

## Investigation of effective Microorganisms (EM) Impact in Water Stress Condition on Growth of Almond (*prunus dulcis* Mill) Seedling

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Received: June 10 2013

Accepted: July 10 2013

### ABSTRACT

Almond is cultured at defect water regions as drought farming on the hills. The main problem in these regions is the lack of soil moisture during growth season. Application seedlings, with regards to deeper of root system with comparing to roots produced of vegetative rootstocks, causes to be stable tree in this condition. This study in order to evaluation of water stress effects and Effective microorganisms (Em) on growth and amount of storage nutrition in leaves of almond seedling was conducted in Department of Horticultural Science, agriculture faculty, Ferdowsi University of Mashhad, in 2011. In this research effects of two different Em concentrations (0, 0.5%) by solution in water in 60 day before of drought stress treatments and drought stress treatments with three levels, 100, 66, and 33% of FC on seedling of almond. Using factorial base on randomized complete bloke design (RCBD) in four replications was evaluated. Results showed effects of different Em and irrigation levels on amount of plant growth. It was found that Em increased amount of plant growth, number of leaf, leaf area, fresh and dry weight, storage chlorophyll, N, K and P in leaves. In finally the best plant growth was achieved by treatments of interaction between irrigation level of FC% 100 and Effective microorganisms.

**KEYWORDS:** chlorophyll, fresh and dry weight, plant growth, seedling,

### INTRODUCTION

Almond is cultured at shortage of water regions as dry farming on the hills. The main problem in these regions is the lack of soil moisture during growth season. Application seedlings, with regards to deeper of root with comparing to roots produced of vegetative rootstocks, causes to be stable tree in this condition[1]. Drought resistance in almond may be due to leaves or roots are in adaptive ways. Falling leaves, reduced leaf size and photosynthesis, stomatal transpiration loss and change to the root directory, there are some ways that may face water stress in plants are used [2,3,4]. Short periods of water stress on plants for several weeks to study the physiological and morphological in juvenile phase and water relations provides[2]. Plant health is influenced by many factors, biological and non-biological interactions in soil and root surfaces[5]. Em is a mixture of effective microorganisms combination that can increase the biological activity of the soil and plants. Studies have shown that this compound can affect soil quality, plant growth, yield and product quality to be effective and other operations will be significantly enhanced performance[6]. When the combination of Em spray on plants or whit soil be used to can increase the population of photosynthetic bacteria and the nitrogen stabilizer. This phenomenon causes plant growth, yield and higher quality by increasing the efficiency of photosynthesis and nitrogen fixation levels[5]. Reports has been showed to improve productivity effective microorganisms effect on rice, sugar cane, some vegetables, citrus [7], amarind, mango[8], cotton and corn [9]. Effective microorganisms are increased in soil and plants by combination the Em, this material is used in organic farming to improve the performance and quality of crops[10,11].

The aim of the present studi was evaluation of water stress effects and effective microorganisms on growth and amount of nutrition storage in leaves seedling of almond.

### MATERIALS AND METHODS

This study was conducted to evaluation of water stress effects and effective microorganisms (Em) on growth and amount of storage nutrition in leaves of almond seedling was conducted in department of Horticulture, Faculty of agriculture, Ferdowsi University of Mashhad. in 2011. The soil used was a mixture of sand, soil of earth's surface and leaf mold. This mixture was poured into the pots with openings 30 and 35 cm height (3 stress treatment × 2 concentration × 4 replication and 3 pots without the plant for the removal of plant

