INTERACTION BETWEEN ROOT EXCRETIONS AND RHIZOSPHERE MICROFLORA OF SOME ECONOMICAL PLANTS

By

Tahia Abdul-Aziz Ismaeil Khater

B. Sc. (1960), Cairo Univ.

M. Sc. (1967), Ain Shams Univ.

THESIS

Submitted in Partial Fulfilment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in

MICROBIOLOGY

Agric. Microbiology Dept.

Faculty of Agric. Ain Shams Univ.

1979

DEDICATED TO

THE MEMOBRY OF;

MY FATHER, AND

MY MOTHER

بسم الله الرحمن الرحيم

APPROVAL SHEET

Title of Thesis: Interaction between root excretions and rhizosphere microflora of some economical plants.

Name: Tahia Abdul-Aziz Ismaeil Khater

Approved by:

(Committee in Charge)

Date: / /1979

ACKNOWLEDGMENT

This work was carried out under the supervision of Prof. Dr. S.A.Z. Mahmoud, Professor of Agric. Microbiology, and Dean of the Fac. of Agric. Ain Shams Univ.; Dr. A. Y.T. Hazem, Associate Prof. of Agric. Microbiology; and Dr. E.M. Ramadan, Lecturer of Agric. Microbiology.

The author wishes to express her deepest gratitude to them for suggesting the problem keeping interest and progressive criticism throughout the whole work.

Gratitudes are also to Dr. A. M. Abdel-Hafez Prof. of Agric. Microbiology, Fac. Agric., Ain Shams Univ.; and Dr. A. A. Assey, Associate Prof. of Agronomy Fac. Agric. Zagazig Univ. for help and encouragement.

The writer wishes to thank Prof. Dr. M. B. Moustafa, Dean of Fac. of Agric. Zagazig Univ.; and all members of Botany Dept., Fac. Agric. Zagazig Univ.; for facilities and encouragement during this study especially lab. work.

CONTENTS

1. INTRODUCTION

Since Hiltner (1904) introduced the term rhizosphere numerous publications appeared on the relation between plant root and microflora. According to the accumulated literature, it is now established that there are many factors which influence the numbers and types of organisms colonizing the rhizosphere. The rhizosphere microflora in relation to higher plants was demonstrated. It was also observed the root region differ qualitatively and quantitatively in biological activities as well as chemical characteristics. Many microorganisms are preferentially stimulated in the rhizosphere, others are inhibited while others are not clearly affected. The root debris and root exudates of the growing plant supply the microflora with stream of nutrients that influence the growing plant. The microbial synthesis of organic substances in the rhizosphere has mixed blessing; many of the rhizosphere organisms are able to synthesize special organic substances which in very small amounts stimulate growth but, in higher amounts are inhibitors. Both association and antagonistic forces may be operative.

Microorganisms of the rhizosphere are considered important owing to their function since they were found to be more active than soil organisms in physiological characters. It is fully established that the rhizosphere microflora play an important part in the growth of plants by supplying them with the yproducts of the mineralization of organic matter and also with the plant growth regulators which have profound effect on plant growth. Many workers have shown that microflora of the rhizosphere differ both quantitatively and qualitatively from those in the soil beyond the influence of the root.

This study was carried out in order to investigate the interaction effect between soil microorganisms and the roots of common bean and tomato plants. This work was undertaken to cover these two major points:

a. Microbial studies of soil and rhizosphere including total microbial flora and some specific organisms of major importance in soil fertility such as sporeforms, actinomycetes, fungi, Azotobacter, clostridia, aerobic cellulose decomposers, and anaerobic cellulose decomposers.

b. Determination of some important organic substances in soil and rhizosphere including sugars, organic acids, amino acids, and growth regulators.

The aforementioned points were followed in soil and rhizosphere at different growth stages of both common bean and tomato plants.

II. REVIEW OF LITERATURE

This study was undertaken in order to investigate the interaction between plant root and soil microorganisms at different growth stages in common bean and tomato plants. The review concerning this study could be divided into two concepts, a) Studies on some types of microorganisms i.e., total count, sporeformers, actinomycetes, fungi, Azotobacter, clostridia as well as aerobic and anaerobic cellulose-decomposers. b) Determination of some organic substances i.e., sugars, organic acids, amino acids and growth regulators. Accordingly, the available literature will be reviewed under these two major titles.

A. Microbial Studies

a. The rhizosphere effect on different groups of microorganisms:

1. Total count:

It is almost invariably found that the bacteria in soil respond much more to the presence of plant roots than do the actinomycetes, fungi, algae, and protozoa. Hoffmann (1914) reported that bacteria were generally more numerous adjacent to plant roots than in soil a foot or more distant from plant. West and Lochhead (1940) and Lochhead and Thexton (1947) had observed that bacteria requiring amino acids for optimal growth were preferentially stimulated in the rhizosphere.

Fedorov and Nepomiluev (1954) stated that the rhizosphere flora of clover differ markedly from the soil flora and consists mainly of non-sporeforming bacteria. Voroshilova (1954) found that ammonifying and denitrifying bacteria were stimulated in the rhizosphere.

Rovira (1956c and 1956d) and, Rouatt and Katznelson (1957) reported that the rhizosphere supported greater population of bacteria which were more capable of growing rapidly and of physiologically active types than do non-rhizospheric organisms. These studies together with those of Katznelson and Rouatt (1957a) also suggested that the rhizosphere microflora might be of greater importance to the plant directly or indirectly than the originally soil microflora.

