A Role of Arbuscular Mycorrhizal Fungi in Control The Fusarium Wilt Disease in Tomato

BushraRezk[*] Sabah Al-Moqrabi[**] YaserHammad[***]
[*]*PhD student, Plant Protection Department, Faculty of Agriculture, Tishreen University, Lattakia, Syria*
[**] *Professor, Plant Protection Department, Faculty of Agriculture, Tishreen University, Lattakia, Syria*
[***]*Assistance Professor, Soil and Water sciences Department, Faculty of Agriculture, Tishreen University, Lattakia, Syria*

Abstract:

*Fusarium wilt disease is considered one of important diseases on tomato plant. This diseaseis caused by the fungi Fusariumoxysporum*f. sp. *lycopersici (Fol), so the present research was conducted to study effect of arbuscularmycorrhiza (AM) (Funneliformismosseae, Dentiscutataerythropa, ,Dentiscutatanigra, Glomusambisporum, and Glomus sp.) in decreasing of fusarium wilt disease under greenhouse conditions. Tomato nurslings were infected with pathogenic fungi Fol afterinoculated it with AM. Infection Severity ratio was calculated, in addition to evaluate the following: length of infected region (colored region), the general length of plant, number of leaves and number and weight of fruits.*

Results showed that the inoculation with AM decreased each of infection severity ratio of studied disease, and length of colored infected region.The disease reduction was47.04% and46.13% in its severity andlength of infected region, respectively, whereas, this treatment led to significant increasing in the length of plant, number of leaves, and number and weight of fruits compared to infected plants (which infected withfusarium wilt disease without AM).

This results showed the important role of mycorrhiza as biocontrol agents against fusarium wilt disease in tomato.

Keywords: *Tomato, ArbuscularMycorrhiza, Fusariumoxysporum, Biological Control, and greenhouse.*

I INTRODUCTION

Tomato (SolanumlycopersicumL.) considers one of vegetables that is consumed largely around the world due to its alimentary importance. Tomato consists of vitamins like A, C and E, in addition to anti oxidation [1]. Tomato is infected with many fungal diseases, and the fusarium wilt disease considers the most important among these diseases, it caused by the fungusFusariumoxysporumf. sp. lycopersici(Sacc.)[2].Fusarium wilt is soil bornedisease, the fungus that caused it can continue in soils as saprophytic fungi for many years without host [3]. The suitable conditions for development this disease are in temperate regions where the climate is worm, and soil is sandy. Under these conditions, plants become dwarf and then wilt and die [4].

Plant diseases form a danger on world food because of economical disadvantages that caused to agricultural production [5]. Chemical control is more effective for these diseases when applied by high concentrations, but it has ecological and hygienically hazards, in addition to birth resistance strains to chemicalcompounds, so, biological control agents were development as friendly factors to environment [6]. In this hand, it was notable that micro organs (as Pseudomonas, Bacillus, etc..) which grow in the root region could be used as perfect biological control agents, where it protects the roots from habitant diseases, in addition to stimulation growth of plant [7], [8].

On other hand, Viscular-ArbuscularMycorrhiza(VAM) were found to play a good role in control plant pathogens which live in soil [9].Mycorrhiza is the fungi that associated with plant roots in symbiotic relationship, this symbiotic system is found between 90% of ground plants [10].

Plants benefit from mycorrhiza through increasing extent of absorption surface to improve water and materials absorption by roots [11].The vascular- arbuscularmycorrhiza (VAM) is the most mycorrhiza publicity [12].Mycelium of VAM penetrates plant root cells and grows inside it forming ArbusculandVesicle inside host roots, then fungi hyphae branch in soil outside the roots. Fungi hyphae absorb water and alimentary elements and transfuse it to plant, where plant provide carbon compounds to the fungus [13]. Moreover, mycorrhiza can competition the

pathogens on aliment and infect locations, besides release toxins and antibiotic compounds that protect host plant [14].

Re. [15] found that handling tomato plant which infected with Fol, by the fungusGlomusmacrocarpum or Glomusfasciculatum led to limit infection severity ratio to 75 and 78%, respectively.

The aim of present research was to study role of arbuscularmycorrhiz (Funneliformismosseae, Dentiscutataerythropa, Dentiscutatanigra, Glomusambisporum, Glomus sp.) in control the fusarium wilt disease in tomato.

