STUDIES ON BACTERIAL SOFT ROT

By

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THESIS

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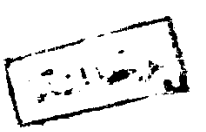

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A C K N O W L E D G E M E N T S

The author wishes to express his sincere thanks to Professor S.H. Elwan, Head of the Botany Department, Faculty of Science, Al-Azhar University for supervision and encouraging support, his continuous discussions contributed a great deal in the presentation of this thesis in its final form. The writer is also deeply grateful to Dr. A.I. Naguib, Professor of Microbiology, National Research Centre for suggesting the problem, supervision of the work. I appreciate very much their combined efforts in rounding up the problem and their endeavour to direct the writing and revise the typescript of this thesis. Thanks are also due to Dr. Y.A. Youssef, Assistant Professor of Microbiology for his kind encouragement.

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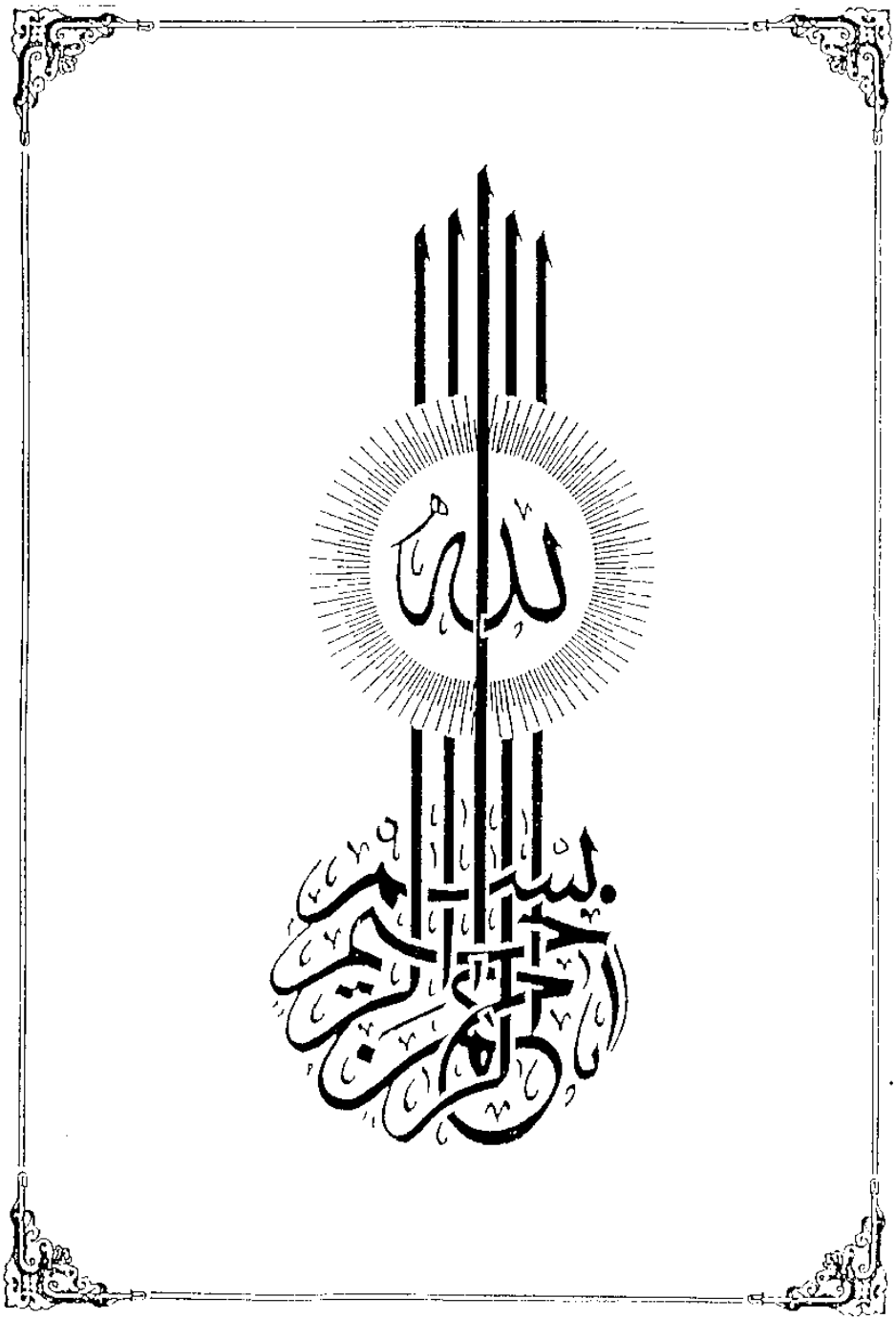
Finally, I am grateful to the authorities of the Desert Institute for all help offered during the work and to "The Agricultural Cooperative Society of Potato Growers" Cairo, A.R.E. for supplying the certified potato cultivars used in this work.



