Biosci. Biotech. Res. Comm. Special Issue No 1:298-305 (2017)

BBRC
Bioscience Biotechnology Research Communications

The effect of drought stress on morphological and physiological traits and essence percentage of medicinal plant, *Nigella sativa*

Fatemeh Soltan Shahattary[1] and Cyrus Mansourifar[2]*

[1]*MSc of Agronomy, Department of Agriculture, Payame-Noor, Karaj, Iran*
[2]*Associate Professor of Agronomy, Department of Agriculture, Payame-Noor, Karaj, Iran*

ABSTRACT

Various environmental stresses are of factors reducing agricultural yields. But applying these stresses on the medicinal plants effects on their active substances (constituents). In the current study, the effect of different amounts of irrigation on some qualitative and quantitative factors of Nigella Sativa was examined. The experimental design was a randomized complete block with three replications. Treatments included 3 levels of drought stress of 50 (severe stress), 70 (medium stress) and 90 (control) percent of field capacity. The required amount of water in each treatment and at any time was calculated through the measurement of soil moisture content using a hygrometer and as a result of water required to reach field capacity. During physiological investigation grain number per follicle, number of grains per plant, grain weight, biological yield, grain yield, harvest index, soluble protein, proline concentration and the essence percent were measured. The results showed that drought stress on morphological, physiological and the essence percent at 1% probability level, there was a significant difference. The results showed that with increasing stress levels, the essence percent, proline, and soluble protein increased. As well as applying moderate drought stress, the largest number of grains per plant, biological yield and grain yield was achieved, but with increasing levels of stress, these traits (characteristics) were decreased. In other traits (characteristics) increasing levels of stress, their amount was decreased.

KEY WORDS: MORPHOLOGICAL, PHYSIOLOGICAL, DROUGHT STRESS, THE ESSENCE PERCENT, NIGELLA SATIVA

ARTICLE INFORMATION:

**Corresponding Author: cyrusamf@yahoo.com*
Received 27th Dec, 2016
Accepted after revision 2nd March, 2017
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007
Thomson Reuters ISI ESC and Crossref Indexed Journal
NAAS Journal Score 2017: 4.31 Cosmos IF : 4.006

Online Contents Available at: http//www.bbrc.in/

INTRODUCTION

Water is one of the most important environmental factors in the production of various products, so that its deficiency can severely damage the growth and development as well as the active ingredient of medicinal plants (Omidbeygi, 2005). Irregular precipitation (rainfall) patterns in arid areas expose plants to different intensities of drought stress. Often, high temperatures and poor nutritional status also makes it more complex effects of drought. Water is not only ecologically but also physiologically is important for plants, because involved in most of the internal processes of plants and almost all metabolic activities of plant cells such as manufacturing of active ingredients in medicinal plants depends on the presence of water (Letchamo et al., 1994). Therefore, one of the most important goals in plant breeding programs is the study of their tolerance facing drought (Yadav and Bhathagar, 2001). Long-term water stress effects on all metabolic processes in plants and thereby reduces plant production (plant throughput). The survival of plant in limiting conditions of drought stress requires its ability to survive in conditions of severe drought (osmotic) caused by drought. Maintenance of optimum moisture and maintaining the structure of biopolymers under stress conditions is crucial for the survival of the plant (Kuzentsov and Shevykova, 1999). Therefore, the optimization of irrigation management due to lack of water along with choice of suitable crops plant to cultivate is particularly important (Ghanbari et al., 2007).

In recent years, herbs have returned again to Iran medicine and have been cultivated in all parts of the country. Medicinal herbs (plants) due to natural essence and similar medicinal compounds together with, better adapt to the body and are usually free of side effects, especially in cases of prolonged use and for chronic diseases, therefore, are very convenient. Thus has a considerable advantages compared to chemical drugs. Also, having good therapeutic effects as well as resistance of some diseases to some medications and sensitizing in people towards some industrial medications have been the factors increased consumption of herbal medicinal products in recent decades. According to the WHO (WHO) 80 per cent of the world people to the early health care has traditionally been dependent on medicinal plants and natural products. Historically, medicinal plants have great importance in the development of communities and extensive research to find herbal medicinal products and natural materials have been done throughout history. The cultivation of these plants requires the evaluation of their capability to produce on a large scale and their resistance to adverse environmental conditions such as lack of irrigation water (Cronquist, 1981).

