ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Interaction Effects of Different Soil Moisture levels, Arbuscular Mycorrhizal Fungi and Three Phosphate Levels on: I- Growth, Yield and Photosynthetic Activity of Garden Cress (*Lepidium sativum* L.) plant.

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Manuscript Info

Manuscript History:

Received: 12 April 2014
Final Accepted: 25 May 2014
Published Online: June 2014

Key words:
soil moisture levels, AM fungi, phosphorus, growth parameters, yield, photosynthetic pigments.

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Abstract

A randomized complete block design 3X5 experiment was designed and conducted to characterize the relationships between three soil moisture levels (W1= 85 % depletion of the available soil water, W2=55% depletion of the available soil water and W3= 25% depletion of the available soil water) and five Vesicular-Arbuscular Mycorrhizal (VAM) fungi treatments which were (control = without AMF and zero phosphorus, P0=zero phosphorus + AMF, P1= 25% recommended phosphorus + AMF, P2= 50% recommended phosphorus + AMF and P3= 100% recommended phosphorus + AMF) on morphological characteristics, yield and its components, photosynthetic pigments concentrations and oil % content. The maximum significant records for growth characters, yield components, oil % and carotenoids concentrations were obtained in plants grown under the highest soil moisture level W3 inoculated with arbuscular mycorrhiza fungi and treated with the highest phosphorus level P3, Followed mostly by the same treatments under the moderate soil moisture level. From this study, it was observed also that the highest chlorophylls content and the highest main root length were drowning under the highest water stress level inoculated with AM fungi and treated with the highest phosphorus level.

INTRODUCTION

Water is one of the resources whose future availability is at risk, and it cannot be replaced by any alternatives (Colombo, 2004). The world is facing serious shortages of fresh water and growing competition for clear water that makes less water available for agriculture. The great challenge for the coming decades will be the task of increasing food production with less water, particularly in countries with limited water and land resources. Serious water shortages are developing in the arid and semi-arid regions as existing water resources are fully exploited (Jafar *et al.*, 2007). Current trends of water availability (FAO, 2002) indicated that agricultural activities are approaching a water crisis in several regions, most notably in the Middle East and North Africa. Water stress is the most influential factor affecting the yield of important crops, it is necessary to get maximum yield in agriculture by using available water in order to get maximum profit form per unit area, because existing agricultural land and irrigation water are rapidly diminishing due to rapid industrialization and urban development (Tawfik, 2008). Plant tissues show several metabolic alterations in response to water deficit. Such alterations can occur at one of the following three levels: i) disturbance of metabolic pathways leading to an accumulation or loss of metabolites; ii) alterations in enzyme activities, and iii) changes in the patterns of protein synthesis.

Lepidium sativum L. or garden cress (Family Brassicaceae) is considered as one of the most important medicinal plants in North Africa (Boulos, 1995). It is an annual herb, common in Nile, Oases and Sinai regions (Tackholm, 1974). The seeds are tonic, have expectorant activity and contain high concentrations of iodine beneficial to peoples suffering from simple goiter. Garden cress is found to contain significant amounts of iron, calcium and folic acid, in addition to vitamins A and C. Moreover, the fresh plant (in the seedling stage) may be eaten as antiscorbutic (Kobasi, 1993). Some Arab scholars have attributed garden cress reputation among Muslims to the fact that it was directly recommended by the Prophet (Shehzad *et al.*, 2011). The importance of modern herbal medicine has encouraged many farmers to start growing this new medicinal plant. Recent researches suggest that this vegetable is effective in preventing chronic diseases such as cancer (Su and Arab, 2006), cardiovascular diseases (Cox *et al.*, 2000) and non-insulin dependent diabetes (Williams *et al.*, 1999). Raw vegetables contain better preserved vitamins C, E, B-6 and foliate recommendations (Su and Arab, 2006). Recently, garden cress has gained more interest from consumers and producers (Zhan *et al.*, 2009), and can be a good choice for salads with its peppery taste, and health promoting substances such as glucotropaeolin, a glucosinolate compound and the precursor of benzyl isothiocyanate (Kassie *et al.*, 2002) and sterols (Conforti *et al.*, 2009).