II MATERIALS AND METHODS

A. *The Pathogenic Fungus Fusariumoxysporum*f. Sp. *Lycopersici:*

The isolate (Fol7) of the fungus F. oxysporumf. sp. lycopersiciwas obtained from plant diseases laboratory at Agriculture faculty in Tishreen University, Syria, where it isolated from tomato plant which has a symptoms of wilt disease. Fol 7 colony characters withcottony growth, whitish to rosy color to up surface, and creamy to rosy to under surface on PDA. Fol 7 isolate showed high pathogenicity to local tomato hybrid(Orgouan) with 100% infection severity ratio [16].

B. *Preparation of Inoculation of the Fungus Fusariumoxysporum*f. Sp. *Lycopersici*:

The fungal inoculation of Fol 7 isolate was prepared by culturing it on sterile wheat grains, whereGrains were boiled to soften, and then cooled and 200 g amounts were placed in Erlenmayer and sealed with cotton and aluminium foil. Then sterile it in autoclave under 110 °C for 30 minutes. After cooling it the grains inoculated with the fungal isolate Fol 7 by adding 5 disks (1 cm for each disk). The fungal disks were fixed well with grains, then incubated under 25 ± 2 ° C for 15 days. Daily mix was done for homogeneous fungal growth on all grains [17].

C. *Preparation of Inoculation of Arbuscular Mycorrhiza:*

The AM inoculation was prepared from soil taken of maize field at Jablah region (Lattakia, Syria). The soil was examined by method of wet sieving and decantingto be sure that it contains spores of mycorrhiza [18].

Then mycorrhiza inoculation was cultured on onion plants for 6 months as [19] and [20]. After 6 months, roots of onion plants were taken and cut to very small pieces and mixed it with soil. The mycorrhiza inoculation saved in refrigerator until use [14].

D. *Classification of Isolated Mycorrhiza:*

Species of mycorrhiza were classified in the agriculture researches center at plant diseases research academe – Department of Fungi Research and Diseases Restriction at Egypt.

E. *Tomato Plants:*

Seeds of tomato hybrid (Orgouan) were used, part of it was inoculated with mycorrhiza by adding mycorrhiza inoculation (50 spore /100 g soil)to the soil which used to culture the seeds. Other part of seeds was cultured just into soil, and considered as control treatment.

F. *Preparation the Soil:*

Soil were collected from region ofTartous-Syria, the soil propertiesshowed in Table (1). The soil were purification and sterilized by adding formaldehyde gas (4.5 L / m^3 soil)and covered with polyethylene for 5 days [21]. Then, it was aeration for a week with continuing stir to carrying gas remain away.

After that, the soil was mixed with sterile turf as (2:1 v/v), and put into black plastic bag that could contain 10 kg. The plastic bags put inside greenhouse during July,August and September 2015.

Table (1): Characters of Used Soil:

Soil type	Sand (%)	Silt (%)	Mud (%)	Organic substance (%)	EC (mellimous)	pH
Sandy silt	56	30	14	1.76	0.55	7.07

G. *Planting the Tomato Seedlings:*

When the tomato seedlings become 35 days after culture (see paragraph E), it removed to plastic bags which prepared in paragraph F, one seedling to each bag, the treatments designed as following:

- Healthy plants non inoculated and non infected (control) (C).
- Plants inoculated with Mycorrhiza (M).
- Plants infected by the fungus *F. oxysporum*f. sp*lycopersici* (Fol).
- Plants inoculated with Mycorrhiza and infected by Fol (M+Fol).

3plants to each treatment were prepared, and 4 replicates were prepared to each treatment. To

prepare the treatment (M), 200 g ofmycorrhizainoculation (which contained about 100 spores) were added to soil of plastic bags. Whereas, in the treatment (Fol), 5 g of fungal were added to soil of plastic bags [17].

Each of length of plant, number of leaves, number and weight of fruits were calculated, in addition to evaluate degree of fusarium wilt infection as following:

0: no symptoms.
1: wilt and yellowish basic leaves.
2: wilt and yellowish middle leaves.
4: wilt and yellowish top leaves.
5: plant death [22].

In according to above ladder, infection severity ratio was calculated depending on the following equation: infection severity (%)=[(number of plants in each degree × value of degree) / total number of plants× 5] × 100 % [23].

H. Statistic Analysis:

Genstate-12 was used to compared the significant differences between means of treatments using LSD test (5%).

