

Alexandria University  
Faculty of Agriculture



# **STUDIES ON SOME SEED BORNE BACTERIAL DISEASES OF SOME SOLANACEAOUS PLANTS**

**A thesis submitted in partial fulfillment of the requirements  
governing the award of the degree of**

**DOCTOR OF PHILOSOPHY IN AGRICULTURAL SCIENCES**

**Department of PLANT PATHOLOGY**

**From**

**Alexandria University**

**BY**

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**2016**

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## LIST OF ABBREVIATIONS

g	Gram
FW	Fresh weight
DW	Dry weight
hrs	Hours
<i>in vitro</i>	In a test tube or petri dish
<i>in vivo</i>	Within the living
FAA	Fatty acid analysis
FAMs	Fatty acid methyl esters
μl	Microliter
mg	Milligram
min	Minutes
μg	Micrgram
mm	Millimeter
ml	Milliliter
RH	Relative humidity
PCR	Polymerase chain reaction
Cfu	Colony forming unit
SA	Salicylic acid
FAO	Food and Agriculture Organization
SDW	Sterile distilled water
spp.	Species (plural)
μm	Micrometer(micron)
(w/v)	Weight per volume
<i>et al.</i>	and others

## 6. SUMMARY

The present study was carried out to investigate seed-borne diseases of Family Solanaceae. The results obtained in this study were summarized in the below points:

- 1-Fourteen isolates from tomato, eggplant, black nightshade and tobacco were obtained from Alexandria, Kalubia and Assiut Governorates in Egypt.
- 2-Artificial infection was carried out by isolated seed-borne bacteria. All the bacterial isolates were tested for their pathogenicity on tomato seedlings Alissa F1 and Gs Nada varieties. Inoculation with isolates of type I, stems appeared shrivel and wither, moreover, discolored water-conducting tissue and chlorosis was appeared on leaves. While, Inoculation with isolates of type II showed necrotic spots surrounded by a chlorotic halo appearing on leaves. In case of inoculation with type III bacterial isolates, leaves appeared water soaked lesions and became brown colour.
- 3- All isolates of type I were tested for their pathogenicity on tomato fruits (Alissa F1) and showed grey areas around the inoculation site. The observations from re-isolation cleared that isolates of type I was an endophytic pathogen, isolates of type II was an epiphytic pathogen and isolates of type III was an epiphytic and endophytic seed-borne bacteria.
- 4- Isolated bacteria were identified according to morphological, motility, Gram stain, and colonial morphology on differential medium [Yeast extract-dextrose-CaCo3 (YDC)] besides the selective media (PA 20, King's B and Tween B ) aspects as well as physiological and biochemical tests. Results indicated that isolated bacteria were suspected to belong to *Pantoea*, *Pseudomonas* and *Xanthomonas* spp.
- 5- Cellular fatty acids composition was investigated of *Pantoea* spp. (3 isolates), *Pseudomonas* spp. (2 isolates) and *Xanthomonas* spp. (2 isolates)
- 6- Partial DNA sequences were analyzed using BLAST tool revealed that the inferred 16S rRNA partial sequences of 7 isolates (registered using accession numbers) showed similarity to *Pantoea ananatis* (3 isolates), *Pseudomonas syringae* pv. *tomato* (2 isolates) and *Xanthomonas vesicatoria* (2 isolates).
- 7- Histopathological studies of cross sections of infected tomato seedlings tissues with *Pantoea* spp. were carried out compared with the control by light microscope and scanning electron microscope. Light microscope observations of infected tissues showed death of some cells in stem tissues and complete colonization of the vascular cylinder by the bacteria was happened and blackness compared with the control. Scanning electron microscope examination of the vascular cylinder of a healthy plant as a control showed normal tissues compared with tomato plant infected by *Pantoea* spp. which revealed the presence of a large number of bacteria, where they fill the vascular cylinder cells and arranged in cell aggregation phase.

