STUDYING THE PROBABLE INTERACTION BETWEEN SOME PATHOGENIC AND VAM LIKE-FUNGI UNDER LABORATORY AND GREENHOUSE CONDITIONS

By

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Title: Studying The Probable Interaction between Some Pathogenic and VAM Like-Fungi under Laboratory and Greenhouse Conditions

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ABSTRACT

Mycorrhizal associations are vary widely in structure and function, but the most common interaction is the vesicular arbuscular mycorrhizal (VAM) symbiosis. This study targeted to isolate the VAM-like, the root endophytes, fungi from healthy roots of lettuce and wheat plants, investigation their antagonistic effects against the causal agents of the tomato fusarium wilt disease, *Fusarium oxysporum* f. sp. *lycopersici* (FOL), and the root-rot disease of eggplant, *Sclerotium rolfsii* (SR). The promotion effects of the isolated root endophytes on growth of wheat plants were investigated. Also, their capabilities to inducing resistance against infections with FOL and SR in plants of tomato and eggplant respectively were studied.

We can concluded that, isolation of the VAM-like fungi from healthy roots of field grown crop plants and applying their inoculants at 1 or 2% particularly under greenhouse conditions resulted in significant improvement in plant growth and increased their resistance against soil-borne pathogens.

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Abbreviations

LVF	Like-VAM fungi
FOL	*Fusarium oxysporum* f. sp. *lycopersici* (Pathogen)
SR	*Sclerotium rolfsii* (Pathogen)
W	LVF isolated from wheat (W) roots
L	LVF isolated from lettuce (L) roots
1%A	LVF inocula used alone at 1% level
1%B	LVF inocula used at 1% level before pathogen-inoculation
1%C	LVF inocula used at 1% level simultaneously with pathogen inoculation
1%D	LVF inocula used at 1% level after pathogen-inoculation
2%A	LVF inocula used at 2% level alone
2%B	LVF inocula used at 2% level before pathogen-inoculation
2%C	LVF inocula used at 2% level simultaneously with pathogen inoculation
2%D	LVF inocula used at 2% level after pathogen-inoculation
3%A	LVF inocula used at 3% level alone
3%B	LVF inocula used at 3% level before pathogen-

	inoculation
3%C	LVF inocula used at 3% level simultaneously with pathogen inoculation
3%D	LVF inocula used at 3% level after pathogen-inoculation
SFW	Shoot fresh weight
SDW	Shoot dry weight
RL	Root length
RFW	Root fresh weight
RDW	Root dry weight
PO	Peroxidase
PPO	polyphenol- oxidase
N	nitrogen
P	phosphorus
K	potassium

INTRODUCTION

Mycorrhizal associations vary widely in structure and function, but the most common interaction is the vesicular arbuscular mycorrhizal (VAM) symbiosis. This interaction is formed between the roots of over 80% of all terrestrial plants include many agriculturally and horticulturally important crop species, and Zygomycetes fungi from the Order Glomales **(Smith and Read, 1997)**. These fungi are obligate symbionts which form endomycorrhizal symbioses (**Frank *et al.*, 1987**) which conferes benefits directly to the host plant's growth and development through the acquisition of P and other mineral nutrients from the soil by the fungus. In addition, they may also enhance the plant's resistance to biotic and abiotic stresses. This relation benefits the plant growth by enabling a greater proportion of available nutrients in the soil to be absorbed into the plant. The fungal partner gets photosynthetic sugars as food from the plant which in turn acquires an array of benefits ranging from better uptake of phosphorus and relatively immobile micronutrients like zinc and copper (**Harrier, 2001**).

The beneficial effects of the VAM symbiosis occur as a result of a complex molecular dialogue between the two symbiotic partners. Identifying the molecules involved in the dialogue is a prerequisite for a greater understanding of the symbiosis.

The mycorrhizal fungi protect the plant from higher tolerance of high soil temperatures and root-borne pathogens.

These fungi are potential 'biofertilizers' and 'bioprotectors' to enhance plant growth, yield (**Bagyaraj and Verma, 1995**). The endomycorrhiza can modify the root architecture to give a root system, which is better adopted for uptake of mineral nutrients and water as well as increasing hormone production (**Shende and Rai, 2010**).

Ultimately, all advances in our knowledge of monoxenic VAM fungi will be useful for the large-scale production of reliable, microbiologically clean inocula. This is likely to permit the reduction of production costs and make VAM fungal inocula as widely available as rhizobia have been for decades. There is little doubt that a more general use of VAM fungal inocula in agriculture could substantially increase financial support for research on this fundamental and universal phenomenon in all natural and managed terrestrial ecosystems.

Boswell *et. al.*, (1998) stated that the autumn-sown winter wheat cover crop increased VAM fungal inoculum potential of a field soil as measured by an in situ maize bioassay during the following growing seasons. Moreover, the mycorrhizal infection degree of maize was correlated with maize growth and yield. They suggested that the management of mycorrhizal fungi by cover cropping may be considered a useful practice in sustainable agriculture.

Thus, this work aimed to isolate the VAM-like fungi from healthy roots of lettuce and wheat plants, investigating the mutual interactive effects between the isolated VAM-like fungi and some plant pathogens *i.e.* the specific (*Fusarium oxysporum* f. sp. *lycopersici*) 'FOL" and the omnivorous (*Sclerotium rolfsii*)

"SR" plant pathogenic fungi in mutual cultures [*in vitro*], Identification and detection of VAM-like fungi using specific primers, investigating the impact of application of different levels of inoculants of the VAM-like fungi on percentages of survived plants, plant height, number of leaves, fresh and dry weights of plant shoots and roots and root lengths in tomato and eggplant plants under greenhouse conditions under stress of infection with FOL or SR, the agents of tomato fusarium wilt and the eggplant root rot diseases, respectively, to determine activities of some oxidative enzymes: chitinase, peroxidase and polyphenol oxidase in both culture filtrates of tested fungi (VAM-like and plant pathogenic fungi) and in shoots of treated plants of tomato and eggplants, to determine the total photosynthetic leaf pigments in addition to the nitrogen (N), phosphorus (P) and potassium (K) contents in shoots of the treated plants.

REVIEW OF LITERATURE

1- The vesicular arbuscular mycorrhizal (VAM) fungi:

Root of most plants form a symbiotic relationship with certain kinds of zygomycetes, ascomycetes and basidiomycetes fungi and the infected roots are transformed into unique morphological structures called mycorrhizae **(Azcon-Aguilar and Barea, 1997; Agrios, 2005)**. The way of the hyphae of the fungi are arranged within the cortical tissues of the root determine the type of the mycorrhizae, namely ectomycorrhizae (intercellularly) or endomycorrhizae (intracellularly **(Agrios, 2005).**

Endomycorrhizae are the most common and their fungal hyphae grow in cortical cells of the feeder roots with specialized feeding hyphae, called arbuscules, or feeding-storing hyphal swellings called vesicles **(Agrios, 2005)**. Some endomycorrhizae contain both these hyphae and are called vesicular arbuscular mycorrhizal (VAM). The mycorrhizae benefit from gaining organic nutrients from the plant and in turn the plant benefits by enhanced water and nutrients uptake, increased growth and yield and protection against soilborne pathogens **(Harley and Smith, 1983 and Linderman, 1994)**.

Prasad *et al.* (2008) recorded that the most common and prevalent VAM fungi play an indispensable role in upgrading plant growth, vigor and survival by a positive impact on the nutritional and hydratic status of the plant and on soil health by increasing the reproductive potential, improving root

performance, and providing a natural defense against invaders, including pests and pathogens. The described species of arbuscular mycorrhizal fungi mainly belong to Zygomycetes placed in the order Glomerales. However, the growing of arbuscular mycorrhizae in pure culture in the absence of living host roots is a matter of global concern. Unfortunately, their biotechnological applications cannot be exploited to the level they deserve due to their axenically unculturable nature. The Glomeromycota have generally coenocytic (occasionally sparsely septate) mycelia and reproduce asexually through blastic development of the hyphal tip to produce spores (Glomerospores) **(Schüßler *et al.*, 2001a)** with diameters of 80-500µm **(Simon *et al.*, 1993).** In some, complex spores form within a terminal saccule **(Schüßler *et al.*, 2001b)**.

Abdel-Fattah and Mohamedin (2000) stated that the VAM-inoculation significantly increased the growth, photosynthetic pigments, total soluble protein and nutrient contents of sorghum compared to non-mycorrhizal sorghum. Analysis of the content of total amino acids and ammonia in leaves on the basis of dry matter production showed that, in most instances, total amino acids of mycorrhizal plants were significantly higher than those of non-inoculated plants. The microflora of the rhizosphere was highly affected by mycorrhizal inoculation. Quantitative changes in acid and alkaline phosphatase activities of the roots in response to the mycorrhizal inoculation are discussed.

Dixon *et al.* (1987) studied the influence of vesicular-arbuscular mycorrhizal (VAM) symbiosis on the transport of

cytokinins from the root to the shoot of *Citrus jambhiri* seedlings inoculated with cultures of *Glomus etunicatum*, *G. fusciculatum*, or *G. mosseae*. Seedlings inoculated with *G. fasciculatum* or *G. mosseae* yielded a greater flux of zeatin, dihydrozeatin and zeatin riboside than non-inoculated seedlings. VAM relationships apparently contributed to, or influenced, the export of cytokinins from the root. The elevated cytokinin flux of inoculated seedlings was associated with improved tissue phosphorus nutrition and a significant increase in seedling biomass.

Almeida and Schenck (1990) established the genus *Sclerocystis* by Berkeley and Broome in 1873 with their description of *S. coremioides* and since then 13 additional species were established. Although some consider *Sclerocystis* allied to the genus *Glomus*, it is concluded that the 2 genera are distinct based on spore ontogeny and sporocarp habit. In *Sclerocystis*, spores are arranged in a hemispherical layer, forming a "head" and a short stalk; no spores are formed at the sporocarp base. Sporocarpic species in *Glomus* exhibit several other spore arrangement patterns. The genus *Sclerocystis* is maintained with one species, *S. coremioides. Sclerocystis coccogena, S. dussii* and *S. alba* are considered synonyms of *S. coremioides.* Five other *Sclerocystis* species are moved to the genus *Glomus*, *G. clavisporum, G. liquidambaris, G. rubiforme, G. sinuosum* and *G. taiwanense.* The remaining 5 described species of *Sclerocystis* are considered synonymous with one of the latter species.

The symbiotic association between the VAM fungi benefits the plant growth by enabling a greater proportion of available nutrients in the soil to be absorbed into the plant. The fungal partner gets photosynthetic sugars as food from the plant which in turn acquires an array of benefits ranging from better uptake of phosphorus and relatively immobile micronutrients like zinc and copper (**Harrier, 2001**). The endomycorrhiza can modify the root architecture to give a root system, which is better adopted for uptake of mineral nutrients and water as well as increasing hormone production (**Shende and Rai, 2010**).

The mycorrhizal fungi protect the plant from higher tolerance of high soil temperatures and root-borne pathogens. These fungi are potential 'biofertilizers' and 'bioprotectors' to enhance plant growth, yield (**Bagyaraj and Verma, 1995**).

2- Molecular identification of VAM fungi using PCR technique:

PCR technique is widely used in assessing AMF diversity. PCR technique is prominent for its efficiency to amplify small quantities of the targeted nucleic acid sequence from extracts **(Schwarzott *et al.*, 2001; Van Tuinen *et al.*, 1998)**. However, PCR inhibitory substances in soils will interfere with amplification and subsequent analysis **(Van Tuinen *et al.*, 1998)**, leading to misleading amplification. Sample purification by polyvinyl polypropylene (PVPP) **(Berthelet *et al.*, 1996)** or dilution of samples **(Schwarzott *et al.*, 2001)** can be employed to reduce the inhibitory effect.

Recently, sequences of the gene coding for the small subunit rRNA (SSU) were obtained from 12 endomycorrhizal

fungal species, and comparisons with other 18S sequences indicated that the Glomales formed a phylogenetically coherent group that originated about 400 million years ago **(Bruns, 1992 and Simon *et al.*, 1993)**.

Simon *et al.* (1993) presented a method to identify arbuscular endomycorrhizal fungi based on the amplification of portions of the nuclear gene coding for the small subunit rRNA. By coupling the sensitivity of the polymerase chain reaction and the specificity afforded by taxon-specific primers, a variety of samples can be analyzed, including small amounts of colonized roots. Family-specific primers as well as generic primers are described and can be used to amplify small subunit rRNA fragments from endomycorrhizal fungi by polymerase chain reaction. This technique should have obvious applications in the study of arbuscular endomycorrhizal fungi populations and allow closer examination of their host specificity.

Redecker *et al.* (2000) showed that phylogenetic analysis of the near-complete 18S rDNA ribosomal subunit from *Glomus sinuosum* (=*Sclerocystis sinuosa)* and S. *coremioides* reveal that both species are each other's closest relatives and fall within a mono- phyletic clade comprising the well-characterized species, *G. mosseae, G. inlraradices* and *G. vesiculiferum,* to the exclusion of several other *Glomus* species. This placement indicates that formation of complex sporocarps is an advanced character of some *Glomus* species, but the sporocarpic trait is not sufficiently unique to group these species into a separate genus *Sclerocystis.* Also, the majority of nucleic acid information derived from the Glomales is from

ribosomal DNA (rDNA), which code for ribosomal RNA (rRNA) **(Redecker, 2000)**. rDNA genes are present in multiple copies arranged in tandem arrays, with each repeat unit consisting of a small subunit (SSU) or 18S rDNA and a large subunit (LSU) or 28S rDNA gene separated by an internal transcribed spacer (ITS), which are located between the 18S and 5.8S rDNA coding regions (ITS1) and between the 5.8S and 28S rDNA coding regions (ITS2) **(Roderick and Edward, 1998)**. The coding regions of 18S, 5.8S and 28S rDNA genes evolved slowly, and are relatively conserved among fungi. The non-coding ITS1 and ITS2 regions evolved more rapidly, leading to sequence variability among genera and species of fungi **(Chen *et al.*, 2000)**.

Simon *et al.* (1992) designed the first specific PCR primer for AMF: VANS1, based on the data obtained from the 18S SSU sequences of ribosomal DNA using PCR fragments generated with universal eukaryotic primers. Later, **Simon *et al.* (1993)** designed other group-specific primers (VAGLO, VAACAU, VALETC and VAGIGG) to amplify unknown taxa from plant roots. As more SSU sequences became available, it was found that the VANS1 priming site was not well conserved in all groups of the AMF **(Clapp *et al.*, 1999; Redecker, 2000)**. The ITS region is extensively used for molecular taxonomy. ITS1 and ITS4, published by **White *et al.* (1990)**, were used to obtain sequence information from spores collected from the field **(Sanders *et al.*, 1995)** and to elucidate the relationship among AMF species **(Redecker *et al.*, 1997)**. Later, **Redecker, (2000)** designed group-specific primers for

five major phylogenetic lineages of AMF to amplify the highly variable ITS. The group-specific primers worked well for AMF collected in field **(Redecker *et al.*, 2003; Wubet *et al.*, 2003; Hijri *et al.*, 2006; Shepherd *et al.*, 2007)**. In my study, Redecker's group specific primers will be used to detect fungi from family level in specimens collected from field soil.

To reveal functional and ecological aspects of distinct AMF communities associated with different plants and/or under different environmental conditions it is essential to detect AMF communities in the field on the species level. However, there are as yet no unbiased methods for this purpose, not only for morphological identification but also for molecular methods. Principally, DNA sequence based methods are most useful for detecting organisms at different community levels, but for ecological work they also depend on reliable baseline databases and tools. For example, fingerprinting methods such as random amplification of polymorphic DNA (RAPD), inter-simple sequence repeat PCR (ISSR) and amplified fragment length polymorphism (AFLP) are expected to be error prone in uncharacterized environments because of too many 'unknowns' in the background, which hampers interpretation of specificity **(Mathimaran *et al.*, 2008)**. A similar problem exists for DNA array techniques. Nevertheless, suitable molecular methods are crucial to overcome the limitations of morphological identification **(Walker & Schüßler, 2004; Walker *et al.*, 2007; Gamper *et al.*, 2009; Stockinger *et al.*, 2009)**.

Molecular characterization of AMF is in most cases achieved by PCR on DNA from roots of host plants, spores or

soil samples. Several primers targeting the rDNA regions as molecular marker were claimed to be AMF specific. Most of these amplify only a restricted number of glomeromycota taxa or DNA of no target organisms. The most comprehensive taxon sampling for the *Glomeromycota* covers the small subunit (SSU) rDNA region (**Schüßler *et al.*, 2001a, b)**, for which a new, AMF specific primer pair was recently published **(Lee *et al.*, 2008).** Unlike the often used AMI primer (**Helgason *et al.*, 1998**) it is perhaps suitable to amplify sequences from all AMF taxa, but the SSU rDNA is inadequate for species resolution of AMF. Inclusion of the internal transcribed spacer (ITS) and the large subunit (LSU) rDNA region allows both robust phylogenetic analyses and species level resolution **(Gamper *et al.*, 2009; Stockinger *et al.*, 2009)**.

The available public database sequences are scattered through SSU, ITS and LSU rDNA subsets with varying lengths, often only 500—800 bp. In most cases this does not allow species level analyses, and short sequences obtained with primers that have inaccurately defined specificity may result in errors. For example, some short database sequences labelled as *Gigaspora* **(Jansa *et al.*, 2003)** cluster with those of *Glomus versiforme* BEG47 *(Diversisporaceae)* **(Gamper *et al.*, 2009)**. Because of the relatively few LSU sequences in the public databases, the design of improved primers is challenging or even impossible. We therefore sequenced the ITS region and the 5' part of the LSU rDNA of a set of well-characterized, but phylogenetically diverse AMF, and designed new primers from the resulting database. These primers are suited to amplify DNA

from members of all known glomeromycotan lineages and by allowing elaboration of a more accurate baseline data set, could be a breakthrough for molecular community analyses of AMF.

Kriiger *et al.* (2009) tested successfully a set of newly designed specific PCR primers of arbuscular mycorrhizal fungi (AMF). They sequenced and analyzed nuclear rDNA fragments from diverse phylogenetic AMF lineages to design four primer mixtures, each targeting one binding site in the small subunit (SSU) or large subunit (LSU) rDNA. They span a fragment covering the partial SSU, whole internal transcribed spacer (ITS) rDNA region and partial LSU to allow species resolution. The new primers are suitable for specifically amplifying AMF rDNA from material that may be contaminated by other organisms (e.g., samples from pot cultures or the field), characterizing the diversity of AMF species from field samples, and amplifying a SSU-ITS-LSU fragment that allows phylogenetic analyses with species level resolution. The PCR primers can be used to monitor entire AMF field communities, based on a single rDNA marker region. Their application will improve the base for deep sequencing approaches; moreover, they can be efficiently used as DNA barcoding primers.

3- Vesicular arbuscular mycorrhizal (VAM) fungi as bioagents:

Krishna and Bagyaraj (1983) found in a groundnut pot test, that the mycorrhizal fungus reduced the number of sclerotia produced by *S. rolfsii* while the root pathogen reduced the percentage of root infection and chlamydospore production by *Glomus fasciculatum*. Root and shoot dry wt. of the host and

their P content were highest in plants inoculated with mycorrhiza only and lowest in plants inoculated with the pathogen only. Simultaneous addition of mycorrhizal inoculum and the pathogen reduced disease severity.

Caron *et al.* (1985 & 1986) grow tomato seedlings inoculated with *Glomus intraradices* [G] and *Fusarium oxysporum* f.sp. *radicis-lycopersici* [F] in pot experiment. The presence of G decreased root necrosis and affected the F population. The interaction between G and F and its effect on tomato plants was investigated. Root colonization by G. was not affected by the presence of F. The number of F. propagules was consistently lower when the plants were inoculated with G. The presence of G. decreased root necrosis caused by F. in wk 5, 11 and 12, but no significant effect was observed for the other 9 wk. The results concluded that the parameters used to study the interaction between a VAM fungus, a fungal root pathogen, and a host plant must be measured at different times after inoculation with the pathogen to make sure that observations are representative of the interaction under study.

Kichadi and Sreenivasa (1998) studied in a pot experiment, the interaction between the VAM fungus, *Glomus fasciculatum*, and the antagonist, *Trichoderma harzianum*, on the soil-borne pathogen *Sclerotium rolfsii* in the rhizosphere of tomato cv. L-15 plants grown in potted soil. The percentage mycorrhizal root colonization and mycorrhizal spore counts were highest in the rhizosphere soil of plants inoculated with both fungi. Disease severity index was least in the dual inoculated

plants. The interaction effects of the fungi not only increased plant growth and yield but also improved P nutrition.

Gardezi *et al.* (1999) investigated the effect of VAM fungi on tomato in soil infested with *Fusarium oxysporum* f.sp. *radicis-lycopersici* in Mexico. VAM fungi had a favourable effect on plant growth both in sterile soil and in pathogen-infected soil. *Glomus* sp. Zac-6 induced the production of the greatest number of fruit. It is concluded that Glomus treatment decreased disease severity and decreased pathogen population as a result of the plants reaction to root colonization by mycorrhizae.

Singh and Kapoor (2000) conducted an experiment under greenhouse conditions to evaluate the effects of different vesicular-arbuscular mycorrhizal (VAM) fungi with and without Mussoorie rock phosphate (MRP) in P-deficient natural non-disinfected soil with mungbean (*Vigna radiata* L. Wilczek) and wheat (*Triticum aestivum* L. emend Thell) as test crops. In wheat, *Glomus* sp. 88 promoted better plant growth and nutrient uptake than the other VAM fungi. There was increase in dry matter production, N and P uptake by 20.2, 110.5 and 160.4% respectively.

Li-Min *et al.* (2000) investigated the relationship between *Gigaspora rosea*, *Glomus mosseae*, *G. versiforme* and wilt of watermelon caused by *F. oxysporum* f.sp. *niveum* under greenhouse conditions. Inoculation with mycorrhiza increased seedling growth and dry weight of watermelon and significantly reduced pathogen propagules in roots and rhizosphere soil. It

was suggested that there is competition between mycorrhiza and *F. oxysporum* f.sp. *niveum*.

Wokocha (2001) screened 37 species of economically important crops and two species of weeds, in 17 plant families, in the screenhouse for reaction to infection by *Sclerotium rolfsii* Sacc. The results established four plant species believed to be new hosts to the pathogen in Nigeria. These were cabbage (*Brassica oleracea* L.), spinach (*Amaranthus* sp.), millet (*Pennisetum typhoides* L.), and sorghum (*Sorghum bicolor*). Dicotyledonous plant species were found to be more susceptible to infection by *S. rolfsii* than the monocotyledonous species, the differences in susceptibility between both were significant. As for the dicotyledonous species, the most susceptible to infection by the pathogen included cassava (*Manihot esculenta* Crantz.), cowpea (*Vigna unguiculata* (L) Walp.), tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.) egg-plant (*Solanum melongena* L.) carrot (*Daucus carota* L.) cabbage (*B.oleracea*) and spinach (*Amaranthus* sp). Disease severity in these crops ranged from 75 to 100%. Monocotyledonous plants in the family Graminae such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), millet (*P.typhoides*) and sorghum (*S.bicolor*), were much less susceptible to infection by *S.rolfsii*, with stem rot severity of 0 to 39%. Bahama grass (*Cynodon dactylon* L.), a monocotyledonous weed, was not attacked by the pathogen in this investigation

Al-Karaki *et al.* (**2004**) studied effects of arbuscular mycorrhizal (AM) fungi inoculation on growth, grain yield and mineral acquisition of two winter wheat (*Triticum aestivum* L.)

cultivars grown in the field under well-watered and water-stressed conditions. Wheat seeds were planted in furrows after treatment with or without the AM fungi *Glomus mosseae* or *G. etunicatum*. Roots were sampled at four growth stages (leaf, tillering, heading and grain-filling) to quantify AM fungi. There was negligible AM fungi colonization during winter months following seeding (leaf sampling in February), when soil temperature was low. During the spring, AM fungi colonization increased gradually. Mycorrhizal colonization was higher in well-watered plants colonized with AM fungi isolates than water-stressed plants. Plants inoculated with *G. etunicatum* generally had higher colonization than plants colonized with *G. mosseae* under both soil moisture conditions. Biomass and grain yields were higher in mycorrhizal than non-mycorrhizal plots irrespective of soil moisture, and *G. etunicatum* inoculated plants generally had higher biomass and grain yields than those colonized by *G. mosseae* under either soil moisture condition. The mycorrhizal plants had higher shoot P and Fe concentrations than non-mycorrhizal plants at all samplings regardless of soil moisture conditions. The improved growth, yield and nutrient uptake in wheat plants reported here demonstrate the potential of mycorrhizal inoculation to reduce the effects of drought stress on wheat grown under field conditions in semiarid areas of the world.

Kumar *et al.* (2004) conducted a pot culture experiment in 1995-96 and 1996-97 to see the efficacy of mycorrhizal fungus (*Glomus mosseae*) in controlling soilborne plant pathogens of chickpea cv. JG-62. Dual inoculations with mycorrhiza and test

pathogens (*R. bataticola* [*Macrophomina phaseolina*], *R. solani* and *F. oxysporum* f.sp. *ciceris*) increased the seed germination, plant height, number of pods, seed weight and biomass production compared to inoculations with pathogen alone. Mycorrhizal inoculations suppressed the incidence of wilt and root rot disease by 54 and 62%, respectively. Sporocarp number and mycorrhizal colonization was also reduced by the dual inoculation as compared to inoculation with mycorrhiza alone. Inoculation with *R. bataticola* + *R. solani* along with *G. mosseae* resulted in highest reduction (65%) in mycorrhizal colonization, while *R. solani* with *G. mosseae* resulted in minimum reduction (39%) in mycorrhizal colonization. Mycorrhizal inoculation resulted in better colonization of roots with VAM and caused reduction in the uptake of nutrients (N, P, K and Zn) in chickpea plants.

Akköprü and Demir (2005) examined the effects of the VAM fungus *Glomus intraradices* and rhizobacteria [RB] (*Pseudomonas fluorescens*, *P. putida* and *Enterobacter cloacae*) which are the important members of the rhizosphere microflora and biological control agents against plant diseases in the pathosystem of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and tomato with respect to morphological parameters (fresh and dry root weight) and phosphorous (P) concentration in the roots. Treatments with single and dual inoculation with *G. intraradices* and RB strains reduced disease severity by 8.6–58.6%. Individual bacteria inoculations were more effective than both the single AMF and dual (*G. intraradices* + RB) inoculations. In addition, the RB and *G. intraradices* enhanced dry root weight

effectively. Significant increases in root weights were recorded particularly in the triple inoculations (FOL + *G. intraradices* + RB) compared with single or dual inoculations. Compared with the non-treated controls all biological control agents increased P-content of treated roots of plants. The results suggest that suitable combinations of these biocontrol agents may ameliorate plant growth and health.

Hashiba and Narisawa (2005) isolated the root endophytic fungus *Heteroconium chaetospira* from roots of Chinese cabbage grown in field soil in Japan. This fungus penetrates through the outer epidermal cells of its host, passes into the inner cortex and grows throughout the cortical cells, including those of the root tip region, without causing apparent pathogenic symptoms. There are no ultrastructural signs of host resistance responses. *H. chaetospira* has been recovered from 19 plant species in which there was no disruption of host growth. *H. chaetospira* has a symbiotic association with Chinese cabbage. The fungus provides nitrogen in exchange for carbon. These associations are beneficial for the inoculated plants, as demonstrated by increased growth rate. When used as a preinoculum, *H. chaetospira* suppresses the incidence of clubroot and *Verticillium* yellows when the test plant is post-inoculated with the causal agents of these diseases. *H. chaetospira* is an effective biocontrol agent against clubroot in Chinese cabbage at a low to moderate soil moisture range and a pathogen resting spore density of 10^5 resting spores per gram of soil in situ. Disease caused by *Pseudomonas syringae* pv. *macricola* and *Alternaria brassicae* on leaves can be suppressed

by treatment with *H. chaetospira*. The fungus persists in the roots and induces systemic resistance to the foliar disease.

Kapoor and Bhatnagar (2011) investigated the effectiveness of *Glomus fasciculatum*, *Trichoderma viride* and *Pseudomonas fluorescens*, alone and in combinations to control disease spread in tomato plants infected with *Fusarium oxysporum* f. sp. *lycopersici*. The three biological control agents were effective in controlling the disease however, the success rate for inhibition varied among the different treatments. Plants inoculated with *P. fluorescens* had higher concentration of phenol and greater activities of phenylalanine ammonia lyase and catalase. Inoculation with *T. viride* led to maximum induction of anti-oxidative enzymes such as catalase and peroxidase. On the other hand, *G. fasciculatum*-inoculated plants showed improved growth and highest phosphorus uptake. A combination of all the three biocontrol agents together, promoted growth and inhibited disease up to 94% in tomato plants. Thus, use of multiple biocontrol agents enhanced level of disease resistance than individual use of bio-inoculants through the induction of multiple defense mechanisms.

4- The biological and physiological effects induced by VAM fungi in plant partner:

A number of regulatory mechanisms of plant defense response have been described during the establishment of the VAM symbiosis, including elicitor degradation, modulation of second messenger concentration, nutritional and hormonal plant defense regulation and activation of regulatory symbiotic gene expression. The functional characterization of these regulatory

mechanisms on AM including cross-talk between them, will be the aim and objective of future work on this topic **(García-Garrido and Ocampo, 2002)**.

Not only a hypersensitive response but also some elements of signal transduction pathways activated after pathogen recognition by the plant have been observed transiently during the early stages of VAM formation. In mycorrhizal tobacco plants, increases of catalase and peroxidase activity were observed **(Blilou *et al.*, 2000a)**. Interestingly, similar results have been shown in onion and bean roots inoculated with AMF **(Spanu and Bonfante-Fasolo, 1988; Lambais, 2000)**. The increase in catalase and peroxidase activity observed in tobacco mycorrhizal roots also coincided with the accumulation of salicylic acid (SA) **(Blilou *et al.*, 2000a)**. SA is a signal molecule involved in the signal pathway activated in plant–pathogen reactions **(Malamy *et al.*, 1990; Métraux *et al.*, 1990)**. Accumulation of SA during the early stages of infection also has been observed in the interaction between rice and *Glomus mosseae*, the accumulation of SA was also correlated to an increase in the expression of genes encoding lipid transfer protein (LTP) and phenylalanine ammonia-lyase (PAL), this provides evidence that induction of *Pal* and *Ltp* is part of the defense pathway **(Blilou *et al.*, 2000b)**.

Linderman and Davis (2004) evaluated the relative responsiveness of a genetically narrow group of marigold plants in order to document differences in host specificity and preference by several VAM fungi. There were, in fact, significant differences in host response as well as extent of root

colonization by the different VAM fungi. One fungus, *Gigaspora albida*, did not colonize any marigold genotype, even though it readily colonized onion roots. These results indicate that there can be considerable variation in host responsiveness due to some genetic control by the host and apparently some preference shown by the fungal symbiont as well. This information underscores the need for caution in predicting benefit from inoculation with VAM fungi under commercial conditions where many different genotypes are grown.

Chaurasia *et al.* (2005) investigated arbuscular mycorrhizal (AM) status of five species of rhododendrons distributed in Kumaun region of the Indian Central Himalaya. Root and rhizosphere soil samples of all the five species of rhododendrons, namely, *Rhododendron anthopogon*, *R. arboreum*, *R. campanulatum*, *R. barbatum* and *R. lepidotum* were collected from temperate, sub-alpine to alpine location in altitudinal range from 1500 to 4500 m amsl (meters height above mean sea level). The AM colonization in root samples ranged from 28 to 42%; and maximum and minimum colonization was observed in *R. arboreum* and *R. lepidotum*, respectively. The highest number of intraradical vesicles (12.5+or-2.8 cm^{-1} root segment) was recorded in *R. arboreum* and the lowest (7.0+or-1.7 cm^{-1} root segment) in *R. barbatum*; vesicles were not observed in *R. lepidotum*. Spores were extracted from the rhizosphere soil by wet sieving to perform microscopic identification of the species. The maximum and minimum populations of spores were detected in the rhizosphere soil samples of *R. anthopogon* (52.0+or-1.5 spores 25 g^{-1} soil) and *R.*

lepidotum (32.0+or-2.5 spore 25 g^{-1} soil), respectively. Spore populations were found to belong to five genera *i.e.*, *Acaulospora*, *Glomus*, *Gigaspora*, *Sclerocystis* and *Scutellospora*; genus *Glomus* was found to be dominant in the rhizosphere soil samples of all the rhododendron species. The most frequent and abundant species was *G. fasciculatum*, however, this species was not isolated from the rhizosphere soil of *R. barbatum*. Finger millet (*Eleusine coracana*) was used to cultivate the trap culture of arbuscular mycorrhizal fungi to confirm the species identity. Spores of *Glomus pustulatum*, not detected in the rhizosphere soil, were recovered from the trap culture. Contrary to this, genus *Gigaspora*, which was present in the rhizosphere soil, did not sporulate in the trap culture. Shannon and Wiener index of diversity and Simpson's index of dominance indicated that the species richness, dominance and diversity of arbuscular mycorrhizal fungi decrease with increasing altitude. In two species of rhododendrons, namely *R. campanulatum* and *R. anthopogon*, dark septate mycelium was also observed.

Wu and Xia (2006) studied the influence of the VAM fungus *Glomus versiforme* on plant growth, osmotic adjustment and photosynthesis of tangerine (*Citrus tangerine*) in potted culture under well-watered and water stress conditions. VAM colonization significantly stimulated plant growth and biomass regardless of water status. The soluble sugar of leaves and roots, the soluble starch of leaves, the total non-structural carbohydrates (NSC) of leaves and roots, and the Mg^{++} of leaves were higher in VAM seedlings than those in corresponding non-

VAM seedlings. The levels of K^+ and Ca^{++} in leaves and roots were higher in VAM seedlings than those in non-VAM seedlings, but differences were only significant under water stress conditions. However, the proline was lower in VAM seedlings compared with that in non-VAM seedlings. VAM seedlings had higher photosynthetic rates than in the non-VAM seedlings.

D'Amico *et al.* (2008) investigated the occurrence of endophytic fungi in fennel, lettuce, chicory, and celery crops in southern Italy. A total of 186 symptomless plants was randomly collected and sampled at the stage of commercial ripeness. Fungal species of *Acremonium, Alternaria, Fusarium,* and *Plectosporium* were detected in all four crops; *Plectosporium tabacinum* was the most common in all crop species and surveyed sites. The effect of eight endophytic isolates (five belonging to *Plectosporium tabacinum* and three to three species of *Acremonium*) inoculated on lettuce plants grown in gnotobiosis was assessed by recording plant height, root length and dry weight, collar diameter, root necrosis, and leaf yellowing. *P. tabacinum* and three species of *Acremonium*, inoculated on gnotobiotically grown lettuce plants, showed pathogenic activity that varied with the fungal isolate. Lettuce plants inoculated with the isolates Ak of *Acremonium kiliense*, Ac of *Acremonium cucurbitacearum,* and P35 of *P. tabacinum* showed an increased root growth, compared to the non-inoculated control. The high frequency of *P. tabacinum* isolation recorded in lettuce plants collected in Bari and Metaponto and in fennel plants from Foggia agricultural districts, suggests a

relationship not only between a crop species and *P. tabacinum*, but also between the occurrence of the endophyte and the crop rotation history of the soil.

Muthukumar and Tamilselvi (2010) surveyed 45 crop species in 39 genera of 21 families to explore the incidence and morphology of endorhizal fungal associations in roots. The survey indicated that 42 of the 45 crop species examined were associated with arbuscular mycorrhizal (AM) fungi. In addition, 20 of the mycorrhizal crop species were also associated with dark septate endophyte (DSE) fungi. Twenty crops had *Arum*-type and 22 had intermediate type AM morphologies. AM morphology has been described for the first time in 27 crop species. Three crop species lacked AM fungal association. *Myristica fragrans* though lacking AM association had DSE fungal association. The extent of colonization in roots ranged from 41% to 97% for AM and <1% to 61% for DSE fungi in the different crop species. Similarly, AM fungal spore numbers in the rhizosphere ranged between 4 and 60 spores 25 g^{-1} of soil. Twelve AM fungal spore morphotypes belonging to *Acaulospora, Gigaspora, Glomus* and *Scutellospora* were isolated from the rhizosphere soils. Although root length colonization by AM fungi was not correlated to AM fungal spore numbers, it was significantly and negatively correlated to DSE fungal colonization. The evidence presented in this paper for the first time revealed 19 crop species to be hosts for DSE fungi.

Mycorrhizal fungi in combination with water supply in pulses drove some of the major changes in leaf area, coarse root mass, and leaf photosynthesis in *Boswellia* seedlings, which

positively influence growth **(Wright *et al.,* 1998)**. **Kaschuk *et al.* (2009)** reported that colonization with AM fungi increased photosynthesis through sink stimulation. The AM symbiosis changed plant growth, biomass (especially below-ground biomass), phosphorus mass fraction in leaves and roots, and photosynthetic performance of *Boswellia* seedlings **(Birhane *et al.,* 2012)**.

Olsson *et al.* (2011) investigated element accumulation in vesicles of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices*, extracted from the roots of inoculated leek plants. In vesicles, P was the most abundant of the elements analyzed, followed by Ca, S, Si and K. We analyzed 12 vesicles from two root systems and found that the variation between vesicles was particularly high for P and Si. The P content related positively to Si, Zn and K, while its relation to Cl fitted to a negative power function. Vesicle transects showed that P and K were present in central parts, while Ca was present mainly near the vesicle surfaces. The results showed that P is an important part (0.5% of the dry weight) of the vesicle content and that the distribution of some elements, within mycelia, may be strongly correlated.

