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THE ENHANCEMENT OF DROUGHT TOLERANCE FOR PLANT ONION (*ALLIUM CEPA* L.) INOCULATED BY ARBUSCULAR MYCORRHIZAL FUNGI

Thamer A.A. Muhsen[1]*, Atyaf S.Hameid[2] and Mahdi S.Y. Al-Attabi[3]

[1,2]College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad, Iraq

[3]Research Department, Horticulture Office, Ministry of Agriculture, Baghdad, Iraq.

*Corresponding author: thamerm555@yahoo.com

Abstract

The current study was aimed to examine the effects of two types of Arbuscular mycorrhizal Fungi (*F. mosseae, C. etunicatum*) on the onion plant under two water conditions (normal irrigation and drought treatment). This study has aspects related to improve tolerance of an onion plant (*Allium cepa* L.) to water stress situations with taking in consideration regulate physiological Growth Parameters PGP of plant and biochemical [fungal root colonization, dry weight of mycorrhizal roots, Spore density of AM fungi, Relative water content, proline content, total carotenoids, Soluble protein content and Phosphorous application] in the existence or lack of AMF. The results indicate that the drought dealing producing increase of spore density of AM fungi, proline content, total carotenoids and soluble protein content except Fugal root colonization, plant root dry weight, Relative water content and Phosphorous uptake which were increased when associating with normal irrigation. The plants inoculated by each *F. mosseae, C. etunicatum* was noted a significant differences ($P < 0.05$) increase in some *PGP* comparing with uninoculated. The highest values of PGP were recorded when onion plant inoculated by two types of AMF. Normal irrigation was showed less enhancement of plants compared with plants that obtained drought stress. The inoculcation by both types of AMF resulted in increasing in an onion plant uptake and protection against drought stress, while the case of relative water content showed relatively similar values in both conditions comparing with non- AMF onion plant.

Keywords: Onion, mycorrhizal fungi, water conditions, Mycorrhizal and Oxiditive stress Measurements.

Introduction

The onion crop (*Allium cepa* L) which belongs to the family Alliaceae is one of the most important vegetable crops in Iraq and the world, which consumed by the individual in large quantities and has a nutritional value and medical, containing good proportions of vitamin C and K, proteins and iron and calcium, and its medical importance because it contains of the *Curtain* Which is of great importance as an anti-oxidant and anti-diseases (Patil *et al*., 1995).

Laniewski and Grayson (2004) note that plant exposure to both biotic and abiotic stress leads to a defect in the chain of transmission of electrons to living cells, which increases the types of Reactive oxygen species (ROS). ROS types are powerful oxidants that have a harmful and toxic influence on living cells for the reason that they attack the constituents of the cell, cause damage such as proteins, oxidation of unsaturated Fatty Acids to carbohydrates, cellular membranes and Photosynthesis, as well as genetic changes to DNA, causing membrane damage and defect in metabolic processes (Tarpey *et al*., 2004; Ozkur, *et al*., 2009). The exposure of the plant to water stress (drought) leads to the development of a mechanism where the concentration of organic and dissolved ions is increased, nitrogen organic compounds, such as proteins, amino acids, ammonium compounds and poly amines, reduce intracellular the drought to allow water to flow from the soil to the roots (Evelin, 2009). The exposure of plants to stress leads to the accumulation of proline, which is an osmotic regulator and reduce the reactive oxygen species (ROS), As the increase of osmotic pressure increases the free radicals oxidizing, which works on oxidation of fat in the cell membrane and increase protein degradation or reduce the number of Polysome responsible for the synthesis of protein so the accumulation of proline has a very important role in reducing the damage ROS (Fattahi *et al*., 2009).

There are many species of arbuscular mycorrhizal fungi, which is the most important fungi, physiologically, economically and environmentally, These fungi are associated with the roots of more than 80% of the higher plants and vegetable crops (Hathout *et al.,* 2010). AM fungi a role in increased nutrient availability for the plants, especially phosphorus in phosphorus-deficient soils, as well as improved water absorption and many minerals like nitrogen, potassium, magnesium, calcium, sulfur, iron, manganese, copper and zinc from the soil works to transfer these elements to the plant by roots (Utobo *et al*., 2011; Muhsen, 2018). In addition, the AM fungi has a much larger surface area than the root capillaries and extends externally around the roots to reach beyond nutrient depletion areas, thus increasing the efficiency of nitrogen absorption and micronutrients(Smith and Read, 2008) and increases the physiological characteristics of plants (AL-Kaisi *et al.*, 2011) also acts as an abiocontrol against diseases and reduce damage caused by pathogenic fungi (Muhsen *et al.,*2015), It helps in stimulating the pathogenic antifungal enzymes like SOD, POD, CAT, PAL, PPO and GPOX (Muhsen and Ali, 2015). Evelin *et al.* (2009) reported that the mycorrhizal plants have a low osmotic pressure that is sustained by the accumulation of fungal secretions,which improves the plant's osmotic regulation and improving the water condition of the mycorrhizal plants efficiently and effectively. Khalafallah and Abo-Ghalia (2008) found that in the study of wheat germination with *Glomus* sp under water stress conditions, the effectiveness of antioxidants and plant resistance to stress increased by significantly increasing their soluble sugar content, proteins and antioxidants in plant leaves, The defense system reduces the damage caused by the oxidation of fatty and proteins in the cell membrane and increases the accumulation of metabolic products (proline, amino acids, soluble and non-soluble proteins, total sugars, soluble sugars and non-soluble). The current study aimed to the

enhancement of drought tolerance for plant onion (*Allium cepa* L.) inoculated by arbuscular mycorrhizal fungi.