Mycorrhizal fungi have a long history in having symbiosis relation with most plants families and they exist in most ecosystems (Saleh, 1998). Nowadays a special attention is paid to the potential role of these fungi in agricultural production due to their ability to increase water and nutrient uptake of agricultural plants (Sardi, 1992). One of the most important effects of Mycorrhizal fungi is increasing the yield of agricultural plants especially in soils of low fertility. Such increase may be due to the increase in the absorption of the roots as a result of the wide extension of fungus mycelium in the soil around the root system that allows the agricultural plant to have access to higher volume of soil (Hayman 1983). Many researchers have indicated that arbuscular mycorrhizal (AM) fungi are capable of alleviating the unfavorable effects of drought on plant growth (Auge, 2001 and Miransari, 2010). Symbiotic relationship between different plants and AM fungi on the exterior part of the root system, resulting in enhanced uptake of water and nutrients by plant's root. Such characters improve plant performance under drought stress, which is believed to increase the absorption of water and some nutrients such as zinc (Zn) and copper (Cu), also improved leaf water turgidity, stomatal activities, root growth and development (Ghazi and Zak, 2003). AM fungi perform as the enhancer of plant-water relationship through increasing stomatal resistance by adjusting plant hormonal balance. Moreover, this chain of improvements enhances plants phosphorous (P) nutrition introduced by AM fungi activities under growth conditions (Elwan, 2001). Phosphorous is one of main elements which have necessary roles for plant flowering, root extension and grain production. Applying AM fungi is one of the benefit methods for increasing the efficiency of phosphorous. Symbiosis between mycorrhizal fungi and plants roots causes better phosphorous absorption by extending hyphae into the soils (Ghorbanian *et al.*, 2011). Tarafdar *et al.* (1994) showed that inoculation of plants with mycorrhiza had a significant effect on phosphorus, zinc, and copper absorption, while it had no effect on Fe absorption. Moreover, Shiranirad (1998) found out that mycorrhizal symbiosis had the statistically significant effect on phosphorus absorption when the amount of phosphorus in soil is low. The objective of the present investigation was therefore to investigate the effect of water stress and the phosphate bio fertilizers dissolving fungi (VAM) (Glomus spp.) on growth, yield, photosynthetic pigments and oil % of *Lepidium sativum* L. plant using three phosphorus levels.

MATERIALS AND METHODES

The experimental site:

Two pot experiments were carried out at the green house of the National Research Center, Dokki, Cairo, Egypt. during two winter seasons of 2012/2013 and 2013/2014.

Soil type:

The mechanical and chemical analyses of the soil were determined according to a standard method described by Klute (1986).

Growth Conditions:

Seeds of Garden cress (*Lepidium sativum* L.) provided from the Egyptian Agricultural Research Center and were thoroughly washed with distilled water then the seeds were sown at a rate of three seeds per hole on the 1st of October in earthenware pots 40 diameter and 40 cm height with perforated bottoms, and were filed with 10 kg of clay loam soil. All pots were weighted every 1 to 3 days on abeam balance. The pots were then irrigated to restore the soil to the appropriate moisture regime by adding a calculating amount of water. The general principal stated by Boutraa and Sanders (2001) was used for the water treatments application. All pots received recommended doses of N and K fertilizers, N 40–50 kg/ha, P 50–60 kg/ha and K 25–30 kg/ha. P fertilizer was added at four rates, P0 (zero phosphorus), P1 (25% recommended), P2 (50% recommended), P3 (100% recommended). Also, K was applied as potassium sulphate (48% K_2O) which was added immediately after thinning, N was added as ammonium nitrate (33.5% N) which was divided into three equal portions the first immediately after sowing, the second after thinning and the third after two weeks from the second, according to the recommendations of Agriculture Ministry.

Table (1): Mechanical and chemical analyses of the soil used during the experiment.

Seasons	1[st]season	2[nd] season
sand %	26	25.7
silt %	36	35.4
clay %	38	37
Texture	clay loam	clay loam
F.C%	31.9	31.2
W.P%	16.2	14.3
CaCO3 %	4.5	4.3
OM %	1.3	1.1
PH	7.7	7.7
EC	0.6	0.55
Na^{+}	2.1	1.90
Mg^{++}	0.82	0.80
Ca^{++}	1.00	0.97
K^{+}	1.11	0.94
$HCO3^{-}$	1.14	1.02
Cl^{-}	0.75	0.64
$CO3^{--}$	2.00	1.99
$SO4^{--}$	1.65	1.44

Preparation of inoculum:

The arbuscular mycorrhiza inoculum was purchased from Agricultural Microbiology Department National Research Centre, Cairo, Egypt. The inoculum was mixture of Glomus spp. (G. mosseae and G. fasciculatum) originating from the rhizosphere soil of maize and alfalfa. The inoculum was multiplied in pot cultures contains peat: vermiculite: perlite mix 1:1:1 by volume with maize and alfalfa (Badr El-Din *et al.*, 1999) and consisted of a mixture of spores, root fragments, hypha and growth medium. The inoculum material contained 275 spores and 240 propagules gm^{-1} oven dry biases using the most probable number test (Mahaveer *et al.*, 2000). The non -inoculated control was without mycorrhizal. Miorrhizal inoculation was done by planting the seed over a thin layer of the mycorrhizal inoculum at the time of sowing at a rate of 50 gm row^{-1}. The inoculum material contained 275 spores gm^{-1} on oven dry bases, whereas 10g of autoclaved soil was used for the non-mycorrhizal treatments. Phosphate treatments were given after 40 days of AM inoculation.

