ROLE OF MYCORRHIZAL FUNGI IN THE GROWTH OF TOMATO PLANT AND IN THE BIOLOGICAL CONTROL OF FUSARIUM WILT

BY

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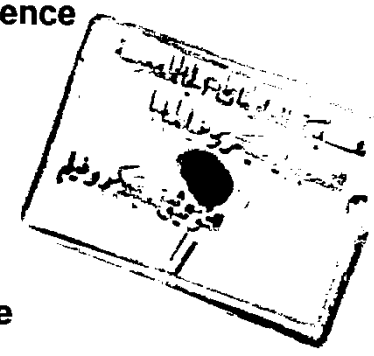

THESIS

Submitted in Partial Fulfillment of the Requirement of the Degree of

MASTER OF SCIENCE

in

MICROBIOLOGY

Department of Microbiology
Faculty of Science
Universityof Ain Shams

(1994)

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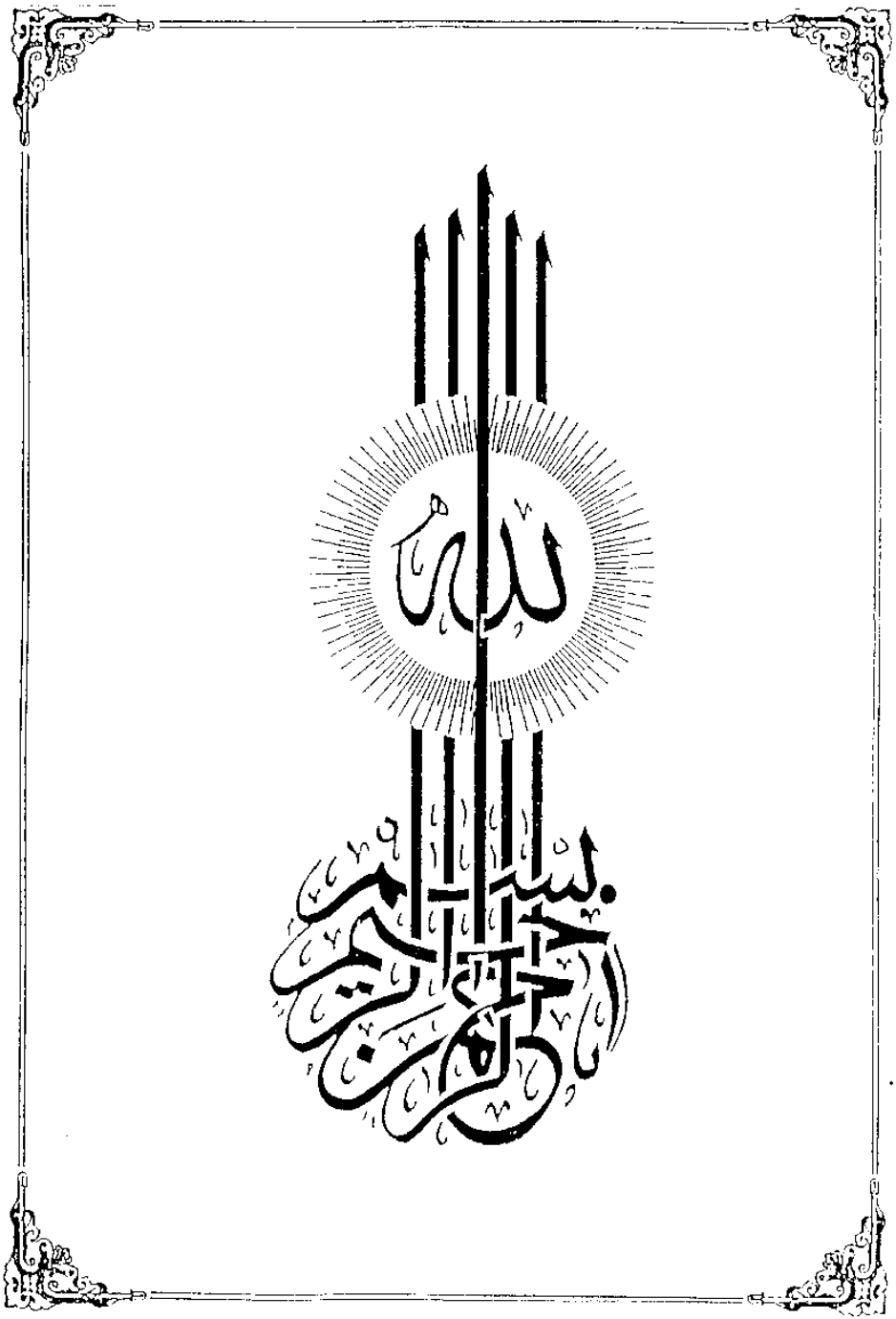

ACKNOWLEDGMENT

My great gratitude and sincere thanks should be submitted to God for his kind and continuous support to me.