Nigella sativa (L.) (black cumin) is an annual plant belonging to the Ranunculaceae family, with a height of 60 to 70 cm, the leaves are gray-green color with notched fiber, white to blue flowers and capsule fruit (follicles) that there are large number of fragrant black grains within. In the Nigella sativa (black cumin) grains, there is 40 percent constant oil and about 1.4 percent essence (Islam et al., 2004). Grains from these plants in medicine are used as a carminative, menstruation facilitator, laxatives, lactate stimulant, anti-constipation and sexual power amplifier in men (Riaz and Chaudhary, 1996). In addition to its automotive growth in various regions of Europe, West Asia and Iran (Esfahan and Arak) , this plant is cultivated for crops (Akbarinia et al., 2005) which meanwhile, the limiting environmental factors can reduce growth and performance of it. The conducted studies showed that appropriate moisture increased the plant height and more foliage and thus increased the number of capsules per Nigella Sativa plant and resulted in increased grain yield (Akbarinia et al., 2005).

Also, the study conducted by Mozzafari et al., (2000) on black cumin (Nigella Sativa) showed the increased drought stress increases grain oil percent but oil yield due to reduced yield in high stresses irrigation decreases. Srivastavs and Misra (2000) has reported effects of sufficient irrigation on growth and essence content of peppermint. Several reports also show an increase in extracted oil per unit area by reducing irrigation water (Mozzafari et al., 2000).

The determination of the most appropriate amount of irrigation water in order to produce the best quality and quantity of the product in the herb lack cumin (*Nigella sativa*) is essential. Therefore, in this study the effects of water shortage in various stages of development on the morphological factors, the amount and main components of essence and physiological traits were evaluated.

MATERIALS AND METHODS

To evaluate the effect of irrigation intervals on morphological, physiological and amount of essence of herb Nigella Sativa in the Deh-Shir-Khan village field of Arak city, an experiment was conducted in the 2016 crop year. The mean of average annual temperature and precipitation have been reported 13.9 ° C and 341 mm, respectively. Texture of the field soil is silt clay.

The experimental design was a randomized complete block with three replications. Treatments included 3 levels of drought stress 50 (severe stress), 70 (medium stress) and 90 (control) percent of field capacity. Grain rate was about 1.5 grams per square meter. The width of each replication was 3 meters and the distance between main plots and subplots was considered about

40 cm. Also, the distance between each repetition 2 m for irrigation water move and walk were considered. In order to study changes in the water content of the soil profile in the various treatments of irrigation, soil moisture content was measured at depths of 15 and 30 cm using hygrometer. To determine soil water holding capacity accidentally six undisturbed samples from the farm were taken from two depths 15 and 30 cm. Sampling was conducted using auger and special capped rings. Samples were taken saturated state after the weighing. The amount of gravity water the moisture contained in the soil at field capacity and permanent wilting point using pressure plates and then drying in oven calculation of the percentage of residual moisture in the soil were determined.

Soil water holding capacity of test run at field capacity points and permanent wilting were calculated. The amount of required water in each treatment and at any time through the measurement of soil moisture content using a hygrometer and as a result of the measurement of required water to reach field capacity was calculated. Irrigation regimes following established plants and thinning were applied. Fighting against the weeds mechanically and hand weeding was done for four times.

Finally, the number of grains per follicle, number of grains per plant, grain weight, biological yield, grain yield, harvest index, soluble protein, proline concentration and essence percent were measured. To measure the percentage of essence water distillation (Clevenger, 1928) was performed using Clevenger apparatus.

For the extraction of soluble proteins in leaves, one gram of frozen leaf samples at -80 ° C using liquid nitrogen in a porcelain mortar was powdered and then adding 4 ml of extraction buffer with the composition: Tris-HCl one molar (pH = 7.5) Na2EDTA + 5% one molar 0.2% + two- Mercaptoethanol 0.4% in distilled water to sample and its homogenization, the mixture was transferred to the capped tubes followed by 13,000 rpm for 20 min was centrifuged and transparent extract was separated from solution and was kept at -20 ° C. The quantitative amount measurement of soluble proteins based on Bradford method (Bradford, 1976) using Bio-Rad reagent a spectrophotometer with wavelength at 595 nm was read and then by depicting curves derived from readings protein standards taken from bovine serum albumin (BSA) in specified concentrations on graph paper were determined.

