APPROVAL SHEET

ENVIRONMENTAL AND MOLECULAR STUDIES ON THE BACTERIUM *Ralstonia solanacearum* AND ITS CONTROL IN EGYPT

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LIST OF APPREVIATIONS

t	**lot(s)**
ha	**Hectar**
PBRP	**Potato Brown Rot Project.**
PCR	**Polymerase Chain Reaction.**
EDTA	**Ethylene diamine tetra acetic acid.**
FITC	**Fluorescin isothiocyanate**
O/F	**Oxidation/fermentation.**

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ABSTRACT

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In this study, a survey on potato brown rot bacterium was carried out during the growing season 2003- 2004 in four Governorates i.e: Gharbia, Menofia, Kalubia and Ismailia. The survey was carried out on potato tubers, soils, water and some weed plants in potato fields. The survey revealed that the high percentage of potato brown rot bacterium was recorded in Menofia followed by Gharbia and Kalubia while, the lowest one was in Ismailia. Also the highest percentage of the bacterium was recorded during March and April followed by November + December and May + Jun while the lowest one was in July + August. On the other hand, the highest percentage of the concerned bacterium in soil was recorded in Menofia while the lowest one was in Ismailia. Also, the Survey revealed that, the highest percentage of the bacterium was on March + April followed by Jan. + Feb. While the lowest one was in July + Aug. Survey of the bacterium in irrigation surface and drainage water confirmed that, the highest percentage was in Menofia followed by Kalubia and Gharbia while the water in Ismailia was free from the bacterium. Also, the survey revealed that, the highest percentage of the bacterium in water was recorded on Mar + Apr while the water was free from the bacterium and/ or found in level below the detection limit of modified (SMSA) medium used for isolation. Also, survey of *R. solanacearum* in six weed plants grown in potato fields and on borders of irrigation canals confirmed that the highest percentage was in *Chenopodium album* followed by *Amaranthus viridis* and *A. sylvestris* while the lowest one was in *Cyperus deformans*. The rest surveyed plants were free from the bacterium. The highest percentage of the bacterium was in Jan. + Feb. while the lowest one was in July + Aug.

ABSTRACT

Approximately, out of 298 bacterial isolates recovered from different sources, only ten isolates were the most pathogenic ones. The most pathogenic isolates varied in their pathogenic potentialities on tomato (Peto 86) and potato (CV. Nicola) according to sources of isolation. The isolate Rs_3 isolated from potato tuber was the most virulent as compared to others, while Rs_7 isolated from soil was the least ones.

Identification of the most virulent isolates according to Bergey's Manual of Systematic Bacteriology showed that the tested isolates have bacteriological characteristics similar to those described as *R. Solanacearum* race (3) bv (2) and no variation could be noticed between the isolates in morphological, cultural and physiological characteristics. Also, the advanced detection methods PCR, Box- PCR and Fatty acids profiling confirmed that, no variations between the tested isolates could be detected using references bacteria (standard known *R. solanacearum).*

Pathogenicity of the tested bacterial isolates on the indicator plants proved that all isolates were able to infect potato and tomato plants causing bacterial wilt symptoms but they were unable to infect pepper, eggplants, tobacco and banana plants. Pathogenicity test plus the ability of the tested isolates to utilize some disaccharides and hexose alcohols confirmed that those isolates belong to *R. solanacearum* race (3) bv (2).

Physical and chemical properties of soil samples collected from Kalubia and Ismailia governorates were analyzed. Soil samples from Kalubia were classified as clay with high content of organic matter, phosphorus, potassium, Iron, manganese and cupper while Ismailia soil samples were classified as loamy sand soil with low content of organic mater and high content of nitrogen and zinc. Also, Ismailia soil showed greater content of anions and cations compared to Kalubia one.

Effect of environmental factors such as soil type, temperature and water holding capacity on survival of *R. solanacearum* in soil, water and compost was studied. Long – term survival time was recorded in sandy soil compared to clay soil and at 15°C compared to 30°C. Meantime, long – term survival time of the bacterium was recorded in the two soil types under 80% water holding capacity compared to 40%. Effect of

water type, temperatures and sterilization of water on survival of *R. solanacearum* under artificial inoculation conditions was studied. Long – term bacterial survival was recorded in sterilized water compared to natural water and in distilled and irrigation, water compared to others. Meantime, long – term bacterial survival was recorded at 15°C compared to 25 and 30°C. New record experiment in Egypt was made to study the efficiency of aerobic composting for eradication of *R. solanacearum* and on population densities of the concerned bacterium and the indigenous bacteria, fungi and actinomycetes activities. Population density of *R. solanacearum* was rapidly decreased and extinct after 10, 20 and 30 days from the beginning of composting at depths 50, 30 and 10 cm. Population of microbial count was decreased with increasing time until 30 days from the beginning of composting where temperatures were increased with increasing time until 30 days (69.2°C), then the population of microbial count was increased again after 40 days from the beginning of composting until maturation.

Population of *R. solanacearum* in rhizosphere of potato plants was reduced with application of compost and with increasing the period after planting compared to population of microbial counts. Meantime, disease severity of potato bacterial wilt on potato plants was decreased and the onset of the disease in the infected plants was retarded until 75 days after planting with application of compost (5% w/w).

Microorganisms (bacteria and actionmycetes) were isolated from the rhizosphere of healthy potato plants and identified then tested against the pathogenic bacteria In vitro and in pots outside the greenhouse. Only two bacterial isolates and four actinomycetes isolates showed the highest antagonistic effect and reduction in growth of *R. solanacearum* In vitro.

Application of bioagents to soil separately or in combination with compost led to reduce the severity of potato bacterial wilt disease. Meantime, application of bioagents as soil treatments was more effective than tuber treatments in reducing disease severity.

Application of *streptomyces antibioticus* was the most effective in reducing potato bacterial wilt disease compared to others.

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