Birhane *et al.* (2012) tested the effects of the AM symbiosis on the performance of *Boswellia papyrifera* seedlings. Mycorrhizal seedlings had higher biomass than control seedlings. Phosphorus mass fraction in shoot and root were also significantly higher for mycorrhizal seedlings. Both a larger leaf area and higher assimilation rates contributed to higher biomass. Because assimilation rates increased even more, mycorrhizal seedlings achieved even higher water use efficiency.

5- The interactions between VAM fungi and plant pathogenic fungi:

Caron *et al.* (1985) mentioned that the presence of *G. intraradices* decreased root necrosis on roots of tomato seedlings inoculated with *Fusarium oxysporum* f.sp. *radicis-lycopersici*. In **1986** they investigated the interaction between the VAM fungus *G. intraradices* and *F. oxysporum* f.sp. *radicis-lycopersici* and its effect on tomato plants. Root colonization by G. was not affected by the presence of F. The number of F. propagules was consistently lower when the plants were inoculated with G. The presence of G. decreased root necrosis caused by F. in wk 5, 11 and 12, but no significant effect was observed for the other 9 wk. The results obtained at any observation time for the endomycorrhizal colonization and the F. population, but not for the percent root necrosis evaluation were consistent throughout the 12-wk experiment.

Ismail *et al.* (2011) recorded that Arbuscular mycorrhizal fungus (AMF) *Glomus irregulare* form symbioses with plant roots, in particular by improving their mineral nutrient uptake and protecting plants against soil-borne pathogens. They found that *G. irregulare* significantly inhibits *Fusarium sambucinum* growth.

6- Production of oxidative enzymes:

Plant chitinases and β-glucanases inhibit fungal growth **(Mauch *et al.*, 1988*;* Arlorio *et al.*, 1992)** and have also been shown to inhibit plant colonization and to reduce the plant disease incidence caused by pathogenic fungi **(Broglie *et al.*, 1991*;* Vierheilig *et al.*, 1993)**. Microorganisms expressing these

activities have also been shown to effectively inhibit fungal growth and to lower disease incidence caused by soilborne pathogens **(Chet *et al.*, 1990*;* Jung *et al.*, 2003*;* Nagarajkumar *et al.*, 2004)**.

The PR-proteins like chitinase and β-1,3-glucanase have the potential to hydrolyze chitin and β-1,3-glucan respectively, which are major components of fungal cell walls. Moreover, the chitinase and glucanase release elicitors from the walls of fungi which, in turn, stimulate various defense responses in plants.

Interestingly, a chitin–binding effector protein Avr4 molecule was found to protect effectively the cell wall of the fungi *Trichoderma viride* and *Fusarium solani* against antifungal activity by basic chitinases in vitro **(Van den Burg *et al.*, 2003)**. Remarkably, and in contrast to plant chitin–binding proteins, positive allosteric interactions were observed between chitin–binding Avr4 molecules **(Van den Burg *et al.*, 2004)**. During growth in vitro, *Cladosporium fulvum* does not produce Avr4 and its chitin is inaccessible. However, during infection of tomato, chitin in the fungal cell walls is accessible and Avr4 is produced **(Van den Burg *et al.*, 2006)**. This all suggests that Avr4 shields fungal cell walls against activated host enzymes during infection.

Ozgonen *et al.* (2001) illustrated that the VAM fungus *Glomus etunicatum* increases the growth of tomato plants and could be used against Fusarium wilt of tomato. While, SA is effective against the pathogen, the root colonization of GE is, however, affected negatively by SA.

Singh *et al.* (2010) assessed the effect of pre-inoculation with vesicular arbuscular mycorrhiza (VAM) on *Fusarium* wilt of tomato in a glasshouse trial. Inoculation with *Fusarium oxysporum* f. sp. *lycopersici* decreased plant growth compared to noninoculated controls, irrespective of the mycorrhizal status of the plant. Mycorrhizal plants were slightly smaller than the non-mycorrhizal ones. Addition of extra phosphorus to the soil enhanced plant growth but this enhancement was totally eliminated by the *Fusarium*. It is not expected, therefore, that VAM could affect the severity of disease caused by *Fusarium* even under conditions where VAM would normally have been beneficial for plant growth. The lowest percentage mycorrhizal infection was found on plants with the most severe disease symptoms.

Jaroszuk and Kurek (2012) detected several enzymes including gluconases, chitinases, xylanases, exocellulases, endocellulases, pictinases and polygalacturonases in 42-day-old cultures of *Fusarium culmorum* isolates with different aggressiveness to rye (*Secale cereale*).

MATERIALS AND METHODS

1- Isolation and description of the targeted VAM-like fungi:

Vigorous grown plants of lettuce (*Lactuca sativa*) and wheat (*Triticum aestivum*) with healthy looking disease-free root systems were collected from the field cultivated crops. Some sampled healthy rootlets were cut from root systems of the collected plants and used for isolation of the target fungi as outlined by **Agrios (1978)**. The root samples were washed in tap water, surface sterilized by dipping in 2% sodium hypochlorite solution for approx. 2-3 min., thoroughly washed with sterilized distilled water, dried between sterilized filter papers, cut into small pieces [0.5-1 cm]. Root pieces were aseptically placed on the surface of Bushnell's agar plates at rate of 3-4 pieces/plate. The Bushnell'**s** agar medium composed of glucose 30g/l, peptone 1g/l, casein hydrolysate 5g/l and the Czapek's minerals in 1000ml distilled water **(Bushnell and Rajendren, 1980)** was prepared and autoclaved as usual. Plates with root pieces were incubated at 25°C for 3-5 days and observed daily. Among fungal mycelia grown out of root pieces, only fungal growth with non-septate hyphal tips were picked up and sub-cultured on fresh Bushnell's agar plates, whereas fungal growth having septet hyphae was ignored **(El-Fiki, et al. 2001)**. The fungi isolated from lettuce and wheat roots were referred herein as root-endophtic-like-VAM fungal L isolates and W isolate, respectively.

2- Molecular identification of isolated VAM-like fungi using nest-PCR technique:

"Nested" means that two pairs of PCR primers are used sequentially for a single locus. The second pair of primers is designed to bind within the first PCR product, so that the second PCR product shorter than the first one. Therefore, if the wrong locus is amplified by mistake, the probability is very low that the wrong product will also be amplified by a second pair of primers. This technique is advantageous for amplifying specific DNA sequences from a complex mixture of DNA. In arbuscular mycorhizal fungi (AMF) studies, nested PCR starts with the first universal fungal primer pair to generate enough general fungal DNA and then second (nested) primers to target particular AMF templates. This technique is useful if the target DNA concentration is relatively low within the general population **(Van Tuinen *et al.* 1998)**.

2.1. DNA Extraction

DNA extraction and purification from the isolated VAM-like fungi (root entophytic Mycorrhiza (L&W isolates) was achieved according to the method of **Appoloni *et al.*, (2008)** using a DNeasy Plant Mini Kit (Qiagen Santa Clarita, CA), this was performed following the manufacturer's instruction as follows: 100 mg fresh mycelia were ground under liquid nitrogen to a fine powder using a mortar and pestle then, the tissue powder and liquid nitrogen were transferred to an appropriately sized tube to allow evaporation of the liquid nitrogen with no allowance of the sample to thaw. A volume of 400 µl of AP1 buffer and 4 µl of RNase A stock solution (100mg

/ ml) were added to a maximum of 100 mg of ground mycellium and vortexed vigorously. The mixture was incubated for 30 min at 65ºC and mixed about 5 times during incubation by inverting the tube. Then, 130 µl of AP2 buffer was added to the lysate, mixed and incubated for 5 min on ice. The lysate was applied to the QIAshredder mini spin column, placed in a 2ml collection tube and centrifuged for 2 min at 14.000 rpm then, the flow-through fraction was transferred to a new tube without disturbing the cell-debris pellet. After that, 1.5 volume of AP3/E buffer was added to the cleared lysate and mixed by pipetting. A volume of 650 µl of the mixture including any precipitate which may have formed, were applied to the DNeasy mini spin column sitting in a 2 ml collection tube then, centrifuged for 1 min at 8000 rpm and the flow-through was discarded. DNeasy mini spin column was placed in a new 2 ml collection tube, 500 µl of AW buffer was added to the DNeasy mini spin column and centrifuged for 1 min at 8000 rpm, the flow-through was discarded and the collection tube was further reused. Then, 500 µl of AW buffer was added to the DNeasy mini spin column and centrifuged for 2 min at 14,000 rpm to dry the membrane. The DNeasy mini spin column was transferred to a 1.5 ml micro centrifuge tube and 100 µl of AE buffer was pipetted directly onto the DNeasy membrane. The micro centrifuge was incubated for 5 min at room temperature (15-25 ºC) and then centrifuged for 1 min at 8000 rpm to elute.

2.2. Estimation of the DNA concentration:

DNA concentration was determined by diluting the DNA 1:5 in dH_2O. The DNA samples were electrophoresed in 1%

agarose gel against 10µg of a DNA size marker (GeneRuler^{TM} 100bp DNA Ladder). This marker covers a range of DNA fragments size between 1031 and 80 bp, and a range of concentration between 95 ng and 11 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

2.3. Polymerase Chain Reaction (PCR):

PCR was performed in a nested procedure as described by **Redecker (2000)**, containing 10X PCR buffer, 2 mM $MgCl_2$, 50 µM (each) of dATP, dCTP, dGTP, dTTP, 0.2 µM each of the primers, 0.1 µl *Taq* polymerase (www.invitrogen.com) and genomic DNA. The first round of amplification was performed using the universal primer ITS4 **(White *et al.* 1990)** as clear in **Table (1)** to amplify the rDNA region. An initial 3 min denaturation at 95 °C was followed by five cycles of 30 s at 95°C, 30 s at 52 °C, and 1.5 min at 72 °C. Thereafter, 25 to 30 cycles with annealing at 51 °C were performed. PCR products were run on agarose gels (0.7 %) to test if it accorded with the expected size of about 1200 bp. Amplified products were used as templates for second round PCR. The second round PCR was conducted seperately with five pairs of Glomales-specific primers **(Redecker *et al.* 2003)**, which specifically targeted at different groups of AMF species **(Table 1)**. Primer pairs were GLOM1310/ITS4i, ARCH1311AB /ITS4i, PARA1313/ ITS4i, and LETC1677/ITS4i. The expect PCR product size was based on the accession from which they were designed **(Table 2)** as described by **Redecker (2000)**. The difference of the

PCR condition compared with the first round is the annealing temperatures at 61°C for 5 cycles then 60°C for 25 cycles. PCR products were electrophoresed on 2% agarose gels. DNA bands were estimated with the aid of a standard DNA ladder (Qiagen, ON, Canada). By comparing the bands of PCR product to the expected size, it provided preliminary classification of fungal groups tested.

Table (1) Primers used in nested PCR and their Tm.

Primers	**Sequence (5'-'3)**	**Tm (^{o}C) [a]**
ITS4i	TTG ATA TGC TTA AGT TCA GCG	56
ARCH1311	TGC TAA ATA GCT AGG CTG C	62
GLOM1310	AGC TAG GYC TAA CAT TGT TA	50
LETC1677	CGG TGA GTA GCA ATA TTC G	60
PARA1313	CTA AAT AGC CAG GCT GTT CTC	58

a All Tms were supplied by Invitrogen, Canada.

Table (2) four primer pairs and the size of expected PCR products in nested PCR.

Primers pair	**Product size (bp) [a]**	**Target group [b]**
ARCH1311/ ITS4i	1052	*Archaeospora gerdemannii / trappei group, or Paraglomus, Glomus occultum*
GLOM1310/ ITS4i	1012	*Glomus group A, Glomus mosseae/ intraradices* group
LETC1677/ ITS4i	676	*Glomus group B Glomus etunicatum / claroideum* group
PARA1313/ ITS4i	1029	*Paraglomus*

a: Expected PCR product size are based on the accession from which they were designed. b: Groups as defined in **Redecker (2000).**

3. LABORATORY STUDIES:

3.1. Antagonistic interaction between pathogenic and VAM-like fungi in dual cultures:

The alternative antagonistic effect of each of L & W isolates, the isolates of the VAM-like fungi, against *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *Sclerotium rolfsii* (SR), the tested pathogenic fungi, was investigated in dual cultures in separate study. In these studies, agar discs (ϕ5 mm) were taken from 7-day-old culture of each given fungal partner grown on Bushnell's agar medium. Two agar discs, one from each fungal partner, were inoculated each opposite the other 1 cm apart from the plate edge in individual plates (ϕ 12 cm) contained 10 ml Bushnell's agar medium. Inoculation of a known fungal partner was conducted either simultaneously with, or 24 hr before or after inoculation of the other fungal partner. Plates of control treatment were inoculated 1 cm apart from the plate edge with a single disc of a known fungus. Three plates were used for each particular treatment. All plates were incubated at 27°C for 7 and 9 days for interactions with FOL and SR pathogenic fungi, respectively. The alternative effects were determined by measuring the growth (mm) of each fungal partner at different intervals i.e. 3, 6 and 9 days [in case of FOL] and 2, 4 and 7 days [in case of SR] after inoculation of the later fungal partner then percentage of reduction in the fungal growth was calculated by using the following formula:

$$\text{Percent inhibition [\%]} = [(C - T) / C] \times 100$$

Where: C = Radial growth of the fungus in control plates (mm) and

T = Radial growth of the same fungus `in dual culture (mm).

4. GREENHOUSE STUDIES:

All experiments in the present work were conducted under greenhouse conditions at the Experimental Farm, Faculty of Agriculture, University of Benha, Egypt, during the 2010-2011 growing seasons.

4.1. Influence of soil inoculation with VAM-like fungal isolates on growth of wheat plants:

The two isolates of the VAM-like fungi isolated from roots of lettuce and wheat plants namely L and W isolates, respectively were investigated if they have pathogenic ability, promote or retarded growth of wheat plants. This study was performed during the winter season of 2010/2011 to investigate effect of inoculation with the obtained VAM-like fungal isolates on growth of wheat plants under greenhouse conditions. Wheat seeds (*Triticum vulgare L*) cultivar Gemmiza were sown in sterilized potted soil, at rate of 6 seeds/pot, 7 days after inoculation the soil with certain level [1, 2 or 3% w/w] of inoculants of a known isolate i.e. L and W isolates of VAM-like fungi. Three pots were used as replicates for each particular treatment. The preparation of used pots and inoculants of the isolated VAM-like fungi was described below.

4.1.1. Preparing used pots and soils:

Loamy sand soil [3clay:1sand w/w] was sterilized by thoroughly mixing with 5% commercial formalin solution (one L of 5% formalin solution/cubic feet of soil mixture) and covered with polyethylene for 2 weeks. Later on, polythene cover was removed and soil was raked for 10 days for ventilation and

formalin evaporation. Similarly, plastic pots (ϕ10 cm) were sterilized by dipping in 5.0% commercial formalin solution for 15 minutes, left to dry for 24 hrs then filled with the previously sterilized soil.

4.1.2. Preparing inoculants of tested VAM-like fungi:

Inoculants of both VAM-like fungal isolates i.e. L & W isolates were prepared separately as following: Each isolate was allowed to grow at 26°C for 2 weeks on Bushnell's agar medium in Petri-dishes (ϕ 7cm) as recommended by **El-Fiki *et al.*, (2001)**. The culture components of a Petri- dish (agar, fungal growth and spores) of a known fungal isolate were transferred to a blender cup containing 50 ml of distilled water and 100 g of fine talcum powder. That mixture was blended in electric blender for 5 minutes to insure complete distribution of the fungal inoculants. The talcum powder formulation was placed on transparent plastic sheet and left to air dried for 24h under room temperature, then it was used to inoculate the sterilized soil [kg soil/pot] at three levels i.e. 1.0% [10g/pot], 2.0% [20g/pot] and 3.0% [30g/pot] and mixed thoroughly with the upper third portion of the potted soil. Un-inoculated sterilized potted soil served as control.

4.1.3. Data collection:

The percentage of survived wheat plants was recorded 15 days after sowing, whereas, number of leaves/plant, shoot length, fresh and dry weight of shoots (g/plant), root length, fresh and dry weight of roots (g/plant) and number of tillers/wheat plant were estimated and recorded 45 days after sowing.

4.2. Studying the non-specific effect of tested VAM-like fungi:

In this study, the VAM-like fungal isolates L and W were investigated for their non-specific protection effects against *Fusarium oxsysporum f. sp. lycopersici* (FOL) and *Sclerotium rolfsii* (SR), the agents of tomato wilt and southern blight on eggplant, respectively. The isolates of the later two pathogenic fungi were kindly obtained from the Plant Pathology Institute at the Agricultural Research Center, Giza.

4.2.1. Preparing inoculants of tested pathogens:

Inoculants for used pathogenic fungi i.e. FOL and SR were prepared using a modified barely-sand (BS) medium **(El-Fiki *et al.* 2001)**. Barely seeds (75g) and the clearly washed sand (25g) were placed in a conical 150 cc flasks. Flasks were supplemented with 50ml of liquid Bushnell's medium and autoclaved as usual then left to cool at room temperature. Flasks were inoculated with equal discs of 7-10 day-old fungal culture (grown on Bushnell's agar medium) of each fungal isolate and incubated for 2 weeks at 25-28°C. The BS-cultures prepared for each fungal isolate was used individually for infestation of sterilized potted soil at level of 3% (w/w) 7 days before sowing. The potted-soil inoculated with 3% of sterilized BS-medium was used control treatment. Five pots were used for each particular treatment.

To verify the non-specificity of the protection induced by the tested VAM-like fungi, two greenhouse experiments were performed. Inoculation of sterilized soils with *Fusarium oxsysporum f. sp. lycopersici* (FOL) or *Sclerotium rolfsii* (SR)

was performed for the 1st and 2nd experiments, respectively. Inoculation with a particular pathogen was performed alone (control treatment), a week before, after or at the same time of inoculation with the VAM-like fungal isolates. Preparation and application of the VAM-like fungal inoculants were done as above described. Thus, the following total of 14 tested treatments for each VAM-like fungal isolate and particular fungal pathogen were performed:

1.1%A (1% inoculants), Alone

2.1%B (1% inoculants), 7 days before pathogen

3.1%C (1% inoculants), simultaneously with pathogen

4.1%D (1% inoculants), 7 days after pathogen

5.2%A (2% inoculants), Alone

6.2%B (2% inoculants), 7 days before pathogen

7.2%C (2% inoculants), simultaneously with pathogen

8.2%D (2% inoculants), 7 days after pathogen

9.3%A (3% inoculants), Alone

10.3%B (3% inoculants), 7 days before pathogen

11.3%C (3% inoculants), simultaneously with pathogen

12.3%D (3% inoculants), 7 days after pathogen

13.Non-inoculated (Control)

14.Pathogen alone inoculated at 3% level

Pots inoculated with FOL were sown with 30-40 days old transplants of tomato (*Lycopersicon esculentum* Mill., cv. hybrid Yasamin 775) whereas, pots inoculated with SR were sown with transplants of eggplant (*Solanum melongena* cv. Tudella). Each

experiment was terminated 45 days after sowing then percentage of survived plants was estimated as following:

Survival % = Survived plants / total cultivated transplants x 100

4.2.2. Data collection:

The potted plants of each treatment were gently removed from pots, washed with tap water, left to air dried at room conditions for about 30-60 minutes then the following parameters i.e., number of leaves/plant, plant height cm/plant, root length cm/plant, root fresh weight g/plant, root dry weight-g/plant, shoot fresh weight g/plant and shoot dry weight g/plant were determined then dry biomass (roots & shoots) was determined after oven drying the samples at 65-70°C for 2–3 days until constant weight gained.

Statistical analysis

All data obtained were statistically analyzed according to the least significant difference (L.S.D.) method described by **Snedecor and Cochran (1982)**.

4.2.3. Diagnosis of infection with the isolated VAM-like fungi:

Root samples of healthy and diseased plants of tomato or eggplant were taken and preserved for 21 days in FAA solution **(Purves *et al.*, 1966)** then examined for VAM colonization as described by **Phillips** and **Hayman (1970)**. For microscopic preparation, the preserved roots were washed several times by tap water to remove the preservative fluid, treated by 10% potassium hydroxide (KOH) in test tubes to remove the host cytoplasm and most cell nuclei then heated in water bath for 10

min at 80-90°C. Root segments were washed with tap water followed by 10% HCl acid. Trypan blue stain (0.5 g/l) was added to the root portions and heated again at 80-90°C for 5 min. Some of treated roots segments chosen at random were picked up and placed on glass slides to which few drops of fresh lactic acid were added then examined microscopically for mycorrhizal infection.

5. Studying some physiological aspects in treated plants:

This study was conducted to identify the probable mechanisms by which the tested VAM-like fungal treatments act in controlling diseases caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *Sclerotium rolfsii* (SR) root pathogens. Thus, the activity of oxidative enzymes i.e., chitinase, peroxidase (PO), and polyphenoloxidase (PPO) were determined in the leaves of treated and untreated healthy 40 days old plants that resulted from the above experiment as following:

5.1. Determination of oxidative enzyme activities:

Samples of plant leaves were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine activities of the following oxidative enzymes **(Tuzun *et al.* 1989)**.

5.1.1 Chitinase activity assay:

The substrate colloidal chitin was prepared from chitin powder according to the method described by **Ried and Ogryd-Ziak (1981).** Twenty five grams of chitin was milled, suspended in 250 ml of 85% phosphoric acid (H_3Po_4) and stored at 4°C for

24 h, then blended in 2 litre of distilled water using a warning blender and the suspension was centrifuged. This washing procedure was repeated twice. The colloidal chitin suspension in the final wash was adjusted to PH 7.0 with (1N) NaoH, separated by centrifugation and the pelted colloidal chitin was store at 4°C. The determination was carried out according to the method of **Monreal and Reese, (1969),** one ml of 1% colloidal chitin in 0.05 M citrate phosphate buffer (PH 6.6) in a test tube, then one ml of enzyme extract was added and mixed by shaking. Tubes were kept in a water bath at 37°C for 60 minutes, then cooled and centrifuged before assaying. Reducing sugar was determined by adding 1 ml of supernatant with 1 ml of dinitrosalicylic acid and 3 ml distilled water in test tubes and tubes were boiled in water bath for 5 minutes, and then cooled. Optical density was determined at 540 nm. Chitinase activity was expresses as mM N-acetyl glucose amine equivalent released gram fresh weight tissue / 60 minutes.

5.1.2. Peroxidase activity assay:

Peroxidase (PO) assay (based on oxidation of pyrogallol to purpurogallin in the presence of H_2O_2) was determined according to the method described by **Allam and Hollis (1972)**. The reaction mixture contained 0.5 ml of 0.1 M potassium phosphate buffer solution at pH 7.0; 0.3 ml enzyme extract; 0.3 ml 0.05M pyrogallol and 0.1 ml 1.0% H_2O_2. The mixture was completed with distilled water up to 3 ml. Enzyme extract was replaced by distilled water in control blank cuvette. The absorbance of 1 ml was recorded and peroxidase activity was

expressed as the change in absorbance at 425nm /15 minute/gram fresh weight.

5.1.3. Polyphenoloxidase activity:

The activity of polyphenoloxidase was measured as mentioned by **Matta and Dimond (1963)**. The reaction mixture consisted of 0.2 ml sample extract, 1.0 ml sodium phosphate buffer (pH 7), 1.0 ml 10^{-3} M catechol and completed with distilled water to 6.0 ml. Enzyme extract was replaced by distilled water in control blank cuvette. The polyphenoloxidase activity was assayed as mentioned above and expressed as the change in absorbency at 420nm /30 minute / 1.0 g fresh weight.

5.2. Determination of the total photosynthetic pigments in leaves of host plants:

The photosynthetic pigments were determined according to **Wettestien (1957).** A fixed fresh weight (0.2 g) samples taken from the top fourth leaf were grind in mortar with 5ml 85% acetone + 0.5 g purified sand being (to facilitate grinding) and CaCO3 salt (0.5 g) was added also to neutralize acidity of the sap for preventing transformation of chlorophyll to pheaphytin. The homogenate was centrifuged for 15 minutes at 4000 rpm, and then it was repeated another time with small volume of acetone in order to get the pigments free. The supernatant was diluted to 25 ml with 85% acetone and its optical density was measured at wavelength (E) of 662, 644 and 440 nm for determination of chlorophyll a, chlorophyll b and carotenoids, respectively, using spectrophotometer (Spectronic 20-D) Concentrations of photosynthetic pigments (mg/g fresh weight) were calculated as follows:

Chlorophyll a = (9.784 x E 662) - (0.99 x E 644)

Chlorophyll b = (21.426 x E 644) - (4.65 x E 662)

Carotenoids = (4.695 x E 440)-[0.263(Chlorophyll a + Chlorophyll b)].

Where E = optical density at the given wavelength. The results were presented as mg/g dry weight of leaves blades.

5.3. Chemical analysis:

Leaf samples for the chemical analysis were taken after 45 days from planting at the rate of three samples per treatment. These samples were washed with distilled water and dried at 65°C for three days. The dry samples were ground in porcelain china motor with pestle and prepared for chemical analysis. The macronutrient elements were determined by the digestion of 0.1g plant material with sulphuric acid and perchloric acid.

5.3.1. Determination of the nitrogen, phosphorous and potassium contents in tissues of the host plants:

5.3.1.1. Nitrogen (N) content:

Nitrogen was determined using micro kjeldahl method **(A.O.A.C., 2005)**.

Total Kjeldahl nitrogen or TKN is the sum of organic nitrogen, ammonia (NH_3), and ammonium (NH_4^+) in the chemical analysis of leaf sampls. To calculate Total Nitrogen (TN), the concentrations of nitrate-N and nitrite-N are determined and added to TKN. The method consists of heating a substance with sulfuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulfate.

In this step potassium sulfate is added to increase the boiling point of the medium (from 337°F to 373°F / 169°C to 189°C). Chemical decomposition of the sample is complete when the initially very dark-colored medium has become clear and colorless. The solution is then distilled with a small quantity of sodium hydroxide, which converts the ammonium salt to ammonia. The amount of ammonia present, and thus the amount of nitrogen present in the sample, is determined by back titration. The end of the condenser is dipped into a solution of boric acid. The ammonia reacts with the acid and the remainder of the acid is then titrated with a sodium carbonate solution by way of a methyl orange pH indicator.

Degradation: Sample + $H_2SO_4 \rightarrow (NH_4)_2SO_4$ (aq) + CO_2 (g) + SO_2 (g) + H_2O (g)

Liberation of ammonia: $(NH_4)_2SO_4$ (aq) + 2NaOH $\rightarrow$ Na_2SO_4 (aq) + $2H_2O$ (l) +$2NH_3$ (g)

Capture of ammonia: B $(OH)_3$ + H_2O + $NH_3 \rightarrow NH_4^+$ + B $(OH)_4^-$

Back-titration: $B(OH)_3$ + H_2O + $Na_2CO_3 \rightarrow NaHCO_3$(aq) + $NaB(OH)_4$(aq) + CO_2(g) + H_2O

5.3.1.2. Potassium (K) content:

Potassium contents were determined using flame photometry method **(Baruah and Borah, 1998)**.

5.3.1.3. Phosphorus (P) content:

Phosphorus (phosphate) was determined using standard colorimetric method **(Thimmaiah, 1999)**.

EXPERIMENTAL RESULTS

1- Isolation and description of the targeted VAM-like fungi:

Isolation trials of the VAM-like (root-entophytic) fungi from strictly surfaced sterilized root segments taken from healthy undamaged roots of wheat and lettuce plants resulted in two VAM-like (root-entophytic) fungal isolates namely W and L for root-entophytes isolated from roots of wheat and lettuce plants, respectively. Both root-entophytic isolates could be grown on Bushnell's agar plates producing coenocytic (occasionally sparsely septate) mycelia with thick irregular branching light-brown hyphae and hyphal swellings. Intercalary and terminally chlamydospores born singly or in chains terminally or intercalary were frequently observed (**Fig.1a**). A structure like sporangium containing sporangiospores was occasionally observed also **(Fig.1b).**

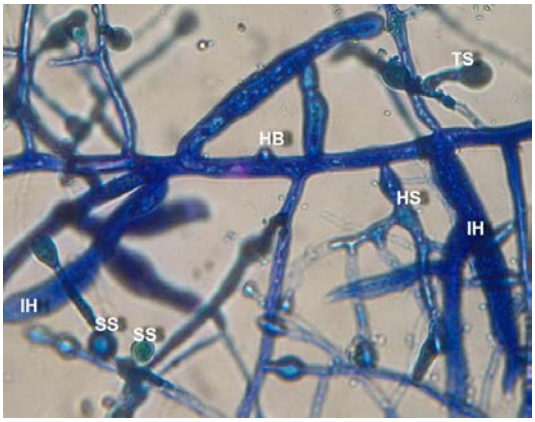


Fig. (1a): Irregular branching mycelial growth with septate and non-septate hyphal branches with different thickness. Note the inflated hyphae (IH), hyphal swellings (HS), intercalary spores (IS), terminal single spores (TS), hyphal Bulge (HB) which developed into a sessile spore (SS).

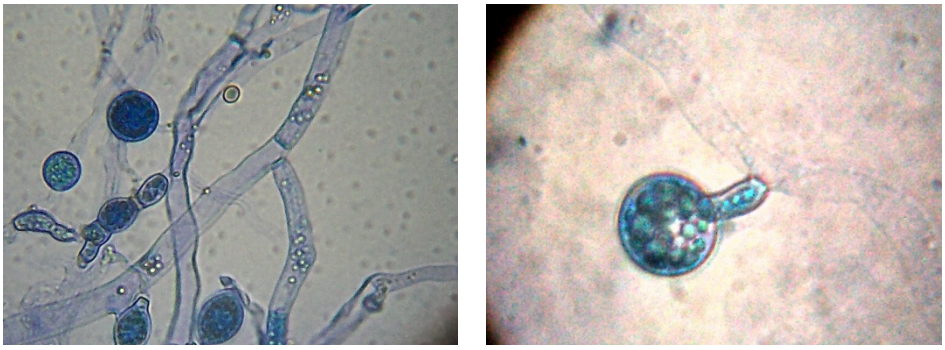


Fig. (1b): A structure like sporangium (upper right) and elliptical and globular chlamydospores formed *in vitro* by the obtained root-entophytic fungal isolates.

2- Nest-PCR technique for identification of the isolated VAM-like fungi:

In this trail, four specific primers *i.e.,* GLOM1310/ITS4i (A), ARCH1311/ITS4i (B), PARA1313/ITS4i (C) and LECT1677/ITS4i (D) were used for detecting and recognizing the isolated VAM-fungi (root entophytic fungi) which isolated from viable lettuce and wheat roots using nested-PCR technique.

The obtained data in **Fig (2)** clearly show that the four tested specific primers *i.e.,* GLOM1310/ITS4i (A), ARCH1311/ITS4i (B), PARA1313/ITS4i (C) and LECT1677/ITS4i (D) were effective in detecting and exhibiting clear intensive amplicons distinguishing the two tested VAM-like fungi (L&W isolates) with very close similarity between them. In this respect, using the specific primer GLOM1310/ITS4i resulted in very intensive two bands with molecular weight ranged between 500-600 bp of the two tested VAM-like isolates (L&W). Meanwhile, using the specific primer ARCH1311/ITS4i exhibited two intensive bands with molecular

weight 700bp of the two tested VAM-like isolates (L&W). Also, using the specific primer PARA1313/ITS4i revealed two clear intensive bands with molecular weight 200bp of the two tested VAM-like isolates (L&W). Therefore, using the specific primer LECT1677/ITS4i exhibited two clear intensive amplicons with molecular weight 350 bp of the two tested VAM-like isolates (L&W). The resulted amplicons of the four tested specific primers with the two tested VAM-like isolates revealed great similarity between the two isolates.

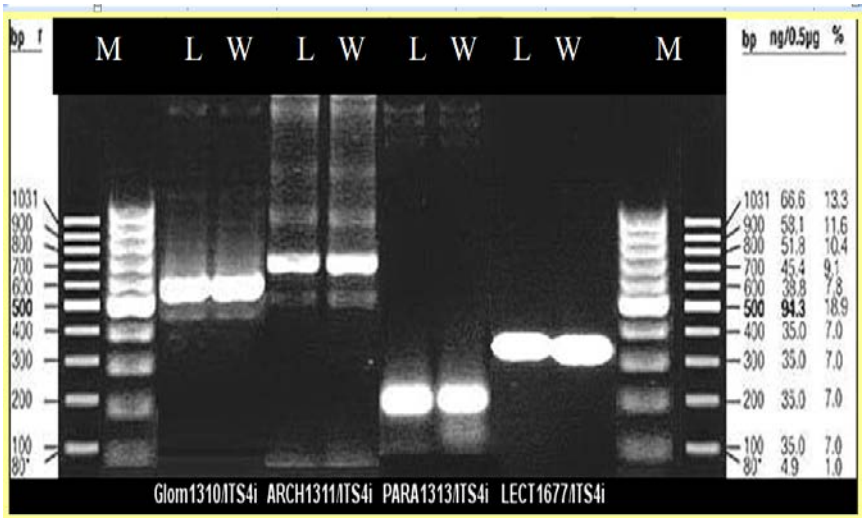


Fig. (2): Nest-PCR - amplicons of the isolated VAM-like fungal isolates L & W obtained with VAM specific primers pairs *i.e.* GLOM1310/ITS4i (A), ARCH1311/ITS4i (B), PARA1313/ITS4i (C) and LECT1677/ITS4i (D) generating fragments of approximate expected size of 600, 700, 200 350 bp, respectively. M = 100bp ladder Marker,

3- Influence of soil inoculation with VAM-like isolates W&L on growth characters of wheat plants:

This study was performed to investigate effect of inoculation with the obtained root-entophytic fungal isolates on growth of wheat plants under greenhouse conditions. Wheat grains (*Triticum aestivum L*) cv. Gemmiza-9 were sown in sterilized potted soil, inoculated with three levels [1, 2 and 3%] of inoculants of tested VAM-like isolates, then the following nine criteria were estimated 45 days after sowing: 1) percentage of survived wheat plants, 2) shoot length, 3) number of leaves/plant, 4) fresh weight of shoots [shoot FW], 5) dry weight of shoots [shoot DW], 6) fresh weight of roots [root FW], 7) dry weight of roots [root DW], 8) root length, and 9) number of tillers/plant [NT]. The obtained results reveal that the three levels of tested inoculants significantly increased all estimated measurements of wheat growth characters compared to the non-inoculated control. Comparisons between levels of the root-entophytic inoculants proved that the lowest level (1%) was the best for improving % survivals and increasing all estimated plant growth criteria followed by the middle level (2%) then the higher one (3%) with clear significant differences between them (**Tables, 3a, 3b** & **3c**) and **Fig., 3**.

It is clear also that, the two tested VAM-like isolates [L & W] were significantly equal regarding six out of the nine estimated measurements *i.e.* [% survival, shoot length (**Table 3a**), shoot FW and shoot DW (**Table 3b**) and root length and root DW (**Table 3c**)] whereas number of leaves/plant (**Table 3a**), number of tillers/plant (**Table 3b**) and root length (**Table**

3c) were significantly higher in case of W than L root-entophytic fungal isolate. However, all estimated criteria were not significantly affected by the interaction between root-entophytic isolates and inoculants levels.

Table (3a): Effect of soil inoculation with the tested VAM-like isolates (W&L) on percentage of survived wheat plants, shoot length and number of leaves/plant.

Inoculants level & root-entophytic	**Survivals %**		Mean	**Shoot Length**		Mean	**No. of leaves**		Mean
	L	W		L	W		L	W	
1%	88.9	88.9	88.9	35.95	37.50	36.72	8.9	9.1	9.0
2%	88.9	77.8	83.3	33.83	33.22	33.53	7.0	8.1	7.5
3%	83.3	72.2	77.8	27.94	25.84	26.89	6.8	7.8	7.3
Control	72.2	72.2	72.2	16.56	16.56	16.56	6.7	6.7	6.7
	83.3	77.8		28.57	28.28		7.3	7.9	
LSD. At 5% for:									
Isolate		NS			NS			0.06	
Level		0.89			0.338			0.09	
Interaction		NS			NS			0.17	

Table (3b): Effect of soil inoculation with the tested VAM-like isolates (W&L) on the shoot fresh & dry weights of wheat plants and number of tillers/plant.

Inoculants level	**Shoot FW**		Mean	**Shoot DW**		Mean	**Number of Tillers**		Mean
	L	W		L	W		L	W	
1%	3.68	4.12	3.90	0.725	0.819	0.772	1.67	1.67	1.67
2%	2.85	3.25	3.05	0.554	0.579	0.567	1.28	1.28	1.28
3%	2.12	2.26	2.19	0.395	0.379	0.387	0.95	1.33	1.14
Control	2.19	2.19	2.19	0.363	0.363	0.363	1.56	1.56	1.56
	2.71	2.95		0.509	0.535		1.36	1.46	
LSD. At 5% for:									
Isolate		NS			NS			0.015	
Level		0.023			0.006			0.022	
Interaction		NS			NS			0.044	

Table (3c): Effect of soil inoculation with the tested VAM-like isolates (W&L) on the root length, fresh & dry weights of roots (g) of wheat plant.

Inoculants level	Root Length		Mean	Root FW		Mean	Root DW		Mean
	L	W		L	W		L	W	
1%	24.3	24.7	24.5	8.52	6.77	7.64	2.07	1.63	1.85
2%	23.0	21.7	22.3	6.37	5.31	5.84	1.61	1.36	1.49
3%	21.0	19.0	20.0	4.77	4.20	4.48	1.21	1.03	1.12
Control	15.3	15.3	15.3	3.97	3.97	3.97	0.76	0.76	0.76
	20.9	20.2		5.91	5.06		1.41	1.20	
LSD. At 5% for:									
Isolate		NS			0.064			NS	
Level		0.14			0.097			0.032	
Interaction		NS			NS			NS	

Fig (3a): Effect of soil inoculation with the tested VAM-like inoculants (W&L isolates) on growth characters of wheat seedlings 45 days after sowing.

