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The Effect of Drought Stress and Micronutrient of Zn and Mn on yield and Essential Oil of (Cuminum cyminum)

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ABSTRACT: In order to study the effect of drought stress and application of micronutrient of Zn and Mn on yield, yield components and essential oil of cumin medicinal plant, a experiment in split plot arranged in randomized complete block design with three replications conducted in Zahedan city, Iran, in 2012. Drought stress in three levels (including control and stopping irrigation at vegetative and reproductive stages) as main plot and four levels of micronutrients (including control, Zn, Mn and Zn+Mn) as sub plot investigated. Plant height, number of umbrella per plant, number of seed per umbrella, seed weight, seed yield, biomass, essential oil yield and its percentage were measured. Results of statistical analysis showed that stopping irrigation at vegetative stage led to significant reduction in yield and its components. The highest seed yield and biomass with averages of 438.37 and 828.72 $kg.ha^{-1}$ achieved from control treatment respectively, which had no difference with stopping irrigation at reproductive stage; stopping irrigation at vegetative stage led to decrease of seed yield and biomass about 28.2 and 21.6 percent against control treatment. Essential oil yield of cumin decreased significantly with increasing of drought severity. While micronutrients have no effect on yield of essential oil, but results showed an increase of 22.1% in Zn+Mn against control treatment.

***Keywords*:** Cuminum cyminum, Drought stress, Essential oil, Mn, Yield, Zn.

INTRODUCTION

One of the medicinal plants which can be placed in the cultivation pattern of dry and semi-dry zones under water shortage conditions is cumin. Cumin with scientific name *Cuminum cyminum* is an annual plant of *Umbelliferae*. Shape of leaves, plantes, color, and cover of plant organs all indicate the adaptability of the plants to dry conditions. The depth of root of the plant is between 12 and 15cm showing that the water required for the plant growth is not provided from the soil depth rather the main part of it is supplied from the shallow soil alternatively got wetted by the light precipitations (Kafi, 2002).

Researches done on the water requirement and irrigation frequencies of *Cuminum cyminum* for gaining maximum yield indicate that principally the water requirement of the plant is so small and the humidity limits the growth and yield (Kafi, 2002). In studying the effects of different saline levels and irrigation frequencies on *Cuminum cyminum* yield, Tatari, (2004) reported that increasing the number of irrigation leads to the enhancement of biological yield, yet a small reduction is seen in seed yield as well as number of seeds in umbrella, 1000-seed weight, and yield index. Also, the best treatment was gained with twice irrigations by sweet water and then twice irrigations (once by sweet water and once by irrigation at each of the saline levels). Jangir and Singh (1996) studied the effect of 4, 5, and 6 times irrigation on *Cuminum cyminum* yield; again their results showed that the irrigation diet had significant effect on seed yield and the yield components and employing five times of irrigation led to the increase of yield compared to four irrigations, but more irrigations (six times) had no useful effect. Patel et al., (1991) observed that increasing the number of irrigation based on the increase of irrigated water to total evaporation from basin ratio (0.3 to 0.6) had positive effect on all growth and yield indices of *Cuminum cyminum.* Rahimian Mashhadi, (1991) concluded that in Mashhad maximum *Cuminum cyminum* yield is gained by complete irrigation diet. While, in a three-year study in Mashhad climate, Sadeghi, (1991) observed the reverse; that is, in ordinary years considering precipitation (250mm a year), the effect of irrigation on increasing the yield of *Cuminum cyminum* not only is insignificant but also decreases the crop. Indigenous experience of the farmers of Torbat-e Jam, Sabzevar, and Torbat-e Heidariaeh were also in accordance with Sadeghi's idea.