Measurement of proline, according to Bates Method (Bates et al., 1973) was performed. To measure proline of the leaf, firstly, 0.2 g of leaf blade was cut then it was in a porcelain mortar with good liquid nitrogen was crushed. Then 10cc of Sulfosalicylic acid 3% was poured into a mortar and leaf sample was crushed good and then was passed through filter paper and cc2 of resulting solution was removed and with cc2 ready-made solution Nine dimenhydrinate (how to prepare it will be mentioned) and cc2 acetic acid was poured into the test capped tube and was placed for 1 hour in a warm bath. Then, the samples were removed from the bath and placed in the ice and to its temperature reach the temperature of room, so that if you touched it does not feel the heat. At this stage under the hood, cc4 amount of toluene was added to each sample and shaken well until completely blended. After a few moments, two phases were resulted that the low (bottom) phase was more transparent and higher (top) phase was red colored (depending on the amount of leaf proline). The toluene as a witness (control) in a spectrophotometer was used to measure the wavelength of light. Then cc4 of top phase was removed and placed into the spectrophotometer under the wavelength of 520 nm and read number rather than variable X was placed in the equation (6). The obtained Y is placed in Equation 7 to obtain the leaf proline in terms of micromoles per mg.

$$Y=42/281X+4/6698 \tag{1}$$

$$\text{Proline rate} = \frac{Y \times 5}{200\,(\text{leaves mg})} \tag{2}$$

The data were analyzed using SAS 9.1 software. Also means comparison using test LSD (at 5%) was performed.

RESULTS AND DISCUSSION

THE NUMBER OF GRAINS PER FOLLICLE

Analysis of variance showed that between levels of drought stress in terms of the number of grains per follicle, there was a significant difference in 1% level (Table 1). Also, the results of mean comparison showed that the control treatment (90% FC) had the highest amount of grain in the follicles (Table 2). The severe drought treatment (50% FC) has the lowest number of grains in follicles (Table 2). Effect of drought stress caused the number of grains per follicle at 70 and 50 percent of field capacity, respectively, to the 22.08 and 29.40 percent decrease compared to the control treatment. The results were in line with many studies. For example, in a study by Rezapour and colleagues (2012) Nigella sativa grain yield in irrigation treatment after 150 mm compared to treatment of 50 mm evaporation from pan surface treatment decreased 22.8 percent, so that the drought stress had a significant impact on all components of grain yield including biomass, number of capsules per plant and grains per capsules and reduced them. Koochaki and colleagues (2007) reported that

Table 1. Analysis of variance Nigella sativa morphological traits under drought stress

Mean of squares						Degrees of Freedom	Sources of changes
The number of grains per follicle	number of grains per plant	1000-grain weight	biological yield	grain yield	harvest index		
30.76**	127.45**	0.007**	94.34**	67.46**	69.33**	2	Block
144.43**	2593.78**	0.120**	4033.00*	3238.78**	212.34**	2	Drought stress
0.44	4.11	0.005	324.32	13.76	3.16	4	Pilot error
1.77	1.23	3.06	10.33	3.63	5.74	Coefficient of variation	

Table 2. The comparison of mean of morphological traits of Nigella sativa under drought stress

Mean of squares						Drought stress (Field Capacity)
The number of grains per follicle	number of grains per plant	1000-grain weight	biological yield	grain yield	harvest index	
45.33a	172.66b	2.55a	188.65a	116.33b	32.33b	90 percent
35.32b	190.00a	2.23b	201.61a	125.66a	38.66a	70 percent
32.00c	132.66c	2.15c	132.60b	64.66 c	22.00 c	50 percent

water stress (drought) by failing to provide photosynthetic inputs reduced yield components of the fennel. It seems that these factors have been effective in reducing some yield components of Nigella sativa (black cumin).