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overweight). Almond seeds were in order to provide the required cold temperatures in January at 4 ° C in the wet sand and in mid-March planted in pots mentioned. Pots were kept in the garden of the College of Agriculture, Ferdowsi University of Mashhad whit longitude 59° 53' 3, latitude 36° 31' 2 and the altitude of 1023 meters above sea level with a full supply of irrigation until early July (end of primary growth stage). The Em treatments were applied during the growth period of 60 days before of water stress treatments. 50% of the pots were sprayed with the prepared solution to a concentration of 0.5% of Em and other pots were irrigated with ordinary water. The three levels of stress, was applied, control (100% Field Capacity), 66% and 33% of Field Capacity. It is worth noting in the control treatment, soil was kept at field capacity. By measuring weight of pots was trying to stress the desired level of moisture in the pots are kept constant. Their moisture status was determined. Water status of Pots was determined by measuring the weight of them every day. Thus, decrease their moisture was compensated by adding water to the intended of stress. For removal of the weight of the plant and reduce the amount of error in soil water was used pots lack of plant containing only soil testing and similar to main pots. After the treatments, to evaluate the treatments, various traits were measured for example total chlorophyll using Arnon (1949) [12] methode Total nitrogen was after distillation titration method using an automatic system (Kjeldahl Auto Analyzer) and nitrogen content of the extracts was determined by Emami device [13]. Phosphorus was measured, the method yellow color Ammonium Molybdate Vanadate by 470 nanometers with a wavelength of spectrophotometer [13]. Potassium with a flame photometer apparatus and method for measuring flame spread [13]. In order to determine the growth rate per plant, total branches in each treatment was recorded in November. The length of each plant was carefully measured and the growth rate notes. This experiment was arranged in a factorials design based on randomized blocks with three water stress treatments and two concentration levels (0 and 0.5 per cent) Effective microorganism in four replications. Statistical analysis was performed by the JUMPS software and comparison of averages whit test LSD (01/0P <) and drawing diagrams was done by Excel software.

## RESULTS AND DISCUSSION

Analysis of variance showed that the effect of microorganisms are in compared to control the level of a percent increase in the amount of plant growth, leaf area, leaf number, fresh weight and dry weight leaves, the amount of chlorophyll, the amount of stored nitrogen, potassium and phosphorus (Table 1 and Figures 1 to 9). Khan et al (2007) [14] reported that the use of Em in the tree *Albizia saman* increase growth factors such as fresh weight and dry weight amount of plant and amount of chlorophyll are in the leaves. According to this report, Em increased growth of seedlings in nursery. The similar results have been reported by Toch et al (1999)[15], Xu (2000) [10], Wang et al (2000) [11] and Khaliq et al (2006) [16]. Also base on reports, Em increased leaf area, leaf chlorophyll, the amount of potash, nitrogen, phosphorus, zinc and manganese in compared to control of apple trees [17],. Rose and Gerbera[18]. The results are listed in terms of increased the material absorption and growth factors evaluated is in accordance with the present study. Increased plant growth with the use of Em to the deep effects the material growth regulators, produced by microorganisms (bacteria, yeasts and fungi) and to improve the usability of the nutrients in the soil is to be cited[19]. Jagnow .et al, (1991) [20] reported that bacteria, an effective amount of Auxin and cytokines production are the cause of leaf area, increased levels of root hairs, absorption more nutrients from the soil and increase be metabolism and provides the conditions for plant growth. This result could be due to more than 60 different breeds of effect of microorganisms. Most important of which are, bacteria, yeasts, viruses and fungi in the mixture Em [21,22]. Results of this study showed different levels of water stress treatment, a difference in the level of one percent (Table 1). The results suggest that in conditions full irrigation (of available water maintain at 100%) growth was highest, but between 66 and 33 percent levels of available water maintain was not significant (Figure 1).

**Table 1.** Analysis of variance of the studied traits

Sources of variation	DF	Mean Squares								
		Mean LN	Mean LA	Mean chloro	Mean growth	Mean P	Mean K	Mean N	Mean WF	Mean WD
EM	1	42884**	1350**	5.005**	294**	0.015**	0.293**	0.194**	26.59**	0.207**
Water stress	2	179026**	1934**	9.7**	337.1**	0.0045**	0.724**	0.18**	145.44**	17.73**
Water stress × EM	2	10942**	356**	18.2**	242.4**	0.017**	0.423**	0.04**	3.97**	0.092**
Error	15	2.6	0.43	0.003	12.41	0.00001	0.0017	0.0008	0.0435	0.00456
Coefficient of (%)variation		0.39	0.99	0.44	3.74	0.43	0.43	2.58	2.01	1.91