Both tested VAM-like isolates (L&W) could be colonized roots of wheat plants and enhanced formation of the infection structures characterizing the VAM (vesicular arbuscular mycorhyzal) fungi *i.e.* arbuscule, vesicle, intracellular hyphae in root cortex and extrametrical mycelium and attached VAM spores (**Fig., 3b)**.

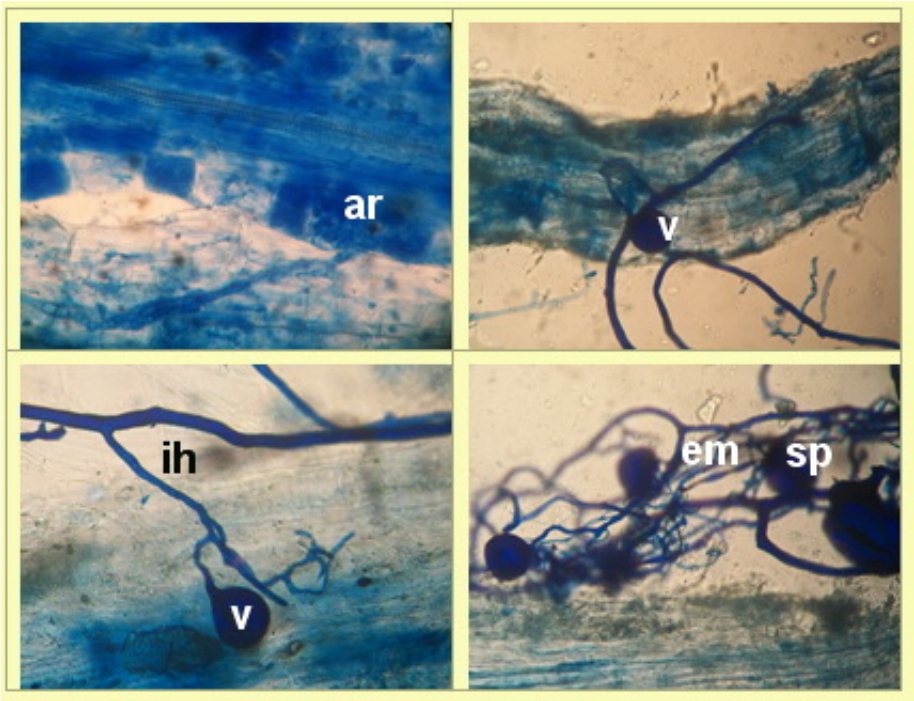


Fig. (3b): Colonization of wheat roots with the tested VAM-like fungi. Note the arbuscule (ar), vesicle(v), intracellular hyphae(ih) in root cortex as well as extrametrical mycelium(em) and attached VAM spores(sp).

4- LABORATORY STUDIES:

4.1. Effects of reciprocal interactions between the tested VAM-like fungi and some pathogenic fungi *in vitro*:

4.1.1. Interaction between the tomato wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and tested VAM- like fungi:

The obtained results, in general, proved that in the mutual cultures (two fungal partners grow together), the growth of a known fungal partner was affected to different extents by growth of the other fungal partner. As for interaction between the causal of tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and VAM-like isolate L, the obtained results (**Table 4a and Figs., 4 and 4a**) state that, the highest antagonistic effect (after 9 days) *i.e.* the reduction % in growth of FOL in case of FOL/L interaction was recorded when isolate L inoculated simultaneously with FOL (36.9%) or 24h before FOL inoculation (31.9%) for the two combined inoculation treatments, respectively. However, inoculation of isolate L 24h after FOL inoculation resulted in the lowest reduction in FOL growth (18.7%) after 9 days. On the other hand, growth of isolate L was greatly affected by growth of FOL in the FOL/L interaction. In such interaction, growth of isolate L after 9 days was reduced by 13.4, 15.0 and 27.1% when isolate L was inoculated 24h before, simultaneously with, and 24h after FOL inoculation, respectively.

Table (4a): Mutual growth effects between FOL and VAM-like isolate L.

Fungi	Inoculation status	Radial growth (mm)			% reduction compared to the non-inoculated control (alone *)[a]		
		3d	6d	9d	3d	6d	9d
FOL	Alone (control) *	40.3	57.3	80.3	0.0	0.0	0.0
	L before FOL	30.0	41.7	54.7	25.6	27.2	31.9
	L simultaneous with FOL	30.0	43.7	50.7	25.6	23.7	36.9
	L after FOL	42.0	54.0	65.3	-4.2	5.8	18.7
L isolate	Alone (control) *	75.3	90.3	104.7	0.0	0.0	0.0
	L before FOL	65.0	84.3	90.7	13.7	6.6	13.4
	L simultaneous with FOL	60.3	79.3	89.0	19.9	12.2	15.0
	L after FOL	42.0	64.7	76.3	44.2	28.3	27.1

a = (Non-inoculated control * – Treatment)/Non-inoculated control * x 100

Approximately, similar trend was observed also in case of FOL/W interaction (**Table 4b and Figs., 4** & **4b**). In such interaction, the growth of VAM-like isolate W was affected negatively more than growth of FOL particularly when inoculated 24h after FOL inoculation. After 9 days incubation, the growth of FOL was reduced by 36.1, 38.1, and 35.2% when inoculated 24h after (W before FOL), simultaneously with FOL and 24h before inoculation with isolate W (W before FOL), respectively, whereas, the growth of isolate W after 9 days was reduced by 36.5, 34.7 and 52.8% for the latter three inoculations, respectively.

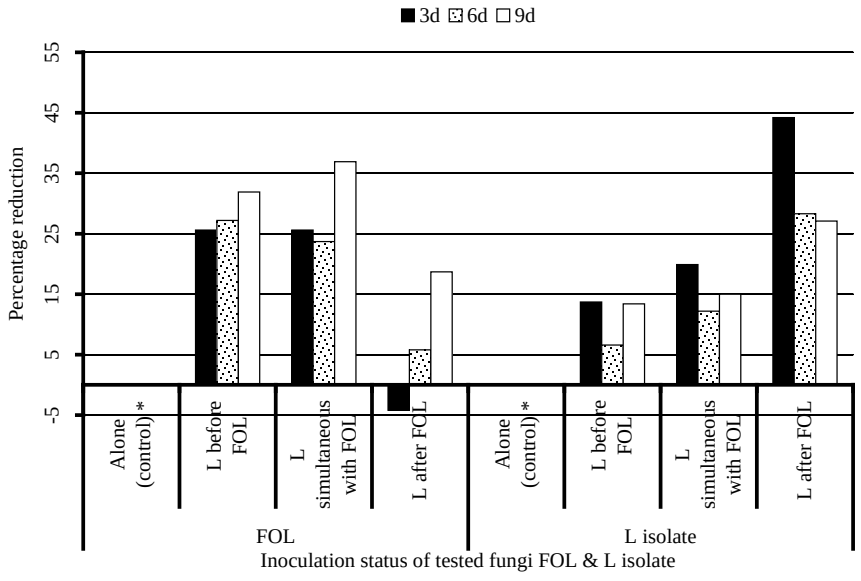
In general, the antagonistic effect of a known fungal partner in most interactions between isolate W and FOL was increased by prolonging the incubation period. However, in case of simultaneous inoculation with FOL, the antagonistic effect of isolate W against FOL was raised by time *i.e.* 28.0, 26.2 and

38.1% whereas the antagonistic effect of FOL against isolate W was lowered by time *i.e.* 42.5, 35.6 and 34.7% after 3, 6 and 9 days of incubation, respectively.

Table (4b): Mutual growth effects between FOL and VAM-like isolate W.

Fungi	Inoculation status	Radial growth (mm)			% reduction compared to the non-inoculated control (alone *)[a]		
		3d	6d	9d	3d	6d	9d
FOL	Alone (control) *	40.3	57.3	80.3	0.0	0.0	0.0
	W before FOL	30.0	43.0	51.3	25.6	25.0	36.1
	W simultaneous with FOL	29.0	42.3	49.7	28.0	26.2	38.1
	W after FOL	40.3	43.3	52.0	0.0	24.4	35.2
W isolate	Alone (control) *	60.3	80.3	96.0	0.0	0.0	0.0
	W before FOL	44.7	56.0	61.0	25.9	30.3	36.5
	W simultaneous with FOL	34.7	51.7	62.7	42.5	35.6	34.7
	W after FOL	42.3	43.3	45.3	29.9	46.1	52.8

a = (Non-inoculated control * – Treatment)/Non-inoculated control * x 100



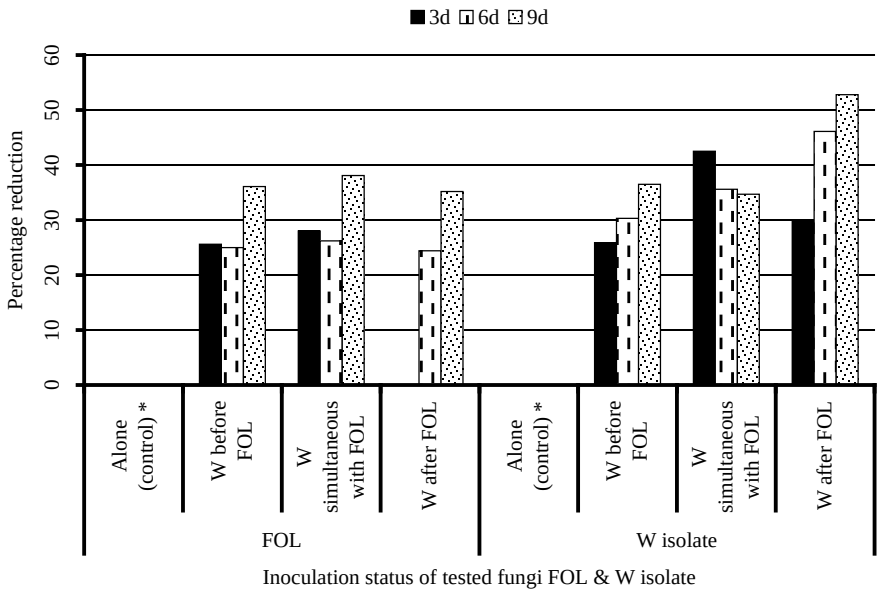


Fig. (4): Mutual growth interactions between FOL (pathogen) inoculated 24h before, after or simultaneously with each of the tested VAM-like isolates.

L isolate (above) and W isolate (below). Data expressed as % reduction between fungal partners after 3, 6 and 9 days.

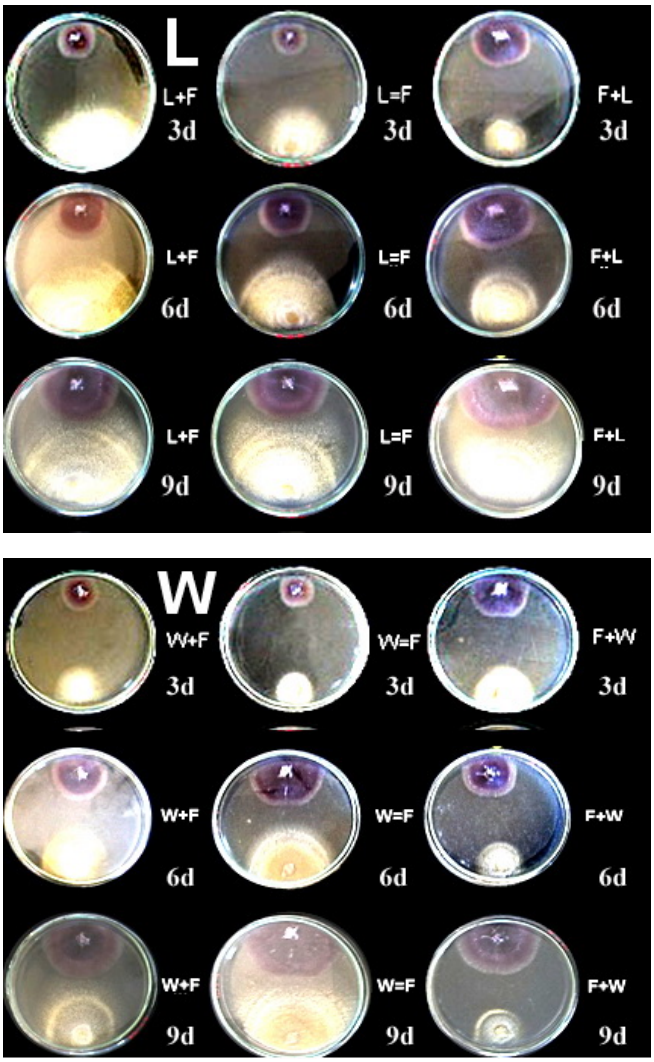


Fig. (4a): Effect of the *in vitro* mutual inoculation (FOL with the tested VAM-like isolates (L and W) on fungal growth after 3d, 6d and 9d from inoculation.

L or W isolate was inoculated 24h before FOL (+ left column), simultaneously with F (= middle column) or 24h after F (+ right column). In each plate, F was the upper whereas the lower was the tested VAM-like isolate.

4.1.2. Interaction between *Sclerotium rolfsii* (SR) and the tested VAM-like isolates:

The obtained results proved that the growth of the fungal partners in the tested interactions between *Sclerotium rolfsii* (SR) (pathogen) and each of the tested VAM-like isolate L (L/SR interaction) and isolate W (W/SR interaction) was responded differently based on isolate of the VAM-like isolates and inoculation status. In the L/SR interaction, the obtained results showed that the antagonistic effect of isolate L against SR pathogen was progressively increased as incubation period increased (**Table 5a and Figs., 5 & 5a**). Inoculation of isolate L 24h before inoculation of SR resulted in the highest reduction in growth of SR isolate *i.e.* 67.3, 80.3 and 95.1% after 2, 4 and 7 days post inoculation of SR, respectively. Growth of SR after 7 days in the L/SR interaction was reduced by 77.0 & 73.0% when isolate L inoculation occur simultaneously with, and 24h after SR inoculation, respectively compared with SR alone.

Table (5a): Mutual growth effects between *Sclerotium rolfesii* (SR) and VAM-like isolate L.

Fungi		Radial growth (mm)			% reduction compared to the non-inoculated control (alone *)[a]		
	Inoculation status	2d	4d	7d	2d	4d	7d
SR	Alone (control) *	51.0	87.7	108.7	0.0	0.0	0.0
	L before SR	16.7	17.3	5.3	67.3	80.3	95.1
	L simultaneous with SR	41.0	39.7	25.0	19.6	54.7	77.0
	L after SR	38.0	44.3	29.3	25.5	49.5	73.0
L isolate	Alone (control) *	64.3	89.3	108.0	0.0	0.0	0.0
	L before SR	57.0	84.3	104.7	11.4	5.6	3.1
	L simultaneous with SR	58.7	80.0	94.7	8.7	10.4	12.3
	L after SR	36.0	66.0	90.3	44.0	26.1	16.4

a = (Non-inoculated control * – Treatment)/Non-inoculated control * x 100

On contrary, the SR pathogen in the L/SR interaction showed little negative effect on growth of L isolate. The growth of isolate L after 7 days was reduced by 3.1, 12.3 and 16.4% when it was inoculated 24h before, simultaneously with, and 24h after SR inoculation, respectively. The adverse effect of SR on growth of isolate L was progressively decreased by prolonging incubation periods particularly when isolate L was inoculated 24h before SR inoculation. Similar trend was observed concerning the W/SR interaction (**Table 5b and Figs., 5 & 5b**). The highest efficacy was recorded when isolate W was inoculated 24h before SR pathogen, growth of SR pathogen was reduced by 57.5, 73.4 and 60.7% while growth of isolate W was reduced by 9.0, 2.6 and 5.3% after 2, 4 and 7 days of incubation, respectively.

Table (5b): Mutual growth effects between *Sclerotium rolfsii* (SR) and VAM-like isolate W.

Fungi		Radial growth (mm)			% reduction compared to the non-inoculated control (alone *)[a]		
	Inoculation status	2d	4d	7d	2d	4d	7d
SR	Alone (control) *	51.0	87.7	108.7	0.0	0.0	0.0
	W before SR	21.7	23.3	42.7	57.5	73.4	60.7
	W simultaneous with SR	38.3	40.0	51.7	24.9	54.4	52.4
	W after SR	32.7	35.0	52.3	35.9	60.1	51.9
W isolate	Alone (control) *	63.0	87.3	94.7	0.0	0.0	0.0
	W before SR	57.3	85.0	89.7	9.0	2.6	5.3
	W simultaneous with SR	58.7	76.0	80.7	6.8	12.9	14.8
	W after SR	38.3	66.0	74.7	39.2	24.4	21.1

a = (Non-inoculated control * – Treatment)/Non-inoculated control * x 100

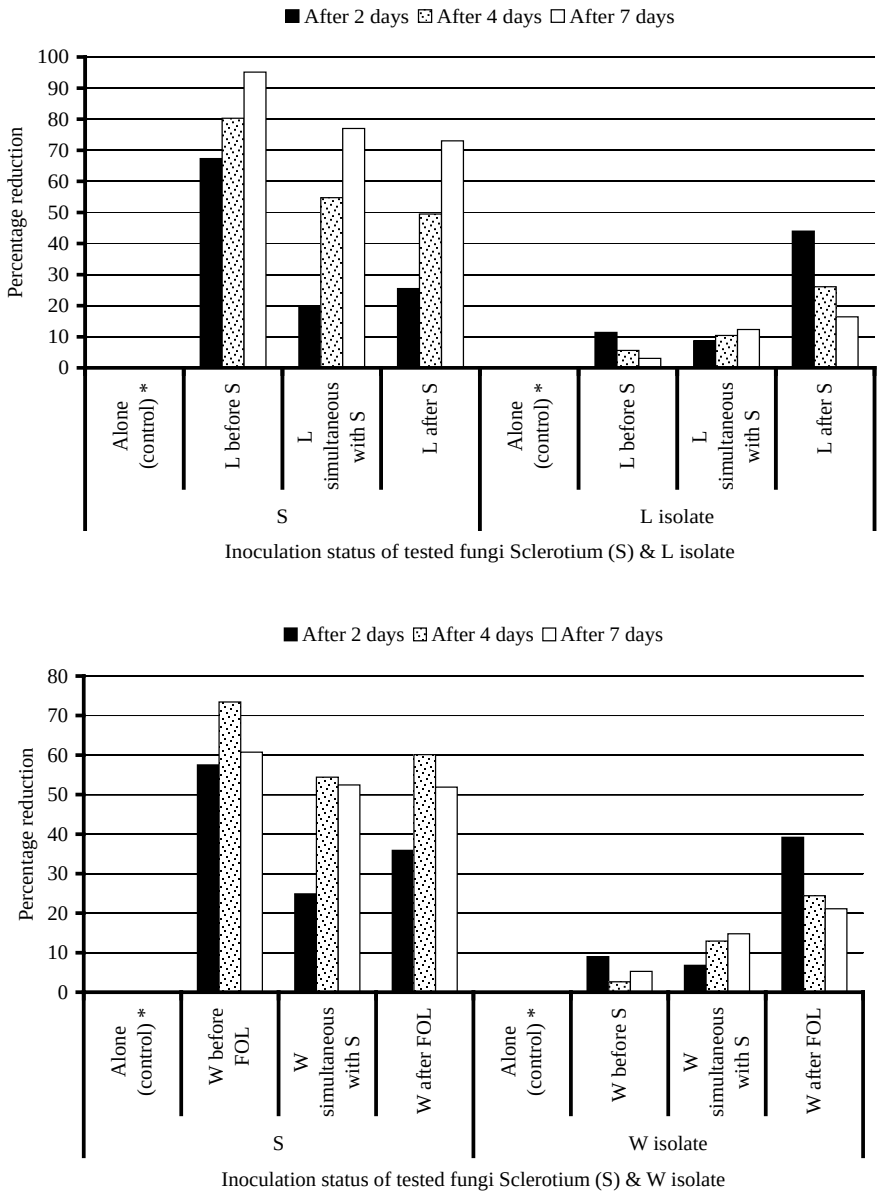


Fig. (5): Mutual growth interactions between (SR) (a pathogen) inoculated 24h before, after or simultaneously with the tested VAM-like isolates.

L isolate (above) and W isolate (below). Data expressed as % reduction between fungal partners after 2, 4 and 7 days.

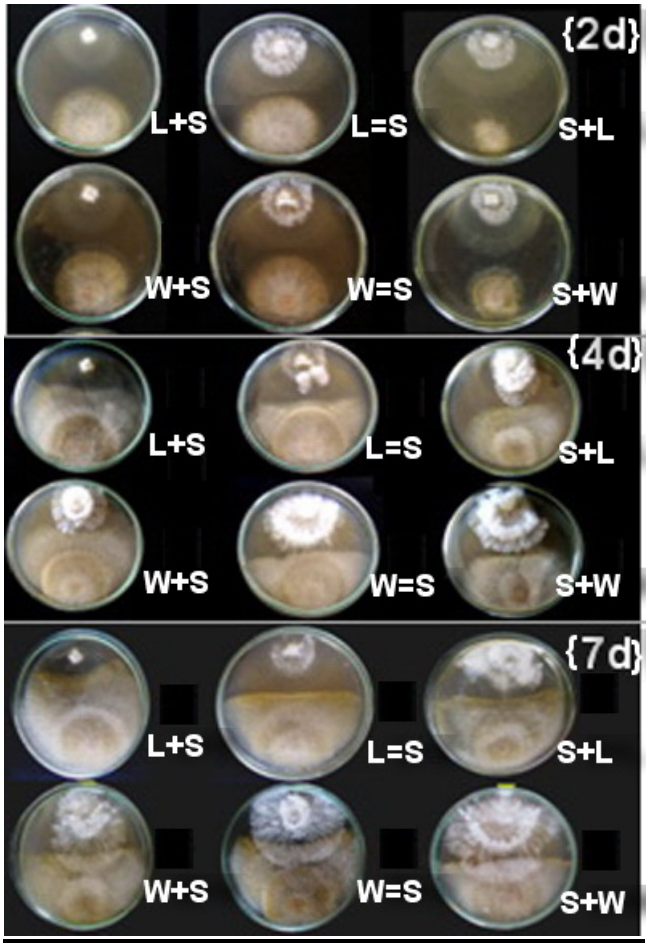


Fig. (5a): Effect of the *in vitro* mutual inoculation *Sclerotium rolfsii* (SR) with the tested VAM-like isolates L or W at different times, on fungal growth after 2d, 4d and 7d from inoculation.

L or W isolate was inoculated 24h before (SR) (+ left column), simultaneously (= middle column) or 24h after (SR) (+ right column). In each plate, (SR)was the upper whereas the lower was the root-entophytic isolate.

4.2. Oxidative enzymes activities of tested VAM-like and pathogenic fungi in the cultural filtrates:

The obtained data in **Table (6) and Fig. (3)** prove that, the three oxidative enzymes chitinase, peroxidase (PO) and polyphenol- oxidase (PPO) were detected in the cultural filtrates of tested pathogenic fungi (FOL and SR) as well as in those of the tested VAM-like isolates (L&W). The activity of all tested enzymes was obviously higher in culture filtrates of the tested VAM-like isolates (L&W) than in culture filtrates of the two investigated pathogenic fungi FOL and SR. In all fungi, the highest activity of any one of the determined oxidative enzymes was detected in filtrates of 7 days-old cultures then successively decreased to different extents as age of culture filtrates increased to 14 and 21 days, respectively.

Table (6): Oxidative enzymes activities (O.D./min/1ml) **of tested VAM-like and pathogenic fungi in the cultural filtrates**.

Enzyme	Age of culture (days)	Tested fungi			
		L isolate	W isolate	(FOL)	(SR)
Chitinase	7	19.68	16.40	6.00	4.28
	14	14.52	11.30	5.50	2.72
	21	11.10	7.86	5.26	2.10
PO	7	20.12	42.27	15.99	8.81
	14	16.07	35.69	11.62	6.16
	21	12.32	31.43	11.15	2.22
PPO	7	27.90	26.25	11.93	9.15
	14	9.00	8.55	5.51	5.70
	21	7.20	8.10	5.40	5.25

As for isolates of tested VAM-like isolates, filtrates of W isolate showed higher PO activity and lower activities of

chitinase and PPO compared with those of L isolate. This trend was true in different ages of the culture filtrates. Although PO activity at any age of culture was higher in filtrates of W isolate but it seems to be decreased at faster rates by ageing than PO activity in filtrates of L isolate. As for the pathogenic fungi, the FOL pathogen shows higher PO activity in its culture filtrates than the SR one. Aging of cultures caused quick decline in activity of any tested oxidative enzyme in filtrates of SR fungus compared with the FOL fungus.

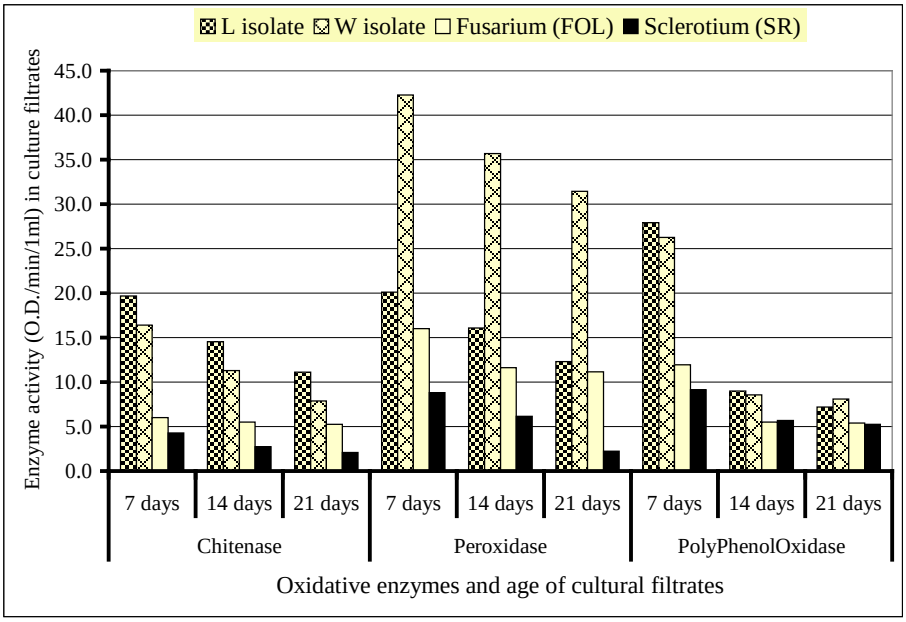


Fig. (6): Activity of the oxidative enzymes (chitenase, peroxidase and polyphenol oxidase) in cultural filtrates of the tested VAM-like isolates (L&W) and the tested pathogenic fungi (FOL and SR).

5- GREENHOUSE STUDIES:

5.1. Effects of reciprocal interactions between the tested VAM-like fungi and some pathogenic fungi on plant survivals% *in vivo*.

In this study, sterilized potted soils were inoculated at 3% (w/w) inocula of either FOL or SR, the casuals of tomato wilt and root-rot of eggplant, respectively. The potted soil infested with FOL or SR was inoculated also by inoculants of VAM-like isolates (W or L) at three levels *i.e.* 1, 2 and 3% (w/w). Inoculants of the latter fungal isolates were added either alone (A), before (B), simultaneously (C) or after inoculation with a known pathogen. Pots containing only sterilized soil were served as control. After 40 days from planting tomato (cv. Hybrid Yassmin-775) or eggplant (cv.Tudella) transplants, the following criteria were determined.

5.1.1. On percentage of survived tomato plants under stress of infection with FOL pathogen:

As for tomato, the data in **Table (7)** and **Fig. (7)** indicate that the survived tomato plants did vary significantly between the tested inoculation treatments, but no significant differences were detected between VAM-like isolates or their interactions with inoculation treatments. On the other hand, inoculation with FOL alone had significantly lower maximum decrease in % survived tomato plants than those in the other combined inoculation treatments.

Table (7): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on survived plants% of tomato and eggplant, respectively.

Treatments	Survival of tomato plants % (FOL)		Mean	Survival of eggplant plants % (SR)		Mean
	L isolate	W isolate		L isolate	W isolate	
1% A	100.0	100.0	100.0	100.0	100.0	100.0
1% B	100.0	100.0	100.0	100.0	100.0	100.0
1% C	66.7	100.0	83.3	100.0	100.0	100.0
1% D	50.0	83.3	66.7	100.0	88.9	94.4
2% A	100.0	100.0	100.0	100.0	100.0	100.0
2% B	100.0	100.0	100.0	100.0	88.9	94.4
2% C	100.0	100.0	100.0	100.0	100.0	100.0
2% D	100.0	66.7	83.3	77.8	88.9	83.3
3% A	50.0	50.0	50.0	77.8	77.8	77.8
3% B	50.0	50.0	50.0	66.7	77.8	72.2
3% C	50.0	66.7	58.3	77.8	77.8	77.8
3% D	50.0	66.7	58.3	77.8	66.7	72.2
Non-inoculated	83.3	83.3	83.3	66.7	66.7	66.7
Pathogen alone **	33.3	33.3	33.3	33.3	33.3	33.3
Mean	73.8	78.6		84.13	83.33	
L.S.D. at 5% for:						
Isolates		NS			NS	
Treatments		28.27			12.278	
Interaction		NS			NS	

* Treatments = inoculation of tested VAM-like isolate at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

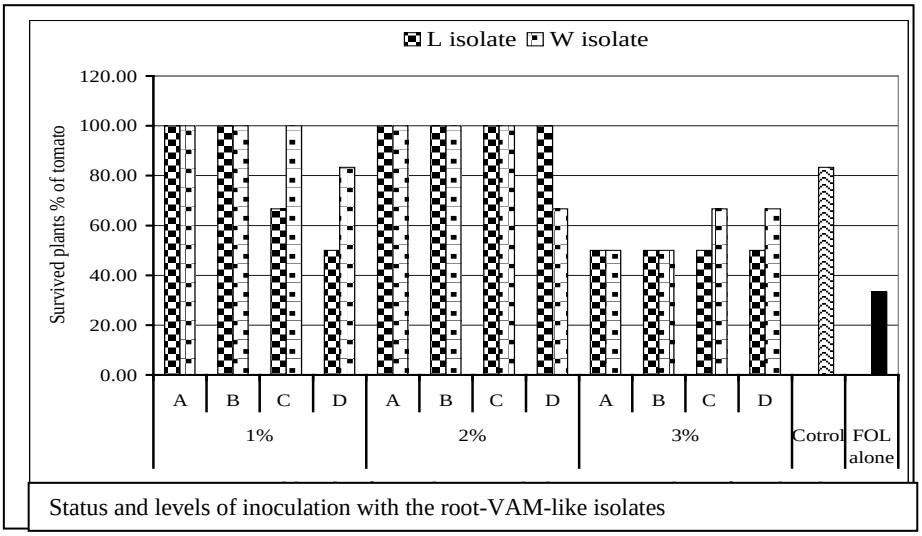
** Pathogen alone = *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.

The tested VAM-like isolates (L and W) recorded 73.8 and 78.6% of survived plants, respectively without significant differences between them. However, the tested treatments significantly affected percentages of survived plants. The highest % of survived plants (83.3-100.0%) were obtained by using treatments of A & B (at 1% level), A, B and C (at 2% level),

1%C and 2%D without significant difference in between. On the other hand, treatments of B and D at 3% level had no significant effect on the survived plants (50.0-58.33%) when compared with inoculation with FOL alone (33.33%). It is interest to state that applying treatments of A and B at 3% level significantly decreased survived plants (50.0%) when compared with the non-inoculated control treatment (83.3%). Percentages of survived plants % were not significantly affected by the interaction between root-entophytic isolates and tested treatments.

5.1.2. On percentage of survived eggplant plants under stress of infection with (SR):

Regarding eggplant, the same data indicate that the survival% of eggplant plants (*Solanum melongena*) was significantly affected only by the tested VAM-like inoculation treatments. Generally, eggplant plants survivals % were ranged between 72.2-100.0% compared with 66.7% and 33.3% for the non-inoculated control treatment and inoculation with (SR) alone, respectively. The highest % of eggplant survived plants (94.4-100.0%) were obtained by using treatments of A, B, C and D (at 1% level) in addition to 2% of B without significant difference in between.



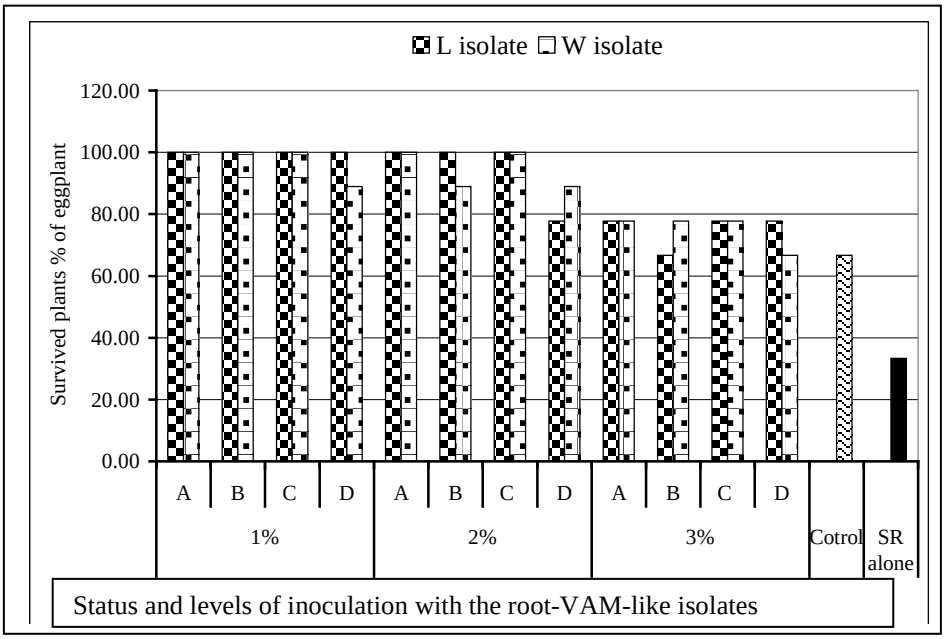


Fig. (7): Survival % of tomato (above) and eggplant (below) plants as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of tested VAM-like isolates L & W.

On the other hand, percentages of survived plants using any treatments of A, B, C and D at level 3% were not significantly varied (72.2-77.8%) comparing with the non-inoculated control (66.7%). As for L isolate, using treatments of A, B, C either at 1% or 2% level, in addition to 1%D were the best of all (100%). Similar results were recorded by isolate W except treatments of 1%D and 2%B which recorded 88.9% of survived plants.

5.2. Effects of reciprocal interactions between the tested VAM-like fungi and some pathogenic fungi on growth characters *in vivo*:

5.2.1. On tomato leaves number/plant under stress of infection with FOL pathogen:

The obtained data **(Table 8 & Fig. 8)** prove that the majority of tested inoculation treatments significantly increased number of leaves/tomato plant (4.0-8.0 leaves) whereas applying 3% of A significantly decreased it compared to inoculation with FOL alone (2.0 leaves). Applying treatments of 1%A, 1%B, 2%A and 2%B induced the highest increase in the number of leaves/plant (7.7-8.0 leaves) without significant differences in between followed by applying 1%C and 2%C (7.3 leaves), 1%D and 2%D (6.0-6.3-leaves) compared to inoculation with FOL alone. In general, all inoculation treatments increased number of leaves/plant to different extents depending on the tested levels of VAM-like isolates (LV) compared to inoculation with FOL alone and the non-inoculated control, respectively.

On contrast, treatments of 3%A, 3%B, 3%C and 3%D decreased number of leaves compared to inoculation with FOL alone whereas applying 3%A decreased number of leaves

compared to the non-inoculated control. Number of leaves/plant, however, was not varied significantly between tested VAM-like isolates and not affected significantly by the interaction between isolates and inoculation treatments.

5.2.2. On eggplant leaves number/plant under stress of infection with (SR):

As for number of leaves/eggplant plant, the same data in **Table (8) & Fig. (8)** clear that number of leaves/eggplant plant was significantly affected by tested inoculation treatments but not by root-entophytic fungal isolates or by the interaction between isolates and inoculation treatments. All inoculation treatments of the tested levels of VAM-like isolates (LV) significantly increased number of leaves/plant (2.00-4.78 leaves) compared to inoculation with (SR) alone (0.89 leaf). Comparing to the non-inoculated control (2.0 leaves), treatments of A, B, C & D at 1 and 2% levels, in addition to 3%A significantly increased number of leaves/plant (3.11-4.78 leaves) while treatments of B, C and D at 3% level had no significant effect (2.11-2.33 leaves).

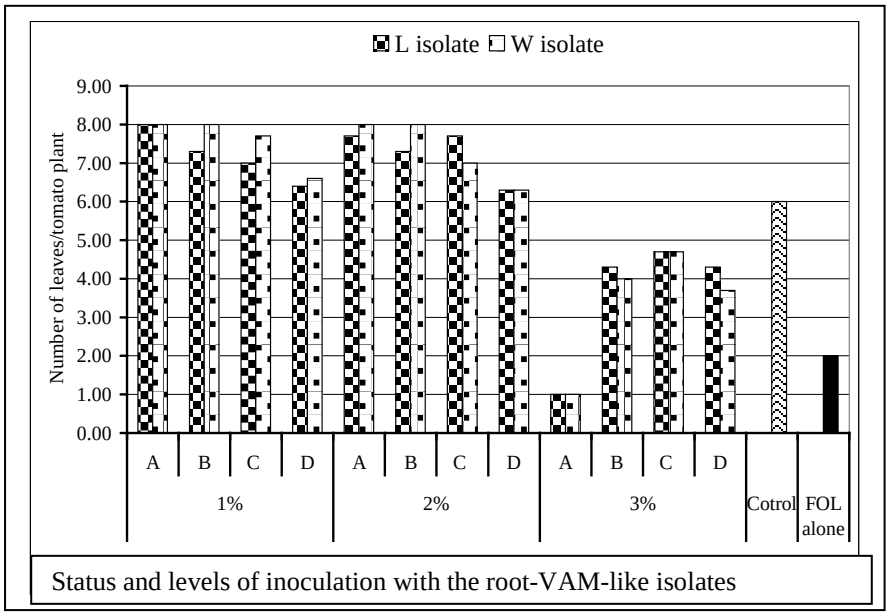
In general, treatments of A and C at 1% or 2% levels for both L and W isolates resulted in the highest number of leaves/plant (4.2-4.9 leaves) either compared to the non-inoculated control or the inoculated with SR alone.