Experimental design and treatments:

This experiment included 15 treatments which were the combination between three soil moisture levels (W1= 85% depletion of the available soil water, W2=55% depletion of the available soil water and W3= 25% depletion of the available soil water) and five treatments of arbuscular Mycorrhizal (AM) fungi which were (control without mycorrhiza and phosphorus,, zero phosphorus (P0) + mycorrhiza, 25% recommended phosphorus + mycorrhiza (P1), 50% recommended phosphorus + mycorrhiza (P2) and 100% recommended phosphorus + mycorrhiza (P3)), treatments were arranged in a randomized complete blocks design with five replicates. The different soil moisture treatments were assigned at random in the main plots, while sub-plots were devoted to the different arbuscular mycorrhiza inoculum treatments.

Sampling and measurements

Random samples of six plants were taken at random to estimate the following characters: plant height (cm), number of leaves/plant, main root length (cm), fresh and dry weights of the whole plant (g). At maturity the following data were recorded: plant height, number of seeds/plant, 1000 seeds weight, number of siliquae/plant. Fixed oil percent in the seeds was determined according to A.O.A.C. method (1980). The photosynthetic pigments of fresh leaves, chlorophyll a and b as well as total chlorophyll and carotenoids were determined using for such purpose the 4[th] leaf from the growing point of the plant using the spectrophotometric method recommended by

Metzener *et al.* (1965). Content of chlorophyll a, chlorophyll b and carotenoids (μg/g) was calculated according to Lichtenthaler and Wellburn (1983) using the following formulae:
Chlorophyll a = 12.21 OD663 – 2.81 OD646;
Chlorophyll b = 20.13 OD646 – 5.03 OD663;
Total Chlorophyll = Chlorophyll a + Chlorophyll b;
Carotenoids = (1000 OD470 – 3.27 Chlorophyll a – 104 Chlorophyll b) / 229

Statistical Analyses:

The collected data were subjected to statistical analysis of variance using the normal (F) test and the means separation were compared by using Least Significant Difference (LSD) at 5% level according to Snedecor and Cochran (1980).

RESULTS and DISCUSSIONS:

Morphological characteristics:

1-*Effect of water stress:*

The results showed that plant morphological characters were significantly reduced with the reduction in water availability except for root length (Table 2). The shoot length of stressed plants were correspondingly declined below the unstressed ones in both seasons, thus the percentage of decline became 22.4% and 6.8% (For W1 and W2 respectively compared with W3). The decrease in shoot length under water stress conditions was obtained by Yousef (2002) on chamomile plants, Anupama *et al.* (2005) on chrysanthemum plant, Khater *et al.* (2005) on *Mentha piperita,* L., Hussain *et al*. (2008) on *Helianthus annuus* L., Hojati *et al*.(2011) on *Carthamus tinctorius* L. and Yousef *et al.* (2013) on *Echinacea purpurea* L. They all reported that water deficit during the vegetative period (before flowering stage) can result in shorter plants. Such decrease in shoot length in response to drought either due to decrease in cell elongation resulting from water shortage which led to a decrease in each of cell turgor, cell volume and eventually cell growth and/or due to blocking up of xylem and phloem vessels thus hindering any translocation through (Nagarajan and Nagarajan, 2010).

Data presented in the same table revealed also that drought stress significantly affected the number of leaves, while the number of leaves was greater in the well-watered (W3) treatment than in the dry treatment (W1) in both seasons. These results are consistent with the work of Fronza and Folegatti (2002) on *Stevia rebaudiana,* Dore (2004) on rose plants, Moeini *et al.* (2006) on *Ocimum basilicum* plant, Khalil *et al.* (2010) on *Ocimum basilicum* and Hojati *et al.* (2011) on *Carthamus tinctorius* L. Such reduction in number of leaves due to water stress can be attributed to its direct effect on cell division which arose from reduction in nucleic acid synthesis which enhanced its break down (Ashraf *et al.,* 1996).

Data obtained in (Table 2) showed also that there was a direct proportional relationship between the root length and increasing the severity of drought. Where the highest significant increase in root length obtained in plants grown under the most stressed level (W1) as compared to well watered plants (W3), this result was gained in both seasons. Similar results obtained by Rambal and Debussche (1995), Prior *et al.* (1997), Chiatante *et al.* (2000), Tahir *et al.* (2002), Snyman (2006), Jaleel *et al.* (2008a) and khalil and Ismael (2010) where they all reported that the roots of stressed plants often grown deeper into the soil than roots of plants that were watered regularly. Such increase in root length in response to drought was attributed to either the increase in gibberellins and cytokinin content of the roots in response to drought which in turn stimulates roots cell division and elongation (Abdalla and El-Khoshiban, 2007).

It was also clear from the obtained data that fresh and dry weights were progressively reduced by increasing stress conditions, where the lowest means were obtained by the dry treatment (W1) in both seasons. The results reached by Khalid (2006) on Ocimum sp., Said-Al Ahl *et al.* (2009) on *Origanum vulgare*, khalil and Ismael (2010) on *Lupinus termis,* Hojati *et al.* (2011) on *Carthamus tinctorius* L. and Yousef *et al.* (2013) on *Echinacea purpurea* L. confirmed our data. The reduction in fresh and dry weights was attributed to accelerated senescence and shedding of leaves under water stress (Faisal *et al.,* 2000). This result could be also due to that one of the first signs of water shortage was the decrease of turgor which resulted in decrease in growth and development of cell especially in leaves (Alishah *et al.,* 2006).

