

**RHIZOSPHERE MICROFLORA IN RELATION
TO SOME ROOT DISEASES**

By

SAID IBRAHIM AMIN EL-SAID

B. Sc. Agric. Cairo University, 1964

M. Sc. Plant Pathology Al-Azhar University 1968

THESIS

Submitted in Partial Fulfilment of the Requirements for the Degree

Of

DOCTOR OF PHILOSOPHY

in

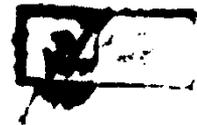
AGRICULTURAL MICROBIOLOGY

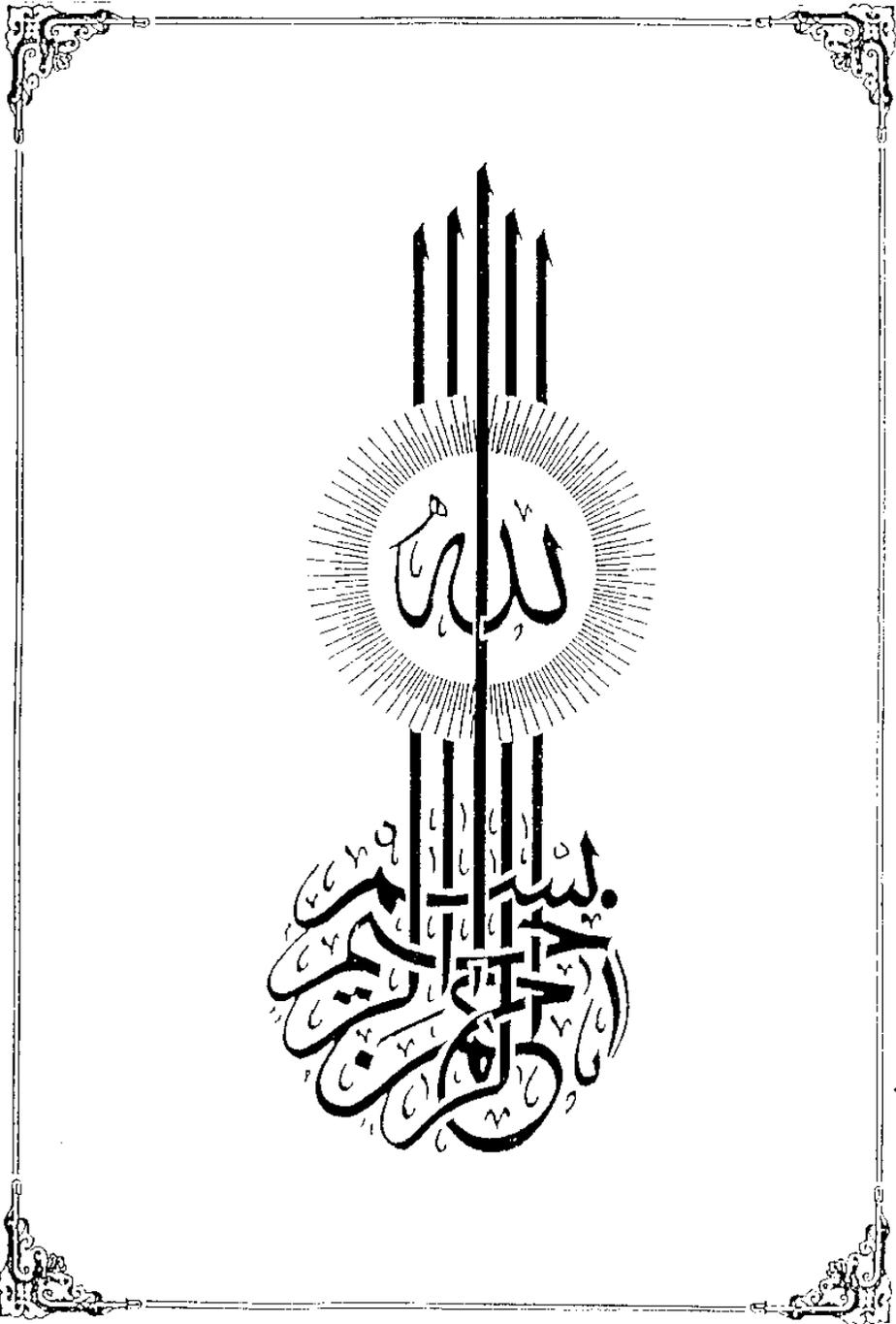
Department of Microbiology

Faculty of Agriculture

Ain Shams University

1976





ACKNOWLEDGEMENT

The author wishes to express his deepest gratitude to Prof. Dr. S.A.Z. Mahmoud, Vice Dean of Faculty of Agriculture, Ain Shams University, Prof. Dr. S. Taha Exhead of Agriculture Microbiology Fac. of Agric. Ain Shams University, and Dr. A.S. Hamed, Associate Professor, National Research Centre, for suggesting the problem, supervision, advice, and encouragement throughout the work.