Table (8): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the number of leaves/plant of eggplant and tomato plants.

Application treatment *	No. of leaves/tomato plant (FOL)			N. of leaves/plant of eggplant (SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	8.0	8.0	8.0	4.9	4.7	4.78
1% B	7.3	8.0	7.7	4.2	4.2	4.22
1% C	7.0	7.7	7.3	4.7	4.4	4.56
1% D	5.7	6.3	6.0	3.2	3.3	3.28
2% A	7.7	8.0	7.8	4.6	4.4	4.50
2% B	7.3	8.0	7.7	4.0	3.8	3.89
2% C	7.7	7.0	7.3	4.4	4.3	4.39
2% D	6.3	6.3	6.3	2.6	3.7	3.11
3% A	1.0	1.0	1.0	2.6	2.3	2.44
3% B	4.3	4.0	4.2	2.3	2.1	2.22
3% C	4.7	4.7	4.7	2.4	2.2	2.33
3% D	4.3	3.7	4.0	2.2	2.0	2.11
Non-inoculated	6.0	6.0	6.0	2.0	2.0	2.00
Pathogen alone **	2.0	2.0	2.0	0.9	0.9	0.89
Mean	5.67	5.79		3.21	3.17	
L.S.D. at 5% for:						
Isolates		NS			NS	
Treatments		0.55			0.542	
Interaction		NS			NS	

* Treatments = inoculation of tested VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at same time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



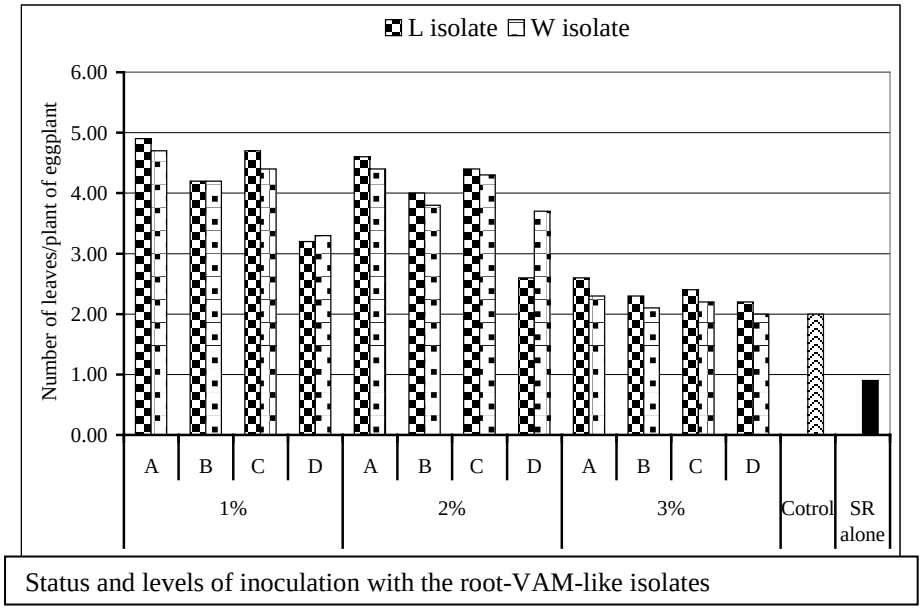


Fig. (8): Number of leaves/plant of tomato (above) and eggplant (below) plants as affected by inoculation with FOL and SR, respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

5.2.3. On plant height of tomato plants under stress of infection with FOL pathogen:

The data presented in **Table (9)** and **Fig. (9)** state that, all the tested VAM-like inoculation treatments significantly increased the heights [cm] of tomato plants (14.8-23.2cm) compared to inoculation with FOL alone (10.3cm). The highest significant increase was produced by applying A & B at 1% or 2% and C at 2% levels (21.3-23.8cm) without significant differences between all. All these treatments in addition to 2%D (19.8cm) were significantly better, in this respect, than the non-inoculated control (17.3cm). In general, applying 2%B recorded the highest increase in plant height for both L and W isolates either compared with inoculation with FOL alone and the non-inoculated control. In this study, the plant height was not significantly varied between the two tested VAM-like isolates as well as, the interaction between them and inoculation treatments.

5.2.4. On plant height of eggplant under stress of infection with SR:

As for plant height [cm] of eggplant, the same data show that it was significantly affected by tested inoculation treatments but not by the tested VAM-like isolates or by the interaction between VAM-like isolates and inoculation treatments. However, all inoculation treatments significantly increased the plant height (10.28-19.89cm) compared to inoculation with SR alone (5.3cm). The highest increase was produced by applying treatments of 1%A (19.89cm) followed by C and B at 1% level and A & C at 2% level (18.22-18.56cm), 2%B (17.06cm), 1%D (16.72 cm) and 2%D (14.56 cm).

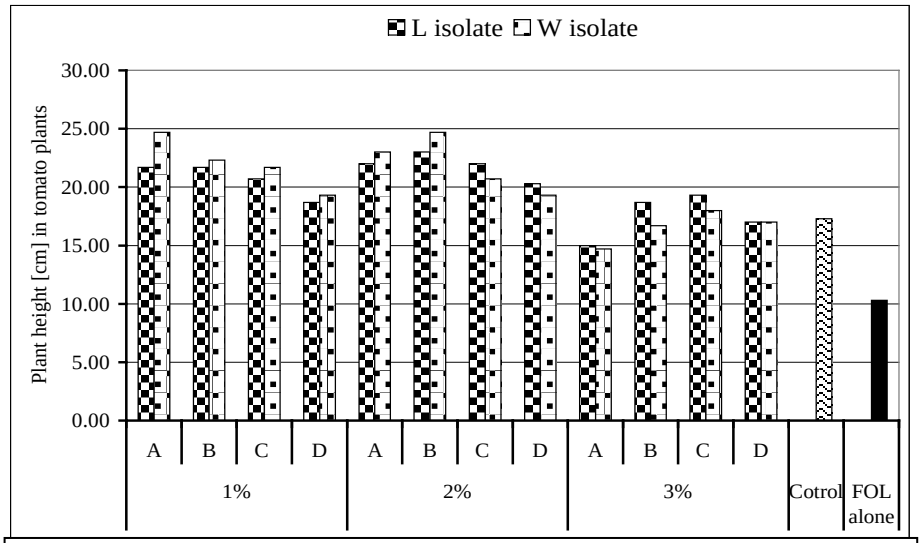
Table (9): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the plant height [cm] of tomato and eggplant.

Application treatments *	Plant height (cm)/tomato plant (FOL)			Plant height (cm)/plant of eggplant (SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	21.7	24.7	23.2	19.7	20.1	19.89
1% B	21.7	22.3	22.0	18.2	18.7	18.44
1% C	20.7	21.7	21.2	18.8	18.3	18.56
1% D	18.7	19.3	19.0	17.6	15.9	16.72
2% A	22.0	23.0	22.5	18.4	18.7	18.56
2% B	23.0	24.7	23.8	17.9	16.2	17.06
2% C	22.0	20.7	21.3	18.4	18.0	18.22
2% D	20.3	19.3	19.8	13.3	15.8	14.56
3% A	15.0	14.7	14.8	11.9	11.7	11.78
3% B	18.7	16.7	17.7	10.1	10.8	10.44
3% C	19.3	18.0	18.7	11.6	11.6	11.56
3% D	17.0	17.0	17.0	10.7	9.9	10.28
Non-inoculated	17.3	17.3	17.3	10.7	10.7	10.67
Pathogen alone **	10.3	10.3	10.3	5.3	5.3	5.33
Mean	19.1	19.3		14.47	14.40	
L.S.D. at 5% for:						
Isolates		NS			NS	
Treatments		2.11			2.145	
Interaction		NS			NS	

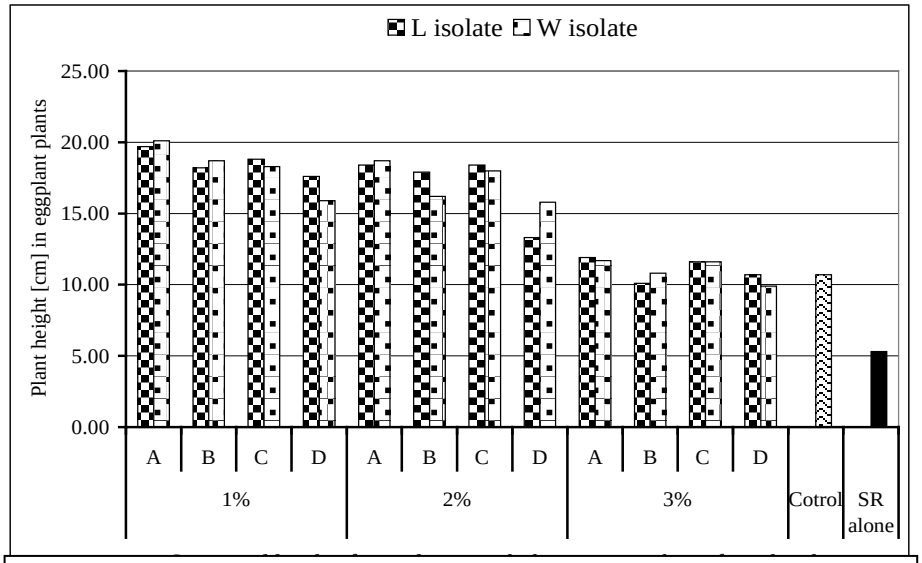
* Treatments = inoculation of tested VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.

Status and levels of inoculation with the root-VAM-like isolates



Status and levels of inoculation with the root-VAM-like isolates



Status and levels of inoculation with the root-VAM-like isolates

Fig. (9): Plant height [cm] of tomato (above) and eggplant (below) plants as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

On the other hand, treatments of A, B, C & D either at 1% or 2% levels significantly increased plant height (14.56-19.89cm) whereas treatments of A, B, C & D at 3% level had no significant effect (10.28-11.78cm) compared to the non-inoculated control (10.7cm). Although plant height was not varied significantly between tested root-entophytic isolates, the present results proved that the treatments of 1 and 2% levels for both L and W isolates and at all statuses i.e. A, B, C and D produced higher plant height compared to the control as well as inoculation with SR alone.

5.2.5. On fresh weight of tomato shoots under stress of infection with FOL pathogen:

The data outlined in **Table (10)** and **Fig. (10)** prove that, the fresh weight of shoot [g/plant] of a tomato plant was significantly affected by tested VAM-like isolates (L&W), inoculation treatments but not by the interaction in between. In this respect, the isolate W was significantly better for increasing fresh weight of shoot (4.56g) than the isolate L (4.20g). Most of tested treatments significantly increased the fresh weight of shoot (3.26-6.51g) compared with inoculation with FOL alone (1.97g). The highest significant increase in fresh weight of shoot (6.03-6.51g) was produced by applying treatments of A at 1% level, A, B and C at 2% level whereas, the least significant increase (3.19g) was induced by using 3%B. Only applying treatment of 3%A had no significant effect on the fresh weight of shoot (2.24g) compared with inoculation with FOL alone. On the contrary, all statuses at level 3% i.e. A, B, C and D for isolate L

and A, B and D for isolate W decreased fresh weight of shoot compared with the non-inoculated control (Fig. 7).

Table (10): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the fresh weight (FW) of shoots (g/plant) of tomato and eggplant.

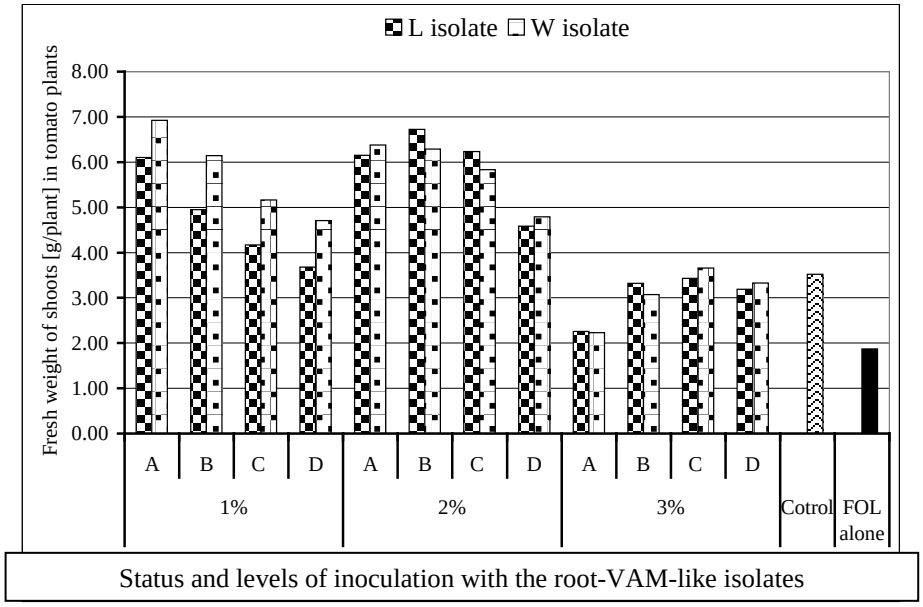
Application treatments *	FW of shoot (g/tomato plant) (FOL)			FW of shoot (g/plant of eggplant (SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	6.10	6.92	6.51	4.10	3.58	3.84
1% B	4.95	6.14	5.54	3.23	3.05	3.14
1% C	4.17	5.16	4.67	3.76	3.06	3.41
1% D	3.68	4.71	3.53	2.11	1.92	2.02
2% A	6.15	6.38	6.26	2.95	2.74	2.85
2% B	6.72	6.29	6.51	2.59	2.51	2.55
2% C	6.23	5.83	6.03	2.77	2.71	2.74
2% D	4.58	4.79	4.68	2.12	1.99	2.06
3% A	2.26	2.23	2.24	1.69	1.50	1.59
3% B	3.32	3.07	3.19	1.32	1.27	1.30
3% C	3.43	3.66	3.55	1.66	1.44	1.55
3% D	3.19	3.33	3.26	1.02	0.91	0.96
Non-inoculated	3.52	3.52	3.52	1.75	1.75	1.75
Pathogen alone **	1.87	1.87	1.87	0.37	0.37	0.37
Mean	4.20	4.56	4.30	2.25	2.06	
L.S.D. at 5% for:						
Isolates		0.040			0.026	
Treatments		0.561			0.369	
Interaction		NS			NS	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.

5.2.6. On fresh weight of eggplant shoots under stress of infection with SR:

Regarding shoot fresh weight [g/plant] of eggplant plants, the same data show that it was significantly affected by the tested VAM-like isolates and inoculation treatments but not by or the interaction between them. The L isolate recorded higher fresh weight of shoot (2.25g) than isolate W (2.06g). All tested inoculation treatments significantly increased the fresh weight of shoot (0.96-3.84g) compared to inoculation with SR alone (0.37g). Comparing to the non-inoculated control (1.75g), the highest significant increase in the fresh weight of shoot was produced by applying treatments of 1%A (3.84g), 1%C (3.41g), 2%A (3.14g), 2%A (2.85g), 2%C (2.74g) and 2%B (2.55g), respectively. On the other hand, treatments of 2%D, 1%D, 3%A and 3%C had no significant effect on the fresh weight of shoot (1.55-2.06g) meanwhile 3%B and 3%D significantly decreased it (0.96-1.3g) compared to the non-inoculated control.



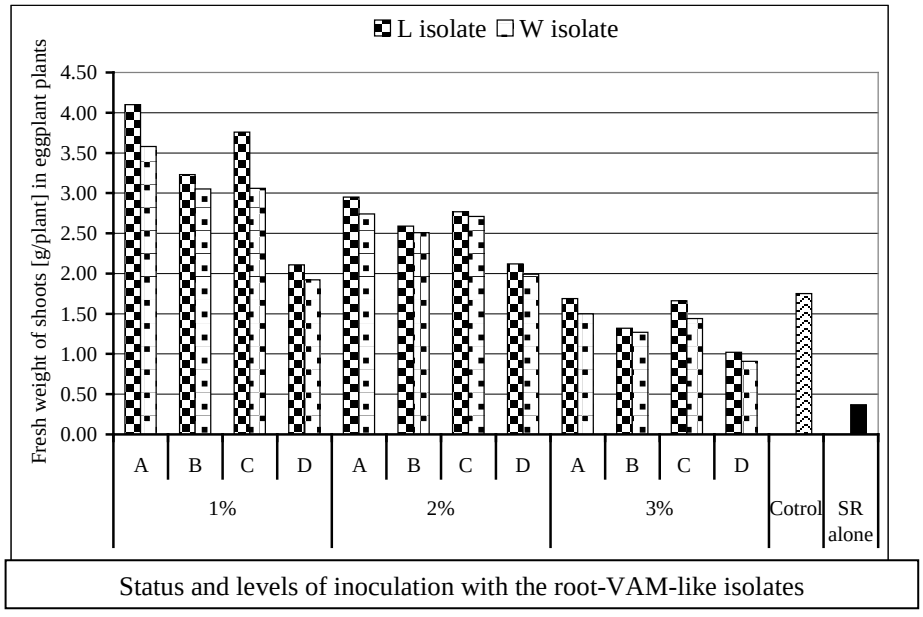


Fig. (10): Fresh weight of shoots [g/plant] for plants of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

5.2.7. On dry weight of tomato shoots under stress of infection with FOL pathogen:

The obtained data **(Table 11** and **Fig. 11)** prove that, all tested inoculation treatments significantly increased the shoot dry weight (SDW) [g/plant] of tomato plants (0.33-0.84g) compared with inoculation with FOL alone (0.24g). Applying treatments of 2%B recorded the highest significant increase in the SDW (0.84g) followed by 2%A (0.83g) and 2%C (0.78g) without significant variations between them. However, applying 3%A significantly decreased the SDW (0.33g) compared to the non-inoculated control (0.43g). Among tested inoculation treatments, using 2%A, 2%B or 2%C were the best treatments, increased the SDW compared to inoculation with FOL alone and the non-inoculated control, respectively **(Fig. 8)**.

5.2.8. On dry weight of eggplant shoots under stress of infection with SR:

Concerning shoot dry weight (SDW) [g/plant] of eggplant plants, the same data indicated that it was significantly affected by the tested VAM-like isolates and inoculation treatments but not by the interaction between them. The L isolate recorded higher SDW (0.45g) than isolate W (0.39g). All tested inoculation treatments significantly increased the SDW (0.17-0.81 gm) compared to inoculation with SR alone (0.06g). In this regard, the highest significant increase in the SDW was produced by treatment of 1%A (0.81g) followed by 1%C (0.61g), 2%A (0.60g), 1%B (0.58g), 2%C (0.57g), 2%B (0.53g) and 1%D (0.43g) compared to the non-inoculated control as well as to inoculation with SR alone. However, the SDW recorded by

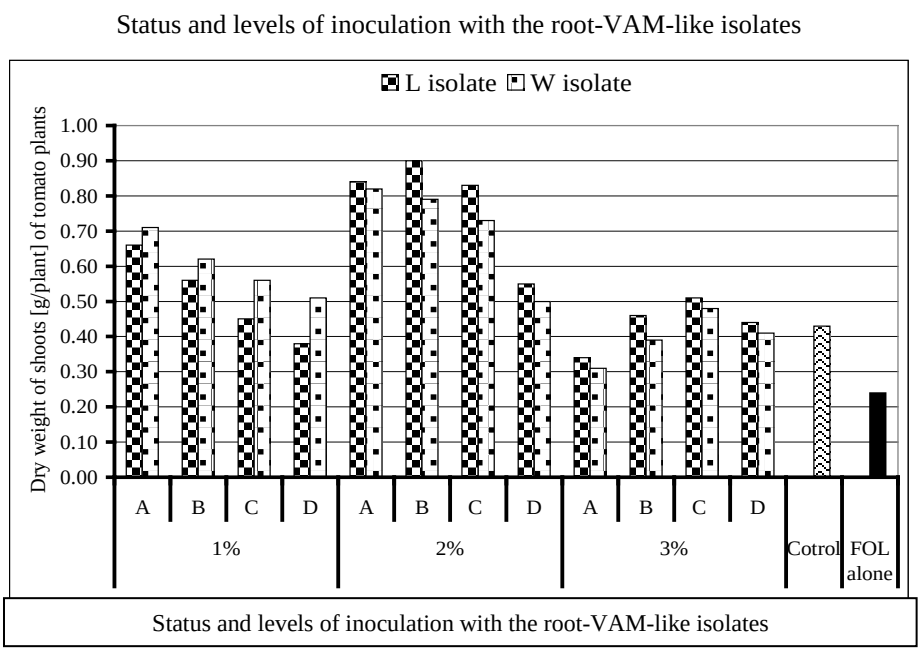
treatments of 2%D (0.41gm), 3%A (0.28gm) and 3%C (0.25gm) was not significantly varied between treatments of 3%B (0.22g) and 3%D (0.17g) compared to the non-inoculated control.

Table (11): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the dry weight (DW) of shoots (g/plant) of tomato and eggplant.

Application treatments *	DW of shoot (g/tomato plant) (FOL)			DW of shoot (g/plant of eggplant (SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	0.66	0.71	0.69	0.95	0.66	0.81
1% B	0.56	0.62	0.59	0.61	0.55	0.58
1% C	0.45	0.56	0.51	0.66	0.57	0.61
1% D	0.38	0.51	0.44	0.52	0.34	0.43
2% A	0.84	0.82	0.83	0.62	0.58	0.60
2% B	0.90	0.79	0.84	0.57	0.50	0.53
2% C	0.83	0.73	0.78	0.58	0.57	0.57
2% D	0.55	0.50	0.53	0.40	0.43	0.41
3% A	0.34	0.31	0.33	0.32	0.24	0.28
3% B	0.46	0.39	0.43	0.24	0.20	0.22
3% C	0.51	0.48	0.50	0.28	0.22	0.25
3% D	0.44	0.41	0.43	0.18	0.15	0.17
Non-inoculated	0.43	0.43	0.43	0.33	0.33	0.33
Pathogen alone **	0.24	0.24	0.24	0.06	0.06	0.06
Mean	0.54	0.54		0.45	0.39	
L.S.D. at 5% for:						
Isolates		NS			0.007	
Treatments		0.068			0.091	
Interaction		NS			NS	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



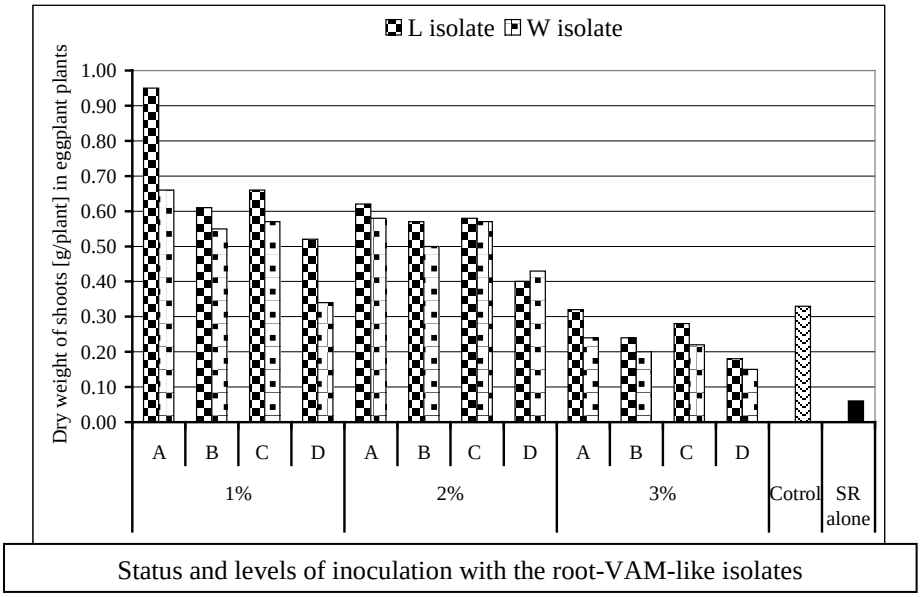


Fig. (11): Dry weight of shoots [g/plant] for plants of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

5.2.9. On tomato root length under stress of infection with FOL pathogen:

The obtained data (**Table 12** and **Fig. 12)** prove that, the isolate W significantly increased root length (RL) of tomato plant more than the L isolate, the RL recorded by the two isolates was 8.14 and 7.74 cm, respectively. However, all tested VAM-like treatments significantly increased RL (5.67-10.83cm) compared with inoculation with FOL alone (2.0cm). Among tested treatments, the highest significant increases in the RL (10.50-10.83cm) were recorded by using 1%A, 1%B, 2A or 2%B without significant differences in between. In general, treatments of 1%A, 1%B, 2%A, 2%B, 2%C and 1%C recorded the highest increase in the RL compared to the non-inoculated control and inoculation with FOL alone, respectively. However, the least significant increase in the RL (5.33cm) was recorded by applying 3%A compared to the FOL inoculation alone (2.0cm). The RL was significantly lower in case of applying 3%A, (5.33cm), 3%D (5.67cm), 3%B (6.0cm) and 3%C (6.17cm) compared to RL in the non-inoculated control treatment (7.0cm).

5.2.10. On eggplant root length under stress of infection with SR:

Regarding root length (RL) of eggplant plants, the same data stated that it was significantly affected by the tested VAM-like isolates and inoculation treatments but not by the interaction between them. The L isolate recorded higher RL (8.6cm) than isolate W (7.71cm). All tested inoculation treatments significantly increased the RL (4.39-13.83cm) compared to inoculation with SR alone (2.33cm). Comparing to the non-

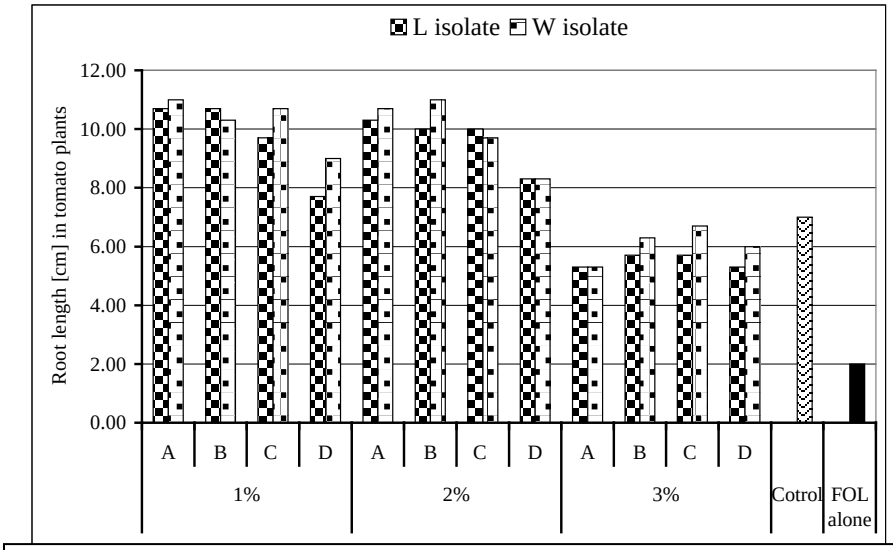
inoculated control (5.67cm), the highest significant increase in the RL was recorded by treatment of 1%A (13.83cm) and 1%C (12.72cm) followed by 2%A (11.61cm), 1%B (11.44cm), 2%C (11.00cm), 2%B (9.89cm), 1%D (7.72cm) and 2%D (7.33cm). On the other hand, the RL recorded by treatments of 3%A, 3%B, 3%C and 3%D (4.39-5.5cm) was not significantly varied when compared to the non-inoculated control.

Table (12): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the root length (cm/plant) of tomato and eggplant.

Application treatments *	Root length (cm)/tomato plant) (FOL)			Root length (cm)/plant of eggplant (SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	10.7	11.0	10.83	13.9	13.8	13.83
1% B	10.7	10.3	10.50	12.2	10.7	11.44
1% C	9.7	10.7	10.17	13.1	12.3	12.72
1% D	7.7	9.0	8.33	8.6	6.9	7.72
2% A	10.3	10.7	10.50	12.8	10.4	11.61
2% B	10.0	11.0	10.50	11.8	8.0	9.89
2% C	10.0	9.7	9.83	11.6	10.4	11.00
2% D	8.3	8.3	8.33	6.7	8.0	7.33
3% A	5.3	5.3	5.33	5.8	5.2	5.50
3% B	5.7	6.3	6.00	5.2	5.4	5.33
3% C	5.7	6.7	6.17	5.8	5.1	5.44
3% D	5.3	6.0	5.67	5.1	3.7	4.39
Non-inoculated	7.0	7.0	7.00	5.7	5.7	5.67
Pathogen alone **	2.0	2.0	2.00	2.3	2.3	2.33
Mean	7.74	8.14		8.60	7.71	
L.S.D. at 5% for:						
Isolates		0.044			0.108	
Treatments		0.613			1.512	
Interaction		NS			NS	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



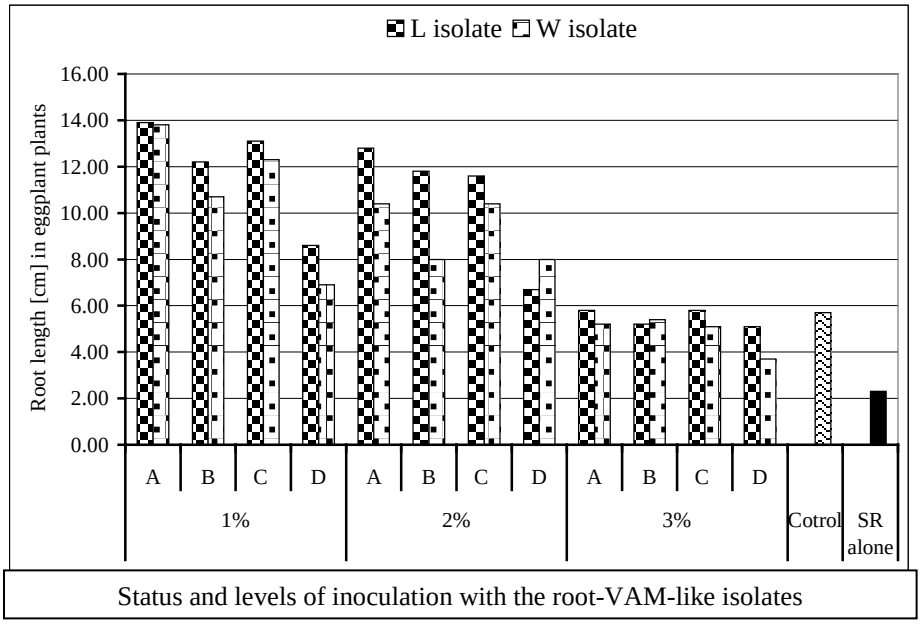


Fig. (12): Root length [cm] for plants of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

5.2.11. On tomato root fresh weight under stress of infection with FOL pathogen:

The obtained data **(Table 13 and Fig. 13)** prove that, the tested VAM-like isolates (L&W) were not significantly varied concerning their effects on the Root fresh weight (RFW) of tomato plants [g/plant] while, it was significantly affected by tested inoculation treatments as well as by the interactions between root-entophytic isolates and inoculation treatments. All tested inoculation treatments significantly increased the RFW (0.29-1.12g) compared with FOL inoculation (0.15g). The highest significant increase in the RFW was recorded by applying 2%A (1.12g), 2%B (1.07g), 1%A (1.06g) followed by using 2%C (0.98g), 1%B (0.84g), 1%C (0.78g), 2%D (0.73g), 1%D (0.67g), while it was significantly decreased by applying 3%B, 3%C, 3%D and 3%A (0.43, 0.42 0.33 and 0.29g, respectively) compared to the non-inoculated control (0.55g).

Concerning interactions, the highest significant increase in the RFW was produced by treatments of A, B and C at 2% level (1.03-1.09g) and 1%A (1.02g) in case of isolate L, whereas the highest significant increase in the RFW in case of isolate W was produced by treatments of A at 1% level and A & B at 2% level (1.10-1.14g) without significant differences in between followed by B, C and D at 1% level and C & D at 2% level (0.72-0.90 g) compared to the non-inoculated control as well as inoculation with FOL alone. However, all inoculation treatments at 3% level *i.e.* A, B, C and D of both L & W isolates slightly decreased RFW compared to the non-inoculated control

meanwhile slightly increased it compared to inoculation with FOL alone **(Fig. 10)**.

5.2.12. On eggplant root fresh weight under stress of infection with (SR):

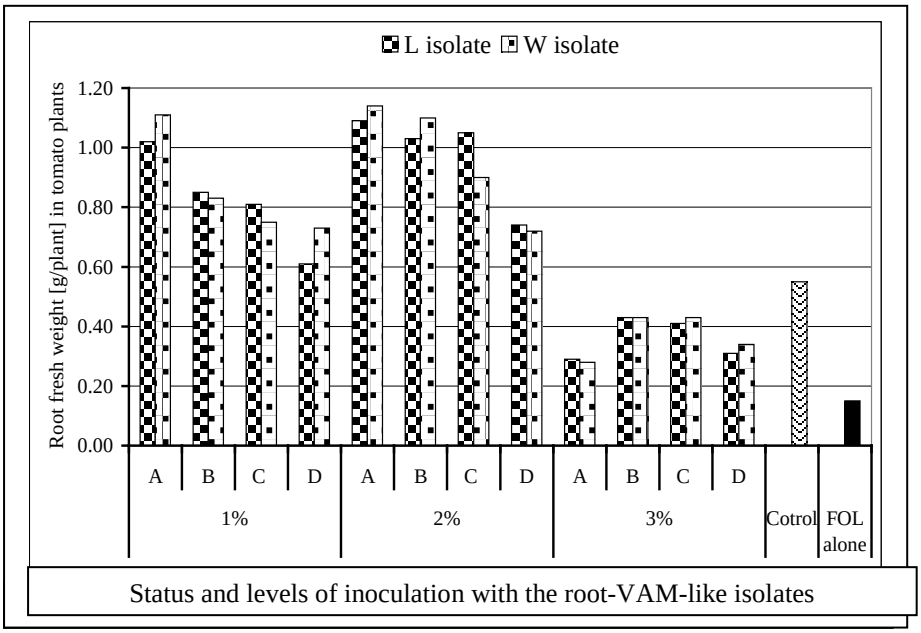
As for root fresh weight (RFW) [g/plant] of eggplant plants, the same data in **Table (13) and Fig. (13)** state that it was significantly affected by tested inoculation treatments whereas it was not affected significantly by the tested VAM-like isolates or the interaction between isolates and inoculation treatment. All tested inoculation treatments significantly increased the RFW (0.4-2.04g) compared to the inoculation with SR alone (0.11g). However, the highest increase in the RFW was recorded by applying 1%A (2.04g) followed by 2%A (1.70g), 1%C (1.62g), 1%B (1.49g), 2%C (1.45g), 2%B (1.25g), 1%D (1.01g) even compared to the non-inoculated control (0.72g) comparing with the non-inoculated control. On contrary, the RFW recorded by treatment of 3%D was significantly lower (0.4g) while it was not significantly varied in case of treatments of 2%D, 3%A, 3%B and 3%C (0.55-0.7g) compared to the non-inoculated control.

Table (13): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the FW of tomato or eggplant roots (g/plant).

Application treatments *	FW of roots g/tomato plant) (FOL)			FW of roots g /plant of eggplant (SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	1.02	1.11	1.06	2.21	1.87	2.04
1% B	0.85	0.83	0.84	1.49	1.49	1.49
1% C	0.81	0.75	0.78	1.66	1.57	1.62
1% D	0.61	0.73	0.67	1.10	0.93	1.01
2% A	1.09	1.14	1.12	1.81	1.59	1.70
2% B	1.03	1.10	1.07	1.35	1.15	1.25
2% C	1.05	0.90	0.98	1.43	1.47	1.45
2% D	0.74	0.72	0.73	0.67	0.73	0.70
3% A	0.29	0.28	0.29	0.63	0.60	0.61
3% B	0.43	0.43	0.43	0.51	0.58	0.55
3% C	0.41	0.43	0.42	0.58	0.58	0.58
3% D	0.31	0.34	0.33	0.41	0.39	0.40
Non-inoculated	0.55	0.55	0.55	0.72	0.72	0.72
Pathogen alone **	0.15	0.15	0.15	0.11	0.11	0.11
Mean	0.67	0.68		1.05	0.98	
L.S.D. at 5% for:						
Isolates		NS			NS	
Treatments		0.066			0.181	
Interaction		0.066			NS	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



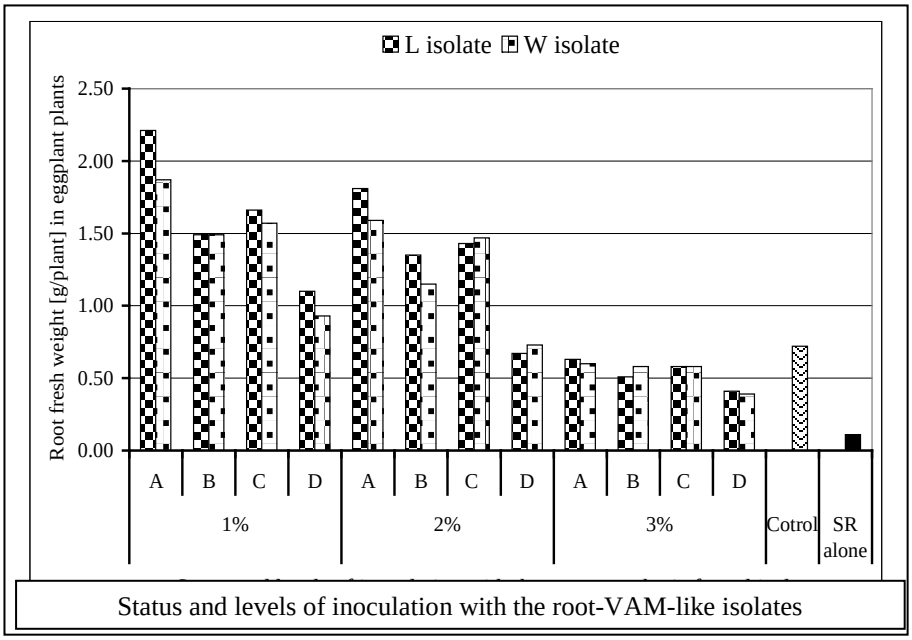


Fig. (13): Root fresh weight [g/plant] for plants of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

5.2.13. On tomato root dry weight under stress of infection with FOL pathogen:

The obtained data **(Table 14 and Fig. 14)** prove that, the root dry weight (RDW) [g/plant] was significantly varied between VAM-like isolates. It was significantly higher in the VAM-like isolate W (0.13g) than VAM-like isolate L (0.12g). The RDW was significantly affected also by tested inoculation treatments as well as by the interactions between VAM-like isolates and treatments. All tested inoculation treatments significantly increased the RDW (0.08-0.20g) compared to inoculation with FOL alone (0.040g). Comparing with the non-inoculated control, the following treatments caused the highest significant increased in the RDW: 2%A (0.20g), 2%B (0.20g), 1%A (0.19g) without significant differences between them followed by 2%C (0.16g), 1%B 0.15g), 1%C (0.13g) and 2%D (0.13g). The RDW, however, was significantly decreased (0.08-0.09g) by applying treatments of 3%A, 3%B and 3%D while it was not affected significantly by using 1%D and 3%C (0.10-0.11g) compared to the non-inoculated control.