In general water stress reduces plant growth through inhibition of various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones (Khalil and El-Noemani, 2012 and Bahreininejad *et al.* 2013)

2-*Effect of AM fungi and different phosphate levels*:

In the present investigation AM fungi was found to have a significant effect on the growth and development of *Lepidium sativum* plant. The difference between the mycorrhizal and non mycorrhizal garden cress plants was visible just after 35 days of AMF inoculation, before phosphate was being applied. Inoculation of garden cress plants with AM fungi significantly increased all growth parameters compared to the control plants (Table 2). The positive response of AM fungi inoculation was in harmony with many others as Mosse *et al.* (1981), Ba *et al.* (2001), Wu and Xia (2006) and Abdul- Wasea, Ibrahim *et al.* (2010), Elhindi (2010) and Abdel-Rahman *et al.* (2011). The increase in growth characters of AM fungi inoculated plants may be due to the ability of AMF to produce hyphae, which were microscopic tubes colonize plant roots and grown out into the soil further than root hairs. Nutrients were taken up by the hyphae to the plant, which lead to a very efficient mobilization and uptake of phosphate, nitrogen, potassium, magnesium, copper, zinc, boron, sulphur and other elements that were transported to the plant. On comparing between the different AM fungi treatments in their effectiveness in promoting growth parameters, it was found that P3 treatment revealed the highest significant means where the percentage of increase reached to (23.34,63.2,25.75,117.8 and 75.34%) for plant height, number of leaves/plant, main root length, fresh and dry weights of the whole plant respectively compared with control plants. Increasing growth parameters due to mycorrhizal inoculation have been reported earlier in other medicinal plants by Sena and Das (1998) and Matsubara and Sakurai (2000) who reported that plant growth was highly variable with or without mycorrhiza. After 60, 90 and 120 days of AM inoculation, fresh and dry weights in mycorrhizal Catharanthus plants at all levels of phosphate was found to be significantly higher as compared to non mycorrhizal Catharanthus plants. They returned this increase to the formation of external mycelium around the roots by AM fungi and the increase in photosynthetic rate. A similar result has been also reported in other medicinal plants like Palmarosa (Gupta and Janardhanan, 1991) and Coleus forskholii (Boby and Bagyaraj, 2003). Furthermore, Ezawa *et al.* (2001) suggested that the higher uptake of phosphorus in mycorrhizal plants and release of inorganic phosphate from arbusculer to root cells which may be due to the increased level of phosphatase enzymes which ultimately resulted in enhanced growth response. Phosphorus translocate from external hyphae which was normally present in the form of polyphosphate can be hydrolyzed by alkaline phosphatase. Similar report was presented by Earanna *et al.* (2002) on *Rauvolfia tetraphylla* and Karthikeyan *et al.* (2008) on *Catharanthus roseus* inoculated with *Glomus mosseae*. Moreover, Vijaya and Ramkrishnaiah (2006) studied the effect of AM fungi and nitrogen fixing bacteria on growth and biochemical aspects of *Stevia rebaundiana*, results showed that treated plants with bio fertilizers increased plant height and leaves biomass. Moreover, Oyun *et al.* (2010) reported that inoculated *Acacia senegal* (L.) plants with AM fungi enhanced leaf number, increased shoot height and other growth parameters. Also, Ghorbanian *et al.* (2011) mentioned that Mycorrhizal fungi extending their root absorbing area through their mycelium network and changing unavailable Phosphorus to available form and translate to root system cause increase in plant height and growth parameters.

3-*Effect of interaction between water stress, AM fungi and different phosphate levels:*

Combination of different stress levels with mycorrhiza and different phosphorus levels were found to have significant influence on all morphological characters (Table 2). It was deduced from the data that inoculated plants under moderate soil moisture level combined with the highest phosphorus treatment (W2XAMFXP3) was mostly effective in promoting plant morphological characters significantly compared to the control and to other treatments in the lowest and highest moisture levels except (W3XAMFXP3) treatment which revealed the highest significant records in all treatments. As for root length the highest significant means were observed in plants inoculated with AM fungi and treated with the highest phosphorus rate under the lowest soil moisture level (W1XAMFXP3) compared with the other treatments. In conformity with the findings of the present study, Awotoye *et al.* (1992) and Osundina (1995) who worked on *Parkia biglobosa* observed that AMF had enhanced leaf number, increased collar diameter, shoot height and other growth parameters. This was due to the association between the plants and the mycorrhizae which enhanced growth and plant vigor and improved the plant physiological activities like stomata conductance and xylem pressure potential which were directly related to soil water balance and growth determinant in water-stressed environment. Also, the root systems of plants inoculated with AMF were often more finely divided and thus have more absorptive surface area (Okon *et al.*, 1996). The AM hyphae also help in retaining moisture around the root zone of plants (Morte *et al.*, 2001). Also, these results were documented by many researches done in this field e.g. Ghorbanian *et al.* (2011) who indicated that AM fungi had abilities to enhance plant growth under water stress and had significant effect on the absorption of phosphorus.