Material and Methods

Stimulating the AM Fungi and Soil Preparation

Funneliformis mossesae and *Claroideoglomus etunicatum* fungi were obtained from plant protection office / Ministry of Agriculture, hat was inspected to confirm that the spores were wet and filtered based on Gerdemann & Nicolson (1963) method. The soil was washed for low the nutrients and then sterilized to eliminate all micro-organisms, after that *Funneliformis mossesae* and *Claroideoglomus etunicatum* were cultured by insertion the mycorrhiza pad in a pot containing soil, then placed the phosphate rock 12% and the sterile using onion bulbs *Allium cepa* L. was cultured based on Owusn,-Bennoah & Mosse (1979) method. The nutrient solution was added as mentioned (Davies *et al*., 2002), The following treatments (control, *F. mosseae*, *C. etunicatum* and *F. mosseae* + *C. etunicatum*) were subjected to drought stress and other normal irrigation conditions after 50 days of planting.

Quantification the Spores Density of AM Fungi

Sporocarps and Spores of AM fungi were isolated by wet sieving and pouring method (Gerdemann & Nicolson, 1963) and quantification of spores density of AM fungi was conceded by using Gaur & Adholeya (1994) method.

Frequency % of mycorrhiza in the root system

The percentage of the mycorrhiza frequency of the root system according to (Biermann and Linderman, 1981).

The dry weight of mycorrhizal roots

Calculated from the the percentage of the Frequency of mycorrhiza × the dry weight of the root according to (Pairunan *et al*., 1980).

Relative Water Content (RWC)

The leaf relative water content (RWC) was accounted in the plants as equation (Aroca *et al*. 2003): RWC = (FW – DW)/ (TW- DW) × 100

where FW= fresh weight, DW= dry weight, and TW=turgid weight.

Oxidative Stress Measurement:

- **Proline:** Free proline was extracted and identified with acid ninhydrin as designated by Bates *et al.* (1973). The absorbance at 520 nm was identified using L-proline as standard.
- **Total Carotenoids:** Total carotenoids were extracted by using 100% methanol from 2 g of fresh leaf samples. Extinction coefficients and equations described by Lichtenthaler (1987) was used to estimate the pigment concentrations.

Content of Soluble Proteins

Soluble protein content was identified by extraction method based on Zhang (1990).

Phosphorus Determination in root:

The phosphorus concentration in plant root was evaluated by the molybdate blue ascorbic acid method based on Murphy & Riley (1962) when the plant material was air dried and digested by perchloric and nitric acids for spoken as P uptake mg/g.

Statistical Analysis

The data were evaluated using the statistical analysis system to study the enhancement of drought tolerance for plant onion (*Allium cepa* L.) inoculated by arbuscular mycorrhizal fungi, The variances between the mean were compared with the least significant difference in (LSD 0.05).

Results

- Percent of root colonization by *F. mosseae, C. etunicatum* is displayed as a percentage interaction of root colonization has been Observed. As seen in figure 1, the interaction between different types of fungi and root colonization showed significant differences under normal irrigation and drought treatment conditions at 50 dpp Showing 88.30% and 70.41% respectively, This result shows that the interaction of *F. mosseae, C. etunicatum* and root colonization was upregulated under both conditions. Whereas, the high colonization showed increased of root colonization under normal irrigation with all the types of fungi.
- Mycorrhizal root development was conducted under normal irrigation and drought treatment for 50 dpp, There is a significant increase. Onion plant roots before the irrigation and drying phase were treated with different types of fungi. Figure 2 shows that inoculated onion plant with *F. mosseae, C. etunicatum* had relativity similar percent of dry weight. Nevertheless, the inoculation of both *F. mosseae, C. etunicatum* improved the drying weights of plants by 2.33g/plant and 2.11g/plant under normal irrigation and drought treatment respectively under both treatments compared to1.22 g/plant and 0.21 g/plant respectively for uninoculated plants.
- Interestingly, this result shows that inoculation with two species of fungi *F. mosseae, C. etunicatum* has resulted in significant increase of spore density. Considering aridity, the drought treatment had higher mycorrhizal intensity compared with normal irrigation treatment during infection by *F. mosseae, C. etunicatum*. A higher intensity of AMF was observed specially under water stress. In addition, colonization with both types of fungi was recorded 950 spore/100g dry soil when exposed to water stress. However, uninoculated roots did not show any present of spores Fig. 3.
- The infulenc of interaction between inoculation of *F. mosseae, C. etunicatum* and RWC, at drought treatment was examined and the result showed that the ultimate RWC was increased in simultaneous inoculation of both types of AMF after 50 dpp. The data showed that RWC increased reaching 96.95 and 78.73 with two types of fungi under normal irrigation and drought treatment respectively, and no significant differences were noticed. Whereas, significant differences were identified, When observing the differences between the control and other treatments at drought treatment, The lowest control treatment recorded WRC was 50.37, results have shown that there was no significant differences were pronounced Fig. 4.