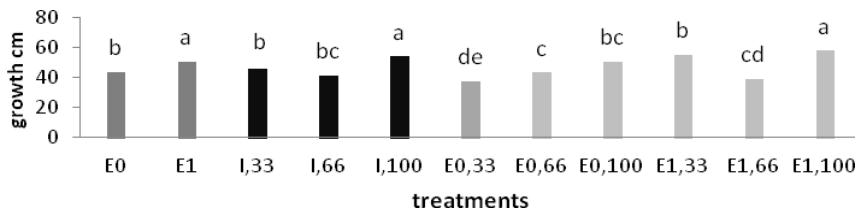
Respectively non-significant and significant at the 5% level and 1% , \*\* and \* ns

Results on the characteristics of leaf area, leaf number, leaves fresh weight and dry, the amount of chlorophyll, the amount of stored nitrogen, potassium and phosphorus represents significant increase in the level of a percent of irrigation treatments complete than other levels (Table 1 and Figure 2 to 9). According to report

of Romero et al (2004)[23], severe water stress caused by early deciduous leaves and almond trees will reduce the growth rate, the result is a decrease in leaf area. In conditions hard stress, declined the amount of carbohydrates in the stem of almond trees, this reduces the efficiency and durability of the leaf. Based on results of this study, the amount of nitrogen stored in high-stress conditions (33% of available water maintains) than moderate stress (66% of available water maintains) increased (Figure 9) that with the results of Esparza et al (2001) [24] is consistent. The amount of leaf area, leaf number, total amount of chlorophyll, the amount of nutrients, potassium and phosphorus in the leaves of pear trees[25], Olive[26], almond[27] and apple [28] under water stress is reduced. Results of this study showed that, in conditions water stress declined sharply, leaf area and growth; the reason is that the loss of early leaves (Fig. 2, 1 and 3) that is consistent with the results mentioned. Dlkhovsh et al (2007) [29] reported that the amount of chlorophyll in safflower under water stress increased. According to reports of Gyrona et al (1993) [30], effects of water stress on the amount of chlorophyll is very diverse and variable and is dependent on environmental conditions and plant genotypes, water stress is followed in some species a decrease in the content of chlorophyll and some of the increase [31]. In this study was observed in conditions 66% stress of available water maintenance, the total amount of leaf chlorophyll a decrease and in 33 percent rise (Figure 5). In this regard, the results of this study are similar to the reports pointed out. The interaction between two factors of irrigation and effect of microorganisms was observed at the level of 1 percent difference (Table 1). This review showed that in water stress conditions 33 percent of water stored, the seedlings been faced with severe water stress, EM effect on growth was more pronounced in the case of complete irrigation. The interaction effect the use of effect of microorganisms and irrigation was evident in leaf traits and leaf number that important factors are development. The growth and leaf number has increased with the Em in stress condition (Figures 1, 2 and 3). The use of microorganisms in different products is increased the growth branches, leaf area and fresh and dry weight and total biomass. It could be due to better root development as a result of biologically active substances in the composition of the Em[32] Em with produced growth hormones (Auxin and Gibberellin) has a positive effect on plant growth[33]. The microorganisms in the conditions of severe water stress are caused more the absorption of nutrient elements (nitrogen and phosphorus) (Figures 7 and 9). During stress increased soil nutrient concentration and osmotic pressure, therefore absorption of nutrients such as nitrogen decreases, accordingly, greater nutrient uptake by plants under stress could be a marker associated with drought resistance[2,34,35]. Thus, the combination of Em has increased resistance to drought almond trees compared to controls.