Table (2): Effects of different soil moisture levels, arbuscular mycorrhizal fungi, three phosphate levels and their interactions on morphological characters of *Lepidium sativum* L. in the two seasons of 2012/2013 and 2013/2014 (combined analysis of two seasons).

Treatments		Plant height (cm)	No of leaves/plant	Main root length (cm)	F.W/plant (g)	D.W/plant (g)
Effect of different soil moisture						
W1		45.00	24.40	13.23	6.55	2.05
W2		54.20	25.67	10.90	8.23	3.29
W3		58.00	27.80	9.87	9.51	3.52
$LSD_{0.05}$		0.49	0.71	0.63	0.23	0.06
Effect of AM fungi and three phosphate levels						
Cont.		40.33	19.00	9.94	4.77	2.19
P0XAMF.		47.67	24.67	11.11	7.63	2.63
P1X AMF		53.67	26.78	11.28	8.57	2.71
P2X AMF		56.67	28.33	11.83	9.12	3.39
P3X AMF		63.67	31.00	12.50	10.39	3.84
$LSD_{0.05}$		0.64	0.91	0.82	0.29	0.08
Interaction effect of different soil moisture X AM fungi						
W1	Cont.	34.00	17.00	12.17	4.10	1.24
	P0XAMF	41.00	24.00	13.00	5.80	2.11
	P1X AMF	44.00	25.00	13.00	6.40	2.04
	P2XAMF	49.00	28.00	14.00	6.90	2.32
	P3X AMF	57.00	28.00	14.00	9.57	2.52
W2	Cont.	43.00	20.00	9.50	5.63	2.63
	P0X AMF	45.00	23.00	10.50	6.90	2.77
	P1XAMF	57.00	26.33	11.00	8.70	3.06
	P2XAMF	60.00	28.00	11.00	9.80	3.93
	P3X AMF	66.00	31.50	12.50	10.80	4.06
W3	Cont.	44.00	20.00	8.17	4.57	2.71
	P0X AMF	57.00	27.00	9.83	10.20	3.00
	P1X AMF	60.00	29.00	9.83	10.60	3.04
	P2X AMF	61.00	29.00	10.50	10.67	3.92
	P3X AMF	68.00	34.00	11.00	11.50	4.94
$LSD_{0.05}$		1.11	1.58	1.42	0.50	0.14

Cont.= zero phosphorate and zero AMF. W1=85% depletion W2=55% depletion W3=25%depletion
P0=zero phosphate P1=25% phosphate P2= 50% phosphate P3= 100% phosphate.

Yield and yield components:
1-*Effect of water stress*:

Evaluating of the results during maturity stage showed that irrigation treatments had significant effect on yield and its components (Table 3). Significant higher means for plant height, number of seeds /plant, 1000 seeds weight and number of silique/plant were recorded with the highest water treatments (W3) compared with the other treatments. This supports the results of Ahmed *et al.* (2000) who found that the increase in yield was directly related to the increase in number of irrigations; this is because the highest water quantities had a better performance for growth and yield components. Halepyati *et al.* (2002) worked on tuberose plants and illustrated that increasing irrigation water recorded an increase in spike length, spike fresh weight, number of opening flower and number of flowers/spike as compared to other treatments. Furthermore, Gaballah *et al.*, (2007) on *Helianthus annuus* L. plants, showed that increasing the amount of irrigation water improved plant yield. Also, Khalil and El- Noemani (2012) on *Lepidium sativum* recorded significant lower number of siliquae/plant, number of seeds/plant and 1000 seeds weight with the longest irrigation intervals they returned this decrease in yield attributes under the longest irrigation interval

Table 3: Effects of different soil moisture levels, arbuscular mycorrhizal fungi, three phosphate levels and their interactions on yield and its components of *Lepidium sativum* L. in the two seasons of 2012/2013 and 2013/2014 (combined analysis of two seasons).

