SZENT ISTVÁN UNIVERSITY

IMPACT OF MYCORRHIZAL FUNGI AND OTHER SYMBIOTIC MICROBES AS BIOCONTROL AGENTS ON SOIL BORNE PATHOGENS AND SOME ECOPHYSIOLOGICAL CHANGES IN TOMATO ROOTS

PhD Theses

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THESES OF PH. D. DISSERTATION

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1. Introduction

1.1. Mycorrhizae in general

The feeder root of most flowing plant growing in nature are generally infected by symbiotic fungi that do not cause root disease; however, instead, are beneficial for their plant hosts. The infected feeder roots are transformed into unique morphological structures called mycorrhizae. That is called 'fungus roots' (Agrios, 1988).

The essential structure and functioning of asymbiotic relationship between trees and fungi is hetermeda 'Mycorrhiza' from the Greek word - 'fungus-root' (Frank, 1885).

The mycorrhizal literature of conventional agriculture (Bagyaray, 1992; Ferrera-Cerrato, 1987; et. al.) has traditionally focussed on the potential of mycorrhizae fungi to improve crop yield and to produce the use of fertilizers. Although the close relationship between the mycorrhizal soil mycelia and the soil biota is well-known (Curl & Truelove, 1986; Linderman, ?). The root system is typically colonized by more than one VAM-fungal species, while mutual exclusion has not been observed; the success in occupancy varies and is not necessarily related to host-response (Lopez-Aguillon & Mosse, 1987).

The VAM-fungi absolutely lacks host-plant specification, but conditions for their utilization are modified by ecological specificity (McGonigle & Fitter, 1990). The transfer of nutrients among the root-zones of associated plant is influenced by VAM fungi, whose hyphae colonized and connect the roots of adjacent plants (Francis & Read, 1984).

A Mycorrhiza can be in a mutualistic symbiosis between plant and fungus localized in a root or a root-like structure in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant.

Several criteria can be used to distinguish mycorrhizae form other plant-fungus associations. The mutualistic nature of the interaction is a critical character that differentiates a mycorrhiza from other plant-fungus associations. Although the line between parasitism and mutualism is fine, and negative interactions between plant and fungus can occur, if environmental conditions change it is positive for both symbionts in a general sense, regarding both given species.

1.1.1. Morphology

The mycorrhizal morphology is the basis for grouping them into two major groups - ectomycorrhizae and endomycorrhizae. The latter group is further divided into ericoid, orchid and the ?ubiquitons?, and large group of vesicular-arbuscular (VA) mycorrhizae. A few plant families

are facultative and conform either ecto- or endomycorrhizae; and some form no mycorrhizae. Endomycorrhizal fungi penetrate root cortical cells in tracellularly, and form special houstoria, like structures interfacing with the host cytoplasm, separated only by the host plasma membrane with the host cytoplasm, or separated only by the host plasma membrane and the fungal cell wall.

Ectomycorrhizal fungi form external fungal mantle and on intercellular hyphal network in the cortex, called - 'Hartignet'. External structures of VAM fungi are the hyphae which penetrate the soil, and the individual resting spores. The latter ones are produced asexually on straight, subtended hyphae, and are known as chlomydospores.

Some VAM fungi have an aggregation of spores in asporocarp. Structures on the exterior of plant roots that had been identified as vesicles are now considered to be chlomydospores in the formative stage. The term 'vesicle' should be restricted to the expanded hyphical tips within the plant cortex. Five major genera of the family Endogonaceae form VAM. These include Glomus, Gigaspora, Acaulospora, and Scutellospora. They are distinguished according to the morphology of their resting spores. The genus *Glomus* contains a number of species that have individual thick-walled terminal chlomydospores.

Also, *Glomus* produces sporocarps that are multiple sporing structures having individual chlomydospores within them. At maturity, spores separate from the hypha. The genus *Gigaspora* rarely forms vesicles but has spores budded from a bulbous, suspensor-like tip of a hypha. The spores have a resemblance to zygospores, the sexual stages of zygomycetes. Due to the asexuality of *Gigaspora*, its spore has hitherto been termed an azygospore, but many mycologists refer to it simply as a spore. The genus *Acaulospora* also produces resting terminal spores. These are formed from a previously developed however short-lived mother spore. The spores are found singly in soil or sometimes within the roots. The genus sclerocytis contains a number of chlomydospores arranged around a central mass of interwoven hyphae that form a sporocarp. Then, the hypha produces a fan-shaped complex of narrow (2 to 7μm) branches that produce infection in the root. Individual VAM spores are from 10 to 400 μm in diameter, but mostly are in range of 40 to 200 μm. A fine endophyte with hyphae of 1 to 3 μm in diameter are rather exist than the 5 to 10 μm of other VAM fungi are characterised by small spores of approximately 10 μm in diameter.

The mycorrhizal hyphae - the small diameter hyphae - increase the surface area of the root system, but cost ten percent more to constract than the equivalent mass of roots, and omitted any discussion of mycorrhizae in their plant nutrition. Similar statements are commonly take place, despite the importance of mycorrhizae in such processes as short circuiting the N-mineralization process in heath lands and the dominant role of mycorrhiza in p nutrition of plants ( ). These include the mycelial network extending into the substrate as well as the root, and the essential role

of the fungus in providing nutrients and water to the plant (in this case the mycorrhiza functions in nutrition relationship as a wet-nurse of the tree) (Peterson, et al., 1991).

1.2. Rhizosphere microbe

The interaction with general Rhizosphere Microflora and Mycorrhizae effects are discussed in many research papers on interactions with specific microbia groups, such as free-living nitrogen fixers.

Five bacterial sp. isolated from blue grama were added back to non-inoculated and VAM-inoculated pot-grown plants. In the rhizosphere soil, population of an *actinomycete*, a *fluorescent pseudomonand*, and a gram-positive non-matile rod were lower in the treatments inoculated with *Glomus mosseae*; on the other hand, a species of caryne bacterium and a gram-negative rod were more numerous in the mycorrhizal treatment. The authors suggested that the species composition of the rhizosphere microflora may be altered by the formation of mycorrhizae.

The hypothesis was supported by Meyer and Linderman, who demonstrated differences in populations of taxonomic and functional groups of bacteria associated with VAM and non-VAM plants. The microbal populations in the rhizosphere are influented by mycorrhizal fungi. The effects vary with time, the species of mycorrhizal fungus, the host, and other environmental parameters.

1.3. The basics of tomato plant culture

Undoubtedly, tomatoes are one of the most important and popular salad crops in many countries world-wide. Indeed, a salad bowl would be definitely poor without tomato. There are many details surrounding their culture which, if understood, help to produce bigger and better crops of fruit. Every time, when an author writes about tomato growing recently, it is obvious that draws on the experience of others, because there are so many ways in cultural techniques. The precise genealogy of the tomato, if one may use that word in the botanical field, is something which fascinates many gardeners. In the 16th century, it had been known in Europe mainly as an ornamental plant, and this persisted until the latter part of the 18th century, when it became recognised as an edible vegetable. Tomatoes belong to the natural order *Solanaceae* and belong to the genus *Lycopersion*. There are several varieties of *Lycopersicon esculetum*: *commune,* common tomato, grandiflorum, large-leaved tomato, *validium,* upright tomato, cerasiforme, cherry tomato, and pyriforme pear tomato. In recent years, plant breeders have been concentrated on the breeding of high-yielding varieties with specific shapes or sizes of fruit, with special habits of growth, and with introduced resistances to various

pests and diseases (Ian G. Walls, 1989). The most important factors having effect on the tomato plant growth are: temperature, light, moisture, nutrient, the type of soil. The tomato plant is unfortunately prone to a wide variety of troubles, many of them appear asserting themselves with some regularity. However, the most important and serious trouble is caused by the diseases due to many microbal pathogens such as: fungus, bacteria, nematodes and varuis. In addition to control measures for specific problems, the following general practices can help to reduce losses from diseases:

- Buying treated seed only from a reputable dealer;
- Sterilizing soil for seedling and transplant production, and for greenhouse production;
- Spraying plants with appropriate pesticides;
- Using resistant cultivars or a soilless medium such as rockwool or NFT;
- Practising certain sanitary measures (W.R.Jarvis, 1991).

1.3.1. Tomato plant in Hungary

Tomato plant fruit is a very important crop vegetable in Hungary in terms of economic and human consumptional concerns.

The varieties cultivated in Hungary in the field or greenhouse are of more than 65 varieties, but the most important varieties cultivated in field and greenhouse are the followings:

Field cultivated are Red Hunter, peto 95, Corall, Mobil, Slager and Kecskemet 579; and all these varieties are for industrial purposes. For fresh market, the following varieites are existing: Kecskemet 3, Savor, Myto and Suso. The next varieties are for greenhouse cultivation: Falcato, Fino, Gala, Primato, and Celsus.

The total production of tomato plant crop in Hungary for 2002 is 118 000 t, and the best area for the growing tomato plant in Hungary is town Szentes. The most important areas for tomato plant in Hungary are the followings: Mosonmagyaróvár, Környe, Balatonboglár, Gödöllő, Kecskemét, Karcag, Békés, Szentes.