As for interactions, the same results proved that applying 1%A or 1%C DW recorded significantly higher RDW in case of isolate W than isolate L but this trend was reversed when 2%C treatment was used. However, no significant differences was observed between the two isolates when 1%C, 1%D, 2%A, 2%B, 2%D were used. All these treatments significantly increased RDW compared to the non-inoculated control while, applying 3%A, 3%B, 3%C or 3%D significantly decreased it.

Table (14): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the DW of tomato or eggplant roots (g/plant).

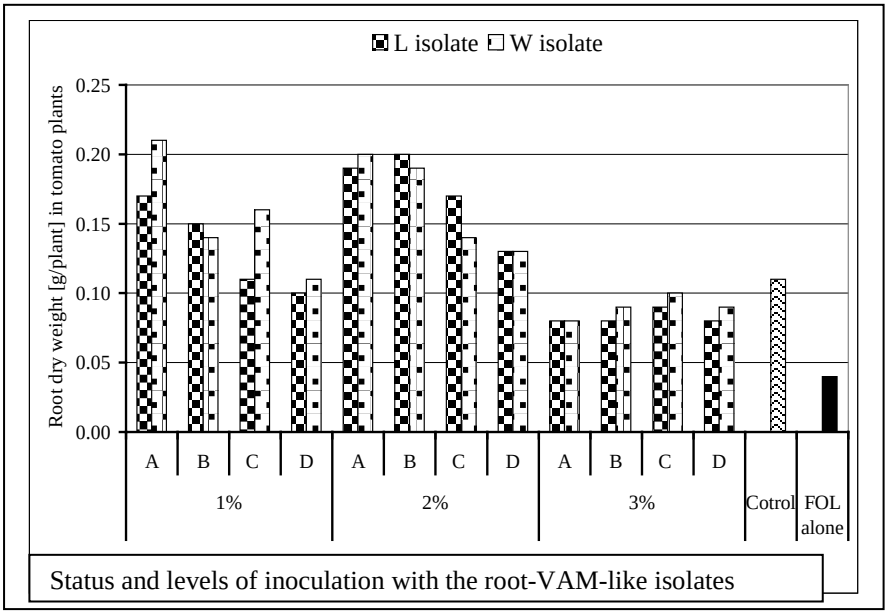
Application treatments *	DW of roots g/tomato plant) for			DW of roots g /plant of eggplant for		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	0.17	0.21	0.19	0.50	0.42	0.460
1% B	0.15	0.14	0.15	0.28	0.30	0.294
1% C	0.11	0.16	0.13	0.37	0.33	0.349
1% D	0.10	0.11	0.11	0.18	0.14	0.158
2% A	0.19	0.20	0.20	0.30	0.25	0.277
2% B	0.20	0.19	0.20	0.20	0.17	0.187
2% C	0.17	0.14	0.16	0.21	0.24	0.226
2% D	0.13	0.13	0.13	0.13	0.12	0.127
3% A	0.08	0.08	0.08	0.11	0.11	0.108
3% B	0.08	0.09	0.09	0.09	0.10	0.094
3% C	0.09	0.10	0.10	0.10	0.10	0.096
3% D	0.08	0.09	0.09	0.09	0.06	0.075
Non-inoculated	0.11	0.11	0.11	0.10	0.10	0.103
Pathogen alone **	0.04	0.04	0.04	0.03	0.03	0.029
Mean	0.12	0.13		0.193	0.176	

L.S.D. at 5% for:

Isolates	0.001	0.003
Treatments	0.014	0.036
Interaction	0.014	NS

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



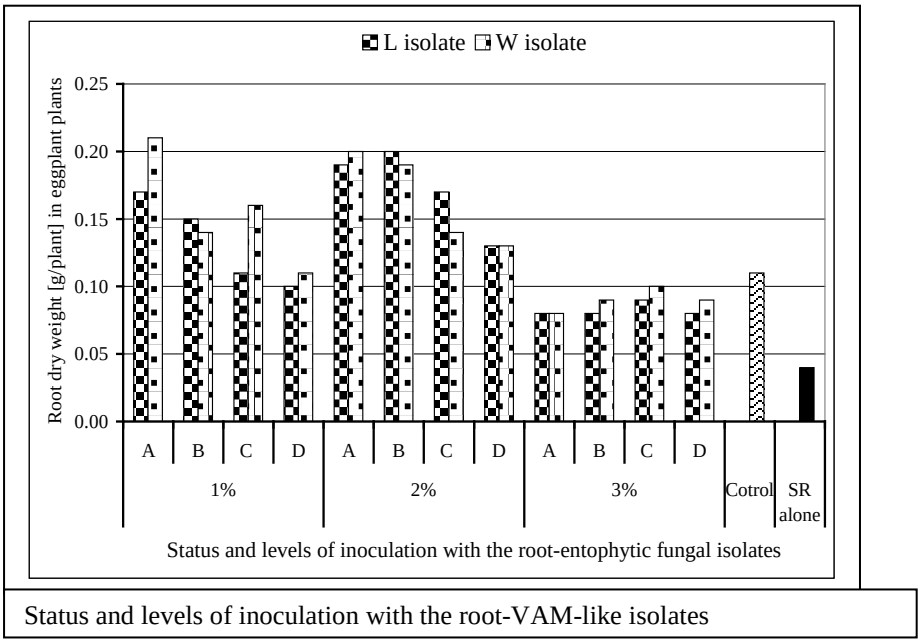


Fig. (14): Root dry weight [g/plant] for plants of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

5.2.14. On eggplant root dry weight under stress of infection with SR:

With regard to the root dry weight (RDW) [g/plant] of eggplant plants the same data stated that it was significantly affected by the tested VAM-like isolates and inoculation treatments but not by or the interaction between them. The L isolate recorded higher RFW (0.193g) than isolate W (0.176g). All tested inoculation treatments significantly increased the RFW (0.075-0.46g) compared to inoculation with SR alone (0.029g). Regarding inoculation treatments, the highest RFW was recorded by applying 1%A (0.46g) followed by 1%C (0.349g), 1%B, 2%A and 2%C (0.294-0.226g) and 2%B and 1%D (0.187-0.158g). However, treatments of 2%D, 3%A, 3%B, 3%C and 3%D had no significant effect on the RDW (0.127-0.075g) when compared to the non-inoculated control.

6. Effects of reciprocal interactions between the tested VAM-like fungi and some pathogenic fungi on total photosynthetic pigments in plant leaves:

6.1. Total photosynthetic pigments in tomato leaves under stress of infection with FOL pathogen:

The obtained results shown in **Table (15)** and **Fig. (15)** illustrate that the synthesis of total photosynthetic pigments in tomato leaves [mg/g FW] seems to be helpful by VAM-like isolate L (5.16 mg) more than by VAM-like isolate W (4.93 mg). It is clear that, all tested inoculation treatments induced obvious increase in the total amount of leaf pigments (4.92-10.05 mg) compared to inoculation with FOL alone (3.56 mg). The highest amount of leaf pigments was induced by using treatments of

1%A (10.05 mg) and 2%A (9.36 mg) whereas, the lowest was recorded by using 3%D treatment (4.92 mg). On the other hand, all treatments of 1% level (i.e. A, B, C & D) and 2% level (*i.e.* 2%A, 2%B, & 2%C) increased the total leaf pigments to different extents (8.08-10.05 mg) while all treatments of 3% level (*i.e.* A, B, C & D) in addition to 2%D caused appreciable decrease (4.92-6.47 mg) compared to the non-inoculated control.

In case of isolate L, applying 2%A recorded the highest increases in leaf pigments (9.96 mg) followed by 1%A (9.46 mg), 2%B (9.14 mg), 1%B (8.96 mg), 2%C (8.60 mg), 1%C (8.38 mg) and 1%D (7.96 mg), all these treatments increased total leaf pigments either compared to the non-inoculated control (7.42 mg) as well as inoculation with FOL alone (3.56 mg). On the other hand, applying treatments of 2%D, 3%A, 3%B, 3%C and 3%D slightly decreased the total leaf pigments compared to the non-inoculated control while slightly increased it compared to inoculation with FOL alone. About inoculation with isolate W, the highest amount of leaf pigments were recorded by the following treatments: 1%A (10.64 mg), 1%B (8.79 mg), 2%A (8.77 mg), 1%C (8.48 mg) comparing to the non-inoculated control (7.42 mg) as well as inoculation with FOL alone (3.56 mg). It is interest to state that applying all inoculation treatments at 3% level for both isolates *i.e.* 3%A, 3%B, 3%C and 3%D caused obvious decreases in amounts of the total leaf pigments *i.e.* 4.91-5.88 mg (for L isolate) and 4.93-6.11 mg (for W isolate) comparing to the non-inoculated control (7.42 mg) but increased it compared to inoculation with FOL alone (3.56 mg).

Table (15): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the total leaf pigments (mg/g FW) of eggplant or tomato plants.

Application treatments *	Total photosynthetic pigments (mg/g FW) in leaves of tomato(FOL)			Total photosynthetic pigments (mg/g FW) in leaves of eggplant(SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	9.46	10.64	10.05	8.84	8.16	8.50
1% B	8.96	8.79	8.87	6.47	6.35	6.41
1% C	8.38	8.48	8.43	7.6	6.64	7.12
1% D	7.96	8.11	8.03	5.52	5.08	5.30
2% A	9.96	8.77	9.36	8.51	7.89	8.20
2% B	9.14	8.26	8.70	5.79	7.17	6.48
2% C	8.60	7.55	8.08	6.51	6.66	6.59
2% D	5.85	7.08	6.47	4.55	4.24	4.40
3% A	5.88	6.11	5.99	4.59	4.92	4.76
3% B	5.53	5.82	5.67	4.51	3.97	4.24
3% C	5.07	5.69	5.38	3.96	4.28	4.12
3% D	4.91	4.93	4.92	3.57	3.05	3.31
Non-inoculated	7.42	7.42	7.42	3.9	3.9	3.90
Pathogen alone **	3.56	3.56	3.56	2.89	2.89	2.89
Mean	5.16	4.93		5.52	5.37	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

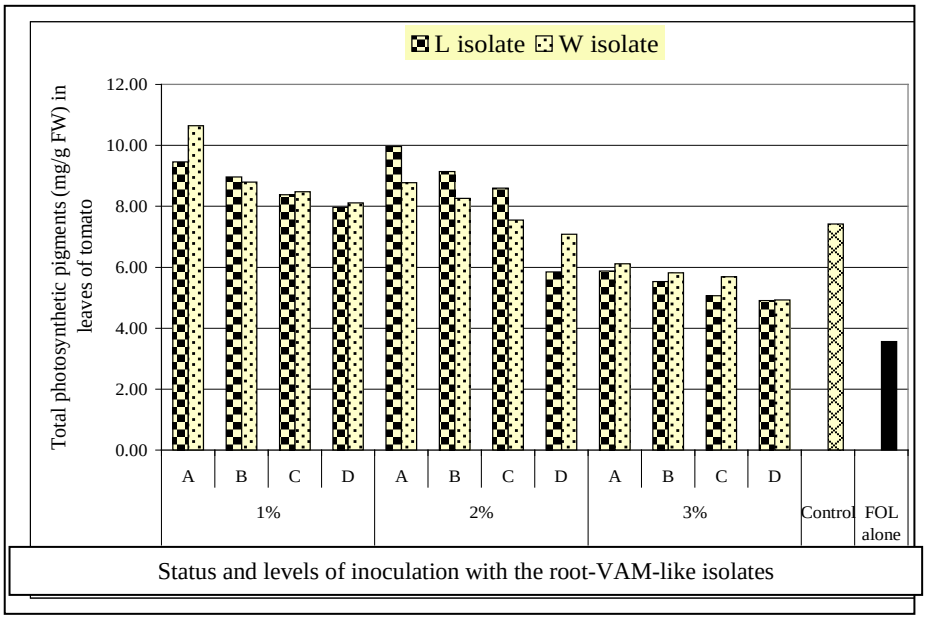
** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.

6.2. Total photosynthetic pigments in eggplant plant leaves under stress of infection with SR:

The same data in **Table (15)** and **Fig. (15)** illustrate that the total amount of leaf pigment (mg/g FW) in leaves of eggplant was slightly higher in case of L isolate (5.52 mg) than W isolate (5.37 mg). In this regard, all tested inoculation treatments induced obvious increase (3.31-8.5 mg) compared to inoculation with SR alone (2.89 mg). The highest amounts were induced by

using treatments of 1%A and 2%A (8.2-8.5 mg) whereas the lowest amount was recorded by 3%D treatment (3.31 mg). All tested treatments, however, increased the total leaf pigments except treatment of 3%D which caused obvious decrease in (3.31mg) compared to the non-inoculated control (3.9mg).

In case of L isolate, using treatments of 1%A, 2%A, 1%C, 2%C, 1%B, 2%B and 1%D, respectively recorded the highest increase in the total leaf pigments compared to inoculation with SR alone as well as to the non-inoculated control. This trend in L isolate was slightly varied in case of W isolate as treatments of 1%A, 2%A, 2%B, 2%C, 1%B, 1%C and 1%D, respectively recorded the highest increase in the total leaf pigments either compared to inoculation with SR alone or to the non-inoculated control. Among all tested treatments, treatment of 3%D only decreased the total leaf pigments (in case of isolates L and W) compared to the non-inoculated control whereas the same treatment increased the total leaf pigments in case of both isolates compared to inoculation with SR alone



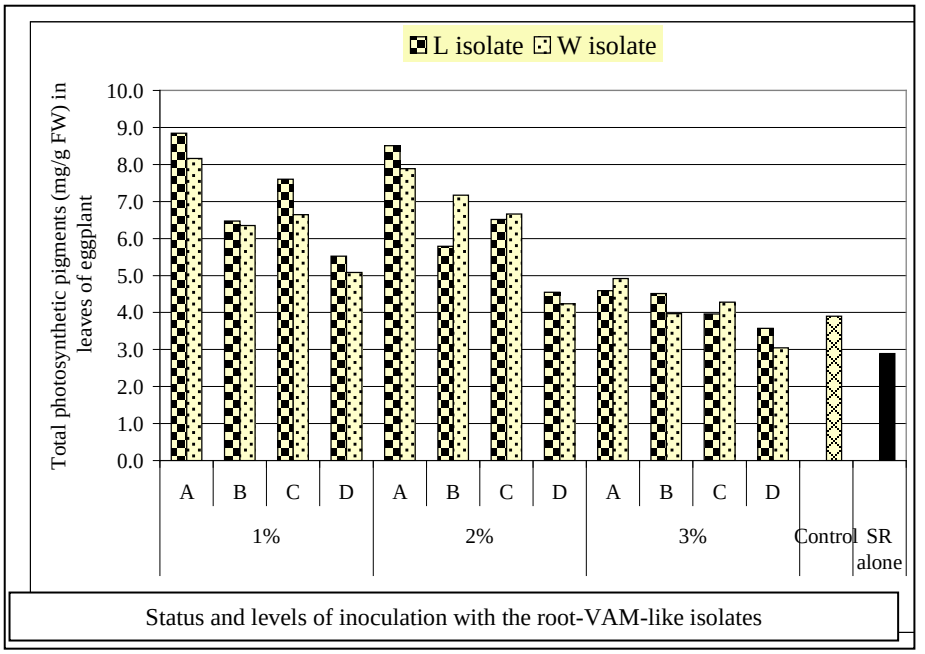


Fig. (15): Total photosynthetic pigments (mg/g FW) in leaves of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

7. Effects of reciprocal interactions between the tested VAM-like fungi and some pathogenic fungi on activities of some oxidative enzymes in plant leaves:

7.1. Chitinase activity in tomato leaves under stress of infection with *FOL* pathogen:

The obtained results **(Table 16 and Fig. 16)** show that, activity (O.D./min/g FW) of the chitinase enzyme in tissues of tomato leaves were affected to different extents by tested inoculation treatments. Isolate L seems to enhance chitinase activity (4.17) more than isolate W (3.04). The highest activity of chitinase enzyme was induced by using the following inoculation treatments: 2%A & 2%C (6.36), 2%B (5.67), 1%A (5.12), 1%B (4.88) and 3%B (4.23) whereas the lowest increase was recorded by using 3%D (1.74) compared to either non-inoculated control (1.70) or inoculation with FOL alone (1.50). As for L isolate, all treatments increased chitinase activity either compared to the non-inoculated control or to inoculation with FOL alone, the highest activity was induced by using 2%A (9.63), 2%C (9.54), 2%B (6.69), 1%A (4.59), 3%B (4.98), 1%B (4.11) whereas the lowest one was recorded by using 3%D (1.98). With regard to isolate W, the highest activity was recorded by 1%A (5.64), 1%B (5.64), 2%B (4.65), 1%C (4.11), 3%B (3.48), 2%C (3.18), 2%A (3.09), 3%C (3.09), and 3%A (1.71), respectively whereas it was obviously decreased by using 2%D (1.65), 1%D (1.65) and 3%D (1.50) comparing to the non-inoculated control (1.70), however, the last treatment 3%D had no effect on chitinase activity compared to inoculation with FOL alone (1.50). It is interest to state that, most treatments of isolate

L recorded higher activity of chitinase enzyme than isolate W except treatments of 1%A, 1%B, 1%C & 3%C as they recorded the reverse trend.

Table (16): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the chitenase activity in plant leaves of tomato or eggplant.

Application treatments *	Chitenase activity (O.D./min/g FW) of tomato leaves(FOL)			Chitenase activity (O.D./min/g FW) of eggplant leaves (SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	4.59	5.64	5.12	42.0	40.6	41.28
1% B	4.11	5.64	4.88	37.4	33.0	35.22
1% C	3.54	4.11	3.83	40.0	40.4	40.22
1% D	2.31	1.65	1.98	17.8	15.6	16.71
2% A	9.63	3.09	6.36	31.2	36.4	33.78
2% B	6.69	4.65	5.67	26.4	22.8	24.60
2% C	9.54	3.18	6.36	29.0	29.3	29.15
2% D	3.18	1.65	2.42	23.2	15.6	19.41
3% A	2.19	1.71	1.95	18.6	12.6	15.60
3% B	4.98	3.48	4.23	7.4	9.6	8.48
3% C	2.40	3.09	2.75	13.8	13.1	13.44
3% D	1.98	1.50	1.74	7.2	7.1	7.14
Non-inoculated	1.70	1.70	1.70	13.2	13.2	13.20
Pathogen alone **	1.50	1.50	1.50	6.6	6.6	6.60
Mean	4.17	3.04		22.41	21.13	

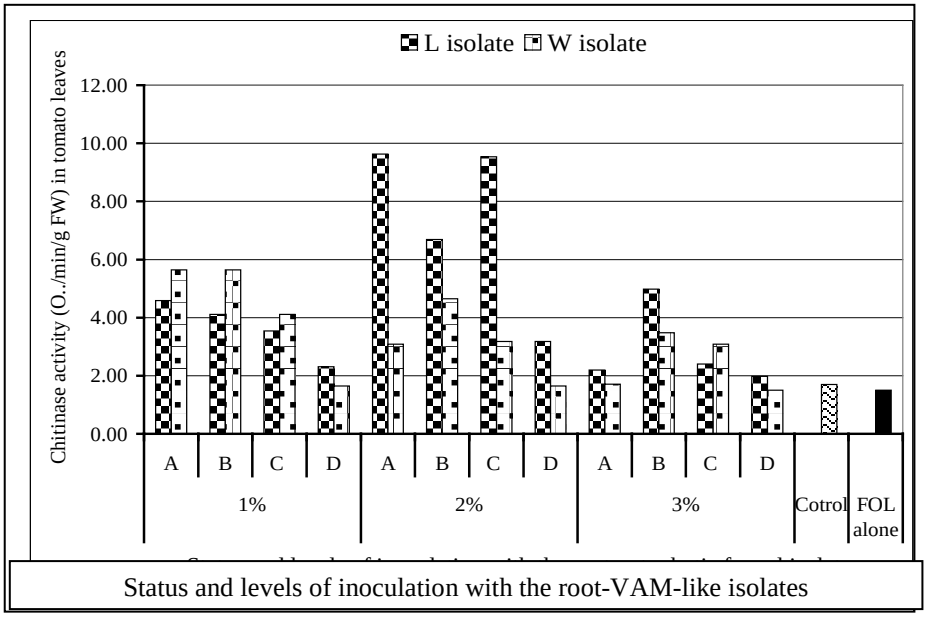
* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.

7.2. Chitinase activity in eggplant leaves under stress of infection with SR:

As for the chitinase activity in eggplant leaves, the same above data in **Table (16) and Fig. (16)** show that it was higher obviously in case of L isolate (22.41) than W isolate (21.13). All

tested inoculation treatments recorded higher chitinase activity (7.14-41.28) than inoculation with SR alone (6.6). Inoculation treatments of 1%A and 1%C recorded the highest chitinase activity followed by 1%B, 2%A, 2%B, 2%C and 2%D, 1%D, 3%A and 3%C, respectively whereas treatments of 3%B and 3%D decreased it compared to the non-inoculated control. Treatments of 1%A, 1%C, 1%B, 2%A, 2%C, 2%B, 2%D, 3%A, 1%D and 3%C, respectively induced the highest chitinase activity *i.e.* 42.0-7.2 in case of L isolate whereas the treatments of 1%A, 1%C, 2%A, 1%B, 2%C, 2%B, 2%D and 1%D, respectively were the best in case of W isolate as they recorded chitinase activity of 40.6-7.1 compared to the non-inoculated control. It is interest to state that the treatments of 3%C, 3%A, 3%B, 3%D decreased chitinase activity (13.1-7.1) in case of W isolate while treatments of 3%B and 3%D only decreased (7.4-7.2) it in case of L isolate compared to their respective non-inoculated control treatments.



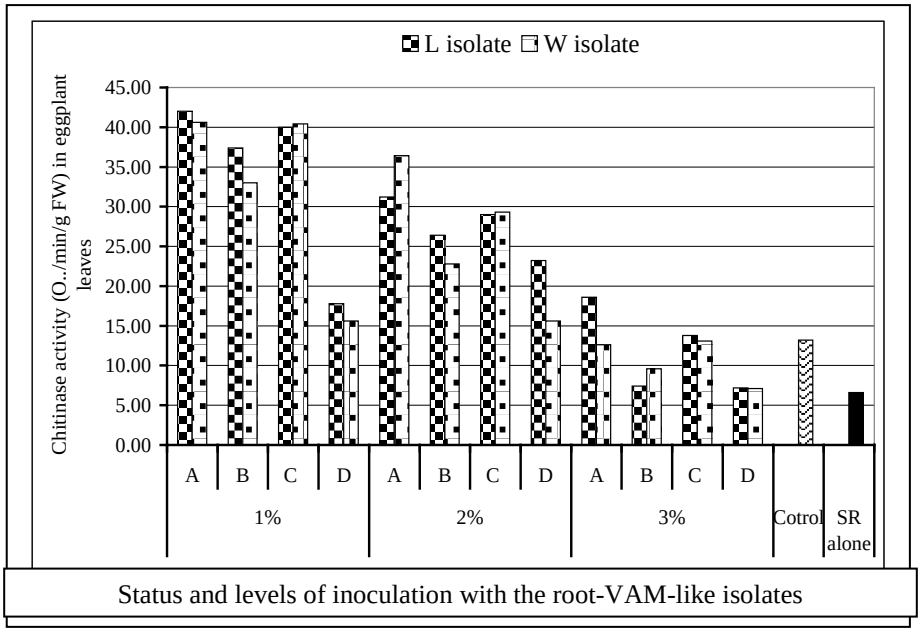


Fig.(16): Chitinase enzyme activity in leaves of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

7.3. Peroxidase (PO) activity in tomato leaves under stress of infection with FOL pathogen:

The data in **Table (17) and Fig. (17)** illustrate that the activity of peroxidase (PO) enzyme (O.D./min/g FW) in tomato leaves was affected to different extents by tested inoculation treatments of VAM-like isolates. Concerning PO activity, the tested inoculation treatments could be arranged in descending order as following: 1%C (16.4), 1%D (14.2), 1%B (14.2), 1%A (13.8), 2%A (12.1), 2%B (12.0), 2%C (11.8), 2%D (10.4), 3%A (8.7), 3%B (8.2), Non-inoculated control (8.2), 3%C (7.2), 3%D (6.6) and FOL alone (5.8). Such arrangement shows, with few exceptions, that most treatments enhanced the PO activity compared to the non-inoculated control. Applying treatment of 3%A showed no effect whereas treatments of 3%C and 3%D decreased PO activity compared to the non-inoculated control, although all these treatments recorded higher PO activity than inoculation with FOL alone. The same data indicated that the PO activity was depending on tested VAM-like fungal isolate. In this respect, the two VAM-like isolates (L&W) gave similar PO activity regarding treatments of 2%A, 2%B & 2%C (11.3-12.7) whereas PO activity recorded by treatments of 1%C, 1%B, 1%D, 1%A & 2%D were higher in the isolate W (11.9-20.7) than the isolate L ((8.9-12.0). In general, the highest PO activity was recorded by 1%C, 1%B & 1%D (17.9-20.7) in case of isolate W while, treatments of 1%C, 1%A & 2%A recorded the highest PO activity (11.6-12.0) in case of isolate L. Furthermore, treatments of 3%C and 3%D in case of isolate W caused appreciable decrease in the PO activity i.e. 6.2 and 5.1 compared to the non-

inoculated control (8.2) and inoculation with FOL alone (5.8), respectively.

Table (17): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the peroxidase activity in plant leaves of tomato and eggplant.

Application treatments *	Peroxidase activity (O.D./min/g FW)of tomato leaves(FOL)			Peroxidase activity (O.D./min/g FW) of eggplant leaves(SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	12.0	15.7	13.8	27.4	22.9	25.15
1% B	9.8	18.5	14.2	26.9	15.6	21.25
1% C	12.0	20.7	16.4	16.4	15.7	16.05
1% D	10.5	17.9	14.2	11.1	15.3	13.20
2% A	11.6	12.7	12.1	15.5	13.5	14.50
2% B	11.3	12.7	12.0	17.7	10.4	14.05
2% C	11.3	12.4	11.8	11.5	9.2	10.35
2% D	8.9	11.9	10.4	13.4	6.4	9.90
3% A	8.2	9.1	8.7	8.9	6.0	7.45
3% B	8.3	8.1	8.2	6.4	5.2	5.80
3% C	8.3	6.2	7.2	5.0	6.4	5.70
3% D	8.1	5.1	6.6	3.9	3.9	3.90
Non-inoculated	8.2	8.2	8.2	10.0	10.0	10.00
Pathogen alone **	5.8	5.8	5.8	3.5	3.5	3.50
Mean	9.59	11.79		12.69	10.29	

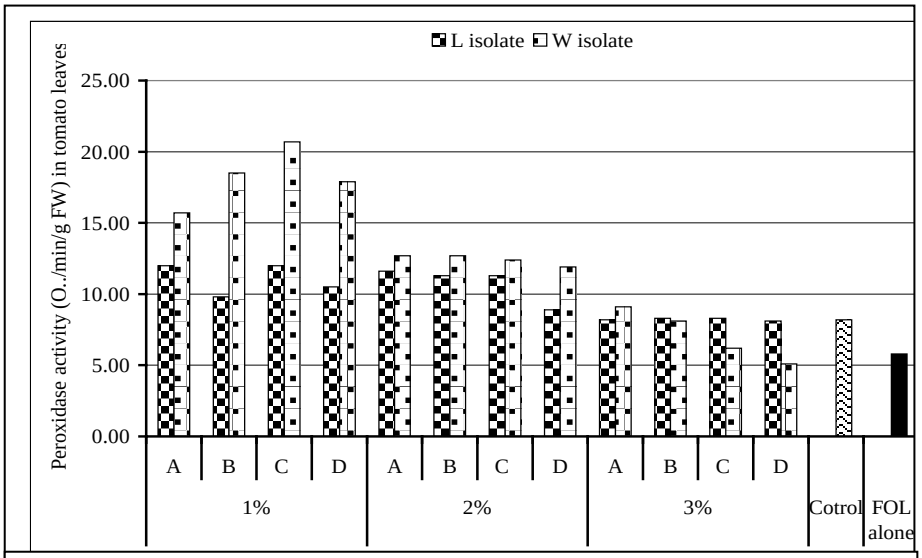
* Treatments = inoculation of VAM-like isolate at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.

7.4. Peroxidase (PO) activity in eggplant leaves under stress of infection with SR:

Regarding PO enzyme activity in leaves of eggplant, the same above data in **Table (17) and Fig. (17)** show that PO was higher in case of L isolate (12.69) than W one (10.29). All tested inoculation treatments recorded higher PO activity (3.9-25.15) than inoculation with SR alone (3.5). Inoculation treatments of 1%A recorded the highest PO activity (25.15), followed by 1%B

(21.25), 1%C (16.05), 2%A (14.50), 2%B (14.05), 1%D (13.20), 2%C (10.35) compared to the non-inoculated control (10.0). With regard to VAM-like isolates, the highest PO activity was induced by treatments of 1%A (27.4) and 1%B (26.9), followed by 2%B (17.7), 1%C (16.4), 2%A (15.5), 2%D (13.4), 2%C (11.5) and 1%D (11.1) respectively in case of L isolate and 1%A (22.9) followed by 1%C (15.7), 1%B (15.6), 1%D (15.3), 2%A (13.5) and 2%B (10.4), respectively in case of W isolate compared to the non-inoculated control (10.0). On the other hand, the PO activity was decreased to different extents by treatments of 3%A, 3%B, 3%C, 3%D (3.9-8.9) for L isolate and 2%C, 2%D, 3%A, 3%B, 3%C, 3%D (3.9-9.2) for W isolate compared to the non-inoculated control.



Status and levels of inoculation with the root-VAM-like isolates

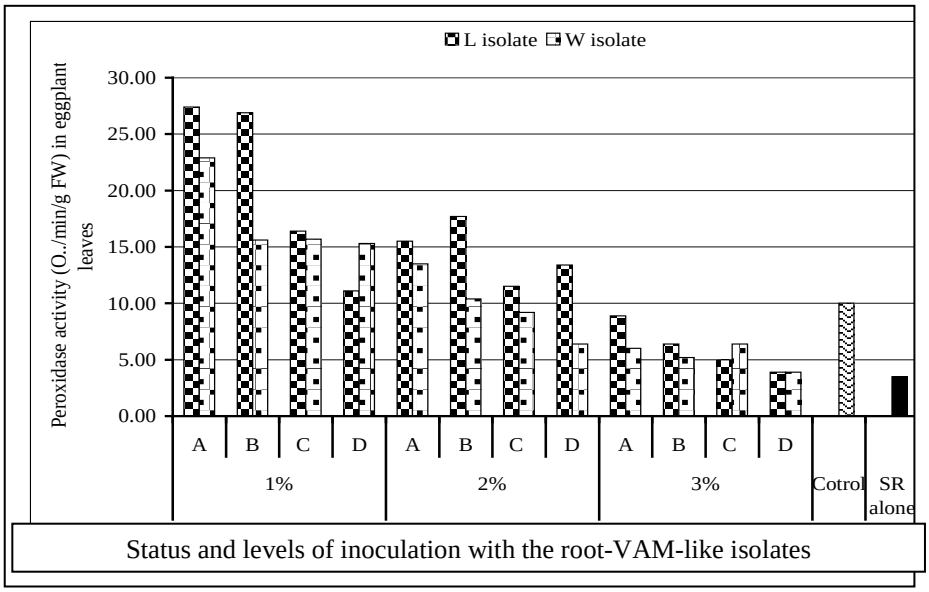


Fig. (17): Peroxidase enzyme activity in leaves of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

7.5. Polyphenol oxidase (PPO) activity in tomato leaves under stress of infection with FOL pathogen:

The data in **Table (18) and Fig. (18)** prove that, the activity of polyphenol oxidase (PPO) enzyme in tomato leaves was increased several times by most of tested inoculation treatments either compared to the non-inoculated control (0.91) as well as inoculation with FOL alone (0.81). The highest PPO activity, in general, was recorded respectively by treatments of 2%C, 1%A, 2%A, 2%D (6.21-6.48) followed by 1%C, 2%B, 1%B, 1%D (4.01-5.18), 3%D, 3%C, 3%B (2.25-2.57) and 3%A (0.95). In case of isolate L, 2%D and 2%C treatments recorded the highest PPO activity (7.92-8.1) whereas, 3%B recorded the lowest activity (2.97). However, applying 3%A decreases PPO activity (0.72) even compared to inoculation with FOL alone. As

for isolate W, the highest PPO activity was recorded by using 2%A treatment (8.82) whereas, 3%A, 3%B, 3%C and 3%D treatments recorded the lowest increases (1.17-1.53) compared to inoculation with FOL alone.

7.6. Polyphenol oxidase (PPO) activity in eggplant leaves under stress of infection with SR pathogen:

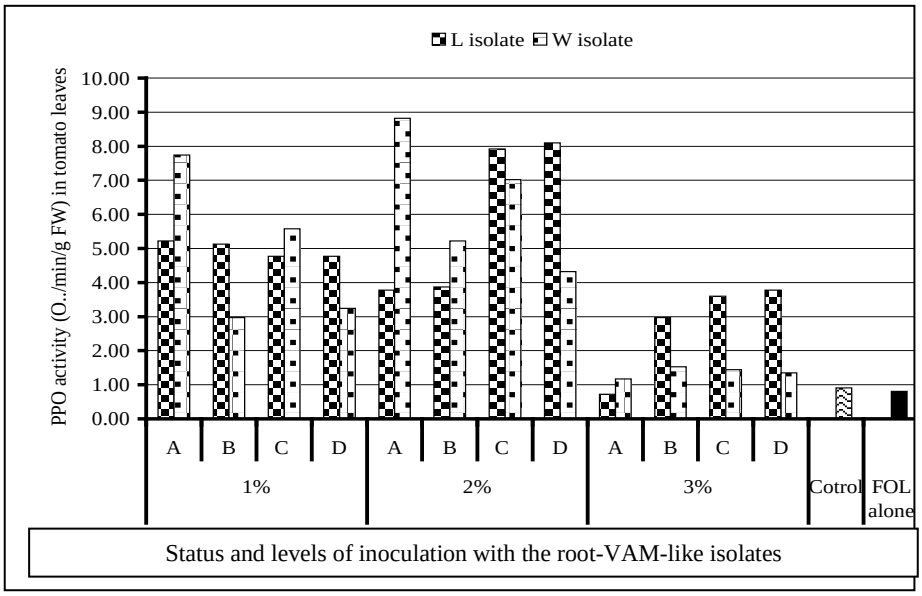
The same data in **Table (18) and Fig. (18)** show that the activity of PPO enzyme in leaves of eggplant was relatively higher in case of W isolate (23.41) than L one (22.76). All tested inoculation treatments recorded higher PPO activity (15.44-39.65) than inoculation with SR alone (11.88). Inoculation treatments of 1%A (39.65), recorded the highest PPO activity followed by 2%A (29.07), 1%B (27.86), 2%C (25.97), 1%C (25.65), 1%D (23.54), 2%B (23.36), 2%D (20.79) and 3%A (20.57), respectively whereas 3%B, 3%C and 3%D slightly decreased PPO activity (15.44-20.21) compared to the non-inoculated control (20.25).

Table (18): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the polyphenol oxidase (PPO) activity in plant leaves of tomato and eggplant.