I wish to express my sincere gratitude and great fulness to Professor Dr. ***Youssef A. Youssef,*** *Professor of Microbiology, Dept. of Microbiology, Faculty of Scinec, University of Ain Shams, and Prof. Dr.* ***Ali El-sayed Tawfik,*** *Agricucltural Research Center, for suggesting the point of research, direct supervision, constructive criticism, valuable discussion and continuous help throughout the present work.*

Faithful thanks and gratitude are also due to Head and staff members of the Microbiology Dept., Faculty of Science, University of Ain Shams .

PREFACE

Mycorrhiza (fungus - root) is the term commonly used to denote the symbiotic association between plant root and the mycelium mycorrhizal fungi. There are two types of mycorrhizas, the ectotrophic or ectromycorrhizas and endotrophic or endomycorrhizas. Mycorrhizal plants increase the surface area of the root system for better absorption of nutrients and water from soil especially when soil is deficient in available phosphorus.

Endomycorrhizas are formed on different plant species of most families of angiosperms and in certain Petridophytes and Bryophytes by fungi in the Zygomycetes and Basidiomycetes.

Endomycorrhizas which have been known to man for more than 100 years probably existed in the earth for 300 million years age. Fungal infections in fossilized roots from the Devonian and carboniferous periods look. Like mycorrhizal infections in root system today.

In endomycorrhizal the fungal hyphae enter the cells of the undamaged root either through cells of the epidermis or root hairs and extend out into the soil for about 2 cm or more from the root surface and thus contribute to the nutritional requirements of the host. Large spores developed on the external mycelium while both mycorrhizal vesicules and arbuscules structures are formed within the cortical root tissue. These structure have led to the name vesicular-arbuscular mycorrhizas (VAM). VA-mycorrhizal fungi are classified on the bases of their spore morphology and attached hyphae into six genera namely *Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocystis*, *Entrophospora* and *Suctellospora*. VA-mycorrhizas are intracellular obligate endosymbionts which have not yet been obtained in pure cultures.

VA-mycorrhizas are wide spread, through the world occurring on different crop plants as graminaceous plants, legumes, cotton, tobacco, potatoes, sugarcane, tomatoes and strawberry.

Because mycorrhizal fungi occur naturally on most crop plants, the effect on plant disease may be considerable. roots are obviously changes by mycorrhizal infection which induced changes in root exudates and alternation in the population of microorganisms in the rhizosphere and could stimulate a microflora antagonistic to plant pathogenic organisms. It is also possible that VA-mycorrhizal fungi can act as mechanical barrier and produce chemical inhibitors which in turn have practical implications in the biological control of some plant diseases, Studying the disease control methods of mycorrhizal fungi will include the host tolerance to pathogens or the production of compounds inhibitory to root infecting fungi. Many root pathogens can be controlled only with expensive physical or chemical soil treatments. Mycorrhizas offer an alternative approach as biological control agents.

Thus the present investigation was carried out to study the following aspects:

1. Extraction of mycorrhizal spores from soil cultivated with tomato plants and their identification.
2. The effect of mycorrhizal fungi in the presence of rock phosphate (unavailable phosphorus) and different levels of superphosphate (available phosphorus on the vegetative growth and yield of tomato plants under natural field conditions). The macro and micro-nutrients of shoots and roots contents was also estimated.

3. The effect of mycorrhizal fungi on the control of tomato vascular wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici* as a new approach of biological control of the disease was also elucidated.

PART I
A. INTRODUCTION
B. MATERIALS AND METHODS

A. INTRODUCTION

A. INTRODUCTION AND HISTORICAL REVIEW

One of the earliest reports of mycorrhizal infection was recorded in 1885 when Frank coned the term mycorrhiza for the fungus-root organs on certain forest trees (**Frank, 1885**) (now called ectomycorrhiza). He recognized the symbiotic relationship between tree roots and fungi. He noticed that the fungus forms hyphal threads within the soil which increase the absorption of water and nutrients from the soil. The relationship is viewed as truly symbiotic in which higher plant gain by improved nutrition, and the fungus by receiving a supply of carbohydrates. This intimate association between the fungus and tree root was regarded on roots of forest trees and also other plants, woody and herbaceous including many crop plants. Both monocotyledons and dicotyledons are now known to posses mycorrhizas. Research workers of **Hatch, (1937); Melin, (1927)** and **Harely, (1959)**, reviewed about this interesting association phenomenon.