He wishes also to express his sincere thanks to Dr. A.M. Abd-El-Hafez, Associate professor the same Department M.D.H. Aly associate professor, National Research Centre, and M. Zaki, associate professors in the same Department Ain Shams University, for their valuable suggestion and sincere help, advice offered especially during the preparation of the manuscript.

The author express his gratitude and appreciation to Prof. Dr. A.M.M. Aly and Dr. A.A. Morsy, associate professors in the National Research Centre and all members of Plant Pathology Unit, Genetic and cytology Unit for kindly help and for supplying all facilities during this investigation.

The writer wishes acknowledge to Dr. I.M. Mahmoud for his kindly help offered in designing a programme for statistical analysis by ICL computer.

	Page
C. Antagonism between rhizospheric microflora of two cotton varieties and cotton wilt.. ..	124
VI- Effect of organic manuring and inoculation with <i>Bocillus subtilis</i> , on the severity of root rot in field bean and squash plants	126
VII- The nature of root exudates of susceptible and resistant cotton varieties.. .. .	129
1- Amino acids	129
2- Sugars	132
3- total free phenols	134
4- Organic acids	136
5- Growth of <i>Fusarium Oxysporum</i> f. <u>vasinfectum</u> on root-exudates.	137
SUMMARY	140
LITERATURE CITED	152

INTRODUCTION

=====

When plants emerged from warm primival seas and invaded the land in the Devonian period, about 300 million years ago, they were undoubtedly accompanied by bacteria and fungi which had been parasitizing them for a million of years. As roots evolved they were invaded in turn. Certainly, fossil roots of the Carboniferous show extensive invasion by fungi. Root-rot and wilt are the most serious diseases invaded root system of many field crops causing a dangerous losses to our crops consequently decreases the national income. Thus, parasitic fungi have been part of the environment of roots for at least as long as the soil itself. It is, therefore, to be expected that the interactions between the parasite, root, and soil, and between parasitic and saprophytic microorganisms, have become extremely complex. Those organisms which did not adjust to this competitive state, by one or another, did not survive. A state of fluctuating biological balance thus developed for each native habitat, and was self adjusting for the relatively slow evolutionary and climatic changes. (Baker and Snyder, 1965).

With intensification of agriculture, losses from root diseases have increased as the buffering effect of biological controls diminished. When biological control

is temporarily or permanently inhibited severe outbreaks of root disease occur. Indeed, biological control of root pathogens must be generally and effectively operative for there to be as little root disease as there is.

Although it is generally recognized that interactions of microorganisms constitute an important limiting factor to survival of disease organisms in soil, widespread utilization of such biological control awaits greater understanding of the processes involved. Biological control, clearly imply control of a disease through some biological agency; the term biological agency are bound to mean a living microorganism or macroorganism other than the diseased or damaged plant acting as host and the pathogen causing disease or damage. Inoculation of soil or plant tissues with antagonistic microorganisms is one method of biological control.

The aim of this study is to reach to the biological control of some root diseases which causes series losses to our crops. The relation between the addition of phytopathogenic fungi, i.e., root rot and wilt and the rhizospheric microflora was investigated. One of the major factors determining the behaviour of plant pathogens is the availability of nutrients, and it is in this respect

that the exudation of organic compounds from roots playing an important role in the problem of biological control of root diseases (Rovira, 1965).

REVIEW OF LITERATURE
=====

General consideration of the rhizosphere

Hiltner (1904) introduced the term rhizosphere to designate that portion of the soil which is subject to the influence of the plant root system and noted that this soil supported greater microbial activity than soil more distant from the roots.

Starkey work in (1929 a,b,c, 1931, 1932 and 1938) indicated the influence of higher plants on the rhizosphere microflora. He stated that among the various modifications of the soil which the plants might provoke are the lowering the concentration of certain nutrients in the soil due to their adsorption, partial desiccation of the soil by adsorption of water, increase in soil carbonates following root excretion of carbon dioxide and contribution of microbial food sloughed off root portions or excretions.

Loehwing (1937) reviewed the relation between plants and their roots including carbon dioxide evolution, adsorption of minerals and root excretions in relation to their influence on the rhizosphere.

Garret (1944) and Wakeman (1945) viewed the increase of microorganisms density in the root zone. It was expected that associative and antagonistic relationships would be particularly pronounced resulting in favourable or unfavourable effects on the plant.