- As seen from figure 5, the results showed that the significant increasing in proline content in an onion plant in the presence fungal infection was occurred within using of drought treatment. The treatment with *F. mosseae, C. etunicatum* with normal irrigation showed lower rate of proline comparing with drought treatment which resulted in remarkable increase of proline level while higher rate of proline was observed with both inoculation around 329.83 (µg /g DW). In contrast, the proline level in uninoculated plant (control) was decreased in both treatments (normal irrigation and drought treatment) as recorded 140.42 (µg /g DW) and 235.61 (µg /g DW) respectively.
- A follow-up experiment to the previous experiments involved looking at the effects of inoculating onion plant with *F. mosseae*, *C. etunicatum* during drought treatment. The results showed that a significant higher rate of photosynthetic pigment contents (Carotenoids) was seen when drought stress comparing with the effect of exposing to the normal irrigation Fig. 6. On the other hand, the effect of inoculation within both types of fungi, total carotenoids showed much higher during water stress recorded 17.79 (mg /g) compared with other treatments and control.
- To evaluate the effect of *F. mosseae*, *C. etunicatum*, with both types of fungi on the content of soluble protein of onion plant, the plant has been inoculated with these types of fungi. In general, the outcomes illustrated that, the content of soluble proteins in an onion plant has been changed slightly during the treatment and higher proteins content compared to uninoculated plants. On the other hand, the result showed that under drougt treatment, there are significant differences, the content of soluble proteins in an onion plant was changed. After inoculation of *C. etunicatum*, *F. mosseae*, the content of soluble proteins increased by 22.69 with *F. mosseae*, 19.94 with *C. etunicatum* and 26.51 with both types of fungi during 50 dpp. Fig. 7.
- To determine phosphorus (p) uptake in plant tissue, the inoculated plant with AMF at different level of infection showed an increase in phosphorus (p) uptake by irrigation treatment. In addition, it has been observed that the value of plant phosphorus Uptake were strongly influenced when there is *F. mosseae, C. etunicatum* by drought treatment and it was recorded decrease in plant phosphorous uptake by 4.16 comparing with the value of irrigation treatment 5.83. Fig.8.

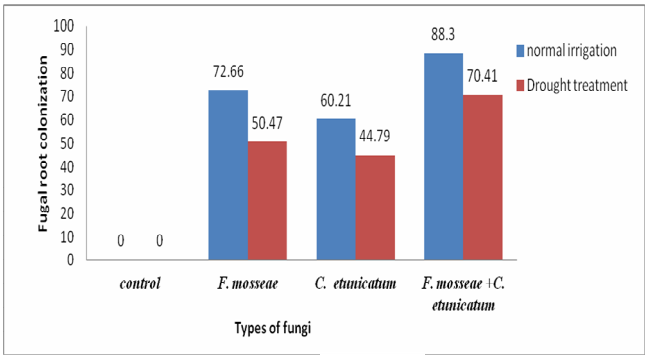

Fig. 1 : Effect of *F. mosseae*, *C. etunicatum* and interaction on root colonization% of onion plants roots under normal irrigation and drought treatment conditions after 50 days of planting. (significantly different at P< 0.05 for all treatments).

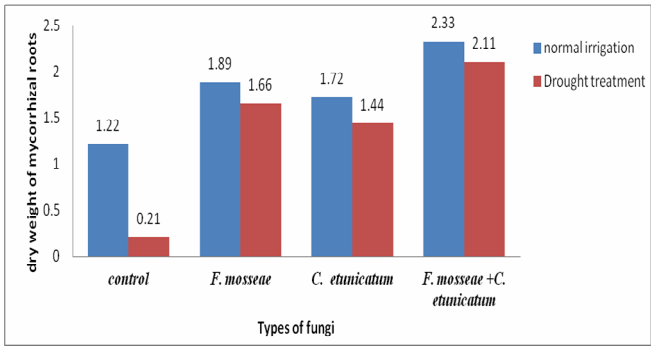

Fig. 2 : Effect of *F. mosseae*, *C. etunicatum* and interaction on the dry weight of mycorrhizal roots(g/plant) of onion plants roots under normal irrigation and drought treatment conditions after 50 days of planting. (significantly different at P< 0.05 for all treatments).