The most important injurious diseases effected by tomato plant in Hungary including the fungus diseases are: Damping-off, septoria leaf spot, Early blight, Gray leaf spot, late blight, Anthracnose, Fusarium wilt, verticillium wilt, soil rot, gray mold rot; the bacterial disease are: Bacterial stemrot, southern bacterial wilt, Bacterial spot, Bacterial speck.

1.3.2. Tomato plant in Libya

Tomatoes are the second most ?importable? crop in Libya with a production area of over .....? ha, yielding 50 000 t. (during what time?) They are grown in the field as fresh-market and processing crops and in greenhouses for the same purpose. The best cultivated area is the coast of North Libya. There are several varieties of tomatoes such as:

a) varieties for open area (in the field) as Roma, Rio Grande, Alwadi (national speciality),

b) varieties for close area (greenhouse) as Falcato, Monte Carlo, Michigan Ohio, Dombo, Dombito, Divista.

1.4. Goals

Respecting the importance of the knowledge of outstanding biological characteristics of tomato plant we wanted to investigate its most important symbiosis arbuscular mycorrhiza. As this symbiosis assures advantages for tomato plant in phosphorous nutrition, in drought tolerance and in resistance to different pathogens, our investigation were planned to clear up:

- The **diversityof the natural arbuscular mycorrhiza fungus communities** in different soil types, and its varieties affected by tomato cultivars.
- Respecting **the role of AM as an agent in biological control** the effect of AM inoculation on the populations of some opportunistic pathogens are to be investigated.
- The possible effectiveness of arbuscular mycorrhiza affected by some technological factors outstanding in tomato production. The **effect of different fungicides** on the development of AM should be examined in details.
- The **interaction between different rhizosphere bacterial populations** (PGPR, phosphate mobilizing, nitrogen fixers) and **AM**. The prospect of AM affected by bacterial preparates applied in chemical-free technologies also should be examined in details.

2. Review of literature

2.1. Biology of VA mycorrhizae

There are two types of mycorrhiza, distinguished by the way the hyphae of the fungi are arranged within the cortical tissues of the root. Ectomycorrhizae roots are usually swollen and, in some host-fungus combinations, appear considerably more forked than nonmycorrhizal roots, ectomycorrhhizae are formed primarily on forest trees mostly by mushroom- and puffball-producing basidiomycetes and by some ascomycetes. The spores are produced above ground and are wind disseminated. The hyphae usually produce a lightly interwoven fungus mantle around the outside of the feeder roots.

Endomycorrhizae roots externally appear similar to nonmycorrhizal roots in shape and colour, but internally the fungus hyphae grow into the cortical cells of the feeder root either by forming specialized feeding hyphae (haustoria), called Arbuscules, or by forming large swollen food-storing hyphal swellings, called Vesicles (Agrios, 1988).

Vesicular-Arbuscular Mycorrhizas (VAM) is the most common of mycorrhizal forms, involve fungi classified as *Zygomycetes.*

Arbuscules are intricately branched 'haustoria-like' structures formed within root cortical cells and surrounded by the plasma membrane which invaginates inside the cells. Repeated dichotomous branching results in a proliferation of fine hyphae. Arbuscules are considered to be a major site of symbiotic exchange with the host plant (M. Brundrett, et. al., 1994). *Vesicles* are intercalary (-o-) or terminal (-o) hyphal swellings formed within the root cortex. These structures accumulate lipids and may become thick-walled spores (M. Brumdertt, et. al., 1944).

Five major genera of the family *Endogonaceae* from VAM are the followings: Glomus, Gigaspora, Acaulospora, Sclerocysitis, and Scutellospora. The genus *Glomus* contains a number of species that have individual thick-walled terminal clamydospores. Glomus also produces sporocarps that are multiple sporing structures having individual chlamydospores within them. At maturity, spores separate from the hypha.

The genus *Gigaspora* rarely forms vesicles but has spores have a resemblance to zygospores, the sexual stages of Zygomycetes. Due to the fact that *Gigaspora* is asexual, its spore has hitherto been termed an azygospore, but many mycologists refer to it simply as a spore.

The genus *Acaulospora* also produces resting terminal spores. These are formed from a previously developed but short-lived mother spore. The spores are found singly in soil or sometimes within roots.

The genus *Sclerocystis* contains a number of chlamydospores arranged around a central mass of interwoven hyphae that form a sporocarp. Spore germination results from the regrowth of attached hyphae in the case of *Glomus* and *Sclerocystis*, and through the wall of the spore in Gigaspora and *Acaulospora*. If the growing hypha of a germinating spore does not contact a susceptible root, it can restrict its growth; the spore then continues in a resting phase. The hypha then produces a fan-

shaped complex of narrow (2 to 7 μm) branches that produce infection in the root. Individual VAM spores are from 10 to 400 μm in diameter, but mostly are in the 40 - 200 μm range. A fine endophyte with hyphae of 1 to 3 μm in diameter rather than the 5 to 10 μm of other VAM fungi is characterized by small spores of approximately 10 μm in diameter.

2.1.1. Taxonomical diversity

The Mycorrhizae fungi are a diverse group. Because of their large number and diversity, only a sketchy classification of some of the most important Mycorrhizae genera will be presented here:

- The lower fungi
- Division 11: EUMYCOTA (eumycetes)-produced mycelium, not plasmodium
- subdivision: 2: ZYGOMYCOTINA
- class: ZYGOMYCETES (The bread moulds)
- order: Endogonales-Mycorrhizal fungi produce spores singly in soil, or in sporocarps containing zygospores, chlamydospores, or sporangia
- family: Endogone
- genus: Glomus, Gigaspora, Acaulospora, Sclerocystis and Scutellospora (Agrios, 1988).

2.1.2. Ecology of VA mycorrhizae

2.1.2.1. Effect of physical factors

2.1.2.1.1. Temperature

The appropriate temperature is very important for colonization and sporution of VAM fungi. Higher temperature of arbuscular development at 30 °C and the mycelial colonization between 28 to 34 °C. The optimum temperature for the germination of Glomus and Acaulo spora spores is around 20-25 °C, whereas *Gigaspora* had a much higher optima. That suggests the increased soil temperature hastening the development of VA mycorrhizae. This may explain the slow development of infection in agricultural crops in temperate soil where soil temperatures are low compared to tropical soils. Since most species of VAM fungi exist world-wide, it is possible that strains and species may be temperature adopted.

2.1.2.1.2. Light

The light can strongly affect the development of mycorrhizae. The stimulatory effect of light on the development of VA mycorrhizae. Shading not only reduces root colonization and spore production but also the plant response to VA mycorrhizae. The day length may play an important role in VA mycorrhiza development. The effect of light on VA mycorrhizae seems to depend on the photosensitivity of the species of host plant. In fact, a photo period of 12 hrs or more may be more important than light intensity in providing high levels of root colonization, but if suitable day-length is provided, the increased light intensity may still increase colonization.

2.1.2.1.3. Water

The VA mycorrhizae occur in a wide range of soil water contents. The spore germination can probably occur at soil moisture levels at which do not normally grow. Colonization has been found in arid regions in xerophytes, very wet soils of marshes, and also in free-floating and submerged aquatic plants, was believed earlier that under saturated conditions, O_2 concentration can inhibit VA mycorrhizae spores germinated in air that their subsequent growth was little affected by lack of O_2 until the oxigen tension was below 3%. The recent observations on mycorrhizae in aquatic plant suggest that perhaps in water there is adequate dissolved O_2, and concentration of toxic substances such as Mn, H_2S, organic acids, developing under anaerobic conditions are probably absent. Soil water may select for certain species of VAM fungi, which adapt to that environment.

2.1.2.1.4. Soil structure

The VA mycorrhizae are important contributors to soil stability. Obviously, by increasing nutrient uptake, they improve plant cover and root proliferation. They also produce large amounts of hyphae that serve to bind soil practicles together (Miller et al., 1990). Erosion losses result in detrimental affects on the chemical, physical, and microbiological properties of soil. Since eroded soils have reduced numbers of mycorrhizal propagules (Powell 1980; Habte, 1989) inoculation with VAM fungi has been proposed to help rehabilitate these areas (Hall, 1980). However, eroded soils often have very low fertility levels, limiting the potential benefits of mycorrhizal inoculation. Aziz and Habte (1989 a, b, c, d; 1990 a, b) quantified the interactive affectsof mycorrhizal inoculation and soil fertility on plant establishment in eroded sites. They reported that selected VAM fungi, along with starter fertilizer can increase plant establishment on eroded soils.

The soil compaction results in reduced plant growth due to decreased root growth, soil water content, and soil aeration. Wallace (1987,b) investigated the effect of compaction on mycorrhizal little bluestem (Schizachyrium scoparium Michx.) plant, finding that compaction reduced plant

tillering, crown exxpansion, and mycorrhizal colonization. (Simmons and Pope, 1988 a, b; Bethlenfalvay et al., 1992).

2.1.2.2. Effect of chemical factors

2.1.2.2.1. pH

The value of pH is very important factor in the development of mycorrhizae. The good germination of VA mycorrhizae spore occur between pH 6 and 7 although there are cases of fungi germination at pH 5 and below as well as pH 8 and above. Optima for germination of *Gigaspora* corraloidea, *Gigaspora heterogama*, and *Glomus mosseae* on the agar have been recorded at pH 5, 6 and 7 respectively. In soil, effects of pH are difficult to evaluate since many chemical properties of soil vary with changes in pH in soil, more than 40% germination of *Glomus epigeum* spores was found over the pH range 4.8-8.0; being the optimum 7.0. No obvious effect of pH on infection in three grassland sites at pH 4.9, 5.9, and 6.2; however, such infection may have been due to different endophyte species. Natural soils of the world cover the pH range 2.8 to >10.0.