Yousef (1946) found that the fungus developed intracellularly within the cortical cells of *Iris germanica albieans* beneath the exodermis and gives rise to arbuscules (highly branched haustoria) in the deeper layer, the other named vesicles (enlarged hyphal swelling) formed intra- or intercellularly. Variation and integrations of the two types of mycorrhizae existed.

Harely (1969) and **Khan (1972)** found that the host plant benefits from the mycorrhizal association due to the enhanced nutrients uptake. **Mosse and Bowen (1963); Khan (1971)** and **Marx (1976)** reported that most higher plants were susceptible to (VA) mycorrhizal infection with all species of *Endogone*. **Haymann (1978)** and **Kruckelmann (1975)** reported that the population of (VA)

mycorrhizal fungi varies with different crops cultivated in the same soil according to the susceptibility of the roots to mycorrhizal infection.

The benefits of mycorrhizal infection are mainly due to the improved uptake of phosphate from soil by plant roots. Most evidence indicate that the mechanism of this response is physical view in which (VA) mycorrhizal hyphae tapping the soluble phosphate beyond the phosphate depletion zone that develops around the root surface in soils of moderate or low phosphorus content because of the lower mobility of phosphate ions in soil (**Haymann, 1978** and **Tinker, 1978**).

Mycorrhizas are generally more abundant and more important in an infertile soil, but germination of (VAM) spores is not inhibited by the presence of phosphorus, nitrogen of potassium nutrients. **Strzemska (1975)** found significant decreasing in mycorrhizal colonization of rye, wheat, barley and oat roots when fertilizers were used. On the other hand, drought also increases colonization but this may result indirectly from reduced phosphate uptake.

The association between mycorrhiza and host plant is finally balanced. The vesicular arbuscular type are intracellular obligate endosymbionts which have not yet been obtained in pure culture (**Mosse, 1973; Mosse and Tinker, 1975** and **Haymann, 1978**). Thus, the (VA) mycorrhiza system appeared to be regarded as consisting of three components: plant, fungal endophyte and soil (**Haymann, 1983**).

Types of Mycorrhizas

Many authors such as **Melin (1927)** and **Siagh (1968)** suggested classification schemes to differentiate various morphological types of mycorrhizas.

Zake (1971) and **(1973)** proposed a classification scheme based on different characteristics such as morphology of the mycorrhiza, the association of a specific fungus in terms of fruit bodies and rhizomorphos or hyphae with the formation of specific types of mycorrhizas regardless the color which differentiate the species of the fungal symbiont involved.

Alexander (1976) classified mycorrhizal fungi into three classes: Endomycorrhizas, Ectomycorrhizas and Ectendomycorrhizas.

Ectomycorrhizas

In ectomycorrhizas, the fungal symbionts do not penetrate the host cells, but form a sheath on the root surface and an intercellular net work between cortical cells, the harting net. There are two separate interfaces between the cortical cells, that with the inner sheath hyphae and that with the harting net.

In the early stage of sheath formation, the hyphae become attached to the root surface by polysaccharide mucilage. The hole of the sheath eventually becomes embedded **(Marks and Foster, 1973; Ling-Lee *et al.*, 1977; Duddridge, 1980 and Duddridge and Read, 1984).**

Scheneck (1981) reported that ectomycorrhiza forms fungal sheath, several thick cells around rootless within the epidermis of mycorrhizal root. The cortical cells are surrounded by hyphae forming harting net which may extent to the endodermis.

Endomycorrhizas

Endomycorrhizas are formed by at least 90% of plant species. In this association, the fungal partner grows mainly inside the root cortex and penetrates into the cells of the host roots. Endomycorrhizas are further divided

into two subgroups based on weather the fungi involved are aseptate zygomycetes or septate endophytes belonging to the Ascomycetes or Basidomycetes.