One of the effects of drought stress is to imbalance the nutrition in plant (Lewis and McFarlane, 1986). By completing the consumption of low consumption foods, it is possible to improve the growth status of the plant in stress conditions. These elements play critical roles in plant nutrition and production. In the mean time, Zn and Mn play catalyst, activator and/or structural role in some enzyme systems (Parker et al., 1992). Among the low consumption elements, lack of zinc creates the furthest problems for crop production (Cakmak, 1999). Zinc plays a key role in the synthesis of proteins, DNA, and RNA (Welch, 2001). Mn is also another essential element for the plant which plays a role in photosynthesis (Hill Reaction), synthesis of protein, carbohydrates, fats, and production of chlorophyll and its lack usually appears early in plant growth season and in young tissues due to its small dynamicity (Marschner, 1995). In their experiment, Bagheri and Mazaherilaghab, (2004) reported that the application of low consumption elements Mn and Zn can have positive significant effect on the growth and chemical compositions of *Cuminum cyminum* essence and the use of their mixture showed further effect. They put it that spraying Zn led to the increase of cuminaldehyde and reduction of pinin. Mn increased benzoic acid but did not have significant effect on the amount of cuminaldehyde. Studying the effect of Mn and Zn spray on three *Carthamus tinctorius* cultivars under drought stress, Movahedi Denavi and Modares Sanavi, (2006) reported that spraying these two low consumption elements compared to the treatment without spraying could compensate for the loss resulted from drought to some extent. In flowering and pollination stage distinguished as the most sensitive stage of *Carthamus tinctorius* growth to drought stress, spraying could not improve the seed and biological yield but the yield elements (including the number of shell in plant, number of seed in each shell and 1000-seed weight under stress conditions) could be improved by spraying Zn and Mn.

MATERIALS AND METHODS

The control was conducted as cut terraces, in terms of complete random blocks in three replications in Sistan & Balouchestan Province, Zahedan city between 2012 and 2013. The farm under study in Kalateh Kambozia, Zahedan in geographical status: E60'51° and N30'29°, 1385m from sea level in loam-sandy soil which is among dry climates with dry and warm summers based on Köppen Climate Classification System. And, also, in 2012, Zahedan is in the warmest (42.5℃) and coldest (-12℃) area with average precipitation in the year 72mm and 36581km^2. The place under study had loam-sandy soil. Results from the chemical analysis of soil showed that the soil had electric conduction of the saturation essential oil 1.9DeciSiemens/m and pH=7.4. The physical and chemical properties of farm soil are presented in Table 1.

Table 1. the physical and chemical properties of farm soil in depth 0-30cm

Texture	Sand (%)	Clay (%)	Silt (%)	Potassium (ppm)	Phosphor (ppm)	Nitrogen (%)	pH	EC (ds/m)
Loam-sandy	41	33	26	210	11	0.07	7.4	1.9

The control was conducted as cut terraces, in terms of complete random blocks in three replicates. Here, drought stress at three levels included control or without stopping irrigation, with stopping irrigation in vegetation stage and stopping irrigation in generation stage as the main factors and four levels of micro nutrient consumption (without consuming fertilizer, Zn, Mn, and Zn+Mn) were sub factors. The treatment with irrigation stoppage in vegetation stage was employed in late vegetation stage and a bit before flowering and the treatment of irrigation stoppage in generation stage was implemented in early seed filling stage. After the land preparation operation (plow, disc and leveler) and the creation of furrow with 60cm width, main terraces were built in each block with 60cm interval. Each secondary terrace also included three leveled hills on both sides of which two rows of cultivation were considered, hence each of the secondary terraces included six cultivation rows with 30cm intervals, 100km phosphor with ammonium phosphate origin at the time of cultivation and 100km nitrogen with urea origin were used. Zn and Mn from zinc sulfate (50km.ha^{-1}) and manganese sulfate (50 kg.ha^{-1}) were applied in strip and soil consumptions. Different amounts of Zn and Mn were mixed with soil before cultivation operation in respective terraces and then cultivation was done in the second half of December manually, or in atmospheric and hill forms. The cultivation operation of *Cuminum cyminum* was done on 25th Dec. 2012 with cultivation depth about

1.5 to 2cm. dimensions of terraces are 2×3m and the interval is considered 2m. The first irrigation was carried out after cultivation using siphon technique and, to ensure the uniform vegetation, second irrigation was done four days after the first one.