Soil pH may affect the distribution of mycorrhizae in a subtle way. The phosphorus has affects on VA mycorrhizae colonization of roots.

2.1.2.2.2. Phosphorus

The addition of phosphorus affects VAM colonization of roots, conclusive recommendations for specific soil p levels for mycorrhizal production cannot be made. There are several reasons for this. First, it is not soil p per se that regulates mycorrhizal colonization, but rather the amount of p absorbed by the host plant. Second, methods for evaluating available soil p often differ greatly, and plant tissue analysis is a far more reliable method for determining available soil p than most methods that analyse soil. Finally, since host plants vary in their ability to absorb p and mycorrhizal fungi, vary in their response to p, each plant-soil-VAM symbiont system must be evaluated separately. For example, in a sandy soil, maximum spore production by *Glomus fasciculatum* on sour orange occurred at 50 ppm added p, but this amount of p applied to a different citrus variety did not encourage sporulation.

Tissue p is also not always a good estimate for mycorrhizal colonization, because the mycorrhizae themselves influence that factor. It is thought that p influences VAM colonization by affecting concentrations of root carbohydrates or the amount of root exudates, and for that reason the effects of p concentration may be partially overcome by other factors such as light intensity. The best indicator for identifying a soil that will provide good VAM colonization appears to be the

percentage of p in plants at the time of VAM colonization. The p-tolerant strains have developed during the 15-year period of superphosphate application.

More work in isolating p-tolerant strains is needed, as they can be used for inoculating high-yielding varieties of field crops and horticultural crops demanding application of heavy doses of fertilisers.

2.1.2.2.3. Nitrogen

The nitrogen fertilizers (188 kg, N/ha as Nitro-chalk) had a large negative effect on the mycorrhizal population.

Reduction in mycorrhizal colonization following application of 300 kg N/ha as $(NH_4)_2SO_4^+$. The NO_3^- salts are to be more inhibitory to VAM development than NH_4^+ comparing to calcium nitrate, urea, and calcium ammonium nitrate at different levels. The nitrogen content of soils could greatly influence the distribution and abundance of VAM (Arora et al., 1992).

2.1.2.2.4. Micronutrients

Micronutrients such as manganese and zink inhibit spore germination of mycorrhizal fungi. Zink and copper was shown to inhibit mycorrhizal colonization in clover, onion, maize, and pinto bean. Zink, copper and manganese applied at the rate of 12, 2.5, and 40 ppm, respectively, along with Ruakura solution improved mycorrhizal root colonization, sporulation and the most probable number of infective propagules of *Glomus fasciulatum*.

Acid soils contain much soluble Al, Mn, and FE, and in neutral soils Mn and Fe can be released in large quantities when reducing condition prevail after waterlogging. The VA endophytes can develop strains adapted to particular soil conditions. Hence, one of the prime strategies for reclamation of mine tailings should be the selection, introduction, and maintenance of suitable mycorrhizal fungi (Arora et al., 1992).

2.1.2.2.5. Pesticides

Most of researches on pesticides have been undertaken with soil fumigants, fungicides and nematicides with exhibit avange of activity toward VAM fungi. Fumigation of soil with biocides such as methyl bromide, chloropicrin, formaldehyde, Mylone, vapam, and vorlex effectively kill endophytes in the treatment zone. Most nematicides such as 1,3-dichloropene, 1,2-dibromo-3 chloropropane (DBCP), and some of the organophosphates (Bhenomiphos) and organocarbamates

(aldicarb) at recommended rates exhibit lack or a slight inhibitory effect. Interestingly, some nematicides, such as DBCP and 1,3-D, can stimulate root infection in host plants.

The systematic fungicides, like thiobendazole, benomyl and triadimefon are more toxic to these fungi. Pentachloronitrobenzene, which is not systemic, is also highly toxic. Many fungicides are probably only fungistatic to these fungi. Limited number of researches have been done on the effect of herbicides and insecticides on VAM.

Many pesticides contain heavy metals, and the presence of such metals in soils may be responsible for poor germination of these fungi (Bharat et al., 1992).

2.1.2.2.6. Salinity

Sodium and chloride ions inhibit spore germination of VAM fungi. There are several reports of VAM in maritime salt marshes. It is not known whether the endophytes involved show a special adaptation to the saline conditions. Hypothesized that VAM fungi could counteract some forms of soil toxicity by absorbing elements harmful to plant or assisting plant tolerance of high alkalinity or high salinity in tropical soils (Arora et al., 1992).

2.1.2.2.7. Organic matter

In general, the organic matter stimulate spore production, and the mycorrhizal root debris can also be an important reservoirs of inoculum and the contact between colonized root debris and uninfected plant may enhanced the mycorrhizal spread with low annual rainfall (Arora et al., 1992).

2.1.2.3. Effect of biological factors

2.1.2.3.1. Host plant

The presence or absence of a host plant obviously plays an important role regardless colonization and subsequent sporulation will occur. Non-host plants such as *Chenopodiaceae* and *Brassicaceae* species can become minimally colonized by VAM fungi, particularly when grown in the presence of host plants. The presence of non-mycorrhizal plants has resulted in reduced colonization of mycorrhizal host plants possibly because of toxic nonhost exudates.

In contrast, detected no reduced colonization of mycorrhizal host plants cropped together with nonmycorrhizal hosts. In fact, onions became more colonized when grown with nonhost swedes than when grown alone, and similar results were observed in barley cropped together with rape. An increase in root colonization might not be unexpected.

The crop species itself can exert a selective effect on which VAM species in a mixed indigenous population become predominant; it is well illustrated in a study. The researchers found marked differences in populations of VAM fungi among different crops grown for 7 years in monoculture.

Although VAM fungi have extremely wide host ranges, the existence of host preference has been suggested by many researchers. The preferential association between certain plant and fungal species can be evaluated with respect to combinations that provide the greatest plant growth stimulation. At present, we do not have a good explanation for the variation in mycorrhyzal dependency of different host plants. One possibility is that plants with coarse and relatively fewer hairs are more dependent on mycorrhiza compared to those plants with fine roots and long root hairs. The mycorrhizal colonization could be treated as a genetic trait. Like resistance or susceptibility of crop plants to infection by fungal pathogens. Reduction in plant size by pruning and defoliation of plant can decrease mycorrhizal root colonization and sporulation (Bharat et al., 1992).

2.1.2.3.2. VA mycorrhizae fungial efficiency

VAM fungi are not always equally infectious to any one plant species, and they certainly vary in their physiological interactions with different plants and hence in their effects on plant growth. Species and strains of VAM fungi have been shown to differ in the extent to which they increase nutrient uptake and plant growth. These observations have led to introduction of the term 'efficient' or 'effective' strains. Generally, those fungi that infect and colonize the root system more rapidly are considered to be 'efficient' strains. The ability to form extensive external hyphae and the ability to absorb p from soil solution and improve plant growth are also important in determining the effectiveness of VAM endophytes. Comprehensive studies are needed with as many species and strains as possible to see whether 'better' ones exist in nature or can perhaps be created by techniques like genetic manipulation (Bharat et al., 1992).

2.1.2.3.3. VAM Fungal dormancy

Dormancy periods were much shorter in dry soils than in most soils. Dormancy can be broken by desiccation or cold treatment. The short dormancy periods could prevent spores from germinating

immediately after formation around the root; such germination would thwart their roles as agents for later crops. Long dormancy may have operated to select such a population of VAM fungi. More studies on dormancy of VAM fungi and its ecological implications are needed (Mukerji et al., 1992).

2.1.2.3.4. Other soil microorganisms

Out of the various microorganisms colonizing the rhizosphere, VAM fungi accupya has unique ecological position, as they are partly inside and partly outside the host.

The part of the fungus within the root does not encounter competition with other soil microorganisms. This advantage enables them to achieve a more functional biomass in intimate contact with the root and thus increases their chances of exerting a greater effect on plants. The different aspects of biological interactions with VAM fungi have been reviewed.

VAM markedly improve nodulation and nitrogen fixation by legume bacteria, mainly by providing the high phosphorus requirement for fixation process. Mycorrhizal colonization also allows introduced populations of beneficial soil organisms like Azotobacter and phosphate-solubilizing bacteria to maintain higher numbers than around non mycorrhizal plants and to exert synergistic effects on plant growth. Generally, VAM decrease the severity of root diseases. The various mechanisms of suppression of pathogens by VAM has also been reviewed. Research on parasites and predators of VAM fungi should be intensified, as they will probably play an important role in the successor failure of VAM inoculum trials in the field, as research should take place on survival and persistence of indigenous and introduced endophytes.

At start, experiments could be done with plants important in agriculture, horticulture, and forestry, which are usually raised in nursery and then planted in the field. As only small quantities of the mycorrhizal inoculum would be required, the method could be applied immediately to practical farming without much difficulty (Knudsen et al., 1992).