Vesicular arbuscular (VA) mycorrhizas are of world wide distribution (**Harely, 1969**). The (VAM) have an extensive hyphal net work (**Mosse, 1959** and **Nicolson, 1959**). The external hayphae grew intercellular or both intra and intercellularly in the root cortex. In some cases the fungus completely colonized the cortical region of the feeder roots but did not invade the endodermis (**Gray, 1971**).

Marx (1976) found that the haustorial cells were thin walled, spherical to oval in shape vesicles may be produced in the cortex. The arbuscules are branched. **Gray (1971)** found that arbuscules were usually produced terminally on hyphae but in some cases formed laterally. It is believed that its function is to exchange the nutrients between the host cells and fungi (**Gianinazzi-Pearson and Gianinazzi, 1988** and **Smith and Gianinazzi-Person, 1988 and 1990**). **Schoknecht and Hattingh, 1976** and **White and Brown, 1979**) suggested that active arbuscules are the probable site of breakdown of polyphosphate granules. However vesicles are of term intercellular and multinucleated having open connection with their parent hyphae. The protoplasm becomes vaculated and numerous oil droplets developed and are thought to act as temporary storage organ (**Mosse and Bowen, 1963**).

Ect-ednomycorrhizas

This type has the feature of both ecto and endo-mycorrhizas, but seems to be less important because it has a limited distribution. This type is known as the "arbutoid" and is belonging to the genera *Arctous*, *Arbutus* and *Arctostophylas*.

Little knowledge are known about their species and their benefit to the plant vegetative growth (**Marx, 1976**).

Mycorrhizal spores

Mature mycorrhizal spores have been described by (**Bulter, 1939**) to have a very resistant walls often consisting of two or more separate detachable layers with different staining properties. Although the spores germinate, hyphae fail to grow on cultural media (**Mosse, 1959** and **Gerdemann and Nicolson, 1963**). identification of Endogone depends on morphological character such as size, shape, color, wall structure of spores and the appearance of the mycelium. **Gerdmann and Nicolson (1963)** identified six distinct spore types, four of these types produce an infection in roots.

Spores can be recognized in a soil suspension (**Mosse and Bowen, 1968**). Endogone spores are not separated after-sieving but they were usually remained attached to the parent hyphae from which they arise. The mycorrhizal spores are larger than the asexual spores of most other fungi existing in the rhizosphere. On the other hand, the subtending hyphae being either simple, swollen or bulbous. Spores with simple attachments have either very narrow channel connecting the spore with the parent hyphae or they may be a thin closing membrane at the level of the spore. However, the base spores may be colorless or black, due to the germination of spore content but mostly there are some shades of yellow or brown colors (**Haymann, 1982**).

Extraction of spores

There are different methods for the extraction of mycorrhizal spores: 1. Wet sieving is the most common extractive method adopted by (**Gerdemann and**

Nicolson, 1963), 2. Sucrose centrifugation method (**Ohomos, 1957**), 3. Adhesion-floating method (**Sutton and Barron, 1972**), 4. Plate method (**Mosse and Bowen, 1968**), 5. Gelatin column method (**Mosse and Jones, 1968**).

Garriet and Skipper (1979) compared different methods for the separation of (VAM) spores from soil by the method of adhesion-floating, sucrose centrifugation, gelatin column, wet sieving and new plate method. They found that spores were of 150 - 500 µm in diameter were higher in the plate method than the adhesion-floating (by 523%), sucrose centrifugation (by 370%) and gelatin column (by 258%). Plate method also showed higher numbers of 100-300 µm in diameter spores as compared with that observed by the wet sieve (by 200%) and modified wet sieve (by 149%). They concluded that the spore counts within different ranges of spores size (50-100, 100-150, 150-300 and > 300 µm in diameter) were greater from the plate method than for the wet sieving approximately four and two times, respectively. The plate method was consistently higher than the wet-sieve method only for small spores viz.: 50-100 µm in diameter. **Mosse (1959)** reported that some spores floated while other sank in solution. This may be led to the loss of some spores when using another methods regardless the plate method.

Classification of mycorrhizal fungi

Mycorrhizas fungi associated with plant roots, belongs to Basidiomycetes, Ascomycetes and Zygomycetes (**Harely, 1969; Hacskaylo, 1971; Marks and Kozlowski, 1973** and **Mikola, 1980**).

VA-mycorrhizal fungi are classified in the family *Endogonaceae*, order: *Endogonales*, class: *Zygomycetes*, subdivision: *Zygomycotina*, division: *Amastigomycotina*, kingdom: *Myceteae*. These fungi are classified on the basis