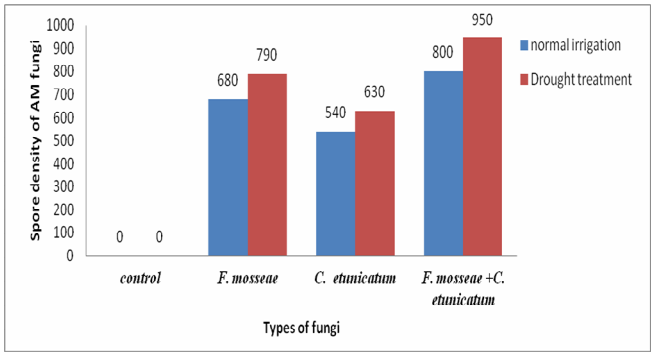

Fig. 3 : Effect of *F. mosseae* , *C. etunicatum* and interaction on Quantification of Spore density for AM fungi (spore/100g dry soil) of onion plants roots under normal irrigation and drought treatment conditions after 50 days of planting. (significantly different at P < 0.05 for all treatments).

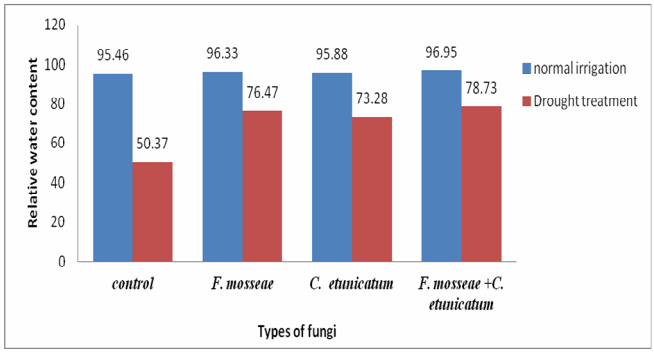

Fig. 4 : Effect of *F. mosseae*, *C. etunicatum* and interaction on the relative water content (RWC) of onion plants under normal irrigation and drought treatment conditions after 50 days of planting (not significant at P<0.05 for all treatments).

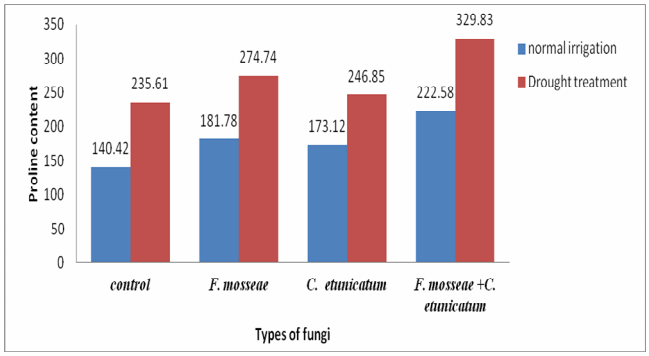

Fig. 5 : Effect of *F. mosseae* , *C. etunicatum* and interaction on the Proline content (µg /g DW) of onion plants under normal irrigation and drought treatment conditions after 50 days of planting. (significantly different at P< 0.05 for all treatments).

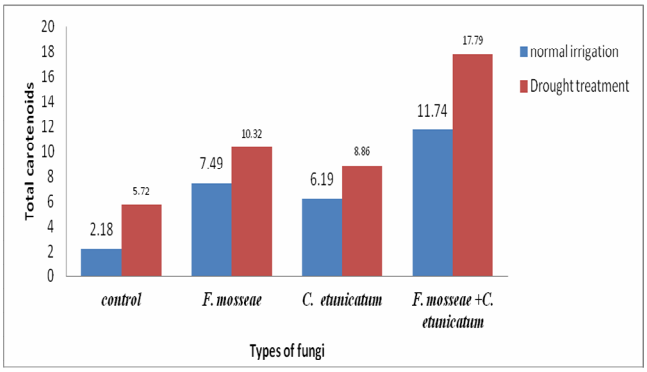

Fig. 6 : Effect of *F. mosseae*, *C. etunicatum* and interaction on the Total carotenoids (mg /g) of onion plants under normal irrigation and drought treatment conditions after 50 days of planting. (significantly different at P< 0.05 for all treatments).

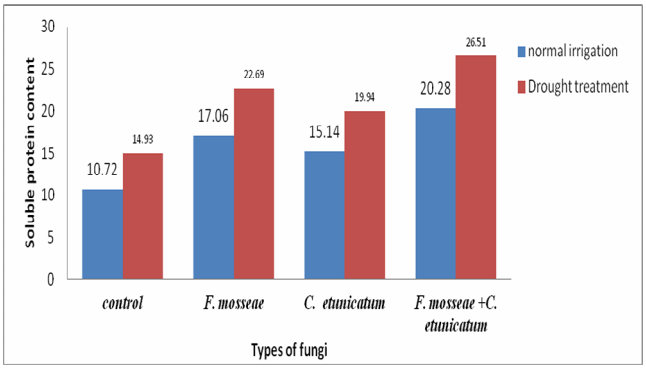

Fig. 7 : Effect of *F. mosseae*, *C. etunicatum* and interaction on the soluble protein content of onion plants under normal irrigation and drought treatment conditions after 50 days of planting. (significantly different at P< 0.05 for all treatments).