2.1.3. The role of VAM fungi in plant growth

The role of a symbiotic relationship between VAM fungi and plant growth are responsible for increasing the development and protection of plant situation.

2.1.3.1. Nutrient uptake

The nutrients phosphorus, nitrogen, zinc, copper, and sulphur have been shown to be absorbed and translocated to the host by mycorrhizal fungi . In addition, the fungus appears to be able to absorb phosphorus at lower solution concentrations than an uninfected plant root.

Mycorrhizal fungi have active phosphatase activity. The phytate, an organic phosphorus source, is decomposed by mycorrhizal fungi, and then the phytate phosphorus is take up. In such soil, phosphorus uptake is often limited without the MF. Generally, positive plant growth responses to mycorrhizal infections are found in soils in which the concentration of a nutrient such as phosphorus is in low concentration in the aqueous phase, but the soil has a reserve of absorbed or other soil forms.

The mechanism of phosphorus translocation within the mycorrhizas is believed to be due to cytoplasmic streaming. Poliphosphate makes up a significant portion of the phosphorus of mycelial strands, and it could be translocated via streaming.

The effects caused by nitrogen have not been noted as often as have those of phosphorus. However, experimentation with nitrogen is not as straightforward as with phosphorus. It has been established that the uptake of NH_4^+ is facilitated by mycorrhizas, especially the ECM of forest trees. There are now two examples (one with ericacous mycorrhiza and one with VAM) of increased availability of otherwise nonutilized organic nitrogen with mycorrhizal fungi. Only some of the mycorrhizal fungi appear to have nitrate reductase. It is a prerequisite for NO utilisation. Ammonium is incorporated into organic compounds prior to transfer out of the root region.

Zink and copper have been shown to be taken up by mycorrhizas; underdeficient conditions. In case of increased plant yields, the occurence of zink and copper deficiencies is not very common, and the deficiency can easily be overcome by the application of low levels of fertilizer, either as a foliar spray or as a soil application.

Heavy metal protection has been shown for plants growing on mine spoils in the presence of large concentrations of zink, cadmium, and magnese. Protection from magnese toxicity in a VAM-infected legume(vicia faba) growing on a high-magnese soil also has been demonstrated. It is thought that the heavy metals are bound by carboxyl groups in the pectic compounds (hemicelluloses) of the interfacial matrices, between the fungus and the host cells. It has also been demonstrated that plants growing on mine spoils with heavy metal contamination have mycorrhizas selected for greater resistance to these metals.

The transfer of nutrients to the plant is across the arbuscule in VAM, through the coiled hypha of orchids and ericoids, and through the Hartig intercellular net of ECM. The affect of mycorrhizae by the mechanism of nutrient uptake in plant growth as: changes in concentrations of growth-regulating compounds such as auxin, cytokinin, Gibberellins, and Ethylene). Photosynthetic retes increase and by change in the partitioning of photosynthetic to shoots and roots increased in the

nutrient absorbation and can change structural and biochemical aspects of root cells that can alter membrane permeability and thus the quality and quantity of root exudation; and increasing in the development of plant growth.(Linderman et al., 1991).

2.1.3.2. Resistance to pathogens

The possibility that antagonistic risosphere bacteria or fungi inhibit mycorrhizal fungi and therefore reduce their effectiveness has been proposed; and that the pathogen antagonist *Streptomyces cinnamoneous* reduced the sporulationand colonization of *Fasciculatum* on finger millet, if it was added 2 wk before the VAM fungus. Mycorrhizae exert a selective pressure on populations of soil microorganisms, some of which can antagonise root pathogens.

These results indicate that VAM fungi are relatively tolerant of antagonists that inhibit fungal pathogens by one or more mechanisms.(Arora et al., 1991).

They further suggest that VAM fungi, having evolved with plants, are highly rhizosphere-competent and are compatible with such antagonists and even function in concert with them. Because of the fact that VAM are major components of the rhizospheres of plants, it is logic that they could effect the incidence and severity of root diseases. The VAM can contribute to root disease suppression in a number of ways, and thus contribute to sustainable agriculture.

The most obvious VAM contribution to reduce root disease is to increase nutrient uptake (?) resulting in more vigorously growing plants in order to be able to ward off or tolerate root disease better. Plants with mycorrhizae may also better tolerate environmental stresses, such as drought, that could predispose them to greater fungal pathogen infections.(Mukerji et al., 1991).

2.2. Interaction between Rhizosphere Bacteria and VAM

2.2.1. Phosphate-solubilizing bacteria

Many bacteria produce organic acids and solubilize inorganic and organic forms of phosphorus that are unavailable to plants. VAM fungi, on the other hand, do not take up insoluble p, but more efficiently utilize labile forms. Regarding the possibility of an interaction between mycorrhizal fungi and phosphate-solubilizing bacteria (PsB) observations showed that asynergistic increase in dry weight and p uptake in maize grown in a low-p soil and dually inoculated with Endogone and p-solubilizing pseudomonas sp. and *Agrobacterium* sp. The study containing the above mentioned observations also showed an enhancement of population of PsB in the rhizosphere VAM-infected plants, although the effect disappeared after 2 months (Guy R. et al., 1991).

Nitrogen and phosphorus levels were also enhanced by the combination of the two organisms. The PsB (a combination of *pseudomonas* sp., *Agrobacterium* sp., and *Bacillus* sp.) increased the VAM infection of plants by indigenous VAM fungi. An interaction between PsB (*Bacillus megaterium var. phosphaticum* and *pseudomonas fluorescens*) and *Glomus macrocarpum* on papper was observed by Krone et.al., in 1991.

The dry weight of plants in the VAM+PsB treatments was higher than in treatments with either alone. VAM enhanced the population densitites of PsB in the rhizosphere, but PsB had no effect on VAM spore production; only VAM parameter was measured. Mycorrhizal infection was significantly improved by PsB inoculation at all levels of added phosphate. Utilizing 32 p form the added insoluble phosphate was improved by the VAM fungi, but not by PsB. The population of PsB declined until a nutrient solution was added to the soil, so the absence of growth-increasing might be due to the inability of the added PsB to grow and multiply in the soil. The interactions observed might be due to the production of plant hormones or vitamins by PsB, rather than to p solubilization.

The PsB produced indoleacetic acid, gibberellins, and cytokinins. Cell-free supernatant from liquid cultures of a PsB (*pseudomonas sp*.) enhanced the growth and VAM infection of tomato, alfalfa, and lavender; an effect mimicked by the addition of pure plant hormones.

Therefore, the possibility exists that a limited root microflora might have developed and contributed to phosphatase activity. Furthermore, the activity of these phosphatase-producing microbes might be enhanced by VAM infection (Dilip K. et al., 1991). These rhizobacteria behaved as 'mycorrhiza-helper' and enhanced root colonization by G. aggregatum in presence of tricalcium phosphate at the rate of 200 mgkg-1 soil (p1 level) giving higher productivity to palimerosa plant. All microbes inoculated together help in the uptake of tricalcium phosphate which is otherwise not used by the plants and their addition at 200 mg kg-1 of soil gave higher productivity to palmarosa plants (Ratti et al., 2001).

An investigation was carried out to assess the role what p solubilizing microorganisms play in the p nutrition of mycorrhizal and mycorrhiza-free Leucaena Leucocephala (Lam.). Soil microorganisms are able to solubilize k phosphate were isolated from the rhizosphere of L. Leucocephala naturally growing in three different soils of Hawaii.

The results suggest the existence of synergistic interaction between p solubilizing microorganisms and mycorrhizal fungi, although the degree of synergism was more pronounced in terms of p uptake than in terms of growth (Osorio. N. W. et al., 2001).

2.2.2. Free-living nitrogen fixers

The symbiotic nitrogen-fixing bacteria account for a majority of the biologically field nitrogen, some free-living bacteria also can fix nitrogen. Free-living diazotrophic species are found in the genera Azotobacter, Beijerinckia, Clostridium, pseudomonas, and Azospirillum. The ecology of nonsymbiotic dinitrogen fixers is reviewed by Subba Rao et al. in 1991. Azospirillum spp. are ubiquitous soil organisms capable of colonizing the roots of tropical grasses and C_3 plants compared to the VAM treatments without bacterial inoculation. Inoculation with A. brasilense or VAM fungi alone did not increase significantly plant growth, and A. brasilense had no effect on VAM infection. The same effects were seen in a low-phosphorus soil fertilized with superphosphate or rock phosphate.

The plants were fertilized with a nutrient solution without N or P (Bharat Rai et al., 1991). Regarding the close spatial association between mycorrhizal fungi and free-living diazotrophic bacteria, it must be stated that these bacteria might utilize p forming mycorrhizal fungi, or mycorrhizal fungi might transport fixed nitrogen form the bacteria to the plant. Azospirillum brasilense has been isolated not only from the rhizosphere, but also from the root cortex (endorhizosphere). This opens up the possibility of a direct interaction between a VAM fungus and Azospirillum within the plant. Interactions might also occur via the production of plant hormones by Free-living nitrogen fixers. Tien et al., in 1991 suggested that plant hormone production by A. brasi might account for the increased growth of inoculated pearlmillet. Azobacter vinelandii and A. beijerinckii stimulated plant growth and VAM infection of lavender, tomato, and alfalfa inoculated with G. mosseae. The same effects were mimicked by the addition of a combination of gibberellic acid, kinetin, and IAA. The involvement of plant hormones, especially cytokinins, in mycorrhizae has been suggested by many researchers.