III RESULTS AND DISCUSSION

A. Classification of Mycorrhiza:

Results showed that the isolated fungal mycorrhiza belong to arbuscularmycorrhiza and to the following species: *Funneliformismosseae,Dentiscutataerythropa,Dentiscutatanigra,Glomusambisporum,Glomus* sp.

B. Infection Symptoms and its Severity:

Symptoms of infection were appeared on infected plants after 29 days of infection. The symptoms were whiteness of under leaves and wilt it, in addition to generalweakness in plant growth. With development the disease, the under leaves turned to yellowish and died, then, the middle become yellowish, and in the severe cases, whole plant become yellow and die. Results in table (2) showed that mean of infection severity was higher in infected control plants(Fol) and reach to 67.66%. whereas, it was less in (M + Fol) which attain 35.83%.That refers to inoculation with mycorrhiza decreased of infection severity in amount 47.04%, as inoculation with mycorrhizadecreased significantly from length of brown colored region, where in (Fol) treatment length of colored region was higher and attained to 11.38 cm, where in(M+ Fol) attained 6.13 cm. This results agree with [24], [15], [25], and [26].

These results due to that mycorrhiza improve alimentary situation of plant and change morphological form of roots and stimulate resistance in plant [27]. in addition to that mycorrhiza competition the pathogens on food and location [28].Also, mycorrhiza stimulate peroxidas enzymes and polyphone Oxidase andchitinase which play an important role in increasing plant resistance to disease [29].

Table (2): Percentage of Infection Severity(%) of Fusarium Wilt Disease, and Length of Brown Region Caused in Tomato Plant Post Treatment with Mycorrhiza After 90 Days From Planting

Treatment	Infection severity (%)	Length of brown colored region (cm)
C	0.00c	0.00 c
M	0.00 c	0.00 c
Fol	67.66a	11.38 a
M+Fol	35.83 b	6.13 b
$LSD_{0.05}$	6.05	0.41

*C. Effect of Arbscularmycorrhiza and F. Oxysporum .F.Sp**lycopersici** in Length and Number of Tomato Plant Leaves:*

Table (3) showed that tomato plants inoculated with mycorrhiza exceeded significantly in plant length and number of leaves compared with infected control. where, mean of plant length and number of leaves were the least in infected control plants (Fol) which attained to 123.2 cm and 6.50 leaves, respectively. whereas in treatment (M+Fol), it attained to 145.5 cm and 12.67 leaves, respectively.

Table (3): Length and Number of Leaves of Tomato Plants Inoculated with Arbuscularmycorrhiza and the Fungus *F.Oxysporum*.F.Sp.*Lycopersici*after 90 Days from Planting

Treatment	Length of plant (cm)	Number of leaves / plant
C	135.5 b	12.17 b
M	150.0 a	14.17 a
Fol	123.3c	6.50c
M+Fol	145.5 a	12.67 b
$LSD_{0.05}$	7.67	1.21

D. Effect of Arbscularmycorrhiza and F.Oxysporum*f.Sp.*Lycopersici in Number and Weight of Tomato Plant Fruits:

In table (4) results showed significant differences between tomato plants inoculated with mycorrhizacompared to infected control treatment (Fol) where mean of fruit number and its weight attained to 6.33 fruit and 22.41 g, respectively, whereas in treatment (M+ Fol), it was notable that fruit number and its weight attained to 8.00 fruit and 35.80g, respectively.

Table (4): Fruitsnumber and Weight of Tomato Plant Post 90 Days From Inoculation with Arbuscularmycorrhiza and *F.Oxysporum*f. Sp. *Lycopersici*

Treatment	Total weight of fruits (g)	Fruits number/plant
C	37.36 b	10.00a
M	39.13 a	10.67 a
Fol	22.41 c	6.33c
M+Fol	35.80 b	8.00 b
$LSD_{0.05}$	1.57	1.53

Results showed that inoculation tomato plants with arbuscularmycorrhizadecreased of infection severity withfusarium wilt disease, this reflect a good growth in tomato plant, this results agree with [30], [2], [25]. this due to ability of mycorrhizato increase plant capability to absorb water and alimentary elements especially phosphor, potassium, etc… [31]. where mycorrhiza increase plantabsorbation of (Ca, Mn, Zn), and improve plant health by increasing amino acids and proteins in roots and leaves, thereby decreaseof wilt symptoms appearance [28]. In addition to mycorrhiza increase plant ability to resistance the disease during compensation root and increasing absorpation surface through spread mycorrhizal hyphae [32].