One of the most important consideration in the study of the microorganisms of the rhizosphere was concerned with the extend of the root influence. Starkey (1929) noted that alfalfa roots slightly stimulated filamentous fungi only, while eggplant exerted an appreciable effect. The latter plant caused the increase in actinomycetes. He added that, in general most of the plants did not exert a great effect at the early stage of growth, and the maximum effect generally appeared after the plant had attained appreciable size. After death of the plant a rapid decline in numbers of bacteria.

Starkey (1931) reported much greater numbers of organisms in the rhizosphere, especially on the immediate plant root, and a marked decline with distance from it. Bacterial population was affected to a much greater extent than actinomycetes and filamentous fungi.

Starkey (1932) comparing rhizosphere counts of various plants grown under identical conditions, found

that they were fairly numerous. Counts, however, were not always comparable, since they were usually obtained from plants of the same age but not at the same stage of development. In so far as the major groups of soil microorganisms were considered, different effects occurred, which were also influenced by stages of growth.

Thom and Hunfeld (1952) found that bacteria were stimulated on roots of alfalfa, rye and vetch more than fungi which were greater more than actinomycetes. In the same soil they found that alfalfa stimulated bacteria more than rye. In another soil, rye was more effective than vetch. Fungi and actinomycetes were stimulated with an equal degree by alfalfa and rye in the first soil, but rhizosphere effect on actinomycetes occurred only in the second soil.

Krassilnikov et al. (1933) noted that the rhizosphere of cultivated peanuts harboured from 10-100 times as many organisms as soil apart from the root. This condition persisted until the flowering stage. Towards maturity, numbers of bacteria diminished and those of fungi and actinomycetes began to increase.

Manzon (1936) in a study on variety of leguminous and non-leguminous plants, concluded that numbers and

activity of bacterial population in the rhizosphere varied greatly, depending on both the species of the plant and on the phase of its development. He added that highest bacterial numbers were obtained with legumes.

Adati (1939) studied the densities of bacteria, actinomycetes and fungi in the rhizospheres of different crops growing in sand, loam, clay and humus soils. He indicated that much higher numbers of all organisms were found in the rhizosphere directly adjacent to the root, decreasing with the distance from it. In general, numbers of microorganisms were highest on legume roots, and a ratio of 197 for bacteria being obtained with peas.

Lochhead (1940) studied the rhizosphere of different crop varieties, and reported that bacteria were stimulated to the greatest extent, fungi usually next and actinomycetes least.

Timonin (1940) comparing the microbial populations of the rhizosphere of seedlings of wheat, oats, alfalfa, and peas, found that bacteria and actinomycetes (together) were 7 to 71 times and fungi were 0.75-3.1 times as great in number in the rhizosphere as in soil. The latter group of organisms was somewhat more abundant in the rhizosphere of oats and barley and the former in that of

alfalfa. In general, an increased rhizosphere effect was observed with advancing age of the seedlings.

Katznelson (1946) reported distinct effects of plant age on bacteria, fungi, actinomycetes, algae and protozoa and non-specific groups of organisms.

Katznelson et al (1948) showed that the influence of root on soil microflora might be expressed by the rhizosphere: soil ratio (R/S), that is the number in the soil at a distance from root. Katznelson (1964) and Katznelson et al. (1948) reported R/S ratio is used for evaluating the influence of soil type, treatments and other factors affecting the root surface microflora.

Clark (1941) suggested that rhizoplane might be used in studying rhizosphere phenomenon. Since it might be a sensitive index of specific qualitative effect of root on soil microorganisms.

Root disease in relation to rhizosphere microflora was also investigated by many workers. Early studies of Lochhead (1940) and Timonin (1940) Lochhead et al. (1940) the relation between the root surface microflora and the resistance or susceptibility to infection with root inhabiting fungi.

Katznelson (1965) suggested for purpose of biological control of soil-borne plant pathogens, an alteration in

the rhizosphere population qualitatively and quantitatively so as to render the root zone inimical to the pathogen. He also added that such changes could be effected by manipulating environmental factors (light, temperature and moisture) and this is obviously difficult under field conditions.

Rhizospheric microflora were affected greatly with the environmental conditions (temperature, moisture, etc.)

Timonin, 1940, Lochhead, 1940, Katznelson et al., 1948, Clark 1948, Lochhead, 1958, Venkateson, 1962, and Rouatt et al. 1963. They studied wheat and soy bean rhizospheric bacteria when grown at three ranges of temperature, i.e., 12.8-15.6°C, 21-23.9°C and 29.4-32.2°C. They found that number of bacteria in the rhizosphere of wheat increased as the temperature of soil decreased. On the other hand, numbers of bacteria in the root free soil on soy bean roots increased with the increasing of soil temperature.

Rhizospheric microflora in relation to plant pathogens

Mitchell (1963) and Mitchell and Alexander (1963) reported that the lysis of several soil borne fungi was associated with chitinase and laminarinase activity of Bacillus cereus, but some factors in addition to these