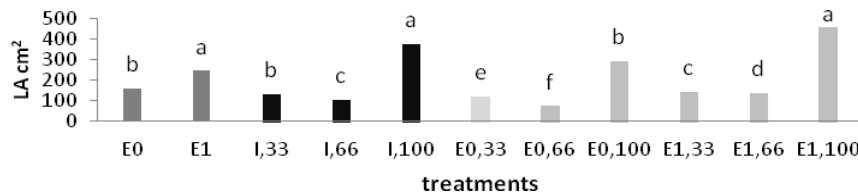
### CONCLUSION

According to survey results, use of combined Em in the almond trees could increase growth and other factors that, it to be also cause efficient use of irrigation water, but is necessary, further studies will be on the effect of different concentrations of this substance on improvement of plant growth and development, for this purpose determine the suitable concentration.



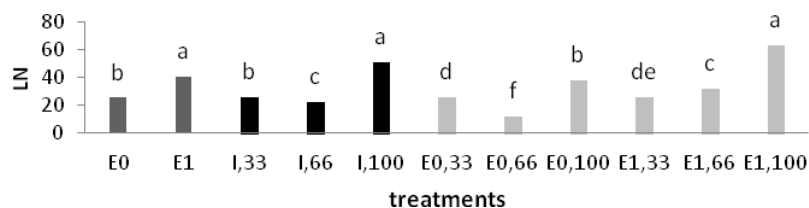
**Figure 1.** Average seedling growth, Influenced by the treatments of Em { E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc) and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 5% using LSD test.



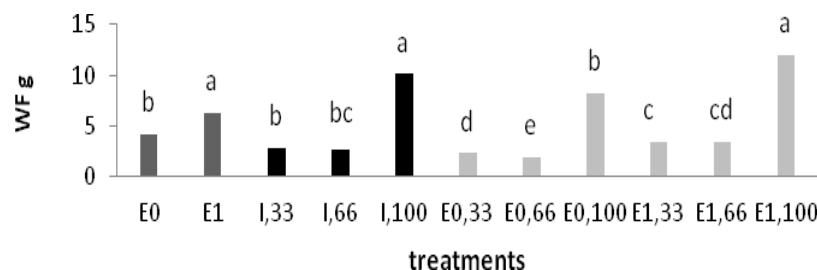
**Figure 2.** Average leaf area, Influenced by the treatments of Em { E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc) and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test.



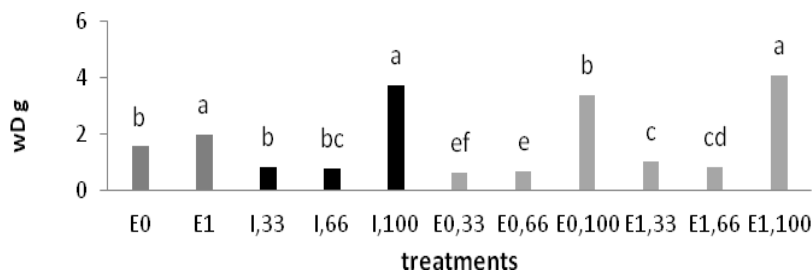
**Figure 3.** Average number of leaves, Influenced by the treatments of Em { E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc )and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test.



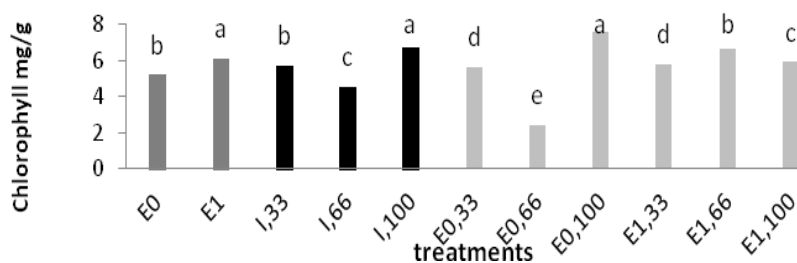
**Figure 4.** Average fresh weight of leaves, Influenced by the treatments of Em { E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc )and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test.