The production of cytokinins by both ectomycorrhizal and VA mycorrhizal fungi has been documented. The stimulation of VAM infection and plant growth by some free-living nitrogen fixers could be due to a hormonal interaction, but more work is needed to prove this hypothesis definitely.

2.2.3. Plant growth promoting bacteria (PGpR)

The PGpR are rhizosphere-inhabiting bacteria that have a beneficial effect on plant growth. Exactly how beneficially rhizobacteria promote plant growth is not totally understood, but they may produce antibiotics or siderophores that suppress minor plant pathogens or produce phytohormones or other growth-enhancing compounds that directly affect the plant.

These rhizobacteria may also interact with mycorrhizal fungi to produce the growth-enhancing effects by as yet unknown mechanisms (Dilip K. et al., 1991).

The involvement of phytohormones in both ecto and VA mycorrhizae has been suggested by many studies. These hormones may be produced by the mycorrhizal fungi themselves or by the plant in response to mycorrhizal colonization. In case of Indole-3-acetic acid (IAA) production by ectomycorrhizal, the bacteria were grown in high concentration of tryptophan. The dichotomous branching and other morphological changes that occur in mycorrhizal roots are induced by the production of auxin by the ectomycorrhizal fungi, suggesting that ethylene, possibly induced by endogenous auxin or auxin produced by the mycorrhizal fungus, was actually responsible for inducing dichotomous branching (Mukerji et al., 1991).

2.3. Diseases of tomato plant caused by soil borne pathogens

Tomato is one of the most important vegetable in the world; and this vegetable is infected by several diseases causing enormous damage. These diseases are caused by parasitic fungi, bacteria, mycoplamsas, viruses, nematodes, and environmental conditions.

2.3.1. Fungal diseases (Damping-off and late damping-off)

The damping-off occurs primarily in the seed bed, when it is too cool and too wet. This disease is caused by various soil-inhabiting fungi, especially *Pythium sp.* and *Rhizoctonia solani* in late damping-off disease; although the affected seedling usually topples over, it dies slowly because the water-conducting vessels remain functional for several days.

Late damping-off is usually caused by *Rhizoctonia solani*, which attacks somewhat older plants in the seed bed as well as transplants in the ground bed (Jarvis W. R. et al., 1991).

The fungi associated with collar rot are *Sclerotinia sclerotioum, S. minor, Botrytis cinerea, Phytophthora parasitica, Alternaria solani,* and *Rhizocotonia solani*. Bacteria may also attack the stem at ground level irrespective of the causal organism. The disease appears as a canker, or rot of the stem at or above the soil line when the stalk becomes girdled, the plant wilts and dies (Agrios, 1989; Jarvis, W.R., 1991).

Anthracnose, caused by *Colletotrichum coccodes*, is a disease of ripe fruit that, if uncontrolled, causes serious losses in the early fresh market. The early symptoms appear as small, slightly sunken circular spots. The spots are usually most numerous on the side of the fruit adjacent to the soil (McKeen, C.D., 1991; Agrios, 1989).

Wilt disease is caused by *Verticillium* albo-atrum and V. dahliae. The symptoms of wilt caused by the two species differ in degree but not in type. The causal fungi inhabit the soil and enter the plants through the roots. From the roots they advance through the woody tissue, where they usually

cause a brownish discoloration in the vascular system; greenhouse plants become infected during January, February, and early March show wilting of several leaves, which become characteristically patterned with bright autumnal colours - yellow and brown (Jarvis, W.R. et al., 1991).

Fusarium wilt is a soil-borne disease caused by *Fusarium oxsporum f. sp. Lycopersici*, which enters the plant through the roots, the fungus grows up through the woody tissue and produces toxic substances that cause the plant turning yellow, wilt and die (Jarvis, W.R., 1991).

Fusarium crown and root rot, caused by *Fussarium oxysporum* f. sp. radicisly copersici is a destructive disease of greenhouse crops. The symptom of fusarium crown and rot is the thinning of the stem at the top of plant when it is 1-2 m high. The wilt progresses from to bottom, till the plant is almost dead. The disease is usually characterised by a remission of wilt becoming clear when the base of the stem is examined at or just below soil level (Jarvis, R., 1991; Agrios, 1989).

Buckeye rot is caused by *Phytothora parasitica*. The symptom of buckeye rot is seen on the fruit. This disease may also produce lesions on the stems and leaves as well as damping-off of seedlings. The seedlings may be killed either before or shortly after the emergence of leaf symptoms appearing as irregular brown lesions on the lower leaves. Either green or ripe fruit, especially when it contacts soil, may become infected through the uninjured skin. Firstly a small, greyish brown spot appears; as it enlarges, dark greenish tan to brown zones appear in the spot (Agrios, 1989).

Soil rot is caused by *Rhizoctonia solani*, which affects the fruit of tomato. Soil rot also tends to occur after overhead irrigation. Some fungus also cause damping-off and girdling of stems of seedling plants (McKeen, C.D., 1991).

Black dot root rot is caused by *Colletotrichum coccodes*, and is characterised by black pin point dots on weak and rotting root systems. The dots are the fruiting bodies of the causal fungus (Agrios, 1989).

Corky root rot caused by pyrenochaeta lycopersici is a disease of greenhouse crops. It has frequently been confused with black dot root rot because the fruiting bodies of *Colletotrichum coccodes.* The first signs of corky root rot are elliptical light-brown areas up to 5 mm long, on the thin roots (McKeen, D.C., 1991).

Feeder root rot disease is caused by genus pythium and cause the destruction of tiny feeder roots (Jarvis, W.R., 1991).

2.3.2. Bacterial diseases

Bacterial canker is caused by *Clavibacter michiganense* ssp. The seedlings are infected occasionally, but they often show no prominent symptoms only several days after being

transplanted to the greenhouse or the field. Wilting of the leaflets is the first symptom. During the hot summer months, the infected plants show the greatest capacity to survive. Also, during July and early August in Ontario, leaf symptoms may appear only as brown areas between the veins. In the early stages of infection the bacteria move through the water and food-conducting strands of the plant stem.

Bacterial canker may hold over from one season to the next on crops infected by plant debris in the soil or by pots, flats, and other containers.

Bacterial stem rot is caused by a soft-rotting bacterium - *Erwinia carotovora ssp. carotovora*, and typically appears in greenhouse tomatoes when the fruit is first picked. The lower leaf scars are surrounded by dark brown lesions. The bacterium may be presented in the soil, and can be splashed on to deleafing wounds by drips from the roof. It can also be carried in irrigation water from wells and outside reservoirs. This bacterium spreads by contact with contaminated hands and tools as well.

Bacterial spots caused by *Xanthomonas campestris pv. vesicatoria* is characterised by spotting on the leaves, stems, and fruit. The most conspicuous spots are on the fruit. The disease spreads during heavy rains. *Xanthomonas vesicatoria* attacks peppers as well, producing lesions, similar to those of tomatoes, on the fruit and leaves.

Bacterial speck is caused by pseudomonas syringae pv. tomato. In recent years, this disease has been much more prevalent than bacterial spot. this disease is the most noticeable on fruit, where it causes numerous dark brown spots, infection occurs most abundantly after heavy rains that splash the bacteria to all parts of the plant.

2.3.3. Tomato plant diseases in Libya

- Bacterial spots, caused by *Xanthomonas campestris pv. vesicatoria,* characterised by spotting on the leaves, stems, and fruit. This disease is very serious in tomato vegetable crop in Libya.
- Southern bacterial wilt; caused by pseudomonas Solanacearum. The affected plants are wilt suddenly and may die. (soft sour)
- Early blight of tomato; caused by Alternaria Solani and cause a serious loss of leaves in some seasons, circular dark brown to black spots of various sizes appear on the leaves and fruit pedicels. It is the most serious disease resulting high level of damage.
- Collar rot of tomato caused by A. alternato. The disease appears as a conker, or rot of the stem at or above the soil line, when the stalk becomes girdled, the plant wilts and dies.

- Late blight on tomato plant, caused by phytophtora infestans. It infects tomatoes under favourable environmental conditions. The disease causes severe defoliation of tomatoes and a destructive rot of the fruit.
- Fusarium wilt of tomato, caused by Fusarium oxysporum. This disease is transferred to the field by seedlings grown in infected soil may contact the disease. However, often they do not show symptoms only after being transplanted.
- Damping-off, on leaf blight, stem conker, and buckeye rot of fruit of tomato. These diseases occur primarily in the seedbed. This disease is caused by various soil-inhabiting fungi, especially phytium sp. The seedlings may be attacked either before they emerge, causing reduction in emergence, or after they emerge cause the plant's wilt, falling over and death.
- Concerning sclerotinia disease the fungus sclerotinia sclerotiorum and some related species over winter as sclorotia on or within infected tissues or as sclerotia that have fallen on the ground and as mycelium in dead or living plants.

The control methods were applied to control all the above mentioned diseases, by using the following methods: pesticides, sterilization of soil in greenhouses, grow resistant cultivars, removing and destroying the first and old affected leaves, roots and fruits from greenhouses, practising crop rotation, avoiding overhead irrigation, if it is possible.

