TX5 of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> using biolog system. | 38 |
| Fig. (8): | Colonies shape of different <i>X. vesicatoria</i> isolates on Nutrient agar medium | 39 |
| Fig. (9): | RAPD-PCR profiles of A-01 primer for selected X. vesicatoria DNA | 41 |
| Fig. (10) | Phylogenic tree of ten selected <i>X. vesicatoria</i> DNA with genetic distance. | 42 |

| • • • | Determination of virulent isolates of <i>Xanthomonas</i> campestris pv. vesicatoria, using tomato plants under | |
|-------|--|----|
| | artificial inoculation conditions | 43 |
| | Susceptibility of some tomato cultivars to <i>X. vesicatoria</i> infection, under greenhouse conditions | 45 |
| | Activity of peroxidase enzyme as optical density (OD) for 5 tomato cultivars at different periods, following inoculation with <i>X. vesicatoria</i> | 47 |
| | Activity of Polyphenol oxidase enzyme as optical density (OD) for 5 tomato cultivars at different periods, following inoculation with <i>X. vesicatoria</i> | 49 |
| | Activity of Phenylalanine ammonia lyase enzyme as optical density (OD) for 5 tomato cultivars at different periods, following inoculation with <i>X. vesicatoria</i> | 51 |
| | Efficacy of some bactericides against growth of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> as optical density and inhibition zone (mm), at different concentrations, <i>in vitro</i> | 53 |
| | Efficacy of some bioagents against growth of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> isolate, <i>in vitro</i> | 54 |
| / | Efficacy of some bactericides on severity of tomato bacterial spot disease, 24 hours before the pathogen, at different concentrations, under greenhouse conditions | 56 |
| | Efficacy of some bactericides on severity of tomato bacterial spot disease, 24 hours after the pathogen, at different concentrations, under greenhouse conditions | 58 |
| | Efficacy of some bioagents on severity of tomato bacterial spot disease, as pre-treatment 24 hours before inoculation with the pathogen, at different concentrations, under | |