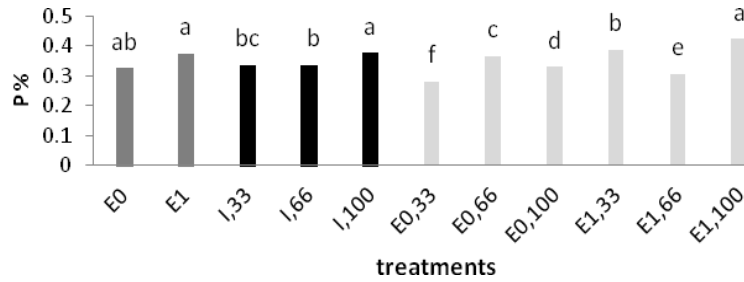
**Figure 5.** Average dry weight of leaves, Influenced by the treatments of Em { E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc )and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test



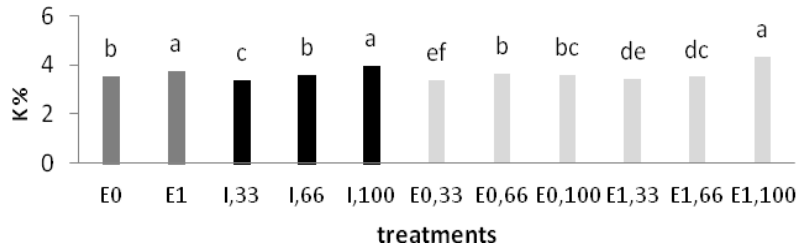
**Figure 6.** Average total chlorophyll in the leaves, Influenced by the treatments of Em { E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc )and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test



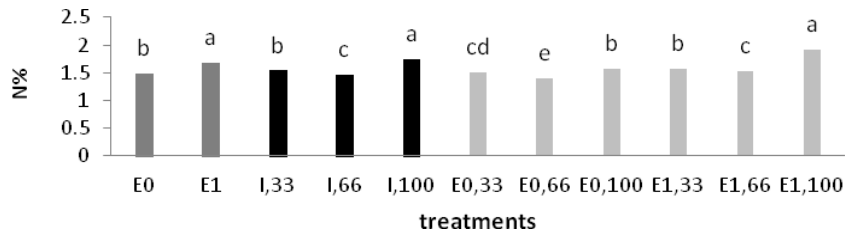
**Figure 7.** Average percentage of available P in leaves Influenced by the treatments of Em {E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc) and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test



**Figure 8 .** Average percentage of available K in leaves Influenced by the treatments of Em {E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc) and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test



**Figure 9.** Average percentage of available N in leaves, Influenced by the treatments of Em {E0(Concentration of 0%) and E1(Concentration of 0.5%) and irrigation{I,100 (the Fc),I66(66% of Fc) and I,33(33% of fc) and Their interaction.

Different letters mean that the significance level of 1% using LSD test

## REFERENCES

- 1- Rom R.C., Carlson R. F. 1987. Rootstocks for fruit crops, John Wiley and Sons, New York.
- 2- Isaakidis A., Sotiropoulos T., Almaliotis D., Therios I., Stylianidis D. 2004. Response to severe water stress of the almond *Prunus amygdalus*. 'Ferragnès' grafted on eight rootstocks, *New Zealand Journal Crop and Horticulture Sci.*, 32: 355–362.
- 3- Torrecillas A., Alarcón J. J., Domingo R., Planesa J., Sánchez-Blanco M. J. 1996. Strategies for drought resistance in leaves of two almond cultivars, *Plant Science.*, 118: 135-143.
- 4- Fereres E., Cruz-Romero G., Hoffman G. J., Rawlings S. 1979. Recovery of orange trees following severe water stress, *Journal of Applied Ecology.*, 16: 833-842.