2.4. Effect of pesticides on VAM fungi symbiosis

The effect of pesticides on VAM fungi exhibit a range of activity toward VAM fungi. Many pesticides contain heavy metals, and the presence of such metals may be responsible for poor germination of these fungi.

2.4.1. Effect of fungicides intensity of VAM fungi

Two effects of fungicides in intensity of VAM fungi are to be mentioned, one inhibits, the other stimulates. The Benomyl, Captan and (PCNB) were tested for their effects on the germination and early hyphal growth of the AM fungi *Glomus etunicatum, G. mosseae* and *Gigaspora rose* in a silty-clay loam soil placed in petri plates. The application of these fungicides inhibited spore germination of the three AM-fungi - Glomus etunicatum, G. mosseae andGigaspora rosea (Schreiner, R.P. et al., 1997). The application of fungicides (prochloraz + Carbendazim, Fenpropimorph, propicorazole, Triadimenol and Anilazine) resulted in minor increases n sporulation in soil (Land, S. et al., 1993). Both - the benomyl and PCNB reduced sporulation by this

fungus (Schreiner, R.P. et al., 1997). The effects of disinfecting agents (Sodium hypochlorite, chloramine, T. mercurium chloride and formaldehyde) and antibiotics (streptomycin, gentamycin, pimaricin and chloramphenicol) were studied on germination of gigaspora margarita and Scutellospora heterogama spores and on incidence of fungal and bacterial contaminants.

Sodium hypochlorite at 0.5 or 1% for 12 min eliminated spore, and formaldehyde and mercurium chloride highly reduced VAM spore viability. Antibiotics reduced VAM spore germination (Colozzi - Filho - A, et al., 1994).

The effects on the crop plant are increased nitrogen (N) fertilization, and fungicide application on the pattern of VA mycorrhizal fungi spore population. The effects of increased N fertilization and fungicide application were investigated on winter wheat in Landreder in 1988 only. The spore density and the MPN increased until harvesting, different spore types increased or decreased according to the plant species; increased N fertilisation slightly inhibited mycorrhizal infection and sporulation on winter wheat, while both - the leaf and the base application of fungicides (prochloraz + carbendazim, fenpropimorph, propiconazole, triadimenol and anilazine) resulted in minor increases in mycorrhizal colonization of roots and sporulation in soil (Land, S., et al., 1993).

2.4.2. Effect of fungicides in colonization

Concerning the effect of fungicides in colonization, they have significant side-effects on the infection ability of colonization. Benomyl, PCNB and captan applied at 20 mg a. i. / kg soil did not effect the root length colonized by *Gigaspora rosea* at 48 and 82 days after transplanting. PCNB also reduced *Glomus mosseae* colonized root length at 48 and 82 days, captan reduced the root length colonized by Gigaspora rosea at 48 days, but not at 82 days, and reduced colonization by *Glomus mosseae* at 82 days, but not at 48 days (Schreiner, R.P., et al., 1997). The fungicides (prochloraz + carbendazim, fenpropimorph, propiconazole, triadimenol and anilazine) resulted in minor increases in mycorrhizal colonization of roots (Land.S. et al., 1993).

Two systemic, anti-oomycete fungicides were tested for their effects on colonisation of leek (Allium porrum) roots by *Glomus* in traracides. Foliar application of the symplastic fungicide fosetyl-Al at concentration of 0.3, 1.0 or 3.0 mg a.i. ml-1 to inoculated leek significantly increased colonization by *Glomus intraracides* (Jabaji-Hare-SH et al., 1987).

Fosetyl-Al reduced growth, especially of roots, and also inhibited mycorrhizal colonization. Dimethyl phosphorate Hnium chloride did not effect the growth of non-mycorrhizal or mycorrhizal plants, or mycorrhizal colonization (Sukarno,N. et al., 1998). After the fungicides applied (Benomyl, propiconazole and fenpropimorph) inhibiting internal and external hyphae; the results showed that the external hyphae were more sensitive than internal hyphae to application of

fungicides (Kjoller, R., 2000). The effect of the fungicide on the AM symbiosis was confirmed on roots of plant ago oanceolata. The direct evaluation of frequency of AM fungal structures in plant species to phosphate application is by Smilauer,P., 2000) - While the fungicide had no effect on ericoid mycorrhizal colonization of root, or symbiotic function inferred from plant N-15 natural abundance (Michelsen,A., 1999).

Metalaxyl and propamocarb are two fungicides added to potting substrates to prevent diseases caused by phycomycetes. The AM fungus used were *Glomus mosseae*. Metalaxyl adversely affected root colonization by *G. mosseae* and decreased rhizosphere activity as measured by esterase activity (Fontanet,., et al., 1998).

The fungicides Terrazole and Terraclor initially inhibited mycorrhizal infection of root of cotton. The inhibition disappeared after 4 weeks, and neither fungicide had a lasting effect (Pattinson, G.S., et al., 1997).

Benomyl and PCNB were AM fungi and fungicides were highly variable; the biological responses depended on fungus fungicide combinations and on environmental conditions (Schreiner, R.P., et al., 1997). Under controlled environmental conditions to determine the effects of the three fungicides - Benlate (R), Aliette (R) and Ridomil (R) on efficiency of p uptake from the soil and transfer across the living plant funngal interface of onion plants (Allium capa. l.) associated with *Glomus sp.* Benlate reduced p inflow and transfer across the interface in one of the experiments. the rate of p uptake per m living external hyphae in the soil was reduced, the contribution of the fungus to p uptake was small. Aliette reduced growth of both shoots and roots, but apparentlyincreased the accumulation of p in the tissues compared with controls. Ridomil reduced p inflow per m of root and p uptake per m living external hyphae, but had no effect on the rate of p transfer across the interface. This led to a reduction in the overall contribution of the fungus to p nutrition (Sukarno, N. et al., 1996).

2.5. Biological control

Biological control means the reduction of the amount of inoculum or diseases producing activity of soil-borne plant pathogen accomplished by or through one or more organisms except man.

The use of microorganisms to control plant pathogenic fungi and bacteria is an active and promising area of research (Guy, R. et al., 1991). The VAM fungi may play role in biological control, but their efforts in combination with bacterial or fungal associates in the mycorrhizosphere have not been documented to any extent. The hypothesis that VAM and rhizosphere associates function in tandem to biologically controlling root diseases has been proposed by R. G. Linderman in 1991.

Mycorrhizae exert a selective pressure on population of soil microorganisms. Those mycorrhizosphere microbes can have specific effects on root pathogens. Biocontrol agents may antagonize not only plant pathogens but also mycorrhizal fungi, which would be self-defeating in terms of plant productivity. Biocontrol agents might also stimulate mycorrhizal spore germination and mycorrhiza formation through the production of growth-promoting substances or indirect effects on the host plant. (Knudsen et al., 1991).

As biological control systems develop for commercial applications, many questions must be answered regarding their interactions with mycorrhizae. At the same time, biocontrol agents should be developed that have neutral or positive effects on mycorrhizae, and vice versa.

By incorporating mycorrhizae into the development strategy of the biocontrol agent, either through selection and screening or genetic engineering, the maximum potential of the system to enhance plant growth can be realized (Dilip, K. et al., 1991). Most of the literature suggests that VAM fungi reduce soil-borne disease or the effects of disease caused by fungal pathogens reduced the disease damage and decreased the disease symptoms (Mukerji et al., 1991).

One hypothesis is that VAM fungi alter host physiology, thereby making the root more resistant to pathogens. VAM fungi influenced the phenol metabolism and lignification of tomato and cucumber root cells. It was speculated that deposition of lignin in the cell walls of the endodermis and stele increased resistance to Fusarium wilt. The localized sites on mycorrhizal onion roots appeared to become more resistant to pink root caused by pyrenochaeta terrestris, apparently by preventing the internal development of the pathogen by cell wall thickenings (callose or lignitubers). Mycorrhizal plants showed increased chitinase activity, which might be effective against fungal pathogens.

Development of biological control for plant diseases is accepted as a durable and environmentally friendly alternative for agrochemicals. Arbuscularmycorrhizal fungi (AMF), which form symbiotic associations with root systems of most agricultural, horticultural and hardwood crop species, have been suggested as widespread potential bioprotective agents. The ability of two AMF (*Glomus mosseae* and *Glomus* intraracides) to induce local or systemic resistance to *phytophthora parasitica* in tomato roots have been compared using a split root.

Glomus mosseae was effective in reducing disease symptoms produced by *p. parasitica* infection, and evidence points to a combination of local and systemic mechanisms being responsible for this bioprotector effect. The biochemical analysis of different plant defence-related enzymes showed a local induction of mycorrhiza-related new isoforms of the hydrolytic enzymes chitinase, chitosanase and beta-1, 3-glucanase, as well as superoxide dismutase, an enzyme which is involved in cell protection against oxidative stress. Systemic alterations of the activity against *phytophthora*

cell wall of root protein extracts also corroborated a systemic effect of mycorrhizal symbiosis on tomato resistance to *phytophthora* (Pozo M., et al., 2002).