THE NUMBER OF GRAINS PER PLANT

Analysis of variance showed that the effect of drought stress on grain number per plant, there was a significant difference at 1% level (Table 1). The results of comparison of mean showed that mild stress had the highest number of grains per plant and severe stress (50% FC) had the lowest number of grains per plant (Table 2). In general, the number of grains per plant in mild drought stress (70% FC) increased 10.04 percent, while applying severe stress the number of grains per plant decreased at 23.16.

Increased number of grains in the less drought stress could be relevant to the more numbers of follicles, bigger and better plant growth. Considering the fact that the number of grains per follicle in fact determines the storage capacity, therefore, the greater the number of grains, plants with larger and more storage has been produced to receive photosynthetic material and increased this trait will improve the yield. In many crop plants, water stress, especially during flowering reduces the number of fertilized flowers, abortion and subsequent reduction of grain and therefore is greatly reduced performance (yield) (Rezaei, 2012). Shabanzadeh and Golvi (2012) showed that the effects of irrigation interval on height, number of branches, number of capsules per plant, grains per flowers and plant, grain (grain) weight, biological yield and harvest index were significant and increasing irrigation intervals, the studied features decreased. The result of this study was consistent with Rezvani and colleagues' one (2012).

THE 1000 GRAIN WEIGHT

Analysis of variance showed that the effect of drought on the w1000 grain weight, there was a significant difference in the 1% level (Table 1). The results of mean comparison showed that the control treatment (90% FC) had the highest amount of 1000 grain weight (Table 2). Also severe stress treatment (50% FC) had the lowest 1000 grain weight (Table 2). In general, drought stress decreased one thousand grains weight at 8.62 and 15.68 percent in the mild and severe drought stress. The results are consistent research conducted by Shabanzadeh and Golvi (2012). The 1000 grain weight indicates the status and reproductive period of any plant and since by beginning of flowering and determination of the number of grains per plant, grains begin to receive and store values of their photosynthesis, there should be a difference between the 1000-grain weight and normal modes when the plant placed under moisture stress. But the 1000 grain weight is among the factors that most is influenced by genetic control and has a high heritability and less affected by environmental factors.

BIOLOGICAL YIELD (FUNCTION)

Analysis of variance showed that the effect of drought on the on biological yield trait, there was a significant difference in the 5% level (Table 1). The results of mean comparison showed that the mild stress level had the highest amount of biological yield and sever stress (50 percent FC) had the lowest biological yield (Table 2). It should be noted that moderate stress level and control had no significant difference. In general biological yield in mild drought stress (70% FC) increased 6.90 percent, while applying severe stress the number of grains per plant was decreased as 29.71 percent.

Plant growth is influenced by a series of biochemical and physiological processes such as photosynthesis, respiration, material transfer, ion absorption and metabolism of food which are also involved in plant dry weight of plant (Kafı et al, 2010). These processes have a direct relationship with the amount of available water and its continuity. With increasing irrigation intervals, these processes are disrupted, and the plant can't produce their maximum potential dry matter (Imam and Zavareh, 2008). On the other hand, the drought stress reduces the uptake of water and nutrients, crop growth rate, growth period, the plant photosynthesis, plant height, growth rate and root development and all of these factors ultimately lead to a reduction in dry matter production (Pessarkli, 1999). According to the Ahmadian and colleagues (2010) increased intensity of water deficiency leads to a reduction in biomass of chamomile in a way that at the 90% treatment of field capacity the most and at 50% treatment of field capacity the least plant biomass was produced, respectively. Rezapour and colleagues (2012) in a study on the Nigella sativa reported that decreased production yield by increase in the drought is related to reduction in plant height, leaf area reduction and enhanced allocation of photoassimilates to the root rather than shoot (aerial part) of plant.

THE GRAIN YIELD (PERFORMANCE)

Analysis of variance showed that the effect of drought stress on the on grain yield trait, there was a significant difference in the 1% level (Table 1). The results of mean comparison showed that the mild stress level had the highest amount of grain yield and sever stress (50 percent FC) had the lowest grain yield (Table 2). In general grain yield in mild drought stress (70% FC) increased 8.02 percent, while applying severe stress the number of grains per plant was decreased as 44.41 percent. Goldani and Rezvani Moghadam (2006) argue that the available accessible soil moisture increases the plant canopy development, thereby absorb more radiation energy plant leading increase yield and its components in the plant. On the other hand, the reduction of photosynthetic area of leaves and shortening the duration of grain filling and prematurity of treatments under drought can be effective in reducing grain yield. Aghayee and Ehsanzadeh (2012) found that drought stress by reducing leaf area, chlorophyll content, openings conductance and ultimately reduce the rate of photosynthesis decreased the function of paper grains pumpkin.

