ECOLOGICAL STUDIES ON RHIZOBIUM TRIFOLII

BY

Reda Abd El-Zaher Abd El-Aziz

B.Sc. Agric. (Soils), Ain Shams University, 1974

M.Sc. Agric. (Agric. Microbiology) Ain Shams University, 1981

THESIS

SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

AGRICULTURAL MICROBIOLOGY

Agricultural Microbiology Department

FACULTY OF AGRICULTURE

AIN SHAMS UNIVERSITY

1987

APPROVAL SHEET

Name : REDA ABD EL ZAHER ABD EL AZIZ.

Title : Ecological studies on *Rhizobium trifolii*.

Approved by:

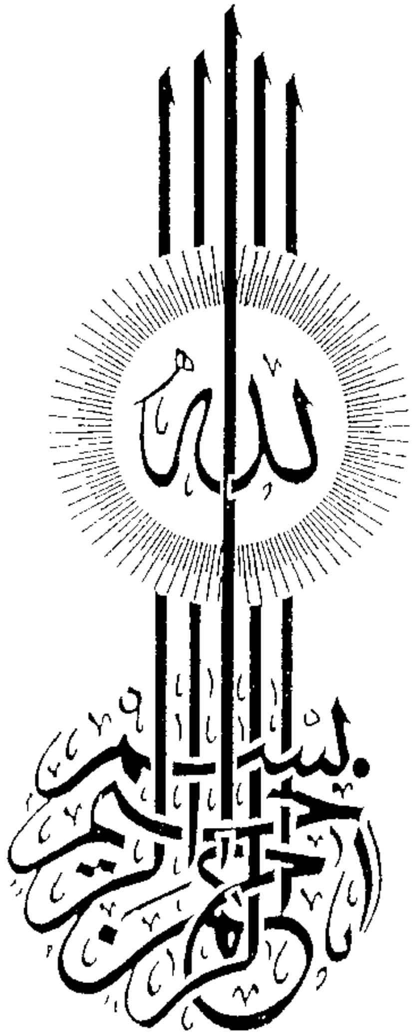
Prof.Dr. ------------------------

Prof.Dr. ------------------------

Prof.Dr. ------------------------

(Committeein charge)

Date: / /1987.


بسم الله الرحمن الرحيم

ACKNOWLEDGMENT

The author would like to express his deepest appreciation and gratitude to Dr. Y.Z. Ishac, Prof. of Agricultural Microbiology and Director of Biofertilizers Unit; Dr. Laila I.Zohdy, Prof. of Agricultural Microbiology, National Research Centre and Dr. M.E. El-Haddad, Prof. of Agricultural Microbiology and the Deputy Director of the Biofertilizers Unit, Faculty of Agriculture, Ain Shams University, for suggesting the problem, supervision, valuable scientific advices, stimulating criticism and giving every possible help throughout the whole work

Thanks are also due to Dr. H. Moawad, Prof. of Agricultural Microbiology, National Research Centre for his appreciable help in the use of fluorescent antibody technique.

Thanks are also due to the authorities of the National Research Centre and the Staff members of Soil and Water Use Laboratory for providing all facilities and materials that made this work possible.

Special regards and best wishes to all the Staff members of the Dept. of Agriculture Microbiology, Ain Shams University for assistance and encouragement.

CONTENTS

Page

Page

INTRODUCTION

INTRODUCTION

Microbial ecology deals with the interactions between microorganisms and their environment. These interactions regulate the biochemical transformations carried out by microorganisms in nature and hence are of great theoretical and practical importance. Information on the ecology of *Rhizobium* with their ability to transform atmospheric nitrogen into biologically useful form is particularly critical to the goal of enhancing biological nitrogen fixation.

The magnitude of the nitrogen added by legumes via N_2-fixation suggests that fixation by these plants could make a significant nitrogen input to an ecosystem, estimated 60-70 Kg N ha^{-1} $year^{-1}$.