Treatments		Plant height (cm)	No of seeds/plant	1000 seeds weight(g)	Number of siliquae /plant
Effect of different soil moisture					
W1		3360.	377.33	1.76	9.07
W2		63.50	420.40	2.40	12.18
W3		66.40	453.20	2.50	16.88
$LSD_{0.05}$		0.61	1.16	0.05	0.22
Effect of AM fungi and three phosphate levels					
Cont.		58. 00	303.33	1.72	8.96
P0XAMF.		58. 67	341.67	2.04	10.61
P1X AMF		64.67	400.67	2.18	12.89
P2X AMF		65.17	492.33	2.57	14.94
P3X AMF		70.56	546.89	2.60	16.14
$LSD_{0.05}$		0.79	1.96	0.07	0.29
Interaction effect of different soil moisture X AM fungi					
W1	Cont.	52.00	250.00	1.48	6.18
	P0X AMF	60.00	315.00	1.72	7.50
	P1X AMF	60.00	350.00	1.74	9.75
	P2XAMF	63.67	447.00	1.90	10.93
	P3X AMF	66.67	524.67	1.96	11.00
W2	Cont.	56.00	320.00	1.83	8.84
	P0X AMF	59.00	340.00	2.16	9.10
	P1XAMF	65.00	382.00	2.27	12.06
	P2XAMF	65.50	500.00	2.87	14.37
	P3X AMF	72.00	559.00	2.94	16.53
W3	Cont.	57.00	340.00	1.85	11.87
	P0X AMF.	63.00	370.00	2.24	15.22
	P1X AMF	69.00	470.00	2.52	16.87
	P2X AMF	70.00	530.00	2.88	19.53
	P3X AMF	73.00	556.00	2.97	20.90
$LSD_{0.05}$		1.37	3.39	0.12	0.50

Cont.= zero phosph and zero AMF. W1=85% depletion W2=55% depletion W3=25%depletion
P0=zero phosphate P1=25% phosphate P2= 50% phosphate P3= 100% phosphate.

to that water stress changing the hormonal balance of mature leaves, thus enhancing leaf senescence and hence the number of active leaves decreased, in addition leaf area was reduced by water shortage, which was attributed to its effect on cell division and lamina expansion, when the leaf level decreased the light attraction and CO_2 diffusion inside the leaf decreased and the total capacity of photosynthesis decreased therefore the photosynthetic materials that transferred to seeds will decrease. Similar results obtained by Houshmand *et al.* (2011) on chamomile and Bahreininejad *et al.* (2013) on *Thymus daenensis* they all reported that drought stress reduced yield and its components of the tested plants.

2-*Effect of AM fungi and different phosphate levels*:

Analysis of variance showed that all yield parameters in this study were significantly affected by the various treatments of Mycorrhiza and phosphorus treatments (Table 3). The mean comparison of data in different treatments showed that the application of AM fungi revealed significant increases in plant height, no of seeds/plant, 1000 seeds weight and no of siliquae /plant compared with control plants in both seasons. However, the maximum records for the previously mentioned characters were obtained in plants inoculated with AMF and treated with the highest phosphate level (P3) compared with the other treatments, where the percentages of increases under this treatment reached to 21.66%, 80.29%, 51.2% and 80.13% for plant height, no of seeds /plant, 1000 seeds weight and no of siliquae /plant seeds respectively compared with control plants. While the lowest means were obtained under control treatment in both seasons. Our results were generally in line with Kuntal *et al.* (2007) who studied the effect of chemical fertilizers N, P and K alone or in combinations with arbuscular mycorrhiza fungi the results showed significant increase in biomass yield due to bio fertilizer applications. This result in accordance with Linderman and Davis (2004), Zandavalli *et al.* (2004), Abdel-Rahman *et al.* (2011) and Asrar *et al.* (2012). Mycorrhizal inoculated plants improved their yield by enhanced translocation of nutrients and water from rhizosphere due to higher root colonization. The plants results higher response with inoculation of mycorrhiza because of more strong relationship with mycorrhiza to fulfill their phosphorus and other nutrients requirement and shared some photosynthetes with fungus to maintain the symbiosis (Hobbie, 2006).

3-*Effect of interaction between water stress, AM fungi and different phosphate levels*:

The effect of bi-interaction between soil moisture levels and AM fungi treatments on plant height, no of seeds/plant, 1000 seeds weight and number of siliquae /plant in the two seasons revealed significant effect (Table 3). The data of interaction illustrated that Mycorrhiza could with symbiosis increasing P nutrient around plant's root and increase root absorption, so under all soil moisture levels the mycorrhizal plants treated with different phosphorus levels had higher yield compared with control plants. According to the results of this experiment, application of mycorrhiza in presence of 100% recommended phosphorus (P3) had the highest performance and yield under different depletion treatments, especially under the highest soil moisture level which revealed the maximum significant values compared with the other treatments (W3XAMFXP3), followed mostly by (W2XAMFXP3) where the difference between the two treatments was mostly insignificant. The positive effects of AMF in plants were attributed to changes in water relations resulting from enhanced P nutrition (Kaya, 2003). Such increase in yield and its components may be due to that mycorrhizal fungi extending absorbing area of the root through their mycelium network and changing unavailable phosphorus to available form and translated it to plant's root system causing increase in plant height, yield and yield components especially under water stress condition (Rejali *et al.*, 2008). Other scientists also showed that under drought stress mycorrhizal fungi extending their hypha in the soil could absorb more water and mineral nutrient such as phosphorus, zinc, copper and iron and in this way could increase yield quality and quantity and help host plant to resist more against drought stress (Miransari *et al.*, 2009). AM fungi develop an extensive network of hypha when in symbiosis with the host plant; this could significantly enhance the absorbing capacity of the host plant roots. It has also been indicated that AM fungi may behave more effectively with increased stress level (Miransari *et al.*, 2007, 2010), Under drought stress, AM fungi were able to decrease abscisic acid concentration and increase IAA and gibberellins concentrations (Liu *et al.*, 2000) and hence alleviate the stress. Similar results obtained by Ghorbanian *et al.* (2011).