THE HARVEST INDEX (HI)

Analysis of variance showed that the effect of drought on the on harvest index trait, there was a significant difference in the 5% level (Table 1). The results of mean comparison showed that the mild stress level had the highest amount of harvest index and sever stress (50 percent FC) had the lowest harvest index (Table 2). It should be noted that moderate drought stress level and control had no significant difference. In general, harvest index in mild drought stress (70% FC) increased 19.57 percent, while applying severe stress, harvest index was decreased as 31.95 percent. The effect of irrigation interval on plant height, number of branches, the number of capsules per plant, number of grains per plant, 1000-grain weight, biological yield and harvest index was significant and with increasing irrigation interval, the studied features were decreased. Most grain yield grain was obtained in irrigation interval of seven days (average irrigation) (Shabanzadeh and Golvi, 2012).

THE PERCENTAGE OF ESSENCE

Analysis of variance showed that between levels of drought stress in terms of the percentage of the essence, there was a significant difference at 1% level (Table 3). Also, the results of mean comparison showed that the control treatment (90% FC) had the lowest percentage of essence (Table 4). The severe drought treatment (50% FC) had the most percentage of essence (Table 4). Effect of drought stress resulted in levels of 70 percent of field capacity, respectively, to the 8.33 and 11.11 percent increased compared to the control.

The plants depending on the plant species and genotypes are different in reactions to drought stress. So water setting and management in medicinal and aromatic plants are important in terms of essence production. Rabiee and colleagues (50) in the study of the effect of drought stress (no stress, moderate stress and severe stress) on cumin showed that the plants under moderate stress compared to the two treatments of no stress (control) and severe stress had higher essence. Rezai Chaîne (2012) in the study of effect of different irrigation treatments on the accumulation of essence, its composition and some eco-physiological traits in fennel found that drought stress increases the fennel essence, but it reduces the essence yield and increased fennel essence due to drought stress has been reported as a result of the higher concentration of essence glands due to reduced

Table 3. Analysis of variance of essence percentage, soluble protein and proline traits of Nigella sativa under drought stress conditions

Mean of squares			Degrees of Freedom	Sources of changes
Essence percentage	soluble protein	proline		
0.00001**	144.10**	2.95**	2	Block
.00001**	6330.08**	9.99*	2	Drought stress
.0000002**	129.64	0.76	4	Pilot error
1.35	15.14	4.93	Coefficient of variation	

Table 4. The mean comparison of percentage of essence, soluble protein and proline of Nigella sativa under drought stress

Traits			Drought Stress (Field Capacity)
Essence percentage	soluble protein	proline	
0.036b	0.37b	15.95b	90 percent
0.039a	3.40b	17.76ab	70 percent
0.040a	81.40a	19.60a	50 percent

leaf area caused by stress and more accumulation of essence. It seems that drought stress increases secondary metabolites (essence) that have protective effect against stress such as drought stress in plants. The results of other studies on the effect of irrigation intervals on the production of secondary metabolites and the essence percentage of savory and rosemary attest (confirm) the results of this trial, so that with increasing irrigation intervals and reduced field capacity, the essence percentage of these plant has also been added (Baher et al., 2002). Rezainejad and colleagues (2002) reported that irrigation interval had a significant effect on the essence percentage of Cumin. Javanshir and colleagues (2002) also examining the effects of irrigation on the amount of essence of anise observed that irrigation treatment had significant effect on the essence percentage.

SOLUBLE PROTEIN

Analysis of variance showed that the effect of drought stress on soluble protein trait, there was a significant difference at the level of 5 percent (Table 3). The results of mean comparison showed that the control treatment had the lowest levels of soluble protein and severe stress (50% FC) had the highest amount of soluble protein (Table 4).