Regarding the influence of moisture and pH on the efficiency of VA-mycorrhiza, it shall be mentioned that *Glomus mosseae* against Meloidogyne incognita on blackgram was studied under potted conditions. The moisture level of 40 to 70 % was found suitable for the mycorrhizal colonization and unfavourable for nematode multiplication. Among the moisture level tested 70 % moisture was found suitable for the well establishment of VAM to control root knot nematode. Higher moisture level (80-100%) was found detrimental to VAM fungus and due to poor establishment of VAM it resulted in increased nematode population. Among the different pH levels tested for interaction studies, pH 7 was suitable for better mycorrhizal colonization and spore production. (SanKaranarayanan, C. et al., 2001).

Two AMF isolated from a pyrethrum-growing region in Kenya were screened efficacy against anematode, *Meloidogyne hapla* in green houses. The fungi were identified at INVAM as (*Glomus etunicatum* "KS18" and *Glomus sp.* "Isolate KS14") significantly suppressed nematode population, growth and development by up to 54%, egg production by up to 75% and disease severity up to 57%. In addition, *G. etunicatum* and *M. hapla* were mutually inhibitory as root colonization by *g. etunicatum* was significantly reduced (up to 24%) by the presence of the nematode. The presence of the nematodes, on the other hand, did not significantly affect root colonization by isolate KS14 (J.W. Wacke, et al., 2001).

Many of the mechanisms described for VAM fungi also applicable to this group. Other mechanisms include the formation of Hartig net and mantle, which provide a physical barrier to the pathogen, production of antibiotics, induction of phenolic compounds and other inhibitory substances in the mycorrhizal root, and a change in root exudates available to the pathogen.(K.G. Mukerji et al., 1991).

The interaction of VAM fungi and fungal pathogens, resulting in the reduction of disease, has been recognised for 50 years. Several reviews have been written on this subject. (Knudsen et al., 1991).

The dissertant believes that VA mycorrhizae can contribute to root disease suppression in a number of ways, and thus contribute to sustainable agriculture.

The most obvious VAM contribution to reduced root disease is to increase nutrient uptake (p and others), resulting in more vigorously growing plants, being able better to ward off or tolerate root disease. Plants with Mycorrhizae may also better tolerate environmental stresses, such as drought, that could predispose them to greater fungal pathogen infections. (Bethlenfalvay et al., 1991).

3. Materials and Methods

3.2.2.2. Separating spores from soil

1. Weigh soil samples and determine their moisture content by oven drying (90°C overnight) a small subsample in a pre-weighed container. This will allow spore numbers to be expressed relative to dry soil weight. You may wish to remove coarse debris and rocks from samples with a 2 mm sieve. Ensure that dry soil samples have been thoroughly wetted for at least 30 min before sieving them.
2. The size of soil samples that can be processed will depend on spore numbers and soil texture. Generally less than 100 g of soil is best, but larger samples of up to 1 kg can be used, if care is atken to ensure that fine screens do not become clogged. A disperasnt, such as Calgon, can be used with clay soils, but this may kill spores. Vigorous washing with water may be necessary to free spores from aggregates of clay or organic materials.
3. Soil is mixed in a substantial volume of water and decanted through a series of sieves after allowing heavy soil practices to settle for a few seconds (Fig. 3.1.). This washing and decanting process is repeated until the water is clear. Roots and coarse debris are collected on a coarse (1000µm) screen, while spores are captured on one or more finer screens. Figure 3.1. shows an example where 80, 200 and 250 µm fractions from a soil are processed separately, but you may wish to use fewer fractions or other screen sizes.
4. The first water centrifugation is optional, but it does remove substantial amounts of floating organic debris (that would otherwise end up with the spores) from many soils. After this step the supernatant and floating debris are discarted. A small amount (± 100mg) of finely powdered kaolin clay can be added to water with the sievings to help to form a stable pellet during centrifugation. Make sure that the centrifuge is balanced before switching it on!
5. The second centrifugation step using 50% sucrose (1 min @ 2000 RPM) separates the spores (and any remaining organic debris) from denser soil components which form a pellet. Immediately after centrifugation, spores in the sucrose supernatant are poured onto the finest sieve (40-50µm) and carefully washed with water to remove the sucrose.
6. After rinsing the spores, they are washed onto a pre-wetted filter in a Buchner funnel before vacuum filtration (Fig. 3.1.). Glass fibre filters (Whatman GF/A) are superior to paper ones. These filters can be stamped with parallel lines 7 mm apart to separate microscope fields for spore counting. Filter papers with spores can be stored in inverted Petri dishes.

3.2.2.3. Spores staining

- After separating spores from soil samples, it is best to avoid the use of dispersants such as Calgon, as these can kill spores. For a fungal isolate 50-1000g of field soil is usually required - depending on the density of its spores in a soil.
- Healthy spores of uniform appearance are selected under the dissecting microscope. The spores can be picked up using a wooden dowel, forceps or a paint brush with vary fine tips and placed on filter paper triangles. Transferred to small filter paper triangles with a very fine paint brush or sharpened dowel. To initiate a pot culture 5-100 large or 50-500 small spores are used. Additonal spores of each type should be mounted in PvlG-A or Melzer's reagent-B or Mounted C (PvlG+Trypen blue) to observe diagnostic features with a compound microscope. These should be presented as a record of the spectes.

3.2.2.4. Spore staining

Reagents of spore mounting media

- Polyvinyl Alcohol-Lacto-Glycerol (PVLG) mountant "A"

- polyvinyl alcohol 8.33g
- distilled water 50ml
- lactic acid 50ml
- glycerine 5ml

24-32 centripose viscosity polyvinyl alcohol should be used and dissolved in water by heating (90°C) overnight.

- Melzer's reagent mixed 1:1 (v/v) with PVLG "B"

- iodine 1.5g
- potassium iodide 5g
- distilled water 100ml

- Mounting media "C"

- PVLG 50ml
- Trypan blue staining 50ml

- Mounting of spores:

- place a drop of mounting mixture "C" on a slide,
- some spores (of proper age) are placed into the drop,
- cover it slightly with cover slides,
- incubate it at 60°C for fortnight.

3.2.2.5. Root-clearing Technique

Using this technique, we chose several samples from the terminal parts of the primary roots 1°-3° lateral branches.(Brundrett et al., 1994).

The reagents are:

- 50% ethanol-water (root preservative);
- 10% KOH (w/v potassium hydroxide) dissolved in water;
- 0.03% w/v chlorazol black E in (CBE) in lactoglycerol (1:1:1 lactic acid, glycerol and water), dissolve CBE in water before adding equal volumes of lactic acid and glycerol;
- 50% glycerol-water (v/v) for destaining and storage of roots.

The roots cleared in a water bath by heating the KOH to 6-90°C. The time required for adequate clearing with this method varies widely. Roots from toung plants will usually only require 1-2 hrs. After clearing, roots are captured on a sieve and rinsed with water (or dilute acid) before transferring them in to the stining solution.

Post-clearing bleaching with alkaline hydrogen peroxide (0.5% NH_4OH and H_2O_2 v/v in water) effectively removes any phenolic compounds left in cleared roots. The time required for roots to discolour in this solution varies between samples. This procedure should be used cautiously because subsequent staining of mycorrhizal fungal hyphae will be reduced or may be eliminated.

3.2.2.6. Root staining (Trypan blue staining)

- The cleared roots were stained in trypan blue staining solution and heated at 90°C for 10 min.
- Roots were differentiated in lactoglycerol at 90°C for 5 min.
- Roots were removed into clear lactoglycerol for storation.

Arbuscularity of the AM colonisation

Fungicide	Duration of breeding (week)			
	2	5	10	15
			2.7	3.4
Benomyl	0	0	0	2.5
			5.9	2.8
			4.1	5.2
Captan	0	0	6.3	4.2
			4.8	4.6
		33.5	55.8	0.9
Ephosite	0	22.0	42.1	0.5
		31.7	49.2	0.2
			44.9	42.3
Propamocarb	0	29.0	39.1	48.7
		40.5	57.8	37.5
		37.2		
ben. + cap.	0	0	0	0
Eph + fo.	0	0	0	0
ben. + propc.	0	0	0	0
		29.3	34.5	48.0
control	0	16.8	40.7	31.6
		20.1	47.2	36.4

Frequency of infection:

Fungicide	Duration of breeding (week) 2	5	10	15
		3.9	6.6	12.7
Benomyl	0	7.6	8.9	15.0
		6.8	7.1	11.4
		7.0	8.5	13.6
Captan	0	8.7	9.7	15.4
		6.8	10.2	12.3
	4.6	19.7	84.2	72.7
Ephosite	2.3	35.4	71.7	87.1
	2.0	34.2	75.9	79.6
		13.8	10.2	28.5
Folpet	0	9.4	15.9	18.8
		9.7	10.6	12.1
	5.4	20.7	85.4	79.0
Propamocarb	1.9	34.6	72.8	86.7
	2.3	34.9	74.9	81.5
ben + cap	0	0	0	0
			2.9	9.3
eph + fo	0	0	6.4	2.9
			2.5	3.8
			2.7	7.7
ben + propc	0	0	6.9	3.6
			2.8	3.4
	3.5	30.6	68.3	83.5
control	7.4	42.8	81.0	86.0
	3.7	35.5	74.2	81.2

Intensity of mycorrhiza colonization (M%)