The irrigations were carried out irregularly and the weeding operation was conducted manually in three stages. At the time of preparing land and also during the growth period of the plant (from cultivation to harvest) no herbicides, pesticides and fungicides were applied. Urea fertilizer was used in two turns during vegetation growth period (four or six leaves) and, at the time when the fast growth begins (stemming), it was consumed in drip irrigation and fertilization. Sampling and cultivations of the secondary terraces was done in the second half of June when the seeds were rape and the branches and leaves went yellow. Before final cultivation, to determine and calculate the yield elements and morphological traits, 10 plant were randomly chosen from each plot and harvested. To calculate seed yield and biological yield with respect to margin effect, $2m^2$ of each plot was harvested and dried in shadow for 72h in farm conditions. The seeds gained were also sampled randomly to measure the essence percentage. After calculating the weight percentage of essence in seeds, its yield in square area (based on $l.ha^{-1}$) was determined.

Results of yield, yield elements, morphological traits, and essence of *Cuminum cyminum* were analyzed in terms of complete random blocks using statistical software SAS ver. 9.0 and MSTATC and variances and means were set via multi-scale Duncan control at P=%5 and the diagrams were drawn in Excel.

RESULTS AND DISCUSSION

Plant Height

Results from variance analysis of the data from the control indicated that stopping irrigation, micro-nutrient fertilizers and interaction effect of these two treatments have significant effect on the height of *Cuminum cyminum* plant (Table 2). Comparing data means showed that stopping irrigation led to the reduction of plant height; maximum plant height (mean=17.7cm) of the treatment without stopping irrigation or control and minimum plant height (mean=10.9cm) of treatment with irrigation stoppage in vegetation stage were gained (Table 3). Plant height reduction as a result of humidity stress depends on the growth stage under stress and water stress early in growth stage can have further effect on decreasing plant height. Using each of the micro-nutrient fertilizers led to the increase of plant height compared to the treatment without fertilizer consumption (control treatment); in the mean time, plant height in Zn consumption treatment (mean=14.6cm) and Mn (mean=14.8cm) had no significant differences and were placed in the same statistical group. Consumption treatment with both Zn and Mn fertilizers (mean=15.3cm) had maximum plant height among the fertilizing treatment which had significant difference with three other treatments and was 11.2% further than control treatment without micro-nutrient fertilizer consumption (control treatment) (Table 3). Also by examining the yield, yield elements and morphological traits wheat in different treatments of micro-nutrient fertilizers, Mostafavi et al., (2008) also reported that simultaneous consumption of Zn and Mn led to the 6.8% increase of wheat plant height compared to control treatment. Comparing the mean interaction effect of irrigation stoppage and micro-nutrient fertilizer treatments indicated that the consumption of each of Mn and Zn micro-nutrient fertilizer leads to the reduction of the effects of drought stress resulted from irrigation stoppage in vegetative and reproductive stages; maximum plant height was gained from Mn+Zn fertilizer treatment in complete irrigation or without irrigation and minimum plant height was related to the treatment without fertilizer consumption under irrigation stoppage in vegetative stage (Table 4).

The Number of Umbrella in Plant, Seed in Umbrella and 1000-Seed Weight

Data variance analysis indicated that irrigation stoppage has significant effect (5%) on the number of umbrellas in a plant and 1000-seed weight and significant effect (1%) on the number of seeds in umbrella. The effect of micro-nutrient fertilizers on the number of umbrellas in plant was also significant (1%) and the interaction effect on none of the traits was significant (Table 2). In this control, the number of umbrellas in a plant was affected more than the number of seeds in umbrella and 1000-seed weight. Comparison of simple effects in Table 3 shows that irrigation stoppage in vegetative stage (mean=12.6) had the minimum number of umbrellas in plant and had no significant differences with irrigation stoppage in reproductive stage (mean=16 umbrellas in plant) and the complete irrigation treatment (mean=19.9) had maximum number of umbrellas in plant (Table 3). Irrigation stoppage also resulted in the reduction of the number of seeds in umbrella; maximum and minimum number of seeds in umbrella (mean= respectively 16.4 and 11.8 seeds) are related to complete irrigation and irrigation stoppage in vegetative stage; also, there was no significant differences regarding the number of seeds in umbrella between treatments complete irrigation and irrigation stoppage in reproductive stage (Table 3). 1000-seed weight in two conditions in both vegetative and reproductive stages was respectively 2.84 and 2.78g and had no statistically significant differences with each other and placed in top statistical group. 1000-seed weight in complete irrigation period (mean=2.48g) was less than both irrigation stoppage conditions (Table 3). Aminpur and Musavi, (1995) and Rahimian mashhahdi, (1991) reported that as a result of low water consumption, the number of umbrellas in *Cuminum cyminum* plant reduced but if the number of irrigations increases, the number of umbrellas will also increase which itself leads to the yield increase. Drought also reduces the number of seeds in umbrella by preventing from the natural growth and development of umbrella and the reduction of seeds in umbrella will finally lead to the reduction of the plant yield. It seems that in irrigation stoppage treatments, the plant spent limited photosynthetic substances for smaller number of umbrellas and as a result 1000-seed weight in umbrella increased but in complete irrigation treatment, when the soil humidity is suitable, the plant could be able to increase the number of umbrellas by further production of photosynthetic substances, and reduce the proportion of photosynthetic substances allocated to each umbrella which leads to the 1000-seed weight decrease (Tatari, 2004).