Also, Ghorbani Javid and colleagues (2007) reported that concentrations of soluble proteins at different levels of drought stress in the tolerant genotypes of alfalfa is almost constant that seemingly has been favorable maintaining the structure of the plant and plant activities, while in sensitive genotype with increasing stress, the concentration of soluble proteins decreased that can be caused by decreased the frequency of precursors producing protein (Substrates) and a decrease in gene expression or manifestation of their origin.

THE PROLINE (AN AMINO ACID)

Analysis of variance showed that between levels of stress in terms of proline, trait there was a significant difference at 1% level (Table 3). Also, the results mean comparison showed that the control treatment (90% FC) had the lowest proline, (Table 4). The severe drought treatment (50% FC) had the most proline (Table 4). The effect of drought stress caused proline at 70 and 50 percent of field capacity, respectively, to the 11.34 and 22.88 percent increase compared to the control.

The study was carried out on eggplant it was shown that applying water shortage stress leads to increased concentration of leaf proline were by re-watering the proline decreased (Taghavi Razavizadeh, 2004). Zeifnejad and colleagues (1997) in their study on sorghum and Griffin and colleagues (2004) in their study on Judas tree and Mehrabi (2009) on sesame reported praline increased under drought stress. Akhondi and colleagues (2007) and Kydamby and colleagues 1990) in their experiments on the alfalfa reported the effect of drought stress on proline. Pagter and colleagues (2005) in their study on the straw plant reported that just under severe stress water, proline increase slightly and thus until the critical level, has little significance. Safarnejad (2004) examining the effects of drought stress on alfalfa genotypes reported that with increasing drought stress, proline concentration increases on leaves. Thus, it can be concluded that genotypes which have produced more proline in the effect of drought stress, this increased proline prevents further decline and loss and show less reduction of yield (performance).

TOTAL CONCLUSION

Totally, the results of this study showed that drought stress reduced number of grain per follicle, 1000-grain weight and grain yield. On the other hand, increased drought stress increased the soluble protein and proline content. For other traits moderate stresses drought increased the traits compared to control.

REFERENCES

Aghayee. A. H and P. Ehsanzadeh. 2012. Effect of irrigation and nitrogen on yield and some physiological parameters of pumpkin paper. Journal of Horticultural Science

Ahmadian, A., A. Ghanbari, A & M. Golvi. 2010. Interaction of drought and manure on yield components, essence and chemical composition of cumin. Iranian Journal of Field Crop Science

Akhoondi, M., P. Safarnejad & M. Lahooti, 2007. Effect of drought stress on Proline Accumulation and changes in Yazdaian alfalfa elements, Nikshahri and Ranger (*Medicago sativa* L.), Science and Technology of Agriculture and Natural Resources, 10: 156-174.

Bates, L. S., R. P. Waldren and I. D. Teare. 1973. Rapid determination of free proline for water- STR Journal 54-23 221

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding. Ann. Biochem., 72:248- 254.

Ghanbari, F., Nadjafı, S., Shabahang, S., and Ghanbari, A. 2007. Effects of irrigation regimes and row arrangement on yield, yield components and seed quality of pumpkin (Cucurbita pepo L.). Asian Journal of Plant Science 6:1072-1079.

Goldani, M. And P. Rezvani Moghaddam. 2006. Effect of planting date and drought levels on yield and yield components of rain fed and irrigated pea in Mashhad. Journal of agricultural research.

Griffın, J. J., T. G. Ranney and D. M. Pharr. 2004. Heat and drought influence photosynthesis, water relations, and soluble carbohydrates of two ecotypes of redbud (*Cercis canadensis*). J. Am. Soc. Hortic. Sci. 129: 497-502.

Imam, Y. And M. Zavaerh.2006. Drought tolerance in higher plants. Publishing Publishing Center of Tehran University, Page 186

Jafarnya, S., S. Khosroshahi, and Ghasemi M. 2012. A comprehensive and illustrated guide of properties and use of medicinal plants, Sohkan Gostar Publications. page 179

Javanshir, A., Zehtab Salmas, S Omid-beigy, R, Alyari, H. and Qasmygolzany, k. 2002; The effects of irrigation and sowing date on the essence percentage and anethole in the herbs anise. National Conference of Iranian Medicinal Plants / Research Institute of Forests and Rangelands) 24-26 Bahman 1380 (in Persian date), pp. 117-118