Photosynthetic pigments content:

1-*Effect of water stress*:

It is evident from the data in Table 4 that water stress had a significant effect on photosynthetic pigments concentration of garden cress leaves. The data illustrated also that, increasing stress level caused significant increase in chla, chlb as well as total chlorophyll content, while it had a depressive effect on carotenoids concentration, where carotenoids showed reversed trend to the chlorophylls content.The maximum values of Chla, Chlb as well as total chl (a+b) were obtained under the lowest soil moisture level (W1) compared with the other soil moisture levels in both seasons. While the highest significant record for carotenoids concentration was observed under the highest soil moisture level W3. Carotenes form a key part of the plant antioxidant defense system, but they are very susceptible to oxidative destruction. Also, it act as an accessory pigment (absorb sun light when chla and chlb cannot absorb sun light) (Havaux, 1998). The increase in chlorophylls concentration as response to increase in drought stress were in accordance with Martínez *et al.*, (2004), Saleem (2007) on tuberose plant, Hessinia *et al.*, (2009) on *Spartina alterniflora* and Jaafar *et al.* (2012) on *Labisia pumila* Benth. Increasing chlorophylls content with increasing water stress, was to escape the stress conditions. The amount of total chlorophyll increased causing more photosynthesis and better food processor which tolerate dry conditions. This condition causes the amount of plant material built to accommodate supply and enhance performance spending to combat drought conditions that will ultimately reduce yield (Mohammad, 2013).

2-*Effect of AM fungi and different phosphate levels*:

Plants inoculated with AM fungi, brought about significant changes in chlorophyll a, b and total chlorophyll concentration as well as carotenoids content compared with control plants. The maximum chlorophyll and carotenoids content was noticed in P3 inoculated plants compared with the other treatments in both seasons (Table 4). While the lowest means were observed under control treatments. The significant increase in chlorophylls content of AM fungi inoculated tissue of garden cress plants was in agreement with results reported elsewhere by Sampath and Kumar (2003), Rooyen *et al.*, (2004) and Huixing (2005)who recorded similar results. This increase may be due to an increase in stomata conductance, photosynthesis, transpiration and plant growth (Sampath and Kumar, 2003; Rajasekaran *et al.*, 2006) or due to the presence of large and more numerous bundle sheath chloroplasts in the inoculated leaves (Arumugam *et al.,* 2010). The most important effect was to promoting the carotenoids content, since carotenoids protected plants against damaging effects of ROS; at this site was essential for chloroplast functioning, acts as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them (Havaux, 1998). A major protective role of carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage (Farooq *et al.,* 2009).

Table 4: Effects of different soil moisture levels, arbuscular mycorrhizal fungi, three phosphate levels and their interactions on photosynthetic pigments (in leaves) and oil % (in seeds) of *Lepidium sativum* L. in the two seasons of 2012/2013 and 2013/2014 (combined analysis of two seasons).

Treatments		Photosynthetic pigments (mg/g)				Oil %
		Chl. a	Chl. b	Total chl. (a+b)	Carotenoids	
Effect of soil moisture levels						
W1		2.11	1.62	3.73	0.75	31.37
W2		1.80	1.36	3.16	1.52	37.96
W3		1.54	1.12	2.67	2.36	41.91
$LSD_{0.05}$		0.23	0.15	0.47	0.11	3.02
Effect of AMF						
Cont.		0.97	0.43	1.39	0.65	34.85
P0XAMF		1.03	1.01	2.04	1.45	36.81
P1X AMF		2.33	1.41	3.74	1.77	37.26
P2X AMF		2.24	1.52	3.76	1.77	37.37
P3X AMF		2.53	2.46	4.99	2.09	39.12
$LSD_{0.05}$		0.33	0.22	0.69	0.16	3.39
Interaction effect of soil moisture levels X AMF						
W1	Cont.	1.00	0.62	1.62	0.49	28.33
	P0XAMF	1.04	1.02	2.06	0.72	31.22
	P1XAMF	2.66	1.70	4.37	0.79	31.53
	P1XAMF	2.66	1.80	4.66	0.80	31.66
	P3XAMF	2.98	2.96	5.94	0.97	34.11
W2	Cont.	0.96	0.36	1.32	0.56	36.00
	P0XAMF	1.03	1.02	2.05	1.33	38.00
	P1XAMF	2.43	1.30	3.73	1.63	38.24
	P2XAMF	1.93	1.51	3.44	1.75	38.44
	P3XAMF	2.86	2.60	5.26	2.35	39.11
W3	Cont.	0.95	0.30	1.25	0.91	40.21
	P0XAMF	1.01	1.00	2.01	2.31	41.22
	P1XAMF	1.90	1.23	3.13	2.89	42.00
	P2XAMF	1.92	1.25	3.17	2.75	42.00
	P3XAMF	1.94	1.83	3.78	2.95	44.13
$LSD_{0.05}$		0.61	0.34	0.77	0.23	4.55

Cont.= zero phosphorus and zero AMF. W1=85% depletion W2=55% depletion W3=25%depletion
P0=zero phosphate P1=25% phosphate P2= 50% phosphate P3= 100% phosphate.