Conclusion: Mycrrhiza fungi play an important role in decreasing of infection of tomato plants byfusarium wilt disease, this reflect a good growth in tomato plant, where it was notable significant increasing in plant length, leaf number, and number of fruits and its weight. That reveal role of mycorrhiza as biological agents against fusarium wilt disease on tomato.

REFRENCES

[1] P. Boonkasem, P. Sricharoen, S. Techawongstein,and S. Chanthai,"Determination of ascorbic acid and total phenolics related to the antioxidant activity of some local tomato (Solanumlycopersicum) varieties".Der Pharma Chemica,Vol.7,No.4, 66-70, 2015.

[2] S. Manila, and R. Nelson, "Biochemical changes induced in tomato as a result of arbuscularmycorrhizal fungal colonization and tomato wilt pathogen infection."Asian Journal of Plant Science and Research, Vol.4. No.1,62-68, 2014.

[3] M. Ignjatov, D. Milošević, Z. Nikolić, Z. J. Gvozdanović-varga, D. Jovičić, and G. Zdjelar, "Fusariumoxysporumas Causal Agentof Tomato Wilt and Fruit Rot." Pestic.Phytomed. (Belgrade),Vol. 27, No.1, 25, 2012.

[4] G. N. Agrios, "Plant Pathology".5rdedition.Academic press,948pp, 2005.

[5] A. Akhter, K. Hage-ahmed, G. Soja, and S. Steinkellner, "Compostand biocharaltermycorrhization,tomatorootexudation,anddevelopmentofFusariumoxysporumf.sp.lycopersici". Frontiers in Plant Science,Vol.6 ,1-13, 2015

[6] B. A. Mcdonald,and C. Linde, "Pathogenpopulationgenetics,evolutionarypotentialanddurableresistance". Annu.Rev.Phytopathol. 40,349–379, 2002.

[7] I. Hmissi, S. Gargouri, and B. Sifi, "Attempt of wheat protection against Fusariumculmorum using Rhizobium isolates."Tunisian Journal of Plant Protection, Vol.6, 75-86, 2011.

[8] M. S. Akhtar, U. Shakeel, and A. Z. Siddiqui, "Biocontrol of Fusariumwilt by Bacillus pumilus, Pseudomonas alcaligenes, and Rhizobium sp. on lentil". Turk J Biol , Vol.34 , 1-7, 2010.

[9] R. M. Muchovej, "Importance of mycorrhizae for agricultural Crops." Institute of Food and Agricultural Sciences/University Of Florida,http://edis.ifas.ufl.edu/BODY_AG116 ,pp1-5, 2002.

[10] O. Angela, and D. Callaghan, "Mycorrhizae.University of Nevada Reno,1-4, 2001.

[11] G. Kernaghan, "Mycorrhizal diversity: Cause and effect?.Pedobiologia 49 pp: 511—520.www. Sciencedirect.com, 2005.