Kafı M. A. Borzooei., Salehi, A. Kamandi, A. Massomi & cJ. Nabati. 2010; The physiology of environmental stress in plants, published by University of Mashhad, Mashhad, page 502

Kafı M. A. Borzooei., Salehi, A. Kamandi, A. Massomi & cJ. Nabati. 2010; The physiology of environmental stress in plants, published by University of Mashhad, Mashhad, page 502

Khoshbin, S. 2010. One hundred miracle plants. New World Press publication, Volume I, page 424

Kidambi, S. P., A. G. Matches and T. P. Bolger. 1990. Mineral concentrations in alfalfa and sainfoin as influenced by soil moisture level. Agron. J. 82: 229-236.

Koochaki, A., M. Nasiri Mahallati, and k. Azizi. 2007. Effect of irrigation intervals and plant density on yield and yield components of two fennel landraces. Journal of agricultural research,

Kuzentsov V.I., and Shevykova N.I. 1999. Proline under stress: Biological role, metabolism, and regulation. Russian Journal of Plant Physiology, 46: 274-287.

Letchamo W., Marquard R., Holz J., and Gosselin A. 1994. Effects of water supply and light intensity on growth and essential oil of two *Thymus vulgaris* selections. Angewandte Botanik, 68: 83-88.

Majnoon Hosseini, n., S. Davazdah Emami. 2008. Agriculture and the production of some medicinal plants and spices. Tehran University Press. Page 300.

Mehrabizadeh, Z., 2009. Survey of chlorophyll fluorescence, proline and agronomic traits in sesame genotypes under different soil moisture regimes, M. Sc Agriculture, Faculty of Agriculture, University of Technology.

Mozzafari, F., S. Ghorbanli., M.Babai, and A.Farzami. 2000; the effect of water stress on the seed oil of *Nigella sativa* L. J. Essential Oil Res. 12:36-38.

Omid-beigy, R 1997. The approaches to production and processing of medicinal plants.Vol 0.1 Publication of Tarrahan Nashr (Publication Designers)

Omidbeygi, R. 2005. Production and Processing of Medicinal Plants. Vol.I. Behnashr Press. Mashhad, Iran. ISBN 9789640208274. (In Persian).

Pagter, M., B. Claudia and H. Brix. 2005. Tolerance and physiological responses of *Phragmites australis* to water deficit. Aquat. Bot. 81: 285-299.

Pessarkli, M. 1999. Handbook of plant and crop stress. Marcel Dekker. New York Inc. pp. 697.

Rezai Chaîne.2013. The effect of various irrigation treatments on the accumulation of essence, its composition and some physiological traits in fennel. Doctoral thesis of Agriculture, Faculty of Agriculture, University of Tabriz.

Rezapour A, Heidari K, Golvi K and Ramroudi K (2012) Effects of drought stress and different amounts of sulfur fertilizer on yield and yield components of black cumin grains and adjustment of osmotic regulators. Medicinal and Aromatic Plants of Iran research. 27 (3): 396-384.

Rezvanydokht Sh, Dashtian, A, Vanjan Anvardokhgt S (2012) the effects of rain fed irrigation on yield of medicinal plants in Shray-Damghan. The first international conference on drought stress 28-29.

Safarnejad, A. 2004. Characterization of somaclones of *Medicago sativa* L. for drought tolerance. J. Agric. Sci. Technol. 6: 121-127.

Shaaban, Sh, Golvi. M. (2012). The effect of foliar application of micronutrients and irrigation on agronomic traits of *Nigella sativa*. Environmental Stresses in Crop Sciences. 4 (1): 9-1.

Taghavi Razavizadeh S. I., 2004. The effects of different amounts of nitrogen, phosphorus and potassium fertilizer use on efficiency, growth and yield of corn, a Master's thesis of Agriculture, Faculty of Agriculture, University of Technology.

Yadav O.P. and Bhathagar S.K. 2001. Evaluation of indices for identification of pearl millet cultivars adapted to stress and non stress condition. Field Crop Science, 70: 201-208.

Zaifnejad, M., R. B. Clark and C. Y. Sullivan. 1997. Aluminum and water stress effects on growth and proline of sorghum. J. Plant Physiol. 150: 338-344.