Egyptian clover is the most extensively cultivated crop in Egypt, occupies more than 35% of total cultivated area in the winter season. As the population and the demand for animal protein is increasing rapidly, pressure on clover as the major winter fooder increases.

It is common that clover plants can grow and nodulate in most of the Egyptian soils, especially in the Nile valley and Delta, without need for inoculation with effective *R.trifolii* strains. In the last decade it has been some problems in the nodulation and nitrogen efficiency of clover/rhizobia system.

The application of high doses of N-fertilizers by the Egyptian farmers as well as the complexity of environmental factors including the changes in some soil properties might be responsible for the deterioration of the N_2-fixation effeciency of native *R. trifolii*. Under these conditions successful seed inoculation with effective rhizobial strains capable of surviving and competing successfuly with native ineffective rhizobial population is an essential factor in order to increase clover production.

Fluorescent antibody technique (FA) is a useful adjunct to serological methods for the rapid identification and characterization of strains in the nodule isolates, but the main promise of FA in *Rhizobium* ecology lies in the study of events prior to nodulation. The FA technique is of especial pertinence to the ecology of free-living rhizobia because it is the only method to provide the potential for direct investigation of the *Rhizobium* in the soil.

In the light of the foregoing statments, it was found of interest to study the serological characteristics of different native *R. trifolii* strains using FA technique aiming at finding a correlation between their serotyping and their N_2-fixation efficiency. Response of Egyptian clover to inoculation with effective *R. trifolii* strains at both normal and salt affected soils as well as the effect of cutting and N-fertilizer addition on the N_2-fixation process were also considered. In addition the competition among the introduced *R. trifolii* strains and native rhizobia for nodulating Egyptian clover was investigated as well.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

It is now clearly established that nitrogen fixation by the symbiotic association of microorganisms with plants is the most important source of N_2-fixation (Warsy et al. 1974). Jordan (1975) reported that root nodule bacteria are the principal means for the incorporation of atmospheric nitrogen into chemical forms which plants can utilize for growth and development without demanding large amounts of nitrogen from the soil. The leguminous plants are, therefore, agents for increasing soil fertility, since about two-thirds of the nitrogen of a nodulated leguminous plants comes from the air and only one-third from the soil and after harvesting the crop, the root residues which constitute about one-third of the plant weight, are ploughed in before the stubble dries and enrich the soil with nitrogen.

The important contribution of leguminous plants to soil fertility is easily seen from the large amount of nitrogen fixed by these plants. Rizk (1962 & 1966) studied nitrogen balance in field experiments in different locations under Egyptian conditions. He found that the amount of atmospheric N_2 (Kg Fed^{-1}) fixed by legumes to be on the average of 58 for lupine, 57 for horse-bean, 33 for groundnut, 44 for fenugreek, 41 for chickpea, 35 for lentil, 17 for soybean, 27 for berseem (Fahl) in a single cut and another variety (miskawi) fixed

99 kg N fed^{-1} in five cuts. Lawrence et al. (1967) found that amount of N_2-fixed under some leguminous plants ranged between 27-179 Kg N ha^{-1}. Mulder et al. (1969) reported that red clover fixed about 300 Kg N ha^{-1} in Western Europe. In Indian soils, 100-300 Kg N ha^{-1} were yearly fixed by various leguminous plants (Dalton and Mortenson, 1972).

Response to Rhizobium inoculation :

Inoculation is recommended for fields which are planted for the first time with legume species. Response to the supplemental bacteria are frequently quite marked in these circumstances. In order to obtain maximal benefits from the activities of the root nodule bacteria, one cannot usually rely on spontaneous infection by the indigenous soil microflora. Many localities contain few fully effective rhizobia and it is not uncommon to observe as many as 25 percent of the bacteria in a given field to have a low degree of effectiveness, 50 percent to have moderate ability, and only 25 percent to be fully effective. Because of the large indigenous population of rhizobia that are not fully effective in N_2-fixation, it is not surprising that supplemental inoculation with selected bacterial strains commonly results in highly significant agronomic responses. A two fold or greater increase in dry weight yield resulting from inoculation is