Studies showed that, among different micro-nutrient fertilizers, there was no significant difference between three treatments control, Zn and Mn yet it was significant with Mn+Zn treatment. Consuming Mn+Zn led to 16.3% increase in the number of umbrellas in plant to the control treatment without consuming fertilizer (Table 3). Statistically, the effect of micro-nutrient fertilizers on the number of seeds in umbrella was significant but Zn-micro-nutrient fertilizer (mean=15.1) had more numbers of seeds compared to other treatments. Namely, it had about 9.9% increase compared to control treatment (Table 3). Zn and Mn are among the essential elements influencing the plant growth which the plant faces the lack of them in most dry zones soils (Malakuti and Lotfollahi, 1999). Consuming Zn and Mn in shortage cases can improve the yield and yield elements. Ziaeian and Malakuti, (2000) also reported that as a result of consuming Zn, Fe, and Mn, the number of seeds in wheat plant significantly increased.

Seed and Biomass Yield

Results showed that irrigation stoppage has significant effect on seed yield (%1) and biomass (5%) of *Cuminum cyminum*, and micro-nutrient fertilizers had also significant effect on seed yield (5%) and biomass (1%) of *Cuminum cyminum*. Yet, the interaction effect of the two treatments on traits was not significant (Table 2). Irrigation stoppage in vegetative stage led to the significant decrease of seed yield and biomass. Comparison between means indicated that maximum seed yield and biomass resulted respectively in means 438.37 and 828.7 $kg.ha^{-1}$ reduced seed yield and biomass respectively as 28.2% and 21.6% compared to complete irrigation (Table 3). Water shortage stress leads to the reduction of photosynthesis by means of reducing leaf area, closing openings, reducing the capability of directing openings, reducing the watering of chloroplast and other parts of protoplasm, and reducing protein and chlorophyll synthesis. Transferring photosynthetic substances is also reduced as a result of water shortage stress and leads to the accumulation and saturation of leaves and consequently limited photosynthesis; the limitation of photosynthesis also reduces the plant growth and yield (Hopkins and Huner, 2004). On the other hand, in drought stress, plant reduces its perspiration organ by reducing the leaf area (Ardakani et al., 2007). This reduction can also be affected by further allocation of the biomass produced by the plant to roots (Albouchi et al., 2003) and/or as a result of chlorophyll reduction and/or photosynthesis yield which has also reported by Viera et al, (1991).

Mean comparison showed that the consumption of micro-nutrient fertilizers leads to the increase of *Cuminum cyminum* seed yield. Minimum seed yield (mean=316.54$kg.ha^{-1}$) was resulted from control treatment and the difference between the treatment and Mn consumption treatment (379.07 $kg.ha^{-1}$) was not significant. Maximum seed yield was also (mean=423.07 $kg.ha^{-1}$) gained from consuming Mn+Zn which the difference between the treatment with Zn consumption treatment (mean=384.12 $kg.ha^{-1}$) was not significant (Table 3). Consuming Mn, Zn, and Mn+Zn also led to respectively 10%, 14.4%, and %20.2 increases of biomass yield compared to not-using fertilizer treatment (control). The statistical difference between Mn and not-using-fertilizer treatments as well as Zn and Zn+Mn treatments were not significant (Table 3). Seed and biomass yield increase when using MnS_2O_4 and Zn S_2O_4 can be attributed to the indirect effect of micro-nutrient elements in increasing the azoth absorption on one hand. That is, the plant uses the micro-nutrient elements to have further and optimum consumption of nitrogen in soil and as a result the yield and biomass increase. On the other hand, micro-nutrient elements like Zn take part in the construction of some proteins and also in azoth metabolism and thereby lead to the yield increase (Parhamfar, 2006).