**8-Treatment of tomato seedlings (Alissa F1) with the selected bacterial isolates on the endogenous salicylic acid (SA) content in inoculated plants was summarized as follow:**

- One week after pathogen inoculation, SA content was significantly increased in inoculated plants compared with control.
- Five weeks after pathogen inoculation, the highest SA level was obtained from control plants followed by the treatment with *P. ananatis* (P1).

**9-Artificial infection test on tomato seedlings were carried out in greenhouse using *P. ananatis*, *P. syringae* pv. *tomato* and *X. vesicatoria* isolates causes graywall, bacterial speck and bacterial spot respectively of tomato (Alissa F1). Results indicated that the untreated tomato seedlings was the highest significant increase in Fresh weight (FW) and dry weight (DW) of whole plant compared with all tested isolates.**

**10-Evaluation of antibiotics *in vitro* on the growth of seed-borne bacterial pathogens at (10µg/mL) indicated that:**

- The best of main effect of antibiotic on the growth of *Pantoea* spp. was Imipenem (IPM) followed by Norfloxacin (NOR) then Pencillin (P) and Gentamycin (GN) compared with the control treatment. No significant differences were showed between the tested bacterial isolates.
- The best of main effect of antibiotics were NOR and IPM followed by P and GN compared with the control treatment. The main effect of bacterial isolates showed that *P. syringe* pv. *tomato* (Ps2) was more sensitive for the tested antibiotics than Ps1 isolate.
- The best of main effect of antibiotics on *X. vesicatoria* were GN and NOR followed by P and IPM. The main effect of bacterial isolates showed significant difference between tested isolates for the previous tested antibiotics.

**11-Evaluation of copper bactericides on the growth of seed-born bacterial pathogens at different concentrations was summarized as follow:**

- The best main effect was Index followed K-mall and Copral which revealed equal significance of tested *P. ananatis* isolates.
- The best main effect was Index followed K-mall and copral which revealed equal significance of *Ps. syringe* pv. *tomato* isolates.
- The best main effect was Index followed by Copral then K-mall which showed a significant difference in their effect of *X. vesicatoria* isolates.

**12-Evaluation of essential oils on the growth of seed-borne bacterial pathogens revealed that:**

- Garlic oil, Caraway oil, Cinnamon oil and Cypress oil followed by Jambul oil revealed that the best main effect in their inhibition of *P.ananatis* growth.
- Cypress oil followed by Jambul oil and Caraway oil showed the best main effect in their inhibition of *Ps. syringae* pv. *tomato* growth.
- Jambul oil followed by Cypress oil showed the best main effect significantly in their inhibition of *X. vesicatoria* growth, while no significant differences between tested isolates in their affected by previous oils.

- 13- Chemical composition of the tested essential oils was carried out by using gas chromatography mass spectrometry (GC-MS).
- 14- Synergistic/antagonistic effect towards combination between NOR, Index and Cypress oil, by using agar diffusion method, was studied.
- 15- Detection of bacteriostatic/bactericidal aspect of tested pathogens after their exposure to antibacterial agents (antibiotics, copper bactericides and essential oils) was determined.
- 16- About the management of greywall disease, pretreatment of tomato seedlings with the tested antibacterial agents alone and in their combinations before inoculation with *P. ananatis* (P1) was performed. Results indicated that:
  - The best treatment in fresh weight (FW) of whole plant was Norfloxacin followed by Index, Cypress oil and Norfloxacin-Cypress oil treatments and there were significant differences between Norfloxacin and the last three treatments.
  - The best treatment in dry weight (DW) of whole plant was Norfloxacin followed by Cypress oil, Index, Norfloxacin-Cypress oil, *P. ananatis*-Norfloxacin and negative control and there were significant differences between Norfloxacin and the last five treatments. The lower treatment in FW and DW of whole plant had a positive control *P. ananatis*.
- 17- Results obtained by Transmission Electron Microscope showed that NOR and Cypress oil caused degradation of the cell wall of *P. ananatis* (P1), which lead to the bacterial cells were apparent irregular in shape and alterations in the cytoplasm density, known as electron-dense material loss subsequently, lead to cytoplasmic vacuolation. Bacterial cells exposed to Index showed the same previous changes, furthermore copper accumulation was happened in bacterial cells.