[12] D. P. Bharadwaj, "The Plant – ArbuscularMycorrhizal Fungi – Bacteria Pathogen System." Doctoral thesis Swedish University of Agricultural Sciences Uppsala, 45pp, 2007.
[13] M. A. Turk, K. M. Assaf, A. K. M. Hameed, andA. M. AL-Tawaha,"Significance of Mycorrhizae. World Journal of Agricultural Sciences,Vol. 2,No. 1, 16-20, 2006.
[14] L. Brooks, D. Brown,S. Smith,and S. Sprenger, "The use of mycorrhizae in native plant production." Native Plant Production,University of Washington. 14pp, 2006.
[15] R. Kapoor, "Induced Resistance in Mycorrhizal Tomato is correlated to Concentration of Jasmonic Acid."OnLine Journal of Biological Sciences,Vol 8No. 3, 49-56, 2008.
[16] S. Al- Moqrabi, Y. Hammad, and B. Rezk, "Effect of some Fusariumoxysporum f. sp.lycopersici isolates in growth and casualty tomato hybrid (Orjouan)." Journal of Tishreen University for Scientific studies and researches, 38, (93): 1- 14, 2016, In Arabic.
[17] H. Ozgonen, and M.Gulcu, "Determination of mycoflora of pea (Pisumsativum)seeds and the effects of Rhizobium leguminosorumonfungal pathogens of peas."African Journal of Biotechnology, Vol 10.No.33, 6235-6240, 2011.
[18] J. W. Gerdemann, and T. H. Nicolson, "Spore of mycorrhizalEndogone species extracted from soil by wet-sieving and decanting."Trans. Br.Mycol. Soc., 46,235-244,1963.
[19] C. F. Scagel,and R. P. Schreiner, "Phosphorus supply alters tuber composition, flower production, and mycorrhizal responsiveness of container-grown hybrid Zantedeschia." Plant and Soil, 283, 323-337, 2006.
[20] C. F. Scagel, "Inoculation with vesicular-arbuscularmycorrhizae fungi and rhizobacteria alters nutrient allocation and flowering of harlequin flower." Hortectunology,Vol.14, No.1,39-40, 2004.
[21] Formalin fungicide.Group fungicide,www.uap.ca, 2010.
[22] K. Yamaguchi, M. Kida, M. Arita,and M. Takahashi, "Induction of systemic resistance byFusariumoxysporumMT00 62 insolanoceous". Ann.phytopath.soc.Japan,vol.58,No.1, 16-22, 1992.
[23] K. Hibar, M. Daami-remadi, W. Hamada, and M. EL-mahjoub, "Bio-fungicides as analternative for tomato Fusarium crown and root rot control". Tunisian Journal of Plant Protection**,** Vol.1, 19-29, 2006.
[24] K. Hage-Ahmed, J. Krammer, and S. Steinkellner, "The intercropping partner affects arbuscularmycorrhizal fungi and Fusariumoxysporum f. sp. lycopersici interactions in tomato".Springer,Vol.23, No.7, 543-550, 2013.
[25] S. M.EL- KHALLAL," Induction and Modulation of Resistance in Tomato Plants Against Fusarium Wilt Disease by Bioagent Fungi (ArbuscularMycorrhiza) And/or HormonalElicitors (Jasmonic Acid & Salicylic Acid):1- Changes in Growth, Some Metabolic Activitiesand Endogenous Hormones Related to Defence Mechanism."Australian Journal Of Basic and Applied.Sciences,Vol.1,No.4, 717-732, 2007.
[26] M. Caron, J. A. Fortin, and C. Richard, "Effect ofGlomusintraradices on infection byFusariumoxysporumf.sp.radiceslycopersiciin tomatoes over a 12-week period."Can. J. Bot. 64, 552-556, 1986.
[27] N. H. El-Batanony, O. N. Massoud, M. M. Mazen, and M. M. Abd El- Monium, "The inhibitory effects of cultural filtrates of some wild Rhizobium spp.on some faba bean root rot pathogen and their antimicrobial synergetic effect when combined with Arbusclarmycorrhizae." World Journal of Agricultural Sciences,Vol.3,No. (6), 721-730, 2007.
[28] T. Muhsen, M. Al-attabi, and B. Ali, "Effect of arbuscularmycorrhizal fungi as a biocontrol agent and organic matter against fusarium wilt in tomato**,"** Journal of Gene c and Environmental Resources Conservaon,Vol.3, No. 3, 237-245, 2015.
[29] M. H. Khan, M. K. Meghvansi, V. Panwar, H. K. Gogoi1, and L. Singh, "Arbuscularmycorrhizal fungi-inducedsignalling in plant defence against phytopathogens".Journal of Phytology, Vol., 2,No.7, 53-69, 2010.
[30] H. Ozgonen,M. Bicici,and A. Erkilic, "The Effect of Salicyclic Acid and EndomycorrhizalFungusGlomusetunicatumon Plant DevelopmentofTomatoes and Fusarium Wilt Caused byFusariumoxysporumf.sp. lycopersici."Turk J Agric For,25:25-29, 2001.
[31] M. ABOHATEM, F. CHAKRAFI, A. DIHAZII,and M. BAAZIZ, "ArbuscularMycorrhizal Fungi Limit Incidence of Fusariumoxysporumf.sp. albedinis on Date Palm Seedlings by Increasing Nutrient Contents,Total Phenols and Peroxidase Activities." The Open Horticulture Journal, Vol.4,10-16, 2011.
[32] J. Morgan, G. Bending,and P. White,"Biological costs and benefits to plant–microbe interactionsin the rhizosphere". Journal of Experimental Botany, Vol. 56, No. 417, 1729–1739, 2005.