Katznelson and Rouatt (1957b) found that the consumption in the rhizosphere soil was considerably higher than that of the control, the increase being greater in the presence of nitrogen substrate. The rate of oxygen uptake in the rhizosphere suggested a physiologically more active microflora.

Elwan and Mahmoud (1960) found that the total microbial flora was much higher in the rhizosphere of some xerophytic plants than in soil apart.

Abdel-Hafez (1966) found that the total microbial flora was stimulated in the rhizosphere of both wheat and broad bean. Also Skyring and Quadling (1969) found that the bacteria isolated from the rhizosphere of flax differed from the population of the corresponding control soil. Assey (1972) found that the total microbial flora was stimulated in the rhizosphere of broad bean and maize.

2. Actinomycetes:

Micer (1956) found that, in the rhizosphere of barley the actinomycetes were more numerous than bacterial spores and heterotrophic anaerobic bacteria.

Starkey (1958) stated that in addition to bacteria, other groups of microorganisms are also more abundant in the rhizosphere than in soil. There is more development of actinomycetes but generally the increase is proportionally less than that of bacteria.

Sperber and Rovira (1959) found that 63% of the clover rhizosphere isolates and 78% of grass rhizosphere isolates showed branched forms.

Yoshida and Sakai (1962) stated that actinomycetes showed no rhizosphere effect in leguminous plant during growth.

In Egypt, Mahmoud et al. (1964) found significantly higher densities of actinomycetes in the rhizosphere of desert plant than in soil at a distant apart.

3. Fungi:

Agnihothrudu (1955) noted that fungi occurred predominantly as spores in soil (70 to 90%) whereas they occurred mostly as vegetative material in the rhizosphere ($>$ 70%).

Thornton (1957) pointed out that there were more fungi in the rhizosphere of wheat and clover than in soil, and the kind of fungi recovered from the roots varied at different stages of plant growth.

Buxton (1957a) found that exudates from roots of three varieties of peas had different effects on spores germination of three strains of Fusarium oxysporum f. pisi, a fungus causing pea wilt. The exudates had a great depressing effect on the strains to which the plants were resistant than on those to which they were susceptible. Similar results were obtained with extracts of the rhizosphere of the three pea varieties (Buxton 1957b).

According to Tollé and Rippel-Balder (1958) the ratio of numbers of fungi in rhizosphere of cereals to numbers in soil varied from 1.4 : 1.0 to 3.0 : 1.0. Similar kinds of fungi were isolated from thoroughly washed roots of oat, wheat, rye, and barley.

Yoshida and Sakai (1962) noted that the rhizosphere fungi increased during legume growth.

Martiniz (1964) stated that a remarkable increase was noted in the number of bacteria in the rhizosphere of maize as compared with those in the control plots. The highest incidence and diversity of bacteria and fungi genera occurred in the most productive soil.

Youssef and Mankarios (1974) reported that there were more fungi in the rhizosphere of cotton and broad bean than in the non-rhizosphere soil. Plant type and age and soil type have a significant influence of the nature and abundance of fungi flora associated with roots. Cladosporium was relatively more abundant in the rhizosphere of broad bean, while penicillium was more abundant in that of cotton.

4. Azotobacter:

Considerable attention has been paid to the abundance of Azotobacter in the rhizosphere of different plants, and also to the use of this organism to increase yield through seed and soil inoculation.

Katznelson (1946) reported that the rhizosphere effect was not apparent, and the rhizosphere did not stimulate these organisms.

Strzelczyk (1958) found that Azotobacter was higher in the rhizosphere of poppy and raddish.
Riviere (1959) reported R:S ratios of 20 and 40 for Azotobacter in the inner rhizosphere of wheat at tiller stage during two seasons.

Vancura and Macura (1959) found a stimulation of Azotobacter by the exudates of barley and wheat, and found that the organic acids were the most active fraction while galactose and fructose were used in preference to other sugars. Amino acids inhibited the growth of Azotobacter under stationary cultures but were slightly stimulatory in stirred cultures conditions.

Katznelson and Strzelczyk (1961) reported the R:S ratio for Azotobacter with several plant species, with a range of 0.2 to 9.8. Vancura et al. (1965) found that the number of Azotobacter reached 10^7/g in the rhizosphere.
A factor to be considered in comparing results of the establishment of Azotobacter in the rhizosphere is that there is evidence that this organism can be "adapted" to colonize root of different plants by successive transfers on their root (Vancura et al. 1961).

In Egypt, some investigators isolated Azotobacter from soil (Ishac 1958; Moubarek 1960, and Abdel-Hafez 1962) and from rhizosphere (Elwan and Mahmoud (1960), and Assey (1972).

Mishustin and Naumova (1962) reported that Azotobacter is an important natural source of soil enrichment with nitrogen. Furthermore, this organism is active in the formation of plant growth stimulators.

Rovira (1962) stated that Azotobacter did not colonize the rhizosphere densely even without competition, he suggested that this organism is unable to utilize organic substrates exuded by roots. Chan et al. (1963) stated that Azotobacter do not colonize densely the rhizosphere.

Radulovic and Hauzer (1967) found a large number of organisms antagonistic to Azotobacter and probable intense competition between many microbes for food, O_2, moisture etc., and few Azotobacter may be suppressed.

Longeri (1968) reported that a possible inhibitory factor for Azotobacter at root surface could be low pH, which was found to be below 4.8, as the organism was absent in acid soils. This may be also related to low available phosphorus and high exchangeable Al content.