Application treatments *	PPO activity (O.D./min/g FW of tomato leaves(FOL)			PPO activity (O.D./min/g FW of eggplant leaves(SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	5.22	7.74	6.48	40.4	38.9	39.65
1% B	5.13	2.97	4.05	25.7	30.1	27.86
1% C	4.77	5.58	5.18	24.1	27.2	25.65
1% D	4.77	3.24	4.01	22.0	25.1	23.54
2% A	3.78	8.82	6.30	27.7	30.4	29.07
2% B	3.87	5.22	4.55	26.2	20.5	23.36
2% C	7.92	7.02	7.47	22.6	29.3	25.97
2% D	8.1	4.32	6.21	21.2	20.3	20.79
3% A	0.72	1.17	0.95	21.2	19.9	20.57
3% B	2.97	1.53	2.25	19.3	18.7	18.99
3% C	3.6	1.44	2.52	22.6	17.8	20.21
3% D	3.78	1.35	2.57	13.6	17.3	15.44
Non-inoculated	0.91	0.91	0.91	20.3	20.3	20.25
Pathogen alone **	0.81	0.81	0.81	11.9	11.9	11.88
Mean	4.03	3.72		22.76	23.41	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



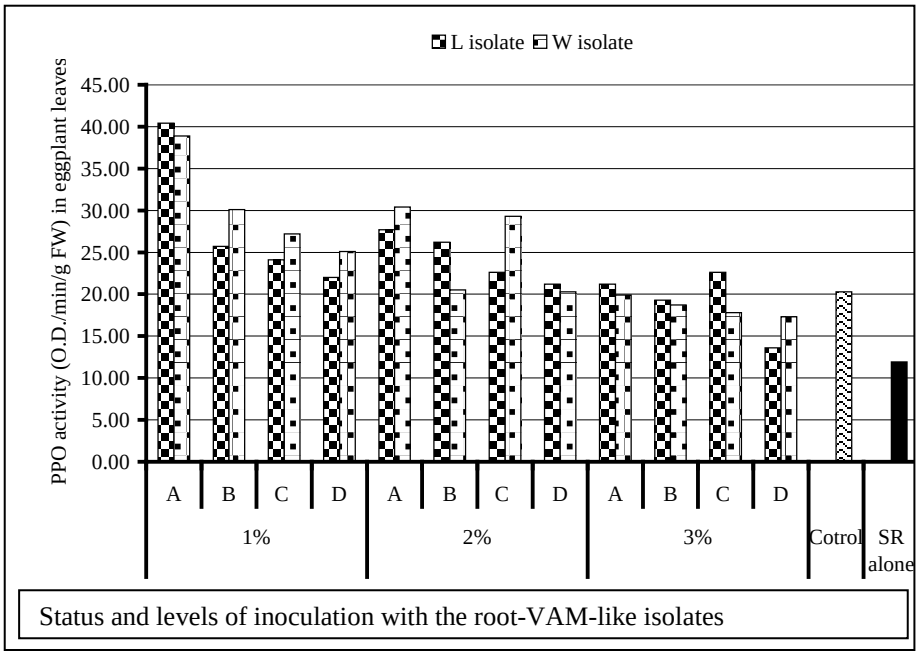


Fig. (18): PPO enzyme activity in leaves of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

In case of L isolate, applying treatment of 1%A recorded the highest PPO activity (40.4) followed by 2%A (27.7), 2%B (26.2), 1%B (25.7), 1%C (24.1), 2%C (22.6), 3%C (22.6), 1%D (22.0), 2%D (21.2) and 3%A (21.2), respectively compared to the non-inoculated control (20.3). On the opposite, the PPO activity seems to be unaffected or might be decreased by treatments of 3%B (19.3) and 3%D (13.6) in case of L isolate and 2%B (20.5), 2%D (20.3), 3%A (19.9), 3%B (18.7), 3%C (17.8) and 3%D (17.3) in case of W isolate compared to the non-inoculated control.

8. Effects of reciprocal interactions between the tested VAM-like fungi and some pathogenic fungi on NPK chemical component in plant leaves:

8.1. Nitrogen (N) content in tomato leaves under stress of infection with FOL pathogen:

The obtained results **(Table 19 and Fig. 19)** indicate that all combined inoculation treatments tested led to obvious increase in the nitrogen (N) content in tomato leaves. Applying treatments of A at 1% level recorded the highest N content (4.78% N) followed by 2%A (3.88%), 1%B (3.85%), 2%C (3.74%) and 1%C (3.64%), 2%B (3.20%), 1%D (2.12%) compared to the non-inoculated control (2.07% N) as well as inoculation with FOL alone (0.95% N).

As for L isolate, using treatments of 1%A recorded the highest N content (4.22%) followed by 1%B (3.98%), 2%A (3.77%), 2%C (3.76%), 1%C (3.29%), 2%B (3.13%) and 1%D (2.81%) except that the treatment of 3%B 3%C, 3%D and 2%D which caused negligible decrease in the N content compared to

the non-inoculated control. While in case of isolate W, using treatments of 1%A recorded the highest N content (5.33%) followed by 1%C or 2%A (3.98%),1%B or 2%C (3.71%) and 2%B (3.27%) except that the treatment of 3%A (2.02%) and 1%D (1.43%) which caused negligible decrease in the N content compared to the non-inoculated control.

8.2. Nitrogen (N) content in eggplant leaves under stress of infection with SR:

As for eggplant, the same results in **(Table 19 and Fig. 19)** indicated that the isolate W enhanced N content in leaves (3.53%) more than the isolate L (3.20%). All tested inoculation treatments, however, led to obvious increase in the N content (1.68-4.70%) compared to inoculation with SR alone (1.01%). On the other hand, the highest N content was induced by treatment of 1%A (4.70%), followed by 2%A (4.42%), 1%C (4.34%), 1%B (4.18%), 2%C (4.08%), 2%B (3.98%), 1%D (3.97%), 2%D (3.53%) compared to the non-inoculated control (3.27%). It is clear that most treatments of isolate W increased N content more than those of L isolate. The following treatments in order list recorded the highest N uptake: 1%A (4.94%), 1%C (4.46%), 2%A (4.23%), 1%D or 2%D (3.98%), 1%B (3.94%) and 2%C (3.70%), in case of L isolate and 2%A (4.61%), 1%A or 2%C (4.46%), 1%B (4.42%), 1%C (4.22%), 2%B or 2%D (3.98%) and 1%D (3.95%) in case of W isolate compared with the non-inoculated control (3.27%). The N content, however, was appreciably decreased in some treatments i.e. 2%D (3.08%), 3%A (3.03%), 3%B (2.65%), 3%C (1.45%) and 3%D (1.03%) in case of (L) isolate and 3%A (2.84%), 3%B (2.81%), 3%C

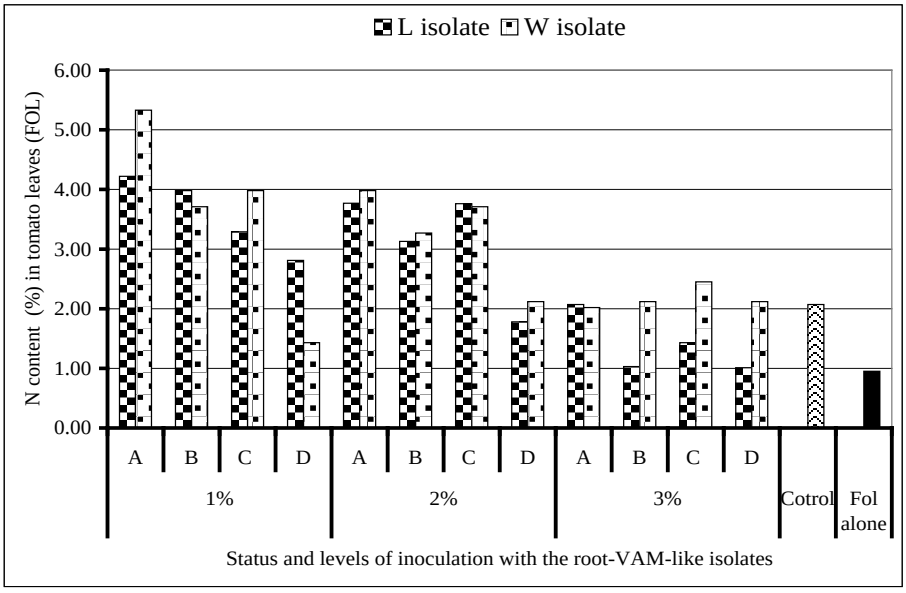
(3.03%) and 3%D (2.32%) in case of (W) isolate compared to the non-inoculated control (3.27%).

Table **(19):** Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the N content in plant leaves of tomato and eggplant.

Application treatments *	N content % in tomato leaves (FOL)			N content % in eggplant Leaves(SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	4.22	5.33	4.78	4.94	4.46	4.70
1% B	3.98	3.71	3.85	3.94	4.42	4.18
1% C	3.29	3.98	3.64	4.46	4.22	4.34
1% D	2.81	1.43	2.12	3.98	3.95	3.97
2% A	3.77	3.98	3.88	4.23	4.61	4.42
2% B	3.13	3.27	3.20	3.98	3.98	3.98
2% C	3.76	3.71	3.74	3.70	4.46	4.08
2% D	1.78	2.12	1.95	3.08	3.98	3.53
3% A	2.07	2.02	2.05	3.03	2.84	2.94
3% B	1.03	2.12	1.58	2.65	2.81	2.73
3% C	1.43	2.45	1.94	1.45	3.03	2.24
3% D	1.01	2.12	1.57	1.03	2.32	1.68
Non-inoculated	2.07	2.07	2.07	3.27	3.27	3.27
Pathogen alone **	0.95	0.95	0.95	1.01	1.01	1.01
Mean	2.52	2.80		3.20	3.53	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



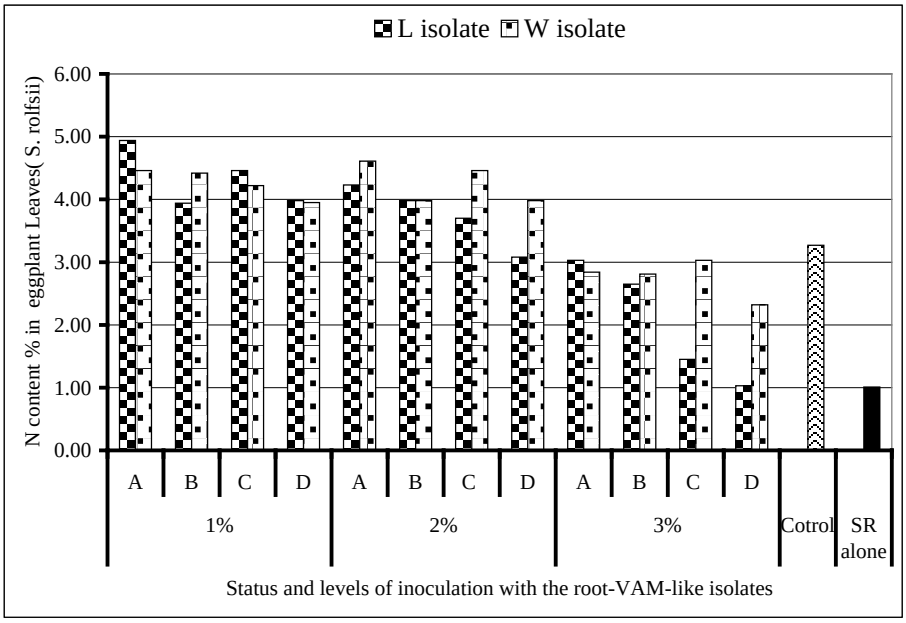


Fig. 19: The N contents in leaves of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested root-entophytic fungal isolates L & W.

8.3. Potassium (K) content in tomato leaves under stress of infection with FOL pathogen:

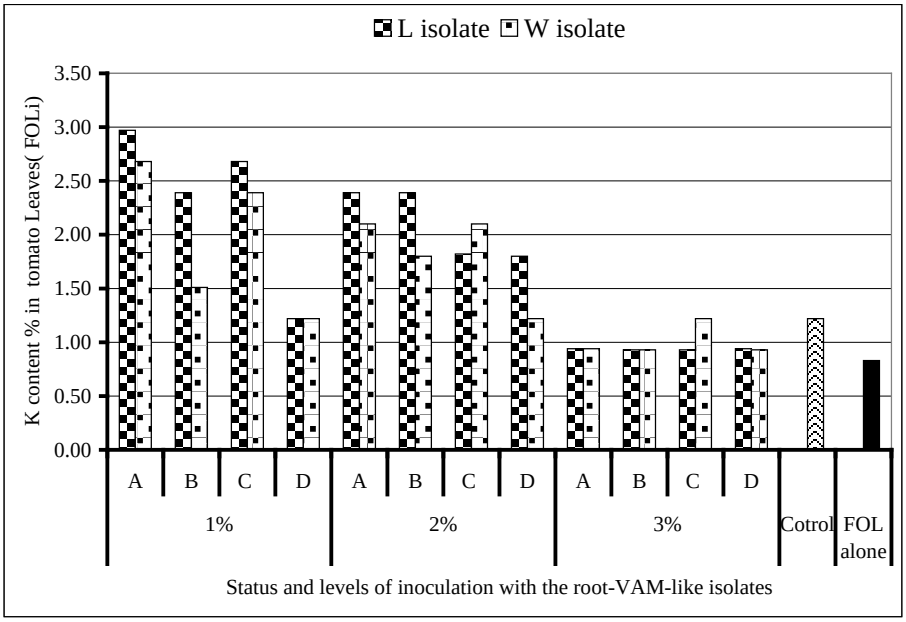
The obtained results shown in **Table (20) and Fig. (20)** indicate that the average of potassium (K) content in tomato leaves was obviously higher in case of VAM-like L isolate (1.68) than VAM-like W isolate (1.51). All tested inoculation treatments increased K content (0.93-2.83%) compared to inoculation with FOL alone (0.83%),while the treatments of 1%A, 1%B, 1%C ,1%D ,2%A, 2%B, 2%C, and 2%D increased K content compared to the non- inoculated control . The highest K content was recorded by using 1%A (2.83%) followed by 1%C (2.54), 2%A 2.25), 2%B (2.10), 2%C (1.96), 1%B (1.95), 2%D (1.51)and 1%D (1.22). Efficacy of a tested treatment for increasing the K contents seems to be varied according tested isolate. For example, treatments of 1%A, 1%C, 1%B, 2%A and 2%B , 2%C and 2%D recorded the highest K contents i.e. 2.97, 2.68, 2.39, 1.82 and 1.80, respectively in case of isolate L while, the treatments of 1%A, 1%C, (2%A or 2%C), 2%B and 1%B were the best for improving K content *i.e.* 2.68, 2.39, 2.10, 1.80 and 1.51 in case of W isolate.

Table (20): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the potassium (K) content in plant leaves of tomato and eggplant.

Application treatments *	K content (%) in tomato leaves (FOL)			K content (%) in eggplant leaves(SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	2.97	2.68	2.83	2.68	2.97	2.83
1% B	2.39	1.51	1.95	2.1	1.51	1.81
1% C	2.68	2.39	2.54	1.8	1.81	1.81
1% D	1.22	1.22	1.22	1.51	2.1	1.81
2% A	2.39	2.10	2.25	2.97	2.39	2.68
2% B	2.39	1.80	2.10	1.51	1.8	1.66
2% C	1.82	2.10	1.96	2.39	1.8	2.10
2% D	1.80	1.22	1.51	2.1	1.81	1.96
3% A	0.94	0.94	0.94	1.51	1.22	1.37
3% B	0.93	0.93	0.93	0.93	1.22	1.08
3% C	0.93	1.22	1.08	1.22	1.51	1.37
3% D	0.94	0.93	0.94	1.22	1.22	1.22
Non-inoculated	1.22	1.22	1.22	1.55	1.55	1.55
Pathogen alone **	0.83	0.83	0.83	0.91	0.91	0.91
Mean	1.68	1.51		1.74	1.70	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



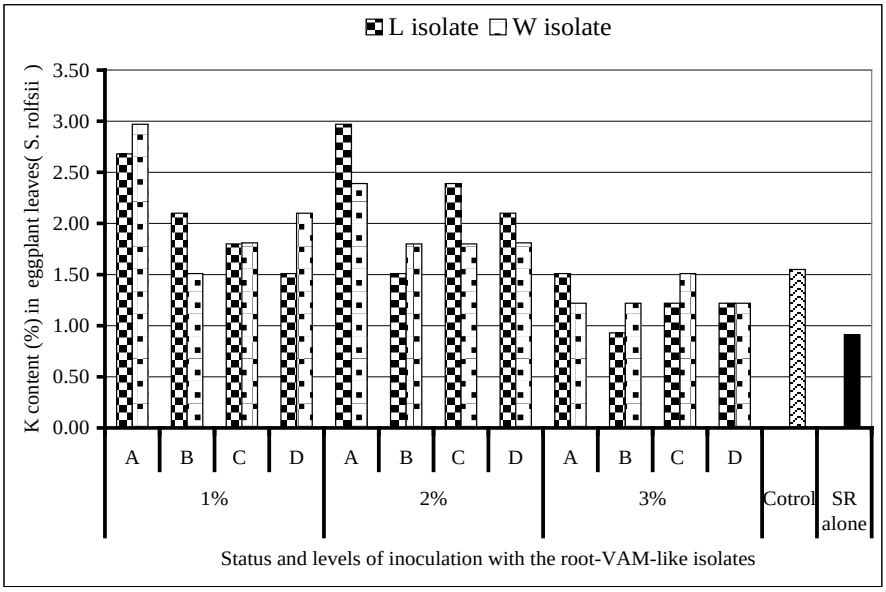


Fig. (20): The K contents in leaves of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

8.4. Potassium (K) content in eggplant leaves under stress of infection with SR:

The same data in **Table (20) and Fig. (20)** state that no detectable variation between the two tested VAM-like L (1.74%) and W (1.70%) isolates regarding their effect on the K content in leaves of eggplant. All tested treatments, however, increased K content (1.22-2.83%) compared to inoculation with SR alone (0.91%). On the other hand, the highest (K) content was recorded by treatment of 1%A (2.83%) followed by 2%A (2.68), 2%C (2.10%), 2%D (1.96), (1%B, 1%C and 1%D (1.88%) and 2%B (1.66%) comparing to the non-inoculated control (1.55%). treatments of 3%A(1.37%), 3%B(1.08%), 3%C(1.37%) and 3%D(1.22%) decreased (K) content compared to the non-inoculated control(1.55%).

It is clear that most treatments of isolate L increased K content more than those of W isolate. The following treatments in order list recorded the highest K content: 2%A (2.97%), 1%A (2.68%), 2%C (2.39%), 1%B or 2%D (2.10%), 1%C (1.80%) in case of L isolate and 1%A (2.97%), 2%A (2.39%), 1%C or 2%D (1.81%), 2%C or 2%D (1.80%) in case of W isolate compared with the non-inoculated control (1.55%). The K content, however, was appreciably decreased in some treatments i.e. 1%D or 3%A or 2%B (1.51%), 3%C or 3%D (1.22%) and 3%B(0.93%) in case of (L) isolate and 3%C or 1%B (1.51%) and 3%A or 3%B or 3%D (1.22%) in case of (W) isolate compared to the non-inoculated control (1.55%).

8.5. Phosphorus (P) content in tomato leaves under stress of infection with FOL pathogen:

The data in **Table (21) and Fig. (21)** indicate that the accumulation of phosphorus (P) content in tomato leaves was higher in treatments of VAM-like isolate W (0.294 %) than those of isolate L (0.249 %). Applying treatments of A,B,C , D at 1% level , 2%A, 2%B and 2%C increased P content (0.406-0.440) either compared to the non-inoculated control (0.251), while all tested inoculation treatments increased P content (0.132-0.440) either compared to inoculation with FOL alone (0.120). Among tested treatments 1%A recorded the highest P content (0.440), followed respectively by 2%A (0.413), 2%C (0.406), 1%C (0.395), 1%B (0.394), 1%D (0.314) and 2%B (0.296). The best five treatments for improving P content were 1%A (0.418), 1%B (0.401), 1%C or 2%C (0.391), 2%A (0.328) and 1%D (0.268) in case of L isolate and 2%A (0.498), 1%A (0.462), 2%C (0.421), 1%C (0.398) and 1%B or 2%C (0.387) incase of W isolate. However, applying treatment of 2%D (0.168), 3%A (0.128), 3%B (0.147), 3%C (0.135) and 3%D (0.128) decreased P content in case of (L) isolate and 2%D (0.225), 3%A (0.148), 3%B (0.167), 3%C (0.161) and 3%D (0.135) in case of (W) isolate compared to the non-inoculated control (0.251%).

8.6. Phosphorus (P) content in eggplant leaves under stress of infection with SR:

The listed data in **Table (21) and Fig. (21)** state that the VAM-like (L) isolate increased phosphorus (P) content in leaves of eggplant (0.328%) more than the (W) isolate (0.290%). All tested treatments, regardless isolates, increased P content (0.178-

0.445%) compared to inoculation with SR alone (0.128%). the P content was higher in treatments of 2%A (0.620%), 1%A (0.445%), 2%C (0.385%), 1%C (0.355%), 2%B (0.354%), 1%B (0.340%), 2%D (0.327%), 1%D (0.313%), whereas it was lower in case of 3%A (0.246%), 3%B (0.214%), 3%C (0.158%) and 3%D (0.178%) comparing to the non-inoculated control (0.266%).

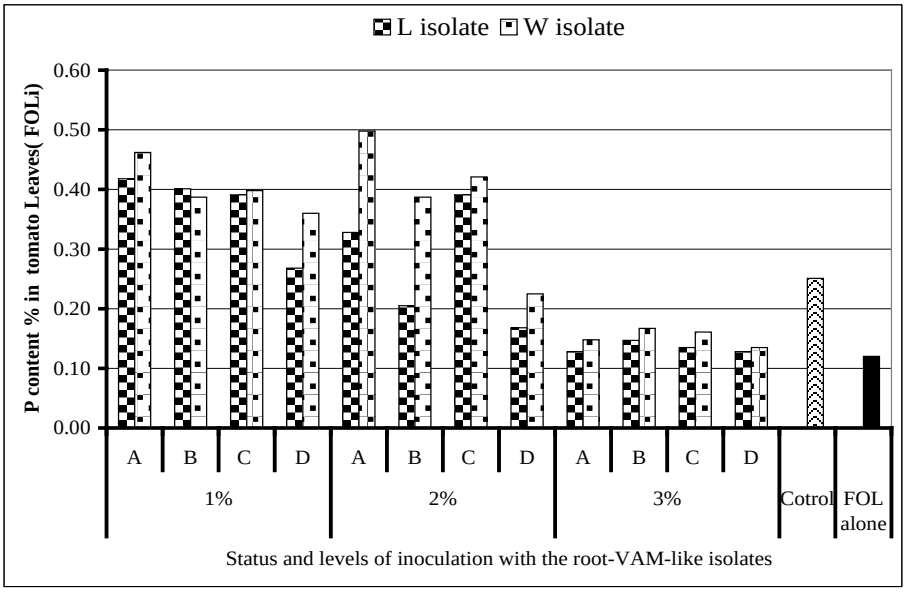
Concerning interactions, the same results proved that, most treatments recorded higher P content in (L) isolate (0.221-0.531%) than (W) isolate (0.135-0.358%), treatment of 2%A for (L) and (W) isolates recorded the highest increase in the P content, whereas treatment of 3%A(0.225%), 3%B(0.188%), 3%C(0.147%) and 3%D(0.221%) decreased P content in case of (L) isolate and 3%B(0.240%), 3%C(0.168%) and 3%D(0.135%) in case of W isolate compared with non-inoculated control(0.266). It is interest to state that, the P content reaches it maximum by using any inoculation level of L or W isolate alone (A), the gradually decreased when each was inoculated before SR (B), simultaneously with SR (C) and after SR, respectively.

Table (21): Effects of reciprocal interactions between tested VAM-like isolates and FOL or SR on the phosphorus (P) content in plant leaves of tomato and eggplant.

Application treatments *	P content mg/g DW of tomato leaves(FOL)			P content mg/g DW of eggplant leaves(SR)		
	L isolate	W isolate	Mean	L isolate	W isolate	Mean
1% A	0.418	0.462	0.440	0.531	0.358	0.445
1% B	0.401	0.387	0.394	0.342	0.338	0.340
1% C	0.391	0.398	0.395	0.315	0.395	0.355
1% D	0.268	0.36	0.314	0.311	0.315	0.313
2% A	0.328	0.498	0.413	0.75	0.489	0.620
2% B	0.205	0.387	0.296	0.359	0.349	0.354
2% C	0.391	0.421	0.406	0.437	0.333	0.385
2% D	0.168	0.225	0.197	0.376	0.278	0.327
3% A	0.128	0.148	0.138	0.225	0.267	0.246
3% B	0.147	0.167	0.157	0.188	0.24	0.214
3% C	0.135	0.161	0.148	0.147	0.168	0.158
3% D	0.128	0.135	0.132	0.221	0.135	0.178
Non-inoculated	0.251	0.251	0.251	0.266	0.266	0.266
Pathogen alone **	0.12	0.12	0.120	0.128	0.128	0.128
Mean	0.249	0.294		0.328	0.290	

* Treatments = inoculation of VAM-like isolates at 1, 2 and 3% levels either alone (A), before (B), at time of (C) and after FOL inoculation (D).

** The tested pathogens were: *Fusarium oxysporum* f. sp. *lycopersici* (FOL), the causal of tomato wilt and *Sclerotium rolfsii* (SR), the causal of eggplant root rot.



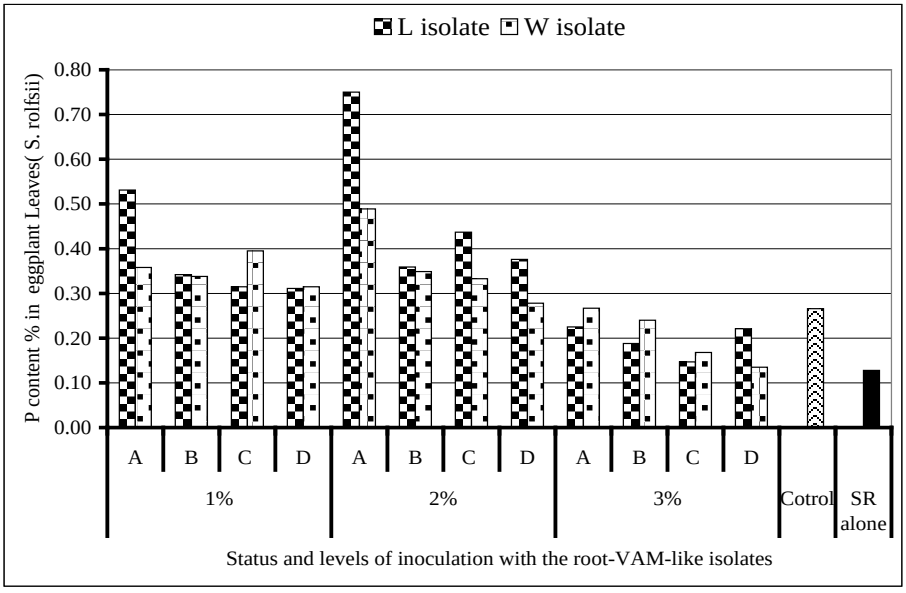


Fig. (21): The P contents in leaves of tomato (above) and eggplant (below) as affected by inoculation with (FOL) and (SR), respectively each alone or combined with different status and inoculation levels of the tested VAM-like isolates (L&W).

DISCUSSION

The VAM fungi are formed by most plant species, including the majority of commercially important crop and horticultural plants **(Hooker *et al.*, 1994)**. Herein, two fungal isolates *i.e.* W & L isolates were isolated from healthy roots of wheat and lettuce plants, respectively. Both isolates could grow and formed coenocytic (non-septate) mycelia, thick irregular branching light-brown hyphae and hyphal swellings. Intercalary and terminally chlamydospores born singly or in chains terminally or intercalary were frequently observed (*in vitro*). The results of PCR technique and specific primers indicated that these fungal isolates might be mixture of VAM fungal species of Archaeospora, Paraglomus, Glomus and Acaulospora which belonging to order Glomerales. **Yang L. (2008)** used five VAM specific primers pairs for amplifying product sizes of dandelions 1, 2 (D1, D2) sampled from garden soil, dandelions 3, 4 (D3, D4) from roadside soil, chive 3 (C) from garden soil, in comparison with expected size [pb] of each primer pair (ES). The ES was 1052 for ARCH1311– ITS4i while the sizes of amplified products were 1300 (D1), 650 (D3), 1200; 980 (D4) and 1200; 1080; 200 (C); As for ACAU1661/ITS4i [*Archaeospora gerdemannii*/ trappei *Glomus occultum* / brasilianum], the ES was 645 pb while amplified products were of 1200, 520 (D1); 1000, 660, 450 (D2), 750 (D3); 650 pb (D4); As for LETC1670/ITS4i [Acaulosporaceae], the ES was 676 pb while amplified products were of 750 (D1), 1000, 800, 680, 350, and 200 (D3); 950,900,800, 680,500,300 (D4); 750,650, 550,

300 (C); As for primer pair GLOM1310/ITS4i [*Glomus etunicatum*/ *claroideum*/ *Glomus mosseae*/ *intraradices*] the ES was 1012 while amplified products were of 850 (D1), 800, 450 (D3), 680, 300 (D4); As for GIGA5.8R/ITS1F [*Gigasporacea*] the ES was 305 pb while the observed were 850,700,600, 500, 450, 200 (D1), 700, 500 (D2), 800, 200 (D3), 650, 500, 300 (D4) and 570 pb (C). **Schüßler *et al.* (2001a)** recorded that, the Glomeromycota have generally coenocytic (occasionally sparsely septate) mycelia and reproduce asexually through blastic development of the hyphal tip to produce spores (Glomerospores) and some complex spores form within a terminal saccule.

In the present study, primary experiment was conducted, under greenhouse conditions, to investigate if our fungal isolates promote growth of wheat (*Triticum aestivum* L.) plants. The wheat seeds were sown in sterilized soil infested with different levels of inoculants (1, 2 & 3% w/w). The obtained results showed that, all inoculants levels, particularly the lowest one (1%) significantly increased all estimated measurements (% survived plants, number of tillers/plant, number of leaves/plant, shoot length, fresh and dry weight of shoots "g/plant", root length, fresh and dry weight of roots "g/plant") of wheat plants compared to the control. In this respect, **Hendrix *et al.* (1992)** stated that the mycorrhizal fungus *Glomus macrocarpum*, among 3 VAM fungi, caused a stunt disease of tobacco (*Nicotiana tabacum*). Rotation and fumigation controlled mycorrhizal colonization of tobacco roots and the build-up of populations of *G. macrocarpum* in the root zones of plants. It is suggested that

mycorrhizal fungi should be considered in research on the effects of crop rotation on productivity. The observed decrease in some of estimated parameters associated with 3% level might be expected. Also, **Hendrix *et al.* (*1992*)** stated that the mycorrhizal fungus *Glomus macrocarpum*, among 3 VAM fungi, caused a stunt disease of tobacco (*Nicotiana tabacum*). Rotation and fumigation controlled mycorrhizal colonization of tobacco roots and the build-up of populations of *G. macrocarpum* in the root zones of plants. It is suggested that mycorrhizal fungi should be considered in research on the effects of crop rotation on productivity. However, both fungal isolates (W & L) could colonize wheat roots and formed structures characterized VAM fungi *i.e.* arbuscule, vesicle, intracellular hyphae in root cortex and extramatrical mycelium and attached VAM spores. In fact, the mycorrhizae benefit from gaining organic nutrients from the plant, and in turn, the plant benefits by enhanced water and nutrients uptake, increased growth and yield **(Harley and Smith, 1983; Linderman, 1994)**. Both W & L fungal isolates could colonize roots of wheat plants forming the infection structures characterizing the VAM (vesicular arbuscular mycorhyzal) fungi *i.e.* arbuscule, vesicle, intracellular hyphae in root cortex and extrametrical mycelium and attached VAM spores. **Agrios (2005)** mentioned that the VAM fungi are the most common and their fungal hyphae grow in cortical cells of the feeder roots with specialized feeding hyphae, called arbuscules or feeding-storing hyphal swellings called vesicles.

The reciprocal antagonistic [interactions] effects between W or L isolates and a particular pathogen *i.e. Fusarium*

oxysporum f. sp. *lycopersici* **[FOL]** and *Sclerotium rolfsii* **[SR]** were investigated in mutual cultures in vitro. Each fungal partner was inoculated alone [control], simultaneously [=S], 24 before [+B] or after [-A] inoculation of the other partner. After incubation for 9 days in the FOL/L interaction, the highest reduction in growth of FOL and L isolate was associated with =S inoculation status comparing to control of each fungal partner. After the same incubation period in FOL/W interaction, growth of FOL was reduced whereas, growth of isolate W was reduced also in statuses of -A, =S and +B inoculations, respectively.

Regarding to SR/L interaction after 7 days incubation period, the inoculation statuses +B, =S and –A reduced growth of SR, whereas, growth of L isolate was reduced also. In the W/SR interaction, the same inoculation statuses reduced growth of SR whereas it reduced growth of W isolate also compared to growth of each partner inoculated alone. These results could attributed to direct competition **(Cordier *et al.*, 1998 a, b)** or to direct inhibition **(St-Arnaud *et al.*, 1995; Filion *et al.*, 1999; Garcia-Garrido and Ocampo, 1989).**

As for the activities of some oxidative enzymes in the fungal filtrates, the oxidative enzymes chitinase, peroxidase (PO) and polyphenol- oxidase (PPO) were detected in the cultural filtrates of the pathogenic fungi (FOL and SR) as well as those of the like-VAM fungal isolates. The activities of all tested enzymes were higher in filtrates of VAM-like fungal isolates than those of tested pathogenic fungi. However, the activity of any enzyme, in general, was gradually decreased as age of filtrates increased. Filtrates of W isolate showed higher PO

activity and lower activities of chitinase whereas, PPO activity was comparable with that of L isolate. In filtrates of W isolate, PO activity decreased at faster rates by ageing than that of L isolate. Filtrates of FOL pathogen shows higher PO activity than those of SR. Aging of culture filtrates of SR fungus caused quick decline in activity of all tested oxidative enzymes compared to FOL. It is well known that, microorganisms expressing these activities have also been shown to effectively inhibit fungal growth and to prevent disease incidence caused by soilborne pathogens **(Chet *et al.*, 1990; Jung *et al.*, 2003; Nagarajkumar *et al.*, 2004)**. Also, **Jaroszuk and Kurek (2012)** detected several enzymes including gluconases, chitinases, xylanases, exocellulases, endocellulases, pictinases and polygalacturonases in 42-day-old cultures of *Fusarium culmorum* isolates.

The biological effect of tested VAM-like fungal isolates (W or L isolates) against infection with tomato fusarium wilt disease [FOL] and root-rot disease of egg-plant [SR] were investigated under greenhouse conditions. Inoculation of sterilized soil with particular pathogen was performed alone at 3% (w/w) or combined with inoculants of particular isolate of the tested VAM-like fungi at 1, 2 or 3% (w/w) and different statuses of inoculation i.e. alone (A), 7 days before pathogen (B), simultaneously with pathogen (C) or 7 days after (D) pathogen inoculation. Percentage of survived plants, fresh and dry weights of shoots and roots, plant height, leaf number, and root length, total photosynthetic leaf pigments, N, P and K contents in treated plants were accomplished 45 days after sowing.

Applying inoculants of the VAM-like fungal isolates at 1 or 2% levels alone (A) or before (B) inoculation with a particular pathogen i.e. FOL or SR resulted in the highest significant increase in % survived plants of tomato and egg-plant compared to the pathogen alone whereas different statuses of inoculants of the VAM-like fungi at 3% levels had no significant or significantly decreased % survived plants depending on the tested pathogen or host plant. Similar trend was observed regarding the different estimated growth and chemical analysis parameters of both host plants that were exposed to different investigated statuses of inoculation. In fact, the VAM fungi, which form symbiotic associations with a wide range of plant species, are interesting group of microorganisms that effectively reduce root disease by a number of soilborne pathogens **(Linderman 1994),** including *Fusarium oxysporum* f. sp. *lycopersici* **(Akköprü and Demir, 2005; Kapoor and Bhatnagar, 2011)** and *Sclerotium rolfsii* **(Kichadi and Sreenivasa (1998).** Different hypotheses have been proposed to explain bio-protection by VAM fungi. These include (i) improvement of plant nutrition and root biomass in mycorrhizal plants, which could contribute to an increased plant tolerance and compensate for root damage caused by a pathogen, (ii) changes in root system morphology, (iii) modification of antagonistic microbial populations in the mycorrhizosphere, and (iv) competition between VAM and pathogenic fungi to colonize root tissues, with the possible induction of resistance mechanisms **(Bethlenfalvay and Linderman 1992; Hooker *et al.*, 1994; Morandi 1996*)***. The VAM fungi improved nutrient status of the host plant **(Smith and Read 1997)** and provided

bio-protection to their host plants against soil-borne fungal pathogens **(Singh *et al.*, 2000; Azcon-Aguilar *et al.*, 2002; Xavier and Boyetchko 2004; St-Arnaud and Elsen 2005; St-Arnaud and Vujanovic 2006)**. However, the herein observed neutral or negative effects of tested VAM-like fungal isolates on the investigated growth parameters when applied at high inoculants level (3%) might be attributed to that high level of inoculants (propagules) produced some undesirable metabolites or toxic substances which could be absorbed by plant roots. **Andrade Linares (2011)** observed that the high level of inoculants of the endophytic fungus *Piriformospora indica* significantly decreased examined growth parameters of tomato plants particularly under low levels of N and P nutrients. She concluded that *P. indica* may become even parasitic under particular nutrient regimes

Applying inoculants of the VAM-like fungal isolates at 1 or 2% levels alone (A) or before (B) inoculation with a particular pathogen i.e. FOL or SR resulted in higher amounts of total photosynthetic leaf pigments and nitrogen (N) potassium (K) and phosphorus (P) contents in plant tissues compared with inoculation with each pathogen alone. In this respect, **Evans (1989)** stated that, the photosynthetic capacity of leaves is related to the nitrogen content. When both photosynthetic capacity and leaf nitrogen content are expressed on the basis of leaf area, considerable variation in the photosynthetic capacity for a given leaf nitrogen content is found between species. **Wright *et al.* (1998)** investigated the influence of VAM colonization on biomass production and photosynthesis of *Trifolium repens* L.