3-*Effect of interaction between water stress, AM fungi and different phosphate levels:*

The plant was stimulated by colonization to a limitation of water stress towards the leaves, and leads to better functioning of chloroplast and photosynthetic efficiency under different soil moisture levels, as shown by Al-Karaki (2000) and Rabie (2005). Our results indicated that after 45 days of AM inoculation in all depletion levels, mycorrhizal cress plants showed higher chlorophyll and carotenoids content as compared to non mycorrhizal cress plants under different depletion levels. Thus, AM symbiosis could enhanced the photosynthetic ability of cress leaves, which was in agreement with the results of other studies (Giri and Mukerji, 2004; Sannazzaro *et al.,* 2006; Colla *et al.,* 2008, Sheng *et al.,* 2008). The maximum chlorophyll a, b and total chlorophyll content were obtained in mycorrhizal plants irrigated with the lowest water level and treated with the highest phosphorus amount (W1XAMFXP3) compared with the other treatments, followed by (W2XAMFXP3) treatment where the difference between the two treatments was mostly insignificant. As for carotenoids content the maximum significant records appeared in inoculated plants under the highest soil moisture level and treated with the highest phosphorus amount (W3XAMFXP3) compared with the other treatments in both seasons (Table 4).

Oil percent:

1-*Effect of water stress:*

By increasing the soil moisture level, the oil percent of L. sativum seeds increased till reached its maximum significant values under W3 treatment in both growing seasons compared with the other treatments (Table 4). While the lowest means were recorded for the highest depletion treatment W1. The present decrease in oil % with increase in water stress were conflicted with that of Chanirar *et al.* (1989) who illustrated that increasing severity of drought above certain limits caused reduction in oil % because there was a relationship between the amount of oil and shoot yield, so when the shoot yield decreases by the drought stress therefore oil percentage reduced. Furthermore, Singh *et al.* (1997) explained that the reduction in oil % of lemongrass plant was due to reduction in herbage yield, this indicated that oil % was positively related to soil water content and herbage yield. These results were fortified by those of El- Sabbagh (2003) on sunflower, Elham and Ibrahim (2009) on sunflower and Khalil and El-Noemani (2012) on garden cress. Also, drought stress leaded to decrease in water and nutrient uptake by plant which caused decrease in carboxylase activity and oil biosynthesis (Aziz *et al.*, 2008).

2-*Effect of AM fungi and different phosphate levels*:

Treated *Lepidium sativum* plants with AM fungi were effective in promoting seeds oil% even before phosphorus was being applied compared to the control plants (Table 4). The highest percentage of oil% was assigned to P3 treatment which inoculated with AM fungi combined with the highest phosphorus level, the percentage of increase reached to 12.25% compared with control plants. While the lowest means were obtained under control treatment. Similar findings were reported by Toussaint *et al.*, (2007) who indicated that the symbiotic AM fungi can induce changes in the accumulation of secondary metabolites, including phenolics in roots and aerial parts and also oil% of host plants. There were several studies on the association of AM fungi with medicinal plants, such as basil *Ocimum basilicum* (Pascual-Villalobos and Ballesta-Acosta, 2003 and Toussaint *et al.*, 2007), *oregano Origanum onites* (Khaosaad *et al.*, 2006), mint *Mentha requienii* (Freitas, 2004) and on garden cress (Khalil and El-Noemani, 2012). Such observation may be due to the ability of VAM fungi to interact with other soil microbes like the free-living nitrogen fixers and phosphate solubilizes to improve their efficiency for the biochemical cycling of elements and supply the host plants with their nutrients requirements (Oyun *et al.*, 2010 and Raei and Weisany, 2013).

3-*Effect of interaction between water stress, AM fungi and different phosphate levels:*

Concerning the effect of bi interaction between different soil moisture levels and different AM fungi inoculation, the data showed that during the two growing seasons the oil % attained their highest mean values in plants inoculated with AM fungi combined with the highest phosphorus level under different soil moisture levels and with significant difference as compared to control treatments. The data also revealed that the highest mean values observed in (W3XAMFXP3) treatment. While, the lowest means of oil% were recorded under (W1XCont) in both seasons. It was also found that there was insignificant difference between plants treated with the moderate soil moisture level and inoculated with AM fungi combined with P3 treatment (W2XAMFXP3) and all treatments of the highest moisture level (W3) except (W3XAMFXP3) treatment. Similar result obtained by Heidari and Karami (2012).

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