Results of interaction effects mean comparison indicate that the consumption of micro-nutrient fertilizers in complete irrigation and also in irrigation stoppage in reproductivestage reduces the adverse effects of drought stress on seed yield to some extent. Mn+Zn consumption in complete irrigation leads to 16.9% increase and in irrigation stoppage in reproductive stage to 21.4% increase of seed yield compared to non-consuming-fertilizer treatment in the two conditions. In irrigation stoppage in vegetative stage again the consumption of micro-nutrient fertilizers has not so much effect on seed yield and then using Mn merely was more effective than other fertilizers and led to the %5.2 increase of the yield compared to control treatment (Table 4). Based on the table of interaction effect means comparison, maximum biomass yield was related to the Mn+Zn treatment in normal irrigation which had no significant statistical difference with Zn consumption in these conditions and Mn+Zn consumption in irrigation stoppage in reproductive stage; minimum biomass was also related to not-using-fertilizer treatments in irrigation stoppage in vegetative and reproductive stages (Table 4).

Essence Percentage and Essence Yield

Variance analysis of the control data showed that none of the irrigation stoppage treatments and micro-nutrient fertilizers and their interaction effects had no significant effect on the percentage of *Cuminum cyminum* (Table 2). Although the effect of irrigation stoppage on essence was not significant, results of means comparison from Duncan Control showed that irrigation stoppage in reproductivestage led to the increase of essence percentage and the treatment had the maximum essence percentage (mean= 2.37) and irrigation stoppage in vegetative stage had also the least essence percentage (mean= 2.10) (Table 3). Drought stress is one the main factors in increasing the essence percentage in most medicinal plants; so that the more the plant is placed in humidity stress and water shortage, the more its essence will increase. Small increase in essence percentage in reproductivestage where the plant undergoes moderate drought stress can be because the higher density of essence secretion tubers as a result of reducing leaf area resulted from stress leads to the higher accumulation of essence (Charles et al, 1990).

Irrigation treatment stoppage had significant effect on *Cuminum cyminum* essence yield (5%) but the treatment consuming micro-nutrient fertilizers and interaction effect of the two treatments on essence yield were not significant (Table 2). Data mean comparison indicated that drought stress intensity increase led to significant decrease of *Cuminum cyminum* yield. Two treatments with and without irrigation stoppage in reproductivestage (means= 9.89 and 9.75 $l.ha^{-1}$ respectively) had no significant differences with each other and placed in top statistical group and treatment irrigation stoppage in vegetative stage (mean=6.59 $l.ha^{-1}$) had minimum essence yield (Table 3). Reduction of essence yield resulted from the reduction of soil humidity is emanated from the adverse effect of drought stress and the yield of the plant vegetative body. Thought the effect of micro-nutrient fertilizers on essence yield was not statistically significant, results of means comparison showed the 22.1% increase of essence yield in Zn+Mn consumption conditions compared to control treatment. The consumption of Zn and Mn separately had not significant differences with using them together (Table 3). In their experiment, Bagheri and Mazaherilaghab, (2004) reported that the application of the low-consumption elements Mn and Zn separately showed positive significant effect on the growth and content of *Cuminum cyminum* essence and the application of their mixture had even better effect.