They observed that the rate of CO_2 assimilation of the youngest, fully expanded leaf of VAM plants was higher compared with non-VAM plants. In addition, VAM plants exhibited a higher specific leaf area compared with non-VAM plants, where this will permit maximizing the area available for CO_2 assimilation per unit of carbon (C). Despite the increased rate of photosynthesis in M plants there was no evidence that the additional C gained was converted to biomass production of M plants. It is suggested that this additional C gained by colonized plants was allocated to the mycorrhizal fungus and that it is the fungus, by acting as a sink for assimilates, that facilitated the stimulation in the rate of photosynthesis of the plant partner. **Abdel Latef and Chaoxing (2011)** reported that the contents of photosynthetic pigments, sugars and soluble protein in leaves were higher in VAM than non-VAM tomato plants. VAM colonization increased the activities of the antioxidant enzymes in leaves. The mycorrhizae benefit from gaining organic nutrients from the plant, and in turn, the plant benefits by enhanced water and nutrients uptake, increased growth and yield and protection against soil-borne pathogens **(Harley and Smith, 1983; Linderman, 1994)**. Inoculation of tomato with AMF resulted in stronger and superior quality seedlings **(Giannuzzi *et al.*, 2001)**, better mineral nutrient uptake **(Chandanie *et al.*, 2009; Marschner and Dell, 1994)**, and improved tolerance to soil borne diseases **(Pozo and Azcon-Aguilar, 2007)**.

The present results proved that, applying inoculants of the VAM-like fungal isolates at 1 or 2% and sometimes 3% levels

alone (A) or before (B) inoculation with a particular pathogen i.e. FOL or SR resulted in the highest activity of chitinase, peroxidase and polyphenol oxidase enzymes in tissues of tomato plants compared to the control or inoculation with FOL alone. As for eggplant and infection with SR, similar trend was observed concerning activities of these oxidative enzymes. Several physiological and biochemical alterations of the host after mycorrhization have been reported by several investigators. Some are possibly linked with a protective effect of the mycorrhizal plant against pathogens, e.g. the induction of hydrolytic enzymes **(Pozo *et al.*, 1999)**, enhanced levels of PR proteins, the accumulation of phytoalexins **(Harrison and Dixon 1993; Morandi 1996; Larose *et al.*, 2002)** and callose **(Cordier *et al.*, 1998b)**, the accumulation of salicylic acid **(Blilou *et al.* 2000a and 2000b; Medina *et al.*, 2003)** and reactive oxygen species **(Salzer *et al.*, 1999)**. During AM development, there is evidence that these defensive responses occur **(Garcia-Garrido and Ocampo, 2002)** and that they are strongly stimulated when a subsequent challenge with a pathogen takes place. Possibly the mechanisms of plant defense are activated faster and to a greater extent in mycorrhizal plants when challenged by a pathogen compared to non-mycorrhizal plants, and it has been suggested that AM colonization acts as a priming system for the process of pathogen resistance **(Azcon-Aguilar *et al.*, 2002; Pozo and Azcon-Aguilar 2007)**. In this respect, elevated JA levels occurring upon mycorrhization, likely associated with a fully established mycorrhiza, may mediate the enhance defense status of the mycorrhizal plant **(Vierheilig and Pich 2002; Hause *et al.* 2007; Vierheilig 2004)**. As the whole metabolism of the plant

is altered by mycorrhization, alterations of the root exudation pattern are no surprise. These alterations could act on the pathogen indirectly, through an altered pH in the rhizosphere and/or directly through an altered composition of the exudates with reduced levels of stimulatory compounds and/or the presence of inhibitory compounds. Changes of the pH in the rhizosphere of the mycorrhizal plant **(Bago *et al.*, 1996; Villegas *et al.*, 1996)** have been reported before, however, no data are available yet how these pH changes of the rhizosphere affect root pathogens. There are a number of reports on root exudates and AMF **(Jones *et al.*, 2004; Nagahashi and Douds 2005; Vierheilig and Bago 2005)**, and more data are accumulated that exudates of mycorrhizal plants affect bacteria **(Sood, 2003),** fungi **(Norman and Hooker 2000; Lioussanne *et al.*, 2003; Scheffknecht *et al.*, 2007)** and nematodes **(Ryan and Jones 2004)** differently then exudates from non-mycorrhizal plants.

Finally, it could be concluded that isolation of the VAM-like fungi from healthy roots of field grown crop plants and applying their inoculants at 1 or 2% particularly under greenhouse conditions resulted in significant improvement in plant growth and increased their resistance against soil-borne pathogens. **Prasad *et al.* (2008)** introduced that, the plants depend heavily on mycorrhizal fungi for many different functions, such as mineral nutrition and abiotic and biotic stress resistance. Substantial evidence has accumulated in the recent past about how the use of the micro-symbiont could significantly contribute in decreasing use of fertilizers and pesticides in agriculture, forestry and flori-hortriculture,

especially if combined with other beneficial soil microorganisms. The most common and prevalent arbuscular mycorrhizal fungi play an indispensable role in upgrading plant growth, vigor and survival by a positive impact on the nutritional and hydratic status of the plant and on soil health, by increasing the reproductive potential, improving root performance, and providing a natural defence against invaders, including pests and pathogens. The described species of arbuscular mycorrhizal fungi mainly belong to Zygomycetes placed in the order Glomerales. However, the growing of arbuscular mycorrhizae in pure culture in the absence of living host roots is a matter of global concern.

SUMMARY

This work targeted to isolate the VAM-like fungi from healthy roots of lettuce and wheat plants, investigation their antagonistic effects against the causal agents of the tomato fusarium wilt disease, *Fusarium oxysporum* f. sp. *lycopersici* (FOL), and the root-rot disease of eggplant, *Sclerotium rolfsii* (SR). The promotion effects of the isolated VAM-like fungi on growth of wheat plants were investigated. Also, their capabilities to inducing resistance against infections with FOL and SR in plants of tomato and eggplant respectively were studied.

The obtained results could be summarized as followings:

1. Two fungi namely W & L isolates were isolated from undamaged healthy roots of wheat and lettuce plants, respectively, both characterized by coenocytic (non-septate) mycelia, thick irregular branching light-brown hyphae and hyphal swellings. Intercalary and terminally chlamydospores born singly or in chains terminally or intercalary were frequently observed. A structure like sporangium containing sporangiospores was occasionally observed also.
2. The four tested specific primers *i.e.*, GLOM1310/ITS4i (A), ARCH1311/ITS4i (B), PARA1313/ITS4i (C) and LECT1677/ITS4i (D) were effective in detecting and exhibiting clear intensive amplicons distinguishing the two tested VAM-like fungi (L&W isolates) with very close similarity between them. In this respect, using the specific primer GLOM1310/ITS4i resulted in very intensive two

bands with molecular weight ranged between 500-600 bp of the two tested VAM-like isolates (L&W). Meanwhile, using the specific primer ARCH1311/ITS4i exhibited two intensive bands with molecular weight 700bp of the two tested VAM-like isolates (L&W). Also, using the specific primer PARA1313/ITS4i revealed two clear intensive bands with molecular weight 200bp of the two tested VAM-like isolates (L&W). Therefore, using the specific primer LECT1677/ITS4i exhibited two clear intensive amplicons with molecular weight 350 bp of the two tested VAM-like isolates (L&W). The resulted amplicons of the four tested specific primers with the two tested VAM-like isolates revealed great similarity between the two isolates.

3. All inoculants levels of the isolated fungi significantly increased all estimated measurements of wheat plants compared to the control. The lowest level (1%) was the best for improving % survivals and increasing all estimated plant growth criteria followed by 2 and 3% levels with clear significant differences between them. However, the isolate W produced significantly higher root length and number of leaves and tillers per plant. Both fungal isolates could colonize roots of wheat plants forming the infection structures characterizing the VAM (vesicular arbuscular mycorhyzal) fungi i.e. arbuscule, vesicle, intracellular hyphae in root cortex and extrametrical mycelium and attached VAM spores.
4. The interactions between each of L & W isolates against *Fusarium oxysporum* f. sp. *lycopersici* (FOL) were

investigated in mutual cultures (two fungal partners grow together). The growth of FOL (after 9 days incubation) was reduced by 31.9, 36.9, and 18.7% whereas, growth of isolate L was reduced by 13.4, 15.0 and 27.1% when L inoculated before FOL, L & FOL inoculated simultaneously and L inoculation after FOL, respectively. This trend was slightly varied in case of FOL/W interaction. The growth of FOL (after 9 days incubation) was reduced by 36.1, 38.1, and 35.2% whereas, growth of isolate W was reduced by 36.5, 34.7 and 52.8% when inoculation with W isolate performed before FOL, simultaneously with FOL and after FOL, respectively.

5. The interactions between each of L & W isolates against *Sclerotium rolfsii* (SR) i.e. L/SR & W/SR were investigated as above mentioned. In case of S/L interaction, the growth of SR (after 7 days incubation) was reduced by 95.1, 77.0, and 73.0% meanwhile, growth of isolate L was reduced by 3.1, 12.3 and 16.4% when L inoculated before SR, simultaneously with SR and after SR, respectively. This trend was slightly varied regarding SR/W interaction. Growth of SR (after 7 days incubation) was reduced by 60.7, 52.4, and 51.9% whereas, growth of isolate W was reduced by 5.3, 14.8 and 21.1% when inoculation with W isolate performed before SR, simultaneously with SR and after SR, respectively.
6. The oxidative enzymes chitinase, peroxidase (PO) and polyphenoloxidase (PPO) were detected in the cultural filtrates of tested pathogenic fungi (FOL and R) as well as in

filtrates of the L and W like-VAM fungal isolates. The activity of all tested enzymes was obviously higher in filtrates of the L & W fungal isolates than in filtrates of the two investigated pathogenic fungi FOL and S. The highest activity of any enzyme, in general, was detected in filtrates of 7 days-old filtrates then successively decreased to different extents as age of filtrates increased to 14 and 21 days, respectively. Filtrates of W isolate showed higher PO activity and lower activities of chitinase and PPO compared with those of L isolate. PO activity in filtrates of W isolate seems to be decreased at faster rates by ageing than PO activity in filtrates of L isolate. As for the pathogenic fungi, the FOL pathogen shows higher PO activity in its filtrates than the S one. Aging of cultures caused quick decline in activity of any tested oxidative enzyme in filtrates of S fungus compared with the FOL fungus.

7. As for greenhouse studies, the VAM-like fungi L and W were used as bio-control agents against infection with tomato fusarium wilt caused by *F. oxysporum f. sp. lycopersici* (FOL) and root-rot of egg-plant caused by *Sclerotium rolfsii* (SR). Inoculants of particular pathogen was used at 3% (w/w) whereas those of a bio-control agent were used at 1, 2, 3% (w/w). Inoculants of the latter were added alone (A), 7 days before pathogen (B), simultaneously with pathogen (C) or 7 days after pathogen inoculation (D). Free- and pathogen-inoculated soils were used as control treatments. The obtained results could be summarized as followings:

8. Using treatments of A & B (1%), A, B and C (2%) recorded the highest % survived tomato plants (100.0%) followed by 1%C and 2%D, respectively. Treatments of B and D (3%) had no significant effect on % survived plants compared with inoculation with FOL alone whereas, A and B (3%) significantly decreased it compared to the control. Regarding with eggplant, treatments of A, B, C and D (1%), A, B and C (2%) produced the highest % survived plants without significant difference in between compared to inoculation with SR alone whereas, B, C and D (3%) showed no significant effect in this respect compared to the control.
9. Applying treatments of A & B (1% & 2%) induced the highest leaf number per tomato plant whereas A, B, C & D (3%) decreased it compared to inoculation with FOL alone. In eggplant, all treatments significantly increased number of leaves per plant compared to inoculation with SR alone. Also, it was significantly increased by using A, B, C, D (1% & 2%) and A (3%) whereas B, C and D (3%) had no significant effect compared to the control.
10. All treatments significantly increased plant height in tomato compared to inoculation with FOL alone. Comparing to the control, treatments of A & B (1%), A, B and C (2%) were the best without significant differences between all. Applying 2%B recorded the highest increase in plant height for both L and W isolates. Similar trend was recorded for plant height of eggplant, the highest increase was produced by treatments of A, C (1%), A (2%), B (1%), C, B, (2%) 1%D and 2%D, respectively compared to inoculation with R alone. On the

other hand, treatments of A, B, C and D (1% & 2%) significantly increased plant height whereas A, B, C and D (3%) had no significant effect compared to the control.

11. Most tested treatments significantly increased fresh weight of shoot in tomato plants compared to inoculation with FOL alone, applying A (1%), A, B and C (2%) recorded the highest increases, using A (3%) had no significant compared with inoculation with FOL alone, however, using A, B and D (3%) for both L & W isolates in addition to C 3%) for W isolate decreased it compared with the control. Concerning eggplant plants, all treatments significantly increased shoot fresh weight compared to inoculation with R alone. Applying treatments of A and C (1%), A, B and C (2%) recorded the highest increase whereas treatments of D (1% & 2%) and A and C (3%) had no significant effect on shoot fresh weight meanwhile B and D (3%) significantly decreased it compared to the control.

12. All treatments recorded significantly higher dry weight of tomato shoots compared to inoculation with FOL alone. The highest increase was recorded by treatments of A, B and C (2%) whereas it was significantly decreased by using 3%A compared to the control. As for eggplant, all treatments significantly increased shoot dry weight compared to inoculation with SR alone. The highest significant increase was produced by treatment of A & C (1%), 2%A, 1%B, C & B (2%) and 1%D, respectively, however, it was significantly decreased by using treatments of B and D (3%) compared to

the control. Isolate L recorded significantly higher shoot dry weight than the W isolate.

13. All treatments significantly increased root length compared to inoculation with FOL alone, the highest significant increases were recorded by using A, B (1% and 2%). However, treatments of A and B (1%), A, B and C (2%) and 1%C recorded the highest increase while A, B, C and D (3%) caused appreciable decrease in this respect compared to the control. As of eggplant, all treatments were significantly better for increasing root length compared to inoculation with SR alone, treatments of A and C (1%), 2%A, 1%B, C & B (2%), 1%D and 2%D, respectively were the best even compared to the control. However, treatments of A, B, C and D (3%) showed no significant effect on the root length in eggplant compared to the control. The isolate W significantly increased root length in plant of tomato and eggplant more than L isolate.
14. In tomato plants, all treatments significantly increased the root fresh weight compared to FOL inoculation alone. The highest significant increases were induced by using treatments of A and B (2%), 1%A, 2%C, B and C (1%), 2%D, 1%D, respectively whereas treatments of A, B, C and D (3%) significantly decreased it compared to the non-inoculated.. As for eggplant, all treatments significantly increased root fresh weight compared to the inoculation with SR alone. The highest increases in root fresh weight of eggplant were recorded by using treatments of A (at 1 and 2%), C and B (1%), C and B (2%) and 1%D whereas

treatment of 3%D significantly decreased it compared to the control.

15. The root dry weight of tomato plants was significantly increased by all treatments compared to inoculation with FOL alone. It was significantly higher in case of isolate W than in isolate L. The highest significant increased was recorded by the following treatments: A & B (2%), 1%A, 2%C, B and C (1%) and 2%D, respectively whereas, treatments of A, B and D (3%) significantly decreased it compared to the control. In case of eggplant, all treatments significantly increased root dry weight compared to inoculation with SR alone. However, the highest increase was recorded by treatments of A, B and C (1% & 2%) in addition D (1%) whereas using 3%D significantly decreased it compared to the control.

16. In tomato, all treatments increased the total amount of leaf pigments compared to inoculation with FOL alone. The isolate L seems to be helpful in synthesis of leaf pigment more than isolate W. The highest increase the total leaf pigments was induced by using treatments 1%A and 2%A. On the contrary, treatments of A, B, C & D (3%) and 2%D caused appreciable decrease compared to the control. Similar trend was observed regarding the total leaf pigments in leaves of eggplant. all tested inoculation treatments induced obvious increase in the total leaf pigments compared to inoculation with SR alone whereas, treatment of 3%D caused obvious decrease compared to the control.

17. In tomato leaves, treatments of isolate L recorded more activity of chitinase enzyme than those of isolate W, the highest activity was induced by treatments of A, C and B (2%), A and B (1%) and 3%B compared to the control or inoculation with FOL alone. Most treatments in case of L isolate, recorded higher chitinase activity more than isolate W except treatments of A, B and C (1%) and 3%C as they recorded the reverse trend. Similar trend was noticed regarding chitinase activity in eggplant leaves. All treatments recorded higher activity than inoculation with SR alone, the highest activity, however, was recorded by treatments of A and C (1%). On the contrary, treatments of A, B, C and D (3%) in case of W isolate and B and D (3) in case of L isolate decreased chitinase activity compared to the control.

18. In tomato, all tested treatments caused appreciable increases in the activity of peroxidase (PO) enzyme in plant leaves compared with inoculation with FOL alone. However, treatments of A, B, C or D at 1% or 2% recorded higher the PO activity more than the same treatments at 3% level. Using treatments of C, D, B, A (1%), A, B, C, D (2%), A and B (3%) increased the PO activity whereas, treatments of C and D (3%) decreased it compared to the control, although all these treatments recorded higher PO activity than inoculation with FOL alone. As for eggplant, the PO enzyme activity was higher in case of L isolate than W one. All treatments recorded higher PO activity than inoculation with SR alone. Treatment of A (1%) recorded the highest PO activity,

followed by B and C (1%), A and B (2%), 1%D and 2%C, respectively compared to the control.

19. The activity of polyphenol oxidase (PPO) enzyme in tomato leaves as affected by the tested treatments was increased, mostly, several times compared to either inoculation with FOL alone or the control. The highest PPO activity was recorded by treatments of A (1%), A, C, D (2%); C (1%), respectively. Using A (3%) particularly in case of L isolate decreases PPO activity compared to inoculation with FOL alone. With regard activity of PPO enzyme in leaves of eggplant, it is clear that it was relatively higher in case of W isolate than L one. All treatments, however, recorded higher PPO activity than inoculation with SR alone. Treatments of A (1%) recorded the highest PPO activity followed by 2%A, 1%B, 2%C, 1%C, 1%D, 2%B, 2%D and 3%A, respectively whereas B, C and D (3%) slightly decreased it compared to the control.

20. All tested inoculation treatments led to obvious increase in the nitrogen (N) content in tomato leaves. Applying treatments of A at 1% level recorded the highest N content followed by 2%A, 1%B, 2%C and 1%C, 2%B, 1%D compared to the non-inoculated control as well as inoculation with FOL alone. As for L isolate, using treatments of 1%A recorded the highest N content followed by 1%B, 2%A, 2%C, 1%C, 2%B and 1%D compared to the non-inoculated control. While in case of isolate W, using treatments of 1%A recorded the highest N content followed by 1%C or 2%A, 1%B or 2%C and 2%B compared to the non-inoculated

control. As for eggplant, it is clear that the isolate W enhanced N content in leaves more than the isolate L. All tested inoculation treatments, however, led to obvious increase in the N content compared to inoculation with SR alone. On the other hand, the highest N content was induced by treatment of 1%A, followed by 2%A, 1%C, 1%B, 2%C, 2%B, 1%D, 2%D compared to the non-inoculated control. Most treatments of isolate W increased N content more than those of L isolate.

21. All treatments increased phosphorus (P) content in tomato leaves compared to the control as well as inoculation with FOL alone. The highest P content was in treatments of A, B, C (1%), A (2% & 3%), D (1%), B, C (2%) compared to the control. Treatments of L isolate were more effective for increasing P content than those of isolate W. Similar trend was noticed regarding P content in leaves of eggplant. All treatments increased P content compared to SR alone. The L isolate was more effective than W isolate. The P content was higher in treatments of A at 1 or 2%, B at 1 or 2%, 1%C, A & B at 3%, 1%D and 2%C comparing to the control.

22. All treatments increased potassium (K) content in tomato leaves compared to the control as well as the inoculation with FOL alone. The highest K content was recorded by using A (2%), A (1%), B (2%), A (3%), B (1%), B (3%), C (2%), D (2%), C (3%), C (1%) D (1%) and D (3%), respectively. Average of K content, in general, was obviously higher in case of L isolate than W isolate. Using treatments of A (2%, 1%, 3%), B (3%, 2% and 1%) in case of isolate L, and A, B

(2% or 1%), A and B (3%) in case of W isolate, respectively were the best in this respect. In eggplant, all tested treatments, however, increased K content in plant leaves compared to inoculation with SR alone, treatment of A (1%) was the best in this respect followed by B and C (1%), , A, B and C (2%) whereas treatments of B, C and D (3%) decreased it compared to the control.

REFERENCES

Abdel Latef, A.A.H. and Chaoxing, H. (2011). Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiol. Plant, 33:1217–1225.

Abdel-Fattah, G.M. and Mohameden, A.H. (2000). Interactions between a vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. Biology and fertility of soils, 32: 401-409.

Agrios, G.N. (1978): Plant Pathology 2nd ed. pp.466-470.

Agrios, G.N. (2005). Plant Pathology. Fifth edition, Academic Press, 925 pp.

Akköprü, A. and Demir, S. (2005). Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. lycopersici by AMF *Glomus intraradices* and some Rhizobacteria. Journal of Phytopathology, 153:544-550.

Al-Karaki, G.; McMichael, B. and Zak, J. (2004). Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza, 14(4):263-9.

Allam , A.I. and Hollis, J. P. (1972). Sulfide inhibition of oxidase in rice roots. Phytopathology, 62: 634-639.

Almeida, R.T. and Schenck, N.C. (1990). A revision of the genus *Sclerocystis* (Glomaceae, Glomales). Mycologia, 82(6): 703-714.

Andrade- Linares, D.R. (2011). Characterization of tomato root-endophytic fungi and analysis of their effects on plant development on fruit yield and quality and on interaction with the pathogen *Verticillium dahliae*. PH.D. Thesis in Biology, University Potsdam, Germany, 212.

A.O.A.C. (2005). Official methods of Analytical, 18th ED. Association of official Analytical Chemists. Gaithersburg, Maryland, U.S.A..

Appoloni, S.; Lekberg, Y.M.; Tercek, T.; Zabinski, C. A. and Redecker, D. (2008). Molecular Community Analysis of Arbuscular Mycorrhizal Fungi in Roots of Geothermal Soils in Yellowstone National Park (USA). Microb. Ecol. (Springer Science + Business Media.

Arlorio, M.; Ludwig, A.; Boller, T. and Bonfante, P. (1992). Inhibition of fungal growth by plant chitinases and β-1, 3-glucanases. Protoplasma, 171: 34–43.

Azcon-Aguilar, C.; Jaizme-Vega, M.C. and Calvet, C. (2002). The contribution of arbuscular mycorrhizal fungi to the control of soil-borne plant pathogens. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture. Birkhuser, Switzerland, pp 187–197.

Azcon-Aguilar, C. and Barea, J.M. (1997). Applying mycorrhizae technology to horticulture: dignificance and potentials. Scientia Horticulturae, 68: 1-24.

Bago, B.; Vierheilig, H.; Piché, Y. and Azcon-Aguilar, C. (1996). Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. New Phytol., 133: 273–280**.**

Bagyaraj, D.J. and Varma, A. (1995). Interaction between arbuscular mycorrhizal fungi and plants, and their importance in sustainable agriculture in arid and semi–arid tropics. In: Jones JG (ed) Advances in microbial ecology, 14. Plenum, NewYork, pp 119–142.

Baruah, A.M. and Borah, R.C. (1998). Practical manual on elementary plant biochemistry and chemistry of plant product (PP.4).BNCA, Assam Agricultural University, Chariali, Assam.

Berthelet, M., Whyte, L.G. and Greer, C.W. (1996). Rapid, direct extraction of DNA from soils for PCR analysis using polyvinylpolypyrrolidone spin columns. FEMS Microbiol. Lett., 138: 17-22.

Bethlenfalvay, G. J. and Linderman, R. G. (1992). Mycorrhizae in Sustainable Agriculture. USA: ASA Special Publication **54**. ISBN 0-89118-112-1.

Birhane, E.; Sterck, F.J.; Fetene, M.; Bongers, F. and Kuyper T. W. (2012). Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth

of frankincense seedlings under pulsed water availability conditions. Oecologia, 169(4): 895–904.

Blilou, I.; Bueno, P.; Ocampo, J.A. and Garcia-Garrido, J.M. (2000a). Induction of catalase and ascorbate peroxidase activities in tobaccco roots inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae*. Mycological Research, 104**:**722–725.

Blilou, I.; Ocampo, J.A. and García-Garrido, J.M. (2000b). Induction of Ltp (Lipid transfer protein) and Pal (phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. Journal of Experimental Botany, 51**:** 1969–1977.

Boswell, E.P.; Koide, R.T.; Shumway, D.L. and Addy, H.D. (1998). Winter wheat cover cropping, VA mycorrhizal fungi and maize growth and yield. Agriculture Ecosystem and Environment, 67: 55-65.

Broglie, K.; Chet, I.; Holliday, M.; Cressman, R.; Biddle, P.; Knowlton, S.; Mauvais, C.J. and Broglie, R. (1991). Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. Science, 254:1194–1197.

Bruns, T.D. (1992). Evolutionary relationships within the fungi: analyses of small subunit rRNA sequences. Mol. Phylogen. Evol., 1:231-243.

Bushnell, W.R. and Rajendren, R.B. (1980). Casein hydrolysates and peptones for artificial culture of *Puccinia graminis f. sp tritici.* Phytopathology, 60:1287. (Abstract)

Caron, M.; Fortin, J.A. and Richard, C. (1985). Influence of substrate on the interaction of *Glomus intraradices* and *Fusarium oxysporum* f.sp. *radicis-lycopersici* on tomatoes. Plant-and-Soil, 87(2): 233-239.

Caron, M.; Fortin, J.A. and Richard, C. (1986). Effect of *Glomus intraradices* on infection by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Canadian-Journal-of-Botany, 64(3): 552-556.

Chandanie, W.A.; Kubota, M. and Hyakumachi, M. (2009). Interact between the arbuscular mycorrhizal fungus *Glomus mosseae* aplant growth-promoting fungi and their significance for enhancplant growth and suppressing damping-off of cucumber (*Cucumsativus* L.). Appl. Soil Ecol., 41: 336-341.

Chaurasia, B.; Pandey, A. and Palni, L.M.S. (2005). Distribution, colonization and diversity of arbuscular mycorrhizal fungi associated with central Himalayan rhododendrons. Forest Ecology and Management, 207(3): 315-324.

Chen, Y.C.; Eisner, J.D.; Kattar, M.M.; Rassoulian- Barrett, S.L.; LaFe, K.; Yarfitz, S.L.; Limaye, A.P. and Cookson, B.T. (2000). Identification of medically important yeasts using PCR-based detection of DNA sequence polymorphisms in the internal transcribed spacer two regions of the rRNA genes. J. Clin. Microbiol., 38:2302-2310.

Chet, I.; Ordentlich, A.; Shapira, R. and Oppenheim, A. (1990). Mechanisms of biocontrol of soil-borne plant pathogens by rhizobacteria. Plant Soil, 129:85–92.

Clapp, J.P.; Fitter, A.H. and Young, J.P.W. (1999). Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. Mol. Ecol., 8:915-921.

Cordier, C.; Gianinazzi, S. and Gianinazzi-Pearson, V. (1998a). Colonization pattern of root tissue by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. Plant Soil, 185:223–232.

Cordier, C.; Pozo, M.J.; Barea, J.M.; Gianinazzi, S. and Gianinazzi-Pearson, V. (1998b). Cell defense responses associated with localized and systemic resistance to Phytophthora parasitica induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microb. Interact, 11:1017–1028.

D'Amico, M.; Frisullo, S. and Cirullia, M. (2008). Endophytic fungi occurring in fennel, lettuce, chicory, and celery — commercial crops in southern Italy, 112: 100–107.

Dixon, R. K.; Garrett, H. E. and Cox, G. S. (1987). Cytokinins in the root pressure exudate of *Citrus jambhiri* Lush. colonized by vesicular-arbuscular mycorrhizae. Oxford Journals, Life Sciences , Tree Physiology, 4(1): 9-18.

El-Fiki, A.I.I.; El-Habaa, G.M.D. and Eid, K.E. (2001). Successful growth and sporulation of the Vesicular Arbuscular Mycorrhizal fungi in axenic cultures. Ann. Agric. Sci., Moshtohor, 39 (2): 933-952.

Evans, J. R. (1989). Photosynthesis and nitrogen relationships in fo- liage of C3 plants. Oecologia, 78:9–19.

Filion, M.; St-Arnaud, M. and Fortin, J.A. (1999). Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol., 141:525–533.

Frank, J.B.; Petrovic, A.M. and Mudge, K.W. (1987). Influence of inoculum placement depth on endomycorrhizal fungal infection and perennial ryegrass shoot growth. Journal of the American Society for Horticultural Science. 112 (3) 282-286.

Gamper, H.; Walker, C. and Schüßler, A. (2009). Diversispora celata sp. nov.: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. New Phytologist, 182: 495-506.

Garcia-Garrido, J.M. and Ocampo, J.A. (2002). Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J. Exp. Bot., 53 (373): 1377-1386.

Garcia-Garrido, J.M., Ocampo, J.A. (1989). Effect of VA mycorrhizal infection of tomato on damage caused by Pseudomonas syringae. Soil Biol. Biochem., 21:65–167.

Gardezi, A.; Espinoza, G.R.; Cerrato, F.R. and Larque-Saavedra, M. (1999). Effect of arbuscular mycorrhizae on tomato (Lycopersicon esculentum Mill) in naturally infested soil with *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Revista-Mexicana-de-Fitopatologia, 17(1): 23-28.

Giannuzzi, S.; Schuepp, H.; Barea, J.M. and Haselwandter, K. (2001). Mycorrhizal technology in Agriculture: From genes to bioproducts. Bikhausser, Bassel, Switzerland.

Harley, J.L. and Smith, S.E. (1983). Mycorrhizal symbioses. Academic Press, New York. pp. 483.

Harrier, L.A. (2001). The arbuscular mycorrhizal symbiosis: a molecular review of the fungal dimension. Journal of Experimental Botany, 52: 90001, 469-478.

Harrison, M. and Dixon, R. (1993). Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular arbuscular mycorrhizal associations in roots of *Medicago truncatula*. Mol Plant-Microbe Interact, 6:643–659.

Hashiba, T. and Narisawa, K. (2005). The development and endophytic nature of the fungus *Heteroconium chaetospira*. 252: (2) 191–196.

Hause, B.; Cornelia, M.; Stanislav, I. and Strack, D. (2007). Jasmonate in arbuscular mycorrhizal interactions. Phytochemistry, 68:101–110.

Hendrix, J.W.; Jones, K.J. and Nesmith, W.C. (1992). Control of pathogenic mycorrhizal fungi in maintenance

of soil productivity by crop-rotation. Journal of Production Agriculture, 5: 383-386.

Hijri, I.; Skorova, Z.; Oehl, F.; Ineichen, K.; Mader, P.; Wiemken, A. and Redecker, D. (2006). Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. Mol Ecol., 15:2277–2289.

Hooker, J. E.; Jaizme-Vega, M. and Atkinson, D. (1994). Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. ALS Advances in Life Sciences, 191-200.

Ismail, Y.; Mc-Cormick, S. and Hijri, M. (2011). A Fungal Symbiont of Plant-Roots Modulates Mycotoxin Gene Expression in the Pathogen *Fusarium sambucinum*. PLoS ONE 6(3):e17990. doi:10.1371/journal.pone.0017990.

Jansa, J.; Mozafar, A.; Kuhn, G.; Anken, T.; Ruh, R.; Sanders, I.R. and Frossard, E. (2003). Soil tillage affects the community structure of mycorrhizal fungi in maize roots. Ecological Applications, 13: 1164-1176.

Jaroszuk-Scisel, J. and Kurek, E. (2012). Hydrolysis of fungal and plant cell walls by enzymatic complexes from cultures of Fusarium isolates with different aggressiveness to rye (*Secale cereale*). Archives of Microbiology, 194 (8): 653-665.

Jones, D.L.; Hodge, A. and Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. New Phytol., 163:459–480.

Jung, W.J.; An, K.N.; Jin, Y.L.; Park, R.D., Lim, K.T.; Kim, K.Y. and Kim, T.H. (2003). Biological control of damping-off caused by *Rhizoctonia solani* using chitinase-producing *Paenibacillus illinoisensis* KJA-424. Soil Biol. Biochem.,35:1261–1264.

Kapoor, R.T. P. and Bhatnagar, A. K. (2011). Functional synergism among *Glomus fasciculatum*, *Trichoderma viride* and *Pseudomonas fluorescens* on Fusarium wilt in tomato. Journal of Plant Pathology, 93(3): 745-750.

Kaschuk, G.; Kuyper, T.W.; Leffelaar, P.A.; Hungria, M. and Giller, K.E. (2009). Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses. Soil Biol Biochem., 41:1233–1244.

Kichadi, S.N. and Sreenivasa, M.N. (1998). Interaction effects of *Glomus fasciculatum* and *Trichoderma harzianum* on *Sclerotium rolfsii* in presence of biogas spent slurry in tomato. Karnataka Journal of Agricultural Sciences, 11(2): 419-422.

Kriiger, M. ; Stockinger, H.; Kriiger, C. and Schüßler, A.. (2009). DNA-based species level detection of *Glomeromycota:* one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist,* 183: 212-223.

Krishna, K.R. and Bagyaraj, D.J. (1983). Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in peanut. Canadian-Journal-of-Botany, 61(9): 2349-2351.

Kumar, R.; Jalali, B.L. and Chand, H. (2004). Interaction between VA-mycorrhizal fungi and soil - borne plant pathogens of chickpea. Legume-Research, 27(1): 19-26.

Lambais, M.R. (2000). Regulation of plant defence-related genes in arbuscular mycorrhizae. In: Podila GK, Douds DD, eds. Current advances in mycorrhizae research. Minnesota, USA: The American Phytopathological Society, 45–59.

Larose, G.; Chenevert-Moutoglis, P.; Gagne, S. and Piché-Vierheilig, H. (2002). Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. J. Plant Physiol., 159:1329–1339.

Lee, J.; Lee, S. and Young, J.P.W. (2008). Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiology Ecology, 65: 339-349.

Li-Min; Meng-Xiang -Xia; Jiang-JiQiang and Liu-Run-Jin (2000). A preliminary study on relationship between arbuscular mycorrhiza fungi and Fusarium wilt of watermelon. Acta-Phytopathologica-Sinica, 30(4): 327-331.

Linderman, R.G. (1994). Role of VAM fungi in biocontrol. In: Mycorrhizae and plant health, Pfleger, F.L. and Linderman, R.G. (eds). APS Press, St. Paul. 1-26.

Linderman, R.G. and Davis, E.A. (2004). Varied response of marigold genotypes to inoculation with different VA mycorrhizal fungi. Scientia Horticulturae, 99: 67-78.

Lioussanne, L.; Jolicoeur, M. and St. Arnaud, M. (2003). Effects of the alteration of tomato root exudation by *Glomus intraradices* colonization on *Phytophthora parasitica* var. *Nicotianae* zoospores. Abstract No. 253, Abstract Book ICOM 4; Montreal/ Canada.

Malamy, J.; Carr, J.P.; Klessig, D.F. and Raskin, I. (1990). Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. Science, 250:1002–1004.

Marschner, H. and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. Plant Soil, 159: 89-102.

Mathimaran, N.; Falquet, L.; Ineichen, K.; Picard, C.; Redecker, D.; Boller, T. and Wiemken, A. (2008). Microsatellites for disentangling underground networks: Strain-specific identification of Glomus intraradices, an arbuscular mycorrhizal fungus. Fungal Genetics and Biology, 45: 812-817.

Matta, A. and Dimond, A.E. (1963). Symptoms of Fusarium wilt in relation to quantity of Fungus and enzyme activity in tomato stems. Phytopathology, 53: 574-587.

Mauch, F.; Mauch-Mani, B. and Boller, T. (1988). Antifungal hydrolases in pea tissue: II. Inhibition of fungal growth by combination of chitinase and β-1, 3 glucanase. Plant Physiol., 88:936–942.

Medina, H.M.J.; Gagnon, H.; Piché, Y.; Ocampo, J.A.; Garcia- Garrido, J.M. and Vierheilig, H. (2003). Root colonization by arbuscular mycorrhizal fungi is affected

by the salicylic acid content of the plant. Plant Sci., 164:993–998.

Metraux, J.P.; Signer, H.; Ryals, J.; Ward, E.; Wyss-Benz, M.; Gaudin, J.; Raschdorf, K.; Schmid, E.; Blum, W. and Inverardi, B. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science, 250**:**1004–1006.

Monreal, J. and Reese, E.T. (1969). The Chitinase of *Serratia marcescens*. Canadian J. Microbiology, 15: 689-696.

Morandi, D. (1996). Occurence of phytoalexins and phenolic compounds on endomycorrhizal interactions, and their potential role in biological control. Plant Soil, 185:241–251.

Muthukumar, T. and Tamilselvi, V. (2010). Occurrence and morphology of endorhizal fungi in crop species. Tropical and Subtropical Agroecosystems, 12: 593 -604.

Nagahashi, G. and Douds, D.D. (2005). Environmental factors that affect presymbiotic hyphal growth and branching of arbuscular mycorrhizal fungi. In: Declerck S, Strullu DG, Fortin JA (eds) In vitro culture of mycorrhizas. Springer, Heidelberg, 95–110.

Nagarajkumar, M.; Bhaskaran, R. and Velazhahan, R. (2004). Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol. Res., 159:73–81.

Norman, J. R. and Hooker, J.E. (2000). Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of non-mycorrhizal than by mycorrhizal strawberry roots. Mycol. Res., 104:1069–1073.

Olsson, P.A.; Hammer, E.C.; Pallon, J.; van- Aarle, I.M. and Wallander, H. (2011). Elemental composition in vesicles of an arbuscular mycorrhizal fungus, as revealed by PIXE analysis. Fungal Biol., 115 (7): 643-8.

Ozgonen, H.; Blclcl, M. and Erkilic, A. (2001). The effect of salicylic acid and endomycorrkizal fungus *Glomus etunicatum* on plant development of tomatoes and Dusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. Turk J. For., 25: 25-29.

Phillips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing roots for rapid assessment of infection. Transactions of the British Mycological Society, 55:158-161.