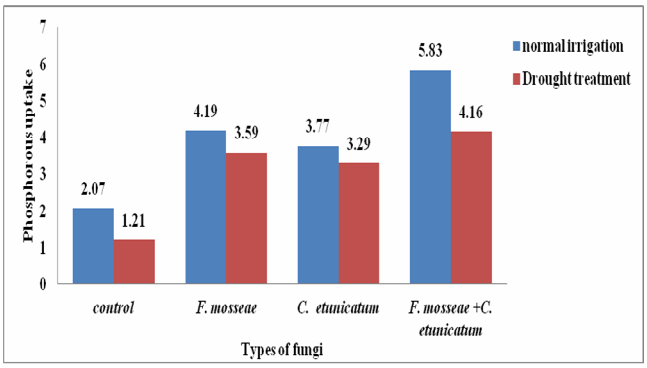

Fig. 8 : Effect of *F. mosseae*, *C. etunicatum* and interaction on the Phosphorous uptake (mg /g) of onion plants under normal irrigation and drought treatment conditions after 50 days of planting. (significantly different at P< 0.05 for all treatments).

Discussion:

Percentage of root colonization by AMF is displayed as a parent of total root segments noticed. This notification was ended specifically in the inoculated plant roots except for high salt concentration treatment that had no calculable the total length, fresh and dry biomass determines that AMF play a significant role in low to moderate salt concentration by declining salt stress on plants, nevertheless AMF did not protect plants from extraordinary salt concentration. Interestingly, the present notification showed poor response of AMF inoculated plants within extraordinary salt concentration, but root colonization was not affected by extraordinary salt level.

In the current study, we identified the influence of arbuscular mycorrhizal fungi (AMF) to increase an onion plant tolerance for water defect. Generally, based on the present findings, the result seems to be in agreement with many previous findings which found that AMF symbiosis has influence on the adaptation and growth of plants (Habibzadeh, 2015) by increasing plant tolerance to the drought stress. Initially, In the irrigation treatment, plant growth parameters increased comparing with drought condition this fact affect in the soil nutrition and its moisture (Abdelmoneim *et al*., 2013). As well as, plant growth parameters increased in the existence of AMF such *G. mosseas* resulting in the improvement of water uptake and nutrients, due to alter physical parameters of the soil and increase root conductance by extension of root surface area (Bethlenfalvay *et al.,* 1988; Pinior *et al*., 2005). A recent study has showed that fungal root colonization was increased under drought condition which assumes to support earlier findings of Abdelmoneim *et al*. (2013); Apostolos *et al*. (2014), who had argued that there is a correlation between increasing root colonization and the adaptation to the effect of drought stress. Drought treatment and mycorrhizal inoculation affected significantly root dry weight and plant growth parameters(Apostolos *et al.,*2014). Under normal irrigation conditions, dry weight of AM onion plants was relatively similar (Figure. 2) however, under drought treatment conditions all AMF plants had higher root dry weights than well-watered and control plants. Our data lend to be supported by earlier findings of (Wu and Xia, 2006) whom also found that root dry weight can be increased under drought condition. Moreover, this observation is in agreement with (Apostolos *et al*., 2014).

Interestingly, our observation showed that root dry weight was increased for onion plant of the mix AMF. This observation is supported by the results of Apostolos *et al*. (2014), who reported that the mix mycorrhizal (*C. etunicatum*, *F. mosseae)* which had higher root dry weight. Different culture techniques by AMF have revealed.

That mix of *C. etunicatum, F. mosseae* richness in drought treatment (Fig. 3) and showed increased of spore density. It is well known that root colonization, spore germination investigations have described the influence of soil treatment (drying or moisture) on hyphal growth (Auge, 2001) Under arid condition, resilient spores and groups of VAM fungi are producing in response declining moisture (Jacobson 1997). It seems that the inoculated onion plants cope with water stress after root colonization with AMF.

The present result can confirm that the increasing spore density by drought treatment and shows that different inoculums can be affected the rates of root infection. The results indicate that under normal irrigation treatment, RWC has not showed any differences between control and inoculated plants. However, RWC rates have been increased by inoculation by AMF during water stress compared to uninoculated plants indicating that AMF inoculums improve the water content under drought treatment condition. Our findings lend to be in consistent with previous study of (Kong *et al.,* 2014). who found that osmotic adjustment substances can be accumulated in the inoculated plants under drought stress resulted in maintaining the water content. This outcome indicates that water stress can decrease water loss and reduce lack of water in the inoculated plant by AMF. It is well known that proline play an important role in prevention