Table 2. Variance analysis of measured factors

SOV	df	Plant height	Number of umbrella in plant	Number of seed in umbrella	1000- seed weight	seed yield	Biomass yield	Essence percentage	Essence yield
					Mean squares				
Replication	2	1.13	5.02	5.65	0.043	4049.1	708.4	0.007	3.09
Irrigation	2	14.13**	160.86*	70.32**	0.440*	49961.8**	96280.5*	0.218 ns	41.66*
Micro-nutrient	4	5.01**	13.64**	3.79 ns	0.116 ns	6060.2*	43636.2**	0.063 ns	7.28 ns
irrigation × Micro-nutrient	3	1.001**	1.39 ns	1.19 ns	0.005 ns	1967.2*	9093.4 ns	0.013 ns	1.26 ns
Error	6	0.24	2.41	3.92	0.141	1614.7	6848.7	0.100	2.59
C.V	18	3.36	9.61	13.60	13.89	10.38	11.15	14.16	18.41

*, ** significant at the 5% and 1% levels of probability respectively and n.s (non-significant)

Table 3. Mean comparison of treatments on measured factors

treatments	Plant height (cm)	Number of umbrella in plant	Number of seed in umbrella	1000-seed weight (g)	Seed yield ($kg.ha^{-1}$)	Biomass ($kg.ha^{-1}$)	Essence percentage	Essence yield ($l.ha^{-1}$)
Irrigation								
Without irrigation stoppage (control)	17.7 a	19.9 a	16.4 a	2.48 b	438.37 a	828.72 a	2.25 ab	9.89 a
Irrigation stoppage in vegetative stage	10.9 c	12.6 b	11.8 b	2.84 a	314.54 b	649.83 b	2.10 b	6.59 b
Irrigation stoppage in reproductive stage	15.1 b	16.0 b	15.4a	2.78 a	407.94 a	747.47 ab	2.37 a	9.75 a
Micro-nutrient								
Without fertilizer (control)	13.5c	14.9 b	13.6 a	2.56 a	361.54 b	654.90 c	2.12 a	7.68 b
Zn	14.6 b	16.0 b	15.1a	2.82 a	384.12 ab	765.53 ab	2.29 a	8.86 ab
Mn	14.8b	15.9 b	14.7 a	2.66 a	379.07 b	726.99 bc	2.25 a	8.57 ab
Zn+Mn	15.3 a	17.8 a	14.8 a	2.76 a	423.07 a	820.61 a	2.30 a	9.86 a

Means with similar letters in each column have no statistic significant difference (p=%5)

Table 4. interaction effect of treatments on measured factors

Treatment		Plant height (cm)	Number of umbrella in plant	Number of seed in umbrella	1000- seed weight (g)	seed yield ($kg.ha^{-1}$)	Biomass yield ($kg.ha^{-1}$)	Essence percentage	Essence yield
Without irrigation stoppage	Without fertilizer	16.4 cd	18.6 bc	15.8 abc	2.29 a	407.20 bc	718.87 bcde	2.06 a	8.38 abc
	Zn	17.2 bc	18.9 bc	16.9 a	2.62 a	438.32 abc	829.11 abc	2.26 a	9.89 a
	Mn	17.8 b	19.8 ab	16.3 ab	2.45 a	417.30abc	806.46 bcd	2.36 a	9.80 ab
	Zn+Mn	19.3a	22.4 a	16.7 a	2.59 a	490.66 a	960.45 a	2.34 a	11.50 a
Irrigation stoppage in vegetative stage	Without fertilizer	9.9 h	11.6 f	11.1 d	2.74 a	312.58 e	618.37 e	2.02 a	6.27 c
	Zn	11.1 g	13.1 f	12.7 bcd	2.49 a	301.63 e	685.54 cde	2.18 a	6.61 c
	Mn	11.3 g	11.8 f	12.4 cd	2.81 a	330.14 de	655.84de	2.04 a	6.74 bc
	Zn+Mn	11.3g	14.0 ef	11.1 d	2.89 a	313.82e	639.55 e	2.16 a	6.77 bc
Irrigation stoppage in reproductive stage	Without fertilizer	14.3 f	14.6 def	14.0 abcd	2.67 a	364.85 cde	627.45 e	2.28 a	8.39 abc
	Zn	15.6 de	16.2 cde	15.8 abc	2.93 a	412.40 bc	781.95 bcde	2.45 a	10.11 a
	Mn	15.4 e	16.1cde	15.4 abc	2.73 a	389.78 bcd	718.65 bcde	2.34 a	9.18 abc
	Zn+Mn	15.2 e	17.2 bcd	16.5 ab	2.81 a	464.72 ab	861.62 ab	2.41 a	11.33 a

Means with similar letters in each column have no statistic significant difference (p=%5)

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