Fungicide	Duration of breeding (week)			
	2	5	10	15
			5.7	10.3
Benomyl	0	0	7.4	6.1
			9.0	7.2
		5.7	9.1	16.9
Captan	0	9.4	15.0	14.6
		6.5	10.7	12.1
	12.0	37.5	52.7	87.9
Ephosite	20.7	29.8	48.1	92.5
	17.5	43.4	62.3	85.4
			7.9	7.5
Folpet	0	0	5.0	6.6
			9.4	10.2
	11.5	44.7	62.6	85.5
Propamocarb	21.8	32.5	49.8	71.9
	18.4	35.0	53.3	87.4
ben + cap	0	0	0	0
eph + fo.	0	0	0	0
			2.4	5.6
ben + propc.	0	0	1.8	3.1
			2.3	3.6
	15.9	11.7	59.7	79.2
control	10.4	25.6	51.5	83.7
	9.8	29.4	60.4	76.9

Spore number:

Fungicide	Number of spores	Mean
	112	

Benomyl	**120** **149**	**127**
Captan	**116** **131** **147**	**131.3**
Ephosite	**215** **260** **293**	**256**
Folpet	**107** **112** **144**	**121**
Propamocarb	**378** **395** **460**	**411**
Ben + Cap.	**108** **124** **139**	**123.7**
Eph + Fo.	**79** **79** **148**	**102**
Ben + Propc.	**62** **68** **107**	**79**
control	**296** **323** **347**	**322**

Intensity of Mycorrhizae colonization	2	5	10	15
Control	12.0	22.2	57.2	79.9
Folpet+Efozite	0	0	0	0
Benomyl+ Propc.	0	0	2.2	4.1

Benomyl	0	0	7.4	7.9
Captan	0	7.2	11.6	14.5
Folpet	0	0	7.4	8.1
Efozite	16.7	36.9	54.4	89.6
Propamocarb	17.2	37.4	43.5	81.6

Infection Frequency	2	5	10	15
Control	5.8	36.2	74.5	76
Folpet+Efozite	0	0	3.9	5.3
Benomyl+Propc.	0	0	4.1	4.9
Folpet	0	11	12.2	19.8
Benomyl	0	11.2	12.1	21.8
Efozite	3	29.8	77.3	79.8
Propamocarb	3.2	30.1	77.7	82.4
Captan	0	7.5	9.5	13.8

Arbuscularity of colonization	2	5	10	15
Control	0	22.1	40.8	38.7
Folpet+Efozite	0	0	0	0
Benomyl+Propc.	0	0	0	0
Benomyl	0	0	2.8	2.9
Folpet	0	7.1	22.9	7.9
Efozite	0	29.1	49	34.9
Propamocarb	0	35.6	47.3	42.8
Captan	0	0	5.1	4.7

Fungicides	**Number of spores**
Control	322
Folpet+Efozite	102
Benomyl+Propamocarb	75
Benomyl	127
Folpet	121
Efozite	256
Propamocarb	411
Captan	131.3
Ben+Cap	123.7

5. DISCUSSION

5.1. Ecophysiological characteristics of rhizobacteria from tomato rhizosphere

It is well known in the special literature that the rhizosphere bacterial populations consist in majority of *Pseudomonas* species. As we isolated our strains from the rhizoplan of tomato roots it is not surprising that only a single Gram positive isolate appeared investigating the cultural the microscopic morphological and physiological biochemical characteristics of the Gram negative isolates we could concluded that six of them belonged to the species *Pseudomonas fluorescens* and two of them is to be ordered to *Pseudomonas putida*. On the bases of the distribution of *P. fluorescens* isolates in point of view of denitrification and pigment production two biotypes could be differentiated. From their physiological, biochemical features they are supposed to become a useful agent of rhizosphere inoculants.

5.2. Impact of arbuscular micorrhiza and rhizobacteria on the growth of tomato plant

Our experiments on the effect of soil inoculations resulted that both AM fungus *Glomus intraradices* and the rhizobacterium (RB) *Pseudomonas fluorescens* significantly stimulated the growth of tomato plants.

During the first two weeks no effect could be experienced because the fungal inoculant colonized the roots not so fast as the bacterial one, because the period infection of mycorrhizal hyphae are longer than the bacteria. The bacterial inoculant, on the other hand, with its fast colonization caused an initial stress for the roots. From the fifth week after the inoculation the stimulatory effect markedly occurred in plant growth. Bacterial hormones produced by *Ps. fluorescens* have a stimulatory effect on the activity of the internal hyphae. By this interaction the result of this inoculant increase the growth of tomato plants. All of this result to affect on ecophysiological character such as the increased root nutrient absorption, root respiration and the rate of photosynthesis. Where enhanced the growth of tomato plant.

5.3. Impact of arbuscular mycorrhiza and rhizosphere bacteria on the growth of tomato plant

Both the AM fungus *Glomus intraradices* and the rhizobacterium (RB) *Pseudomonas fluorescens* significantly inhibited the development of the disease tomato leaf–specks. When the seedlings were planted and inoculated with AM and RB in their age of two-leafs, so the preventive effect hardly appeared in the second leafs. The increasing surfaces of the third, fourth, fifth leafs more and more specks could developed in the uninoculated controls. The inoculation with AM as a single treatment significantly increased the resistance of tomato plants. The appropriate treatment with RB resulted a bigger resistance shown in

every generation of leafs. The combined inoculation with AM and RB resulted a very strong resistance in all parts of each experimental plants. In addition the resistance

maintained even in the further generation of the leafs. It is concluded that a highly effected biological control of the soil-born pathogen *Pseudomonas syringae* pv. *tomato* can be realized only with the combined application of arbuscular mycorhiza and rhizobacterium inoculants.

5.4. Impact of different fungicides on the arbuscular mycorrhiza of tomato plant

We investigated the effect of fungicides types of benomyl, captan, ephosit, folpet, propamocarb, a combination of benomyl plus captan, benomyl plus propamocarb and folpet plus ephosite on the common symbiosis arbuscular mycorrhiza fungi and rhizosphere bacteria of tomato plant. The impact of test fungicides are contraversion in the results. The effect of the chemical control have significant side effect as inhibitor or stimulation the interaction relation between mycorrhiza and rhizosphere bacteria. And increased or decreased the activity of mycorrhiza and RB in the soil or inside the root tissue. Our results in these investigations are discussion as that fungicides captan and folpet which belong to the chloro-alkyl-thio-phtalimides inhibit AM fungi in big dimension. This effect can be explained with their broad acting spectrum, since they contain N-S-bonds in their own molecules which can react with the thiol groups of fungal proteins, especially in the branching hyphal tips as haustoria. For this reason can they inhibit even the frequency of infection. Benomyl being the representative of the benzimidazol-type fungicides inhibits the development of microtubuls in the first stage of the cell division. This result suggest a bigger taxonomical distance between Glomales and Oomycetes. (The members of latter group are resistent to benomyl.)

The effect of ephosit which was the representative of the phosphit – type fungicides remained indifferent to the growth of AM. As it was experienced that its influence on tomato plant does not result any change in the plant fungus interaction, a big distance is to be suggested between the biological features of Oomycetes and AM fungi.

The applycation of the dimethyl amino alkyl carbaminate type fungicide propamocarb had a surprising result. A significant stimulation of the vegetative growth of AM fungi was experienced. This drug which does not contain any sulphur directly influence only the membran function of the plant cells (inclusive the Phytophtora cells) does not affect the cells of higher fungi. In addition the cells of Glomalean fungi, with their special interaction with root cells, can be stimulated by this compound.

With the combinations of antifungal and antioomyceta compounds a total inhibition of the growth of AM fungi was experienced. So that the strong inhibitory effect of the benzimidasol type benomyl and chloro-alkyl-thio-phtalimide type folpet was totally increased by the presence of propamocarb and ephosit. It is conclouded that the side effect of fungicides on AM can be specially dangerous in the case of their combinations.

6. SUMMARY

The our investigation have two big side, one of this side effect of the common symbiosis AM fungi and RB on plant growth and in resistance of disease such as (leaf speck). The other side for investigate the effect of fungicides on the commom symbiosis AM fungi and RB.

The results demonstrated that the effects of *Glomus intraradices* and *Pseudomonas fluorescens* have strong positive effect on tomato plant growth by changes in some ecophysiological characteristics, such as the weight and length of roots and shoot roots as (77% g and 160% cm) from control.

The result of effect the *Glomus intraradices* and *Pseudomonas fluorescens* on leaf specks used by *Pseudomonas syringae pv. tomato*, due to inhibit and reduce the pathogenisity and symptomes.

The investigation of fungides on AM fungi and RB to resulted in three different suggest as:

- **strong inhibitor on the AM fungi activity and on interaction between AM fungi and RB in soil and plant root tissue by fungicides (benomyl, captan, folpet and all combination).**
- **Negative effect by efosite type.**
- **Strong stimulater in activity of AM and the interaction with RB by propamocarb type.**
- **By this results we can suggested that, the interaction between mycorrhiza and rhizosphere bacteria can effect strongly on the plant growth by increase the nutrient uptaken, respiration and photosinthesis rate. The biological control method by use mycorrhiza we can achieved good result for control the soil-borne disease without any contamination. The chemical method have many side effect such as :**
- **Inhibit and reduce microflora in the soil.**
- **Contamination in soil is due to many problems with the plant, human and animals.**
- **Economical part for development the strategy of fungicides is very expensive.**

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