- 5- Higa T. 1991. Effective microorganisms:A biotechnology for mankind.p. 8-14. In J.F. Parr, S.B. scanning electron microscop, Hort Science., 25: 1106. Abstract.
- 6- Imai S., Higa T. 1994. Kyusei nature farming in Japan: Effect of EM on the growth and yield of spinach. p. 92-96. In J.F. Parr., S.B. Hornick and M.E. Simpson (ed.).  
Proceedings of the Second International Conference on Kyusei Nature Farming. U.S.  
Department of Agriculture, Washington, D.C., USA.
- 7- Zachria P. P. 1995. Studies on the application of Effective Microorganisms (EM) in paddy,sugarcane and vegetables in India. Proc, of Second Conference on Effective  
Microorganisms., P: 31-41.
- 8- Keomanichanh K.1995. Effect of EM application on fruit trees and paddy rice. Proc. of Second Conference of Effective Microorganisms., p: 87-89
- 9- Ahmad R., Hussain T., Jilani G., Shahid S.A., Akhtar S.N., Abbas M.A.1995. Use of effective microorganisms for sustainable crop production in Pakistan., Pp. 15-27.
- 10- Xu H.L.2000. Effect of a Microbial Inoculant, and Organic Fertilizer, on the Growth, Photosynthesis and Yield of Sweet Corm, Journal of Crop Production., 3(1): 183-214.
- 11- Wang R., Xu H.L. ,Mridha M.AU. 2000. Effect of Organic Fertilizer and EM Inoculation on Leaf Photosynthesis and Fruit Yield and Quality of Tomato Plants, Journal of Crop Production., 3(1):173-182.
- 12- Arnon D.I .1949. Copper enzymes in isolated chloroplasts and polyphenol oxidase in *Beta vulgaris*, Plant Physiol0gy., 24(1): 1–15.
- 13- Emami A.1997. Methods of plant analysis, Technical Publication, No. 182, Printing in Soil and Water Research Institute of Iran.
- 14- Khan B. M., Hossain M. K., Mridha M. A. U.2007. Effect of microbial inoculants on Albizia saman germination and seedling growth , Journal of Forestry Research ., 17( 2) :99-102.
- 15- Thach N.Q., Long C.A., Liet V.V., Trung N.V., Thanh N. X., Dich T.V., Duong N., Tuna N.K., Xuan L.T.H., Dan P.V.1999. Preliminary Results of Effective Microorganisms (EM) Application in Vietnam., Pp: 254-260. In: Senanayake Y.D.A., Sangakkara U.R., (eds.): Kyusei Nature Farming and Effective Microorganisms, Proceedings of the 5th International Conference on Kyusei Nature Farming and Effective Microorganisms for Agricultural and Environmental Sustainability, Bangkok, Thailand.
- 16- Khaliq A., Kaleem Abbasi M., Hussain T.2006. (EM) on seed cotton yield in Pakistan. Bioresource Technol.,97: 967-972.
- 17- Mohamed F., Sahain M., Elham Z., Motty A., Mohamed H., Shiekh El ., Laila F . 2007. Effect Of Some Biostimulant On Growth And fruiting Of Anna Apple Trees In Newly Reclaimed Areas, Research Journal of Agriculture and Biological Sciences., 3(5): 422-429
- 18- Romuald G ., Kleibier T.2010. Effect of effective microorganisms(EM) on nutrient contents in substrate and development and yielding of Rose(*Rosa x hybrida*) and Gerbera (*Gerbera jamesonii*) Ecological Chemistry and Engineering's., 17( 4).
- 19- Martin P. Glatzle A ., Klob W., Omay H ., Schmidt W.1989. N<sub>2</sub> Fixing bacteria in the rhizosphere: quantification and hormonal effect on root development. Z. p flonzener nahr bodenk.,152: 237-245.
- 20- Jagnow G., Hoflich G ., Hoffmann H .1991. Inoculation of non symbiotic rhizospher bacteria, possibitites of increasing and stabilizing yield, Angew botonic., 65: 97-126.
- 21- Daly M.J., Stewert D.P.C.1999. Influence of effective microorganicms (EM) on vegetable production and carbon mineralization a preliminary investigation, Journal of Sustainable Agriculture., 14: 15-25
- 22- Attala A., Eman S., Amal M.,Seginy E.I., Eliwa G.I .2000. Response of "le-connte" pear trees to foliar application with active dry yeasts. Journal of Agricmansoura University., 25: 7701-7707.
- 23- Romero P., Navarro J.M., García F., Botía Ordaz P . 2004. Effects of regulated deficit irrigation during the pre-harvest period on gas exchange, leaf development and crop yield of mature almond trees,Tree Physiol., 24(3):303-12

