SENSITIVITY OF DIFFERENT DETECTION METHODS OF *Ralstonia solanacearum* IN POTATO TUBERS AND INFECTED SOURCES

By

EMAN ELEIWA ALI

B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Cairo Univ., 2005

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APPROVAL SHEET

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APPROVAL COMMITTEE

Dr. MOHAMED REDA AHMED TOHAMY.....
Professor of Plant Pathology, Fac. Agric., Zagazig University

Dr. NOUR ELDIN KAMEL SOLIMAN .....
Professor of Plant Pathology, Fac. Agric., Cairo University

Dr. MAURICE SABRY MIKHAIL .....
Professor of Plant Pathology, Fac. Agric., Cairo University

Date: 4 / 5 / 2017

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Prof. Dr. MAURICE SABRY MIKHAIL
Professor of Plant Pathology, Plant Pathology Dept., Fac. Agric., Cairo Univ.

Dr. AHMED ISMAIL ABDEL-ALIM
Lecturer of Plant Pathology, Plant Pathology Dept., Fac. Agric., Cairo Univ.

Dr. FAIZA GEBRIEL FAWZI
Head Researcher, Plant Pathology Res. Inst., Agric. Res. Centre, Giza

Name of Candidate: Eman Eleiwa Ali Eleiwa **Degree:** M. Sc.
Title of Thesis: Sensitivity of different detection methods of *Ralstonia solanacearum* in potato tubers and infected sources
Supervisors: Dr. Maurice Sabry Mikhail
Dr. Ahmed Ismail Abdel-Alim
Dr. Faiza Gebriel Fawzi
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ABSTRACT

The efficiency of culturing *Ralstonia solanacearum* on Semi Selective Modified South Africa medium (SMSA), inoculation in SMSA broth (SMSB) enrichment, immunofluorescent antibody staining (IFAS) using polyclonal antibodies, tomato bioassay and conventional polymerase chain reaction (PCR) methods were evaluated for their sensitivity in routine detection of the brown rot bacterium, *Ralstonia solanacearum*, in potato piece. The experiments were performed using potato extracts of two cultivars; Spunta and Cara, also using two types of soil; sandy and clay. Potato extracts were prepared from 200 tubers according to the EPPO procedure. The sensitivity of the different detection methods was compared by adding bacterial suspensions ranging from 10^8 to 10^2 CFU per ml to potato extracts prepared from two cultivars. Also, inoculated two types of soil with bacterial suspensions ranging from 10^8 to 10^2 CFU per ml were used. The results showed that 3.6×10^2 and 3.6×10^3 CFU per ml were the lowest bacterial number detected by isolation on SMSA medium using artificially inoculated potato extracts of cv. Cara and Spunta, respectively. In soil the limit of the detection was 3.5×10^2 CFU per g soil in both types of the soil. In comparison, a tomato bioassay has shown to detect bacterial populations number between 3.6×10^4 and 3.6×10^5 CFU per ml in potato extracts of cv. Cara and Spunta respectively, and the same limit of bacterial detection 3.5×10^4 and 3.5×10^5 CFU per g soil was observed in sandy and clay soil samples, respectively. The detection threshold using IFAS test was 3.6×10^4CFU per ml in potato extracts of two cultivars whereas the sensitivity detection threshold decreased to 3.5×10^6 CFU per g of both soils. Using conventional PCR method, the detection limit of the pathogen in inoculated potato extracts were 3.6×10^4CFU per ml in both cultivars before enrichment, while overnight enrichment in SMSB broth the limit of sensitivity detection did not changed in cv. Spunta while deceased this limit to 3.6×10^5CFU per ml in cv. Cara. In soil, the limit of the detection was decreased to 3.5×10^5 and 3.5×10^6 CFU per g soil of sandy and clay soils respectively before enrichment, and after enrichment did not enhanced the detection ability. Generally, IFSA test was the best detection methods in screening of potato seeds although that, its sensitivity is lower than culturing on SMSA medium. But, it is faster, robust, cheap, screening large numbers of samples and reasonably specific method. In soil, use of modified semi-selective medium is the best in detecting *R. solanacearum* compared to those of other methods.

Key words: *Ralstonia solanacearum*, soil, potato, detection, selective medium, PCR, IFAS.

Dedication

First I would like to express my deepest thanks to Allah for continuous giving and help me to finish this work. Second I dedicate this work to whom my heart felt thanks; to my husband and my daughters Maryam and Sara for their patience and help, as well as to my parents and sisters for all the support they lovely offered along the period of my post graduation.

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