the plant from cells oxidation under water stress (Farahani *et al*., 2008; Girousse *et al.,* 1996). Also, proline protect the plant by acting as an osmosolute, (N)storage compound and hydrophobic preservation for cellular and enzymes structures (Duran *et al*., 2016; Madan *et al.,* 1995). On the other hand, to test the hypothesis that the growth of onion plant can be affected by AMF, the influence of two types of arbuscular mycorrhizal fungi (*C. etunicatum*, *F. mosseae*) has been examined. Accurately, the current study was designed to examine the effect of these two species of AMF on the growth of onion plant under normal irrigation and drought treatment conditions relating to plants free from these mycorrhizal by evaluated proline content. The present result showed that the proline content was increased under drought treatment and in the presence of AMF, this result seems to be in disagreement with the previous finding of earlier studies of Ashraf & Iram, (2005), Mafakheri *et al.* (2010); Din *et al.* (2011) and Karimi *et al.* (2012) who illustrated that proline rate is responsible for serving as nitrogen compound and energy during drought treatment. The present bservations showed that water defect affected plant growth, therefore, the proline content has been increased to result in maintain of osmotic level, as well as, it can be suitable indicator induced during water stress. In these plants, Root Colonization arise with a decrease in drought Treatment (Augé, 2001). Under drought stress conditions, G. *versiforme* fungi improved fresh and dry weight of plant roots and shoots and enlarged the root colonization percentage (Wu & Xia, 2006).

Apart from the monitoring survival and protection of an onion plant against water stress, the result showed that AMF has the advantage that it can also be used to study the importance of increasing of carotenoids to overcome photo break down and photo inhibition of pigments in water defect condition (Apostolos *et al*., 2014). It was found that after testing arrange of AMF inoculated, the carotenoids significantly increased under both water stress and AMF conditions. After the infection with AMF, the onion plant still survived for 50 dpi under water stress condition, contrasting with plants under water irrigation. The nutrition quality of green and red leaf lettuces (*Lactuca sativa* L.) enhanced and increased the level of carotenoids synthesis in the presence of AMF. Indicating that carotenoids are an important molecule to enhance the nutritional value, growth and photosynthesis in the plants as well as protection against harm of single oxygen by improvement specific antioxidant enzymes (gluta-thione, ascorbate peroxidase and catalase) (Duran *et al*., 2016; Baslam *et al*., 2013). In addition, improvement in antioxidant enzymes levels (Glutathione, Catalase and Ascorbate Peroxidase) Meanwhile, the findings showed that presentation of AM assistances the plants to overawed photodestruction and photoinhibition of pigments in the conditions of water stress by cumulative the content of carotenoids, by way of they assistance in safety of photosynthetic apparatus in contradiction of the damage produced by single oxygen. Consequently, put of and extinction of excited triplet state of chlorophyll can be carried by carotenoids (Foyer and Harbinson, 1994).

In the case of water deficit treatment, it was noticed that the content of soluble proteins in an onion plant clearly changed during the time of experiment on 50 dpi and inoculated plants had a high-level compared to uninoculated plants (control). Previous studies by Abdelmoneim *et al*. (2013) have indicated that water deficit had undesirable impact on plant solvable protein in the inoculated or uninoculated plant by *G. mosseae* that appears to remarkable drop in plant photosynthesis.

Another study by Mohammadkhani & Heidari (2008), Karimi *et al.* (2012), also illustrated that chlorophyll content values in the plant leaves were decreased in the water stress condition comparing with water irrigation treatment. These facts indicate that the photosynthesis plays a role in the protection against inhibition of few essential materials which are important for protein synthesis during water stress treatment.

Such finding is corroborated by (Kong *et al*., 2014), who demonstrated the inoculation with AMF can reduce plant's damage caused by water stress. In other word, it can accelerate the absorption of the content of protein and water and adjust contents of osmotic substances to increase of osmotic pressure, reduction of water losing caused by water drought.

AMF was found to be suggested as a suitable technique to protect the plant against water stress and influence on growth parameters. It can be said that, this technique can be used to increase plant photosynthetic and growth rate which in turn resulted in increase of transport and building up of carbohydrates in plant cells which play an important to reduce osmotic potential (Khalvati *et al*., 2005).

The present study suggested that in inoculated plant, phosphorus uptake was mostly increased in the both treatment conditions, whereas almost lower rates were observed within the uninfected controls and AMF inoculated under water stress suggesting an increase of protection against water drought which in turn high level of phosphorus causes inhibition of roots colonization and reduce entry point formation (Amijee 1989).

This result suggests that to increase in growth parameters, up taking of mineral nutrients such phosphorus is required. Former studies have founded that the utility of AMF for improvement hydraulic conductivity and recover from water stress approve the water stress tolerance of the plants the has been recognized to better phosphorus nutrition (Bryla and Duniway, 1997). On the other hand, prior study by Subramanian *et al*. (2006) have noticed that water stress result in increasing of phosphorus uptakes in the shoots and roots of mycorrhizal plants.