- 24- Esparza G., Dejong T. M., Weinbaum S. A., Klein I .2001. Effects of irrigation deprivation during the harvest period on nonstructural carbohydrate and nitrogen contents of dormant, mature almond trees, *Tree Physiology.*, 21(14):1081-6.
- 25- Javadi T., Arzani K ., Ebrahimzadeh H., Moieni A .2004. Effects of drought stress on physiological and biochemical characteristics of nine genotypes of Asian pear (*Pyrus serotona* Rehd, PhD Thesis, Department of Horticulture (Pomology) Tarbiat Modares University, Iran.
- 26- Yazdani N., Arzani K., Arjy A .2006. Moderate drought stress, by Paclobutrazol on olive varieties Blade and Mission, Master's thesis, Department of Horticultural Sciences, Tarbiat Modares University, Iran.
- 27- Rouhi V., Samson R., Van Damme P., Lemeur R.2006. Seed germination of *Prunus scoparia* (Spach) C.K. Schneider and drought stress evaluation based on ecophysiological parameters and growth characteristics for three contrasting almond species (*P. dulcis* (Miller) D. Webb, *P. lycioides* (Spach) C.K. Schneider and *P. scoparia* (Spach) C.K. Schneider), PhD thesis, Ghent University, Belgium.
- 28- Hassani Gh.,Norjo A., Henare M.2008. Effect of different irrigation levels on yield and fruit quality of Golden Delicious apple varieties, *Journal of seedlings and seeds*, 1: 51-62
- 29- Delkhosh B., Shiranirad A.H., Noormohammadi G.H., Darwish F.2007. Effect of drought stress on grain yield and chlorophyll in rapessed cultivars, *Journal of Agricultural Sciences.*, 12 (2): 359-368.
- 30- Girona J., Marsal J., Cohen M., Mata M., Miravete C.1993. Physiological and yield response of almond (*Prunus dulcis* L.) to different irrigation regimes, *Acta Horticulture.*, 335: 389-398.
- 31- Ward K., Scarth R., Daun J., Mcvetty P.B.E.1992. Effects of genotype and environment on seed chlorophyll degradation during ripening in four cultivars of oilseed rape (*Brassica napus* L.),*Canadian Journal of Plant Science.*,72:643-649.
- 32- Lim T.D., Pak T.W ., Jong C.B. 1999. Yields of Rice and Maize as Affected by Effective Microorganisms., Pp: 92-98. In: Senanayake, Y.D.A ., U.R. Sangakkara, (eds.) *Kyusei Nature Farming and Effective Microorganisms*, Proceedings of the 5th International Conference on Kyusei Nature Farming and Effective Microorganisms for Agricultural and Environmental Sustainability, Bangkok, Thailand.
- 33- Chowdhry A.R., Hussain M.M., Mia M.S., Karim A.M.M.S., Haider J., Bhuyan N.I., Shifuddin K .1994. Effect of organic amendments and EM on crop production in Bangladesh. 155-163. In: Parr, J.F., S.B. Hornick and M.E. Simpson, (eds.) *Kyusei Nature Farming*, Proceedings of the Second International Conference on Kyusei Nature Farming, Piracicaba, SP, Brazil.
- 34- Fereres E., Aldrich T. M., Schulbach H., Martinich D. A.1991. Responses of young almond trees to late-season drought, *California Agricultur.*, 1: 1-12.
- 35- Holevas C., Stylianidis D ., Michailidis Z .1985. Nutrient element variability in the leaves of almond trees in relation to variety, rootstock and the vegetative part of the tree, *Options IAMZ.*, 85/1: 111–120.