This Thesis has not been previously submitted to this or any other University. The references mentioned show how far I have availed myself to the work of other researchers.

M. R. Rabie.

C O N T E N T S

P A R T O N E

- General Preface
- Historical Introduction
- Scope of Work
- Material and Methods

GENERAL PREFACE

If pitfalls are to be avoided in soil reclamation programmes, fundamentals based on well-conducted research work should be at hand before execution. Precise data of the chemical, physical and biological characteristics of representative soil samples are indispensable. With regard to biological characteristics, biotic eco-pedological work is inevitable being introductory for studying physiological aspects. Since development and protection of living entities are the items of life relation in a reclaimed area, studies are to be carried out on growth affinities and pathogenic correlations.

In a previous investigation, eco-pedological work was made dealing with the occurrence of potentially-pathogenic pectolytic bacteria in extension areas of the Western Desert, Egypt (Rabie, 1969). Without physiological evaluation of the parasitic significance of isolates of this work on particular hosts, the importance of those eco-pedological studies remains obscure. Therefore, it was planned to study the physiology of parasitism of the most potent strain of fifty pectolytic isolates, causing rot of potato cultivars introduced in Egypt partly for the sake of export.

Admittedly the work was planned to be *in vitro* since *in vivo* work would need tackling of team nature and facilities without considering time as limiting factor. However, *in vitro* work is definitely required to give knowledge which would be more precise in model system conducted in this thesis.

The major aim of the present work is to find out interpretable grades of resistance of individual cultivars under conditions presumably prepondrating in the dynamic environment of the natural habitats. This would - it is hoped - be of important value as regards the indispensable fundamentals required before soil reclamation pregrammes are executed.

HISTORICAL INTRODUCTION

The basic function of Scinece is to serve Society, with the ideal that each generation might progress in terms of standard of living and opportunities for intellectual development.

Plant diseases have indeed played an important role in the social development of the world. Throughout the ages, mankind has learnt to exist with those plant diseases as he failed to eradicate completely any one of them. Efforts have been continuously made for controlling plant diseases, so that mankind no longer lives at their mercy. In order to achieve this goal, one has to study all factors affecting the host-pathogen relationships.

In point of fact, the host-parasite relationships are so complex that one cannot get precise knowledge of this nature without evaluating the role of the factors involved in this complexity. The factors involved are so numerous particularly when taking into consideration the ever-changing environmental, edaphic and biotic factors in the natural habitats. The dynamicity of the factors therein minimizes the precision of the fundamentals concerned. It follows that model systems should be planned in an endeavour to spotlight the role of particular factors. In this sense, the work presented in this thesis is limited

to thorough investigation of certain factors, the indispensability of which in understanding the nature of the host-parasite relationship recieves a general agreement among investigators. These factors are : the host, the parasite and the enzyme(s) concerned as affected by the environment, the most important features of which are the temperature and humidity. Accordingly, in the following historical introduction an attempt is made to review the available literature concerned with the host-parasite relationships within the scope of this investigation. This will be in the following sequence :

- Identity of the host and the parasite.
- Resistance and susceptibility of the host.
  1. Resistance due to the host variations.
  2. Resistance due to inhibitory substances.
  3. Resistance due to host and pathogen metabolism.
  4. Resistance in relation to temperature.
  5. Resistance in relation to moisture.
  6. Relation of resistance to the size and site of infection.
  7. Relation of oxygen to resistance.