References

Abdelmoneim, T.S.; Moussa, T.; Almaghrabi, Q.A.; Alzahrani, S. and Hassan A.I. (2013). Increasing Plant Tolerance to Drought Stress by Inoculation with Arbuscular Mycorrhizal Fungi. Life Science Journal. 10: 3273-3280.

AL-Kaisi, W.A.; Muhsen, T.A.A. and Hamed, A.S. (2011). Effect of mycorrhiza (*Glomus mosseae*) and superphosphate on physiological characters of *Hodeum vulgare* Journal of the College of Basic Education,72: 765-784.

Amijee, F. (1989) Vesicular-arbuscular mycorrhizas: An ubiquitous symbiosis between fungi and roots of vascular plants. Science direct. 3(4): 176-180.

Apostolos, K.; Orfanoudakis, M.; Abraham, E.; Parissi, Z. and Serafidou, N. (2014). Effects of Arbuscular Mycorrhiza Fungi on Growth Characteristics of *Dactylis glomerata* L. under Drought Stress Conditions.

Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 42: 132-137.

Aroca, R.; Irigoyen, J.J. and Sánchez-Díaz, M. (2003). Drought enhances maize chilling tolerance. II. Photosynthetic traits and protective mechanisms against oxidative stress. Physiol Plant. 117: 540-549.

Ashraf, M. and Iram, A. (2005). Drought stress induced changes in some organic substances in nodules andother plant parts of two potential legumes differing in salt tolerance. Flora, 200: 535–546.

Auge, R. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza, 11: 3-42.

Baslam, M.; Esteban, R.; García-Plazaola, J.I. and Goicoechea, N. (2013). Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces Appl Microbiol Biotechnol. 97(7): 3119-3128.

Bates, L.S.; Waldren, S.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205–207.

Biermann, B. and Linderman, R.G. (1981). Quantifying vesicular-arbuscular mycorrhizal: A proposed method towards standardization. New Phytol., 87: 63-67.

Bryla, D. and Duniway, J.M. (1997). Effects of mycorrhizal infection on drought tolerance and recovery in safflower and wheat. Plant and Soil. 197.

Davies, F.T.; Purgear, J.D.; Newton, R.J.; Egilla, J.N. and Saraiva, J.A. (2002). Mycorrhizal fungi increase chromium uptake by sunflower plants: Influence on tissue mineral concentration, growth, and gas exchange. J. Plant, Nutrition, 25(11): 2389-2407.

Din, J.; Khan, S.; Ali, I. and Gurmani, A. (2011). Physiological and agronomic response of canola varieties to drought stress. Journal of Animal and Plant Sciences. 21: 78-82.

Duran, P.; Sobarzo, J.; Armada, E.; López-Castillo, O.M.; Cornejo, P.; Mora, M.L. and Azcón, R. (2016). Inoculation with selenobacteria and arbuscular mycorrhizal fungi to enhance selenium content in lettuce plants and improve tolerance against drought stress. Journal of soil science and plant nutrition. 16.

Evelin, H.; Kapoor, R. and Giri, B. (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann. Bot., 104: 1263–1280.

Farahani, A.; Lebaschi, H.; Hussein, M.; Amir H.S.; Ali Reza, V. and Jahanfar, D. (2008). Effects of arbuscular mycorrhizal fungi, different levels of phosphorus and drought stress on water use efficiency, relative water content and proline accumulation rate of Coriander (*Coriandrum sativum* L.). J. Med. Plants Res. 2.

Fattahi, N.F.; Modarres Sanavy, S.A.M.; Ghanati, F. and Dolatabadian, A. (2009). Effect of foliar application of pyridoxine on antioxidant enzyme activity, proline accumulation and lipid peroxidation of maize (*Zea mays* L.) under water deficit. Nat. Bot. Hort. Agrobot. Cluj., 37(1): 116-121.

Foyer, C.H. and Harbinson, J. (1994). Oxygen metabolism and the regulation of photosynthetic electron transport. In: Foyer CH, Mullineaux PM, editors. Causes of Photooxidative Stress and Amelioration of Defence System in Plants. Boca Raton, FL: CRC. 1–42.

Gaur, A. and Adholya, A. (1994). Estimation of VAM spores in soil a modified method. Mycorrhiza News (9): 10-11.

Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46: 235-244.

Girousse, C.; Bournoville, R. and Bonnemain, J.L. (1996). Water deficit-induced changes in concentrations in proline and some other amino acids in the phloem sap of alfalfa. Plant Physiol. 111: 109-113.

Habibzadeh, Y. (2015). Arbuscular Mycorrhizal Fungi in Alleviation of Drought Stress on Grain Yield and Yield Components of Mungbean (*Vigna radiata* L.) Plants. International Journal of Sciences. 1: 34-40.

Hathout, T.A.; Felaifel, M.S.; El-Khallal, S.M.; Abo-Ghalia, H.H. and Gad, R.A. (2010). Biocontrol of *Phaseolus vulgaris* root rot using arbuscular mycorrhizal. Egypt. J. Agric. Res., 88(1): 15-29.