Pozo, M.J. and Azcon-Aguilar, C. (2007). Unraveling mycorrhiza-induced resitance. Curr Opinion Plant Biol., 4:393–398.

Pozo, M.J.; Azcon-Aguilar, C.; Dumas-Gaudot, E. and Barea, J.M. (1999). β-1,3-glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. Plant Sci., 141:149–157.

Prasad, R..; Sharma, M.; Chatterjee, S.; Chauhan, G.; Tripathi, S.; Das, A.; Kamal, S.; Rawat, A. K. S.; Bhutani, K. K. and Rai, M. K. (2008). Interactions of *Piriformospora indica* with Medicinal Plants. Mycorrhiza. 655-678

Purves, M.J., D.C. Collier and D. Walls, 1966. Laboratory techniques in Botany (2nd Ed.), Butterworth, London (c.f. Fares, Clair, N. 1986)

Redecker, D. (2000). Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza, 10: 73-80.

Redecker, D.; Thierfelder, H.; Walker, C. and Werner, D. (1997). Restriction analysis of PCR-amplified internal transcribed spacers of ribosomal DNA as a tool for species identification in different genera of the order Glomales. Appl Environ Microbiol., 63:1756-1761.

Redecker, D.; Joseph, B. Morton, J.B;Thomas, D. and Bruns, T.D. (2000). Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides*. *Mycologia*, 92(2): 282-285.

Redecker, D.; Hijri, I. and Wiemken, A. (2003). Molecular identification of arbuscular mycorrhizal fungi in roots: Perspectives and problems. Folia Geobotanica, 38:113-124.

Reid, J.D. and Ogryd-Ziak, D.M. (1981). Chitinase over producing mutant of *Serratia marcescens*. Appl. and Eviron.Microbiol., 41: 664-669.

Roderick, D.M. and Edward, C. H. (1998). Molecular evolution: a phylogenetic approach. 352 pages

Ryan, A. and Jones, P. (2004). The effect of mycorrhization of potato roots on the hatching chemicals active towards the potato cyst nematodes. *Globodera pallida* and *G. rostochiensis*. Nematol, 6:335–342.

Salzer, P.; Corbière, H. and Boller, T. (1999). Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza-forming fungus *Glomus mosseae*. Planta, 208:319–325.

Sanders, I.R.; Alt, M.; Groppe, K.; Boller, T. And Wiemken, A. (1995). Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol., 130:419-427.

Scheffknecht, S.; St-Arnaud, M.; Khaosaad, T.; Steinkellner, S. and Vierheilig, H. (2007). An altered root exudation pattern through mycorrhization affecting microconidia germination of the highly specialized tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* is not tomato specific but also occurs in FOL non-host plants. Can. J. Bot., 85:347–351.

Schüßler, A.; Gehrig, H.; Schwarzott, D. and Walker, C. (2001a). Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. Mycological Research, 105: 5-15.

Schüßler, A.; Schwarzott, D. and Walker, C. (2001b). A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycological Research, 105: 1413-1421.

Schussler, A., Schwarzott, D. and Walker, C. (2001). A new fungal phylum, theGlomeromycota: Phylogeny and Evolution. Mycol. Res. 105: 1413-1421.

Schwarzott, D.; Walker, C. and Schüßler, A. (2001). *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. Mol Phylogen Evol., 21:190–197.

Shende, S. and Rai, M. (2010). Role of mycorrhizal fungi in growth promotion of crop plants. Progress in Mycology, 259-292.

Shepherd, M.; Nguyen, L.; Jones, M. E.; Nichols, J. D.; Carpenter, F. L. (2007). A method for assessing arbuscular mycorrhizal fungi group distribution in tree roots by intergenic transcribed sequence variation. PLANT AND SOIL. 290(1-2):259-268.

Simon, L.; Bousquet, J.; Levesque, C. and Lalonde, M. (1993). "Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants". Nature 363, (6424): 67–69.

Simon, L.; Lalonde, M. and Bruns, T.D. (1992). Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endo- mycorrhizal fungi colonizing roots. Appl Environ Microbiol., 58:291-295

Singh, R.; Adholeya, A. and Mukerji, K.G. (2000). Mycorrhiza in control of soil-borne pathogens. In: Mukerji KG, Chamola BP, Singh J (eds.) Mycorrhizal biology. Kluwer, New York, pp 173–196.

Singh, P.K.; Mishra, M. and Vyas, D. (2010). Interactions of vesicular arbuscular mycorrhizal fungi with Fusarium wilt and growth of the tomato. Indian Phytopathology**,** 63 (1): 30-34.

Singh, S. and Kapoor, K.K. (2000). Influence of Inoculation of Different Vesicular Arbuscular Mycorrhizal Fungi on Growth and Nutrient of Mungbean and Wheat. Philippine Journal of science, 129, 1.

Smith, S.E. and Read, D.J. (1997). Mycorrhizal symbioses. Academic Press, San Diego, CA. 605pp.

Snedecor, G. W. and Cochran, W. G. (1982). Statistical methods. The Iowa State University Press. 7th Edit., 2nd Printing. 507 pp.

Sood, S.G. (2003). Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular-arbuscular mycorrhizal tomato plants. FEMS Microbiol. Ecol., 45:219–227.

Spanu, P. and Bonfante-Fasolo, P. (1988). Cell wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. New Phytologist,109:119–124.

St-Arnaud, M. and Elsen, A. (2005). Interaction of arbuscular mycorrhizal fungi with soil-borne pathogens and non-pathogenic rhizosphere micro-organisms. In: Declerck S,

Strullu DG, Fortin JA (eds) In vitro culture of mycorrhizas. Springer, Heidelberg, 217–231.

St-Arnaud, M.; Hamel, C.; Vimard, B.; Caron, M. and Fortin, J.A. (1995). Altered growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in an *in vitro* dual culture system with the vesicular arbuscular mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots. Mycorrhiza, 5:431–438.

St-Arnaud, M. and Vujanovic, V. (2006). Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. In: Hamel C, Plenchette C (eds). Mycorrhizae in crop production: (1) 67-122.

Stockinger, H., Walker, C. and Schüßler, A. (2009). Intraradices DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. New Phytologist, in press.

Thimmaiah, S.K. (1999). Standard methods of biochemical analysis. Kalyani. Pub.,Ludhiana ,44.

Tuzun, S.; Rao, M.N.; Vogli, U.; Schardl, C.L. and KU, J.A. (1989). Induced systemic resistance toblue mold, early induction and accumulation of B,1,3-gluconases chitinases And other pathogenesis –related proteins (b-proteins) in immunized tobac. phytopathology, 79:979-983.

van den Burg, H.A.; Westerink, N.; Francoijs, K.J.; Roth, R.; Woestenenk, E.; Boeren, S.; de Wit, P.J.G.M.; Joosten, M.H..J. and Vervoort, J. (2003). Natural disulfide bond-disrupted mutants of AVR4 of the tomato

pathogen *Cladosporium fulvum* are sensitive to proteolysis, circumvent Cf-4-mediated resistance, but retain their chitin binding ability. J Biol Chem 278:27340–27346

Van den Burg, HA; Harrison, SJ; Joosten, MHAJ; Vervoort, J; de Wit, PJGM. (2006). *Cladosporium fulvum* Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. Molecular Plant-Microbe Interactions 19: 1420.

Van den Burg, H.A.; Spronk, C.A.; Boeren, S.; Kennedy, M.A.; Vissers, J.P.; Vuister, G.W.; de Wit, P.J.G.M. and Vervoort, J. (2004). Binding of the Avr4 elicitor of *Cladosporium fulvum* to chitotriose units is facilitated by positive allosteric protein–protein interactions: the chitin–binding site of Avr4 represents a novel binding site on the folding scaffold shared between the invertebrate and the plant chitin–binding domain. Journal of Biological Chemistry, 279: 16786–16796. .

Van Tuinen, D.; Jacquot, E.; Zhao, B.; Gollotte, A. and Gianinazzi-Pear- son, V. (1998). Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol Ecol., 7:879-887.

Vierheilig, H. (2004). Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. Can J. Bot., 82:1166–1176.

Vierheilig, H.; Alt, M.; Neuhaus, J.M.; Boller, T. and Wiemken, A. (1993). Colonization of transgenic *Nicotiana sylvestris* plants expressing different forms of *Nicotiana tabacum* chitinase by the root pathogen *Rhizoctonia solani* and by the mycorrhizal symbiont *Glomus mosseae*. Mol Plant Microbe Interact, 6:261–264.

Vierheilig, H. and Bago, B. (2005). Host and non-host impact on the physiology of the AM symbiosis. In: Declerck S, Strullu DG, Fortin JA (eds) *In vitro* culture of mycorrhizas. Springer, Heidelberg, 139–158.

Vierheilig, H. and Piché, Y. (2002). Signalling in arbuscular mycorrhiza: facts and hypotheses. In: Buslig B, Manthey J (eds) Flavonoids in cell function. Kluwer, New York, 23–39.

Villegas, J.; Williams, R.D.; Nantais, L.; Archambault, J. and Fortin, J.A. (1996). Effects of N source on pH and nutrient exchange of extramatrical mycelium in a mycorrhizal Ri T-DNA transformed root system. Mycorrhiza, 6:247–251.

Walker, C. and Schüßler, A. (2004). Nomenclatural clarifications and new taxa in the Glomeromycota. Mycological Research, 108: 981-982.

Walker, C.; Vestberg, M.; Demircik, F.; Stockinger, H.; Saito, M.; Sawaki, H.; Nishmura, I. and Schüßler, A. (2007). Molecular phylogeny and new taxa in the Archaeosporales (Glomeromycota): Ambispora fennica gen. sp nov., Ambisporaceae fam. nov., and emendation

of Archaeospora and Archaeosporaceae. Mycological Research, 111: 137-153.

Wettestien, D.V. (1957). Cholorophyll Letal und form wechsel der plastiden Exp. Cel Res., 12:427.(Germany).

White, T.J.; Bruns, T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA et al (ed) PCR protocols, a guide to methods and applications. Academic, London, 315–322.

Wokocha, R.C. (2001). Reaction of monocotyledonous and dicotyledonous plants to infection by *Sclerotium rolfsii* in the Nigerian savanna: implications for control of the basal stem rot disease. Nigerian Journal Of Botany, 14.

Wright, D. P.; Scholes, J. D. and Read, D. J. (1998). Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. Plant, Cell and Environment, 21: 209–216.

Wu, Q.S and Xia, R.X. (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J. Plant Physiol., 163:417–425.

Wubet, T.; Weifi, M.; Kottke, I. and Oberwinkler, F. (2003). Morphology and molecular diversity of arbuscular mycorrhizal fungi in wild and cultivated yew (Taxus baccata). The Canadian Journal of Botany, 81: 255-266.

Xavier, L.J.C. and Boyetchko, S.M. (2004). Arbuscular mycorrhizal fungi in plant disease control. In: Arora DK (ed) Fungal biotechnology in agricultural, food, and environmental applications. Dekker, New York, 183–194.

Yang, L. (2008). Morphology and Diversity of Arbuscula Mycorrhizal Fungi Colonizing Roots of Dandelion and Chive. University of Saskatchewan, Master of Science.102 p

الملخص العربي

الهدف من هذا العمل هو عزل الفطريات المستوطنة للجذور والمشابهة لميكوريزا الحويصلات الشجيرية وعلاقتها بتحسن النمو فى النباتات ودراسة قدراتها على التضاد ضد مسببات أمراض ذبول الفيوزاريوم فى الطماطم (*Fusarium oxysporum* f. sp. *lycopersici* (FOL)) وعفن الجذور فى الباذنجان (*Sclerotium rolfsii* (SR)) فضلا عن دراسة قدراتها على تحفيز واستحثاث المقاومة في نباتات *الطماطم* والباذنجان ضد مسببات أمراض الذبول وعفن الجذور على التوالي ، هذا ويمكن تلخيص أهم النتائج المتحصل عليها فى النقاط التالية:

١. تم الحصول على عزلتين من الفطريات من جذور سليمة ظاهريا لنباتات القمح والخس تم تسميتهما بـ عزلة القمح(W) ، عزلة الخس(L) على التوالي ويتميز كليهما بتكوين ميسيليوم غير مقسم ذو هيفات لونها بنى فاتح وغير منتظمة التفرع تظهر عليها انتفاخات متفاوتة الأحجام كما يتكون على تلك الهيفات جراثيم كلاميدية منفردة أو في سلاسل بينية أو طرفية كما تكون تراكيب تشبه حوافظ الجراثيم الإسبورانجية .

٢. في طريقة nested-PCR كانت الأربعة بادئات المتخصصة المختبرة وهي GLOM1310/ITS4i (A), ARCH1311/ITS4i (B), PARA1313/ITS4i (C) and LECT1677/ITS4i (D) ذات فعالية كبيرة في كشف واظهار درجة التشابه العالية بين اثنين من عزلات الـ VAM (عزلات W & L) من خلال وجود حزم من الـ DNA عند نفس المستوي من الوزن الجزيئ (bp). وفي هذا الصدد فقد اظهر استخدام البادئ المتخصص GLOM1310/ITS4i وجود حزمتين كثيفتين من الـ DNA تراوح الوزن الجزيئي لهما بين 500 bp – 600 للعزلتين المختبرتين من الـ VAM (L & W)، بينما اظهر استخدام البادئ المتخصص ARCH1311/ITS4i حزمتين

مكثفتين كان الوزن الجزيئي لهما 700 bp للعزلتين المختبرتين من VAM (W & L). و قد كشف استخدام البادئ المتخصص PARA1313/ITS4i وجود حزمتين واضحتين من الـ DNA عند الوزن الجزيئي 200bp للعزلتين المختبرتين من VAM (W & L). كما كشف استخدام البادئ المتخصص LECT1677/ITS4i وجود حزمتين واضحتين عند الوزن الجزيئي 350bp للعزلتين المختبرتين من VAM (W & L).

٣. عند زراعة حبوب القمح في أصص محتوية على تربة معقمة ومحقونة بمستويات مختلفة من لقاح عزلتي القمح والخس – أظهرت النتائج أن جميع مستويات اللقاح المستخدمة قد سببت زيادات ملحوظة في كافة القياسات المختبرة مقارنة بالكنترول ، وكان المستوى الأدنى (١%) هو الأفضل لزيادة نسب بقاء للنباتات مع تحسن كبير في جميع المعايير المستخدمة في قياس نمو النباتات ، يليه في ذلك المستوى الأوسط (٢%) ثم المستوى الأعلى (٣%) مع فروق ذات دلالة إحصائية واضحة بين هذه المستويات. كما لوحظ أن عزلة القمح قد أدت إلى زيادات معنوية في طول الجذور وعدد كل من الأوراق والأشطاء على النبات مقارنة بعزلة الخس. كما أظهر الفحص المجهري للجذور المصبوغة قدرة كلتا العزلتين على استعمار أنسجة القشرة في جذور القمح مع تكون تراكيب تشبه تلك التي تكونها ميكوريزا الحويصلات والتراكيب الشجيرية.

٤. تم دراسة التفاعلات المتبادلة بين شريكين فطريين ينموان معا على نفس البيئة – أحدهما يمثله عزلة القمح (W) أو عزلة الخس (L) أما الأخر فيمثله الفطر فيوزاريوم (FOL) (مسبب الذبول في الطماطم) – وقد أظهرت النتائج أن نمو أحد الشريكين قد أثر سلبا على نمو الآخر مقارنة بنمو كل منهما منفردا. ففي التفاعل L/FOL لوحظ بعد تسعة أيام من التحضين تناقص نمو العزلة L بنسبة ٢٧.١ ، ١٥.٠ ، ١٣.٤% بينما تناقص نمو الفطر FOL بنسبة ٣١.٩ ، ٣٦.٩ ، ١٨.٧% وذلك عند حقن L قبل ٢٤ ساعة من حقن FOL ، حقنهما

معا في نفس التوقيت، حقن FOL قبل ٢٤ ساعة من حقن L على التوالي. أما في حالة التفاعل W/FOL فقد اختلف الوضع نسبيا حيث تناقص نمو FOL بعد تسعة أيام من التحضين بنسبة ٣٦.١ ، ٣٨.١ ، ٣٥.٢% بينما نقص نمو العزلة W بنسبة ٣٦.٥ ، ٣٤.٧ ، ٥٢.٨% عند نفس ظروف الحقن السابقة ، على التوالي.

٥. تم أيضا دراسة التفاعلات المتبادلة بين كل من عزلتي الخس والقمح مقابل الفطر اسكليروشيوم رولفزياى (SR) على النحو المذكور أعلاه. ففي حالة التفاعل L/SR لوحظ تناقص نمو S بعد ٧ أيام من التحضين بنسب ٩٥.١ ، ٧٧.٠ ، ٧٣.٠% بينما تناقص نمو L بنسبة ٣.١ ، ١٢.٣ ، ١٦.٤٪ عند تلقيح (L) قبل ٢٤ ساعة من تلقيح (SR) ، تلقيحهما معا في وقت واحد ، تلقيح بعد ٢٤ ساعة من تلقيح (SR) ، على التوالي. أما فى حالة التفاعل W/SR فقد تناقص نمو (SR) بنسبة 60.7 ، 52.4 ، ٥١.٩٪ في حين تناقص نمو العزلة W بنسبة ٥.٣ ، ١٤.٨ ، ٢١.١٪ وذلك بعد ٧ أيام من التحضين وتحت نفس ظروف الحقن الثلاثة السابق ذكرها ، على التوالي.

٦. أثبتت الدراسة احتواء الرواشح المزرعية للفطريات تحت الدراسة على الأنزيمات المؤكسدة بيروكسيديز و بولي فينول أوكسيديز وأيضا الكيتينيز وكان نشاط جميع تلك الإنزيمات أكثر وضوحا في رواشح الفطريات المعزولة من جذور الخس (العزلة L) والقمح (العزلة W) مقارنة بالرواشح المزرعية لمسببات الذبول في الطماطم (FOL) وعفن الجذور في الباذنجان (SR) وفى جميع الأحيان كانت الأنشطة الإنزيمية أعلى في رواشح المزارع الفطرية عند عمر ٧ يوم ثم انخفضت تلك الأنشطة تدريجيا بزيادة عمر المزارع إلى ١٤ ثم ٢٥ يوما على التوالي. ولقد كان نشاط البيروكسيديز أعلى بينما كان نشاط الكيتينيز والبولي فينول اوكسيديز أقل ، كما أنخفض نشاط البيروكسيديز بمعدل أسرع فى رواشح مزارع العزلة (W) مقارنة بالعزلة (L). أما بالنسبة للفطريات المسببة للأمراض ،

فقد كان نشاط البيروكسيديز أعلى في رواشح فطر الذبول مقارنة بفطر عفن الجذور غير أن معدل الانخفاض فى أنشطة جميع الإنزيمات أعلى في رواشح فطر عفن الجذور مقارنة برواشح فطر الذبول عند جميع الأعمار.

٧. هذا وقد أجريت تجربتي أصص تحت ظروف الصوبة لدراسة مدى إمكانية استخدام الفطريات المعزولة من جذور الخس والقمح كعوامل مقاومة حيوية ضد أمراض الذبول الفيوزاريومى (FOL) في الطماطم (التجربة الأولى) وعفن الجذور في الباذنجان (التجربة التالية). وقد تم حقن التربة المعقمة بلقاح مسببات الأمراض (كل على انفراد بمعدل ٣% وزن/وزن) وكذلك لقاح فطريات المقاومة الحيوية بنفس المعدلات السابق ذكرها في التجربة الاستطلاعية غير أن هذا اللقاح قد أضيف إلى التربة إما منفردا (A) ، أسبوع واحد قبل (B) أو بعد (D) أو في نفس وقت حقنها (C) بالمسبب الممرض ، مع استخدام التربة المعقمة غير المحقونة كمعاملة مقارنة – تم انهاء كلتا التجربتين بعد ٤٠ يوما من شتل النباتات ثم تقدير نسب بقاء النباتات الناجية ، الأوزان الجافة للمجموع الخضري والجذور (جم/نبات) ، أطوال النبات ، عدد الأوراق/نبات ، والكمية الكلية لأصباغ التمثيل الضوئي في الأوراق ، نشاط الإنزيمات المؤكسدة ، محتوى الأوراق من عناصر النيتروجين والفوسفور والبوتاسيوم في جميع المعاملات تحت الدراسة – ويمكن تلخيص أهم النتائج المتحصل عليها كما يلي:

٨. تم الحصول على أعلى نسبة بقاء (١٠٠%) لنباتات الطماطم عند استخدام لقاح عوامل المقاومة الحيوية بمعدلات ١ أو ٢% سواء منفردا (A) أو قبل تلقيح التربة بمسبب الذبول (B) وبمعدل ٢% في نفس توقيت الحقن بمسبب الذبول (C) يليه في ذلك استخدام معدلات ١ ، ٢% في نفس التوقيت (C) أو بعد حقن مسبب الذبول (D) ، على التوالي – هذا ولم يكن للمعاملات B أو D (عند مستوى ٣%) تأثيرا معنويا على نسبة البقاء مقارنة بحقن المسبب الممرض (FOL) منفردا بينما أدت المعاملات A ، B بنفس المعدل (٣%) إلى نقص

هذه النسبة معنويا مقارنة بمعاملة الكنترول (الغير محقون) – أما في تجربة الباذنجان ومدى الإصابة بعفن الجذور فقد سجلت المعاملات A ، B ، C (بمعدلات ١ أو ٢%) وكذلك المعاملة D (بمعدل ١%) أكبر زيادة معنوية في نسب البقاء مقارنة بمسبب عفن الجذور SR منفردا – هذا ولم يكن للمعاملات B ، C ، D (معدل ٣%) تأثيرات معنوية على نسب البقاء عند مقارنتها بمعاملة الكنترول.

٩. تم تسجيل أكبر عدد من الأوراق على نباتات الطماطم عند استخدام المعاملات A ، B (بمعدل ١ أو ٢%) بينما أحدثت جميع المعاملات (A ، B ، C ، D) نقصا معنويا في عدد الأوراق/نبات عند مستوى لقاح (٣%) مقارنة بمسبب الذبول FOL منفردا. أما في الباذنجان فقد أظهرت المعاملات(A ، B ، C ، D) زيادة معنوية في عدد الأوراق/نبات عند مستوى لقاح (١ أو٢%) مقارنة بمسبب عفن الجذور SR منفردا، كما أدت نفس تلك المعاملات عند نفس المعدلات بالإضافة إلى المعاملة A (بمعدل ٣%) إلى زيادة معنوية في عدد الأوراق/نبات بينما لم يكن للمعاملات A ، B ، C (بمعدل ٣%) تأثيرا معنويا في هذا الصدد مقارنة بالكنترول.

١٠. أحدثت جميع المعاملات تحت الدراسة زيادة معنوية في أطوال نباتات الطماطم مقارنة بمسبب الذبول FOL منفردا – كما كانت المعاملات A ، B (بمعدل ١ أو ٢%) وكذلك المعاملة C (بمعدل ٢%) الأفضل معنويا (دون فروق معنوية بينها) مقارنة بمعاملة الكنترول – كما سجلت المعاملة B (بمعدل ٢% لكل من العزلتين W & L) أعلى زيادة في ارتفاع النبات – هذا وقد لوحظ اتجاها مماثلا بالنسبة لطول النبات في تجربة الباذنجان ، حيث سجلت المعاملات A ، C (بمعدل ١%) ، A (بمعدل ٢%) ، B (١%) ، B ، C (٢%) ، D (٢ & ١%) ، على التوالي أكبر زيادة فى أطوال النباتات مقارنة بمسبب المرض SR منفردا – ومن ناحية أخرى ، أظهرت جميع المعاملات (A ، B ، C ، D) عند

معدل ١ أو ٢% زيادة معنوية في ارتفاع النبات بينما لم يكن لنفس تلك المعاملات عند مستوى ٣% تأثيرا معنويا على ارتفاع النبات مقارنة بالكنترول.

١١. في تجربة الطماطم ، سجلت معظم المعاملات المختبرة زيادة معنوية في الوزن الرطب للمجموع الخضري للنباتات كما سجلت المعاملات A (١%) ، A ، B ، C (٢%) أكبر زيادة ولم يكن للمعاملة A (٣%) تأثيرا معنويا فى هذا الخصوص عند المقارنة بمسبب الذبول FOL منفردا – ومن ناحية أظهرت المعاملات A ، D & B لكل من العزلتين W & L (بمعدل ٣%) والمعاملة C للعزلة L (بمعدل ٣%) نقصا معنويا في الوزن الرطب للمجموع الخضري لنباتات الطماطم مقارنة بمعاملة الكنترول. وفيما يتعلق بتجربة الباذنجان ، أدت جميع المعاملات تحت الاختبار إلى زيادة الوزن الرطب للمجموع الخضري لنباتات الباذنجان مقارنة بمسبب المرض SR منفردا – ومن ناحية أخرى سجلت المعاملات C & A (بمعدل ١%) ، A ، C & B (بمعدل ٢%) أكبر زيادة معنوية ولم يكن للمعاملات D (بمعدل ١ أو ٢%) ، C & A (بمعدل ٣%) تأثيرات معنوية بينما أحدثت المعاملتين D & B (بمعدل ٣%) نقصا معنويا فى الوزن الطازج للمجموع الخضري للنباتات مقارنة بالكنترول.

١٢. في تجربة الطماطم ، سجلت جميع المعاملات المختبرة زيادة معنوية في الوزن الجاف للمجموع الخضري للنباتات مقارنة بمسبب الذبول FOL منفردا – ومن ناحية أخرى سجلت المعاملات A ، B ، C (٢%) أعلى زيادة بينما سببت المعاملة A (بمعدل ٣%) نقصا معنويا فى الوزن الجاف للمجموع الخضري للنباتات مقارنة بالكنترول – وقد لوحظ نفس الاتجاه بالنسبة للباذنجان حيث سجلت جميع المعاملات المختبرة زيادة معنوية في الوزن الجاف للمجموع الخضري للنباتات مقارنة بمسبب المرض SR منفردا – كما سجلت المعاملات C & A (بمعدل ١%) ، A (٢%) ، B (١%) ، C & B (٢%) ، D (١%) على التوالي أعلى زيادة معنوية بينما سجلت المعاملات D & B (بمعدل ٣%)

نقصا ملحوظا فى هذا الوزن مقارنة بالكنترول – من الجدير بالذكر أن الوزن الجاف للمجموع الخضري لنباتات الباذنجان كان أعلى معنويا فى حالة العزلة L مقارنة بالعزلة W.

١٣. في تجربة الطماطم ، سجلت جميع المعاملات المختبرة زيادة معنوية في طول الجذر مقارنة بالمعاملة بمسبب الذبول FOL منفردا – ومن ناحية أخرى سجلت المعاملات A ، B ، C (١ % ٢%) أعلى زيادة في طول الجذر بينما أدت المعاملات A ، B ، C ، D (بمعدل ٣%) نقصا معنويا فى طول الجذر مقارنة بالكنترول – وقد لوحظ نفس الاتجاه بالنسبة للباذنجان حيث سجلت جميع المعاملات المختبرة زيادة معنوية فى طول الجذر مقارنة بمسبب المرض SR منفردا – كما سجلت المعاملات A & C (بمعدل ١%) ، A (٢%) ، B (١%) ، C & B (٢%) ، D (١%) على التوالي أعلى زيادة معنوية بينما تناقص طول الجذر معنويا بواسطة المعاملات D & B (بمعدل ٣%) مقارنة بالكنترول – من الجدير بالذكر أن طول الجذر لنباتات الباذنجان كان أعلى معنويا في حالة العزلة W مقارنة بالعزلة L.

١٤. في نباتات الطماطم ، سجلت جميع المعاملات زيادة معنوية في الوزن الرطب للجذور مقارنة بمسبب الذبول FOL منفردا – ومن ناحية أخرى سجلت المعاملات A ، B (٢%) ، A (١%) ، C (٢%) B ، C (١%) أكبر زيادة في الوزن الرطب للجذور على التوالي بينما سببت المعاملات A ، B ، C ، D (٣%) انخفاضا معنويا مقارنة بالكنترول .. أما بالنسبة للباذنجان ، سجلت كل المعاملات زيادة كبيرة في الوزن الطازج للجذور مقارنة بمسبب المرض SR منفردا. وسجلت أعلى الزيادات في الوزن الرطب للجذور باستخدام المعاملات A (بمعدل ١ أو ٢٪) ، C ، B (بمعدل ١٪ ، ٢%) في حين سببت المعاملة D (٣%) انخفاضا معنويا مقارنة بالكنترول.

١٥. في حالة الطماطم ، زاد الوزن الجاف للجذور معنويا في جميع المعاملات مقارنة بمسبب الذبول FOL منفردا – كما كانت العزلة W أفضل معنويا في ذلك مقارنة بالعزلة L – ومن ناحية أخرى سجلت المعاملات A ، B (٢%) ، A (١%) ، C (٢%) B ، C (١%) على التوالي أكبر زيادة فى الوزن الجاف للجذور بينما انخفض معنويا في حالة A ، B ، C ، D (٣%) مقارنة بالكنترول. وقد لوحظ نفس الاتجاه في حالة الباذنجان ، حيث سجلت جميع المعاملات زيادة معنوية في الوزن الجاف للجذور مقارنة مع التلقيح بمسبب المرض SR منفردا. هذا وكانت أعلى زيادة مصاحبة للمعاملات A ، B ، C (١٪ ، ٢٪) ، D (١٪) بينما سببت المعاملة D (٣%) انخفاضا معنويا مقارنة بالكنترول.

١٦. في الطماطم ، أظهرت العزلة (L) كمية أكبر في أصباغ التمثيل الضوئي في أوراق الطماطم مقارنة بالعزلة W كما كانت الكمية أكبر في جميع المعاملات مقارنة بمسبب الذبول FOL منفردا – كما سببت المعاملة A (معدل ١ ، ٢%) الكمية الأكبر من الأصباغ بعكس الحال فى المعاملات A ، B ، C ، D (٣%) التي قللت من كميتها مقارنة بالكنترول. وفى أوراق الباذنجان لوحظ اتجاه مماثل حيث زادت كمية تلك الأصباغ في كل المعاملات بوضوح مقارنة مع التلقيح بمسبب المرض SR منفردا بينما سببت المعاملة D (٣%) انخفاضا واضحا بها مقارنة بالكنترول.

١٧. أظهرت معظم معاملات العزلة L نشاطا أكبر لإنزيم كيتينيز في أوراق الطماطم مقارنة بالعزلة W ، كما أظهرت المعاملات A ، C ، B (٢%) ، A ، B (١%) ، D (٣%) على التوالي النشاط الأكبر لهذا الإنزيم مقارنة بكل من معاملة الكنترول ومسبب الذبول FOL منفردا. وقد لوحظ اتجاها مماثلا بشأن نشاط الإنزيم الكيتينيز في أوراق الباذنجان حيث سجلت جميع المعاملات زيادة في النشاط مقارنة مع التلقيح بمسبب المرض SR منفردا كما كان النشاط

الأعلى مصاحبا للمعاملات A ، C (١%) وعلى العكس من ذلك أدت المعاملات A ، B ، C ، D (٣٪) في حالة العزلة W والمعاملات B ، D (٣) في حالة من العزلة L إلى انخفاض واضح فى نشاط الكيتينيز مقارنة بالكنترول.

١٨. في الطماطم ، زاد نشاط إنزيم البيروكسيديز بصورة ملحوظة في جميع المعاملات مقارنة بمسبب الذبول FOL منفردا – ومن جهة أخرى كان النشاط أكبر في المعاملات A ، B ، C ، D (١٪ ، ٢٪) مقارنة بنفس المعاملات عند مستوى ٣٪ – كما أن استخدام المعاملتين C ، D بمعدل ٣٪ فقط سبب انخفاضا في نشاط هذا الإنزيم مقارنة بالكنترول. أما بالنسبة للباذنجان ، فقد كانت العزلة L أكثر فعالية في زيادة نشاط البيروكسيداز مقارنة بالعزلة W – كما سجلت جميع المعاملات زيادة نشاط الإنزيم مقارنة بتلقيح مسبب المرض SR منفردا – هذا وقد سببت المعاملة A (١%) الزيادة الأعلى فى نشاط الإنزيم يليها المعاملات B ، C (١٪) ثم A ، B (٢%) ، D (١%) ، C (٢%) ، على التوالي مقارنة بالكنترول.

١٩. في تجربة الطماطم أدت معظم المعاملات إلى زيادة نشاط انزيم البولي فينول أوكسيديز باستثناء المعاملة A (٣٪) في حالة العزلة L حيث انخفض هذا النشاط مقارنة بكل من مسبب الذبول FOL منفردا وكذلك معاملة الكنترول– وقد كان أعلى نشاط للإنزيم مصاحبا للمعاملات A (١٪)، A ، C، D (٢٪)، C (١٪) ، على التوالي. أما في تجربة الباذنجان، فقد كان نشاط هذا الإنزيم أعلى نسبيا في جميع المعاملات في حالة العزلة W مقارنة بالعزلة L ، كما كان النشاط أعلى في جميع الأحوال بتلقيح مسبب المرض SR منفردا – ومن ناحية أخرى أدت المعاملات B، C، D (٣٪) إلى انخفاض طفيف في نشاط انزيم البولي فينول أوكسيديز مقارنة بالكنترول.

٢٠. أظهرت جميع المعاملات المختبرة خاصة في حالة العزلة L زيادة محتوى أوراق الطماطم من النيتروجين مقارنة بكل من مسبب الذبول FOL منفردا وكذلك

معاملة الكنترول – وسجلت المعاملات A ، B (٢%) أعلى محتوى من النيتروجين يليها المعاملات A (١٪) ، C (٢٪) ، B (١٪) على التوالي. وقد لوحظ نفس الاتجاه فيما يتعلق بتأثير المعاملات المختلفة على محتوى أوراق الباذنجان من النيتروجين.

٢١. أظهرت جميع المعاملات زيادة محتوى الأوراق في الطماطم من الفسفور (P) مقارنة بكل من مسبب الذبول FOL منفردا وكذلك معاملة الكنترول – وسجلت المعاملات A ، B (١%) أعلى محتوى من الفسفور يليها المعاملات C (٢٪) ، (٢٪) مقارنة بالكنترول . وقد لوحظ نفس الاتجاه لوحظ فيما يتعلق بمحتوى أوراق الباذنجان من الفوسفور.

٢٢. كذلك أظهرت جميع المعاملات زيادة محتوى الأوراق في الطماطم من البوتاسيوم مقارنة بكل من مسبب الذبول FOL منفردا ومعاملة الكنترول . وقد سجل أعلى محتوى من البوتاسيوم باستخدام المعاملات A (١ & ٢%) وكان معاملات العزلة L أفضل في هذا الخصوص من معاملات العزلة W. وقد لوحظ نفس الاتجاه فيما يتعلق بمحتوى أوراق الباذنجان من البوتاسيوم.

الخلاصة

مما سبق نستنتج أن استخدام معدل لقاح ١% ،٢% من كلا العزلتين الفطريتين والمعزولة من جذور نباتات القمح والخس السليمة أدى إلى تحسين نمو نباتات القمح والطماطم والباذنجان النامية تحت ظروف الصوبة وزيادة مقاومة نباتات الطماطم النامية في تربة ملقحة بفطر *Fusarium oxysporum f.sp. lycopersici* المسبب لمرض الذبول الفيوزاريومى الطماطم ونباتات الباذنجان النامية في تربة

ملقحة بفطر *Sclerotium rolfsii* المسبب لمرض عفن الجذور تحت ظروف الصوبة.

التوصية

توصى هذه الدراسة باستخدام معدل لقاح١ % أو ٢%من الفطريات المعزولة من جذور نباتات القمح والخس السليمة حيث أدت إلى تحسين نمو القمح والطماطم والباذنجان وزيادة مقاومة نباتات الطماطم والباذنجان النامية في تربة ملقحة بفطر بفطر *Fusarium oxysporum f.sp. lycopersici* المسبب لمرض ذبول

الطماطم ونباتات الباذنجان النامية في تربة ملقحة بفطر *Sclerotium rolfsii* المسبب لمرض عفن الجذور.

دراسات على التفاعل المحتمل بين بعض الفطريات الممرضة للنبات والفطريات الشبيهة بالميكورهيزا الحويصلية الشجيرية تحت ظروف المعمل والصوبة

رسالة مقدمة من

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كلية الزراعة بمشتهر جامعة بنها ٢٠٠٧

للحصول على درجة
الماجستير فى العلوم الزراعية
(أمراض النبات)

قسم النبات الزراعى
فرع أمراض النبات
كلية الزراعة بمشتهر
جامعة بنها

٢٠١٣

العنوان: دراسات على التفاعل المحتمل بين بعض الفطريات الممرضة للنبات والفطريات الشبيهة بالميكورهيزا الحويصلية الشجيرية تحت ظروف المعمل والصوبة.

الاسم: هبه عليوه أحمد أبو المجد

الدرجة: الماجستير

القسم: النبات الزراعى– فرع أمراض النبات

الملخص

تتباين مجموعات الميكروهيزا من حيث التركيب والوظيفة، لكن المعيشة التكافلية هي الاكثر شيوعا في الميكورهيزا الحويصلات الشجيرية. يهدف هذا العمل لعزل الفطريات المستوطنة للجذور والمشابهة لميكورهيزا الحويصلات الشجيرية وعلاقتها بتحسن النمو فى النباتات ودراسة قدراتها على التضاد ضد أمراض الذبول الفيوزاريوم فى الطماطم وعفن الجذور فى الباذنجان (SR) *Sclerotium rolfsii* فضلا عن دراسة قدراتها على تحفيز واستحثاث المقاومة في نباتات الطماطم والباذنجان ضد مسببات أمراض الذبول وعفن الجذور على التوالي .

توصى هذه الدراسة باستخدام معدل لقاح ١ الي ٢% من الفطريات المعزولة من جذور نباتات القمح والخس السليمة حيث أدي ذلك الي تحسين نمو النباتات وزيادة مقاومتها للامراض الكامنة في التربة وذلك تحت ظروف الصوبة.