- Chemical analysis of the potato tubers.
- Studies on pathogenicity.
- Factors affecting the growth of organisms, production and activity of their pectic enzymes.
  1. pH.
  2. Carbon / nitrogen ratio and nature of both sources.
  3. Nutritional requirements.
  4. Temperature.
  5. Inoculum potential.
  6. Mutation and adaptability.
- Pectic enzymes and substrates in hydrolysis of pectic substances.
- Biosynthesis of pectolytic enzymes.
- Pectic enzymes in pathogenesis.

Identity of the Host and the Parasite

In the process of pectolysis "exerting pectolytic activity", infectivity is of the parenchymatous type. Such type of infectivity manifests itself symptomatically in the form of soft rot. With regard to the bacterial parasites involved in this type of rot, both Gram-positive and Gram-negative, spore-forming and non-spore forming, facultative and obligate forms have been reported to be the causal organisms (e.g. Burkey 1928, Sabet and Dowson 1951; Kerr 1953, Paton, 1958, Graham, 1958, Dorey, 1959, Naguib, Elwan and Rabie, 1971). Although the genera Aerobacter, Bacillus, Clostridium, Pseudomonas, Xanthomonas Corynebacterium and Flavobacterium were reported to exert pectolytic activity, yet, the genus Bacillus was the most common among the bacterial genera so far reported in this respect (e.g. Burkey, 1928, Kerr, 1953, Naguib, Elwan and Rabie, 1971). The latter authors reported that 50 pectolytic strains out of 924 isolated from 31 desert soil samples from different localties belonged to the genus Bacillus with species identities related to B. subtilis B. cereus, B. licheniformis, B. megaterium and B. coagulans. In screening experiments, the author selected the most potent strain named B. subtilis, I-E which will be the organism used in all the work presented hereafter.

Resistance and Susceptibility of the Host

The most important point concerning the host in pathogenicity is resistance and susceptibility due to the different factors. Resistance and susceptibility of the host to be invaded by pathogens are expressed in many ways.

1. Resistance due to the host variations :

Brierly (1928) found that the potato varieties, Rusal, New York and Russet Rusal, followed by Irish Cobbler were most susceptible to all types of rot (B. mesentericus, B. phytophthorus, B. carotovorum and B. aroideae). McCormick was most resistant. Rose was resistant to all but black-leg rot. Five other varieties are placed between these extremes.

Müller and Behr (1949) found that the resistance gene "R" which determines the behaviour of the solanaceous species towards the Phytophthra fungus, had several associated alleles and was inherited independently of other economically-important characters, such as "time of maturity" and "yield capacity".

Garber, Shaeffer and Goldman (1956) reported that three types of host response were observed when slices of the fleshy storage organs of varieties of radish and turnip

were inoculated with mutants of Erwinia aroideae. These types of host response were ; uniformly resistant, uniformly susceptible and variable responses.

Walker (1959) noted that host resistance may be inherited as a quantitative or as a qualitative character and its expression may be expected to be influenced by environment. He also noted that varieties of potato, resistant and susceptible, have been known for a long time. Invasion of all varieties does occur, but in case of those which are highly resistant, the pathogen fails to induce any symptoms and no resting spores or prosori are produced.

Barnett (1959) reported that the resistance of a plant to infection by micro-organisms is based upon the genetic composition of the host and expression of the resistance gene may be influenced by numerous factors. The principal factors are: i) aggressiveness of the parasite, ii) presence or absence and availability of specific nutrients at the infection site; iii) presence or absence of specific inhibitory substances, iv) metabolism of the host including the activity of numerous enzyme systems v) host nutrition especially the carbon-nitrogen ratio and vi) environment.