Jacobson, K.M. (1997). Moisture and substrate stability determine VA-mycorrhizal fungal community distribution and structure in an arid grassland. J Arid Environ 35: 59–75.

Karimi, S.; Abbaspour, H.; Sinaki, J.M. and Makarian, H. (2012). Effects of Water Deficit and Chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean (*Ricinus communis* L.). Journal of Stress Physiology and Biochemistry 8(3):160-169.

Khalafallah, A.A. and Abo-Ghalia, H.H. (2008). Effect of arbuscular mycorrhizal fungi on the metabolic products and activity of antioxidant system in wheat plants subjected to short-term water stress, followed by recovery at different growth stages. Journal of Applied Sciences Research, 4(5): 559-569.

Kong, J.; Pei, Z.; Du, M. and Sun, G.Z.X. (2014). Effects of arbuscular mycorrhizal fungi on the drought resistance of the mining area repair plant Sainfoin. International Journal of Mining Science and Technology. 24. 10.1016.

Laniewski, N.G. and Grayson, J.M. (2004). Antioxidant Treatment Reduces Expansion and Contraction of Antigen-Specific CD8+ T Cells during Primary but Not Secondary Viral Infection. J. Virol., 78: 11246–11257.

Lichtenthaler, H.K.(1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol.148: 350-382.

Madan, S.N.H.; Jain, R. and Chowdhury, J. (1995). Proline and Proline Metabolising Enzymes in in-vitro Selected NaCl-tolerant *Brassica juncea* L. under Salt Stress. Annals of Botany, 76(1): 51-57.

Mafakheri, A.; Siosemardeh, A.; Bahramnejad, B.; Struik, P. and Sohrabi, Y. (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science 4(8): 4.

Mohammadkhani, N. and Heidari, R. (2008). Effects of drought stress on soluble proteins in two maize varieties. Turk. J. Biol., 32: 23-30.

Muhsen, T.A.A. (2018). Evalution of the Effect of *Gigaspora Margarita* and *Glomus Desriticola* Fungi in Stimulating the Resistance of the *Capsicum Annuum L.* plant towards Chromium and Lead. Journal of Global Pharma Technology. 10(05):181-192.

Muhsen, T.A. and Ali, B.Z. (2015). Evaluation of the Efficacy of Arbuscular Mycorrhizal Fungi in Enhancing Resistance of *Lycopersicon esculentum* Roots Against

Fusarium oxysporum Wilt Disease. Ibn Al-Haitham J. for Pure & Appl. Sci., 28 (2): 292-306.

Muhsen, T.A.A.; Al-Attabi, M.S.Y. and Ali, B.Z. (2015). Effect of arbuscular mycorrhizal fungi as a biocontrol agent and organic matter against fusarium wilt in tomato. Journal of Gene c and Environmental Resources Conservation. 3(3): 237-245.

Murphy, J. and Riley, I.P. (1962). A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31-6.

Owusn,-Bennoah, E. and Mosse, B. (1979) Components of va mycorrhizal inoculum and their effects on growth of onion. New phytol., 87: 355-361.

Ozkur, O.; Ozdemir, F.; Bor, M. and Turkan, I. (2009). Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought Environmental and Experimental Botany, 66: 487-492.

Pairunan, A.K.; Robson A.D. and Abbott L.K. (1980). The effectiveness of Vesicular–arbuscular mycorrhiza increasing growth and phosphorus uptake of subterranean clover from phosphorus sources of different solubilities. New Phytol. 84: 327-338.

Patil, B.S.; Pike, L.M. and Yoo, K.S. (1995). Variation in the quercetin content in different colored onions (*Allium cepa* L.). J. Amer. Soc. Hort. Sci., 120(6): 909–913.

Khalvati, M.A.; Hu, Y.; Mozafar, A. and Schmidhalter U. (2005). Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. Plant Biology. 7(6): 706-12.

Smith, S.E. and Read, D.J. (2008). Mycorrhizal symbiosis. (2nd ed). London. Academic press, San Diogo, California. U.K. 650.

Subramanian, K.S.; Santhanakrishnan, P. and Balasubramanian, P. (2006). Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Scientia Horticulturae., 107(3): 245-253.

Tarpey, M.M.; Wink, D.A. and Grisham, M.B. (2004). Methods for detection of reactive metabolites of oxygen and nitrogen :in vetro and in vivo consideration .Am. J. Physiol. Regul. Integr. Comp. Physiol., 286: 431-444.

Utobo, E.B.; Ogbodo, E.N. and Nwogbaga, A.C. (2011). Techniques for Extraction and Quantification of Arbuscular Mycorrhizal Fungi. Libyan agric. Res. center J. Internat., 2(2):68-78.

Wu, Q.S.; Xia, R.X. and Zou, Y.N. (2006). Reactive oxygen metabolism in nonmycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. J. Plant Physiol., 163: 1101-1110.

Zhang, Z.L. (1990). Plant physiology experiment manual. 2nd ed. High Educational Press, Beijing, 141-158.