



Citation: H. Fatemi Kiyani, M. Tatari, M.R. Tokalo, M. Salehi, K. Hajmohammadnia Ghalibaf (2022) The effect of deficit irrigation and fertilizer on quantitative and qualitative yield of quinoa (*Chenopodium quinoa*). *Italian Journal of Agrometeorology* (1): 83-99. doi: 10.36253/ijam-1136

Received: November 19, 2020

Accepted: November 29, 2021

Published: July 19, 2022

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

The effect of deficit irrigation and fertilizer on quantitative and qualitative yield of quinoa (*Chenopodium quinoa*)

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Abstract. In order to investigate the effect of deficit irrigation and chemical fertilizers on yield and some physiological traits of quinoa an experiment was conducted in 2019 as split plot based on a randomized complete block design in two locations (Mashhad and Neishabour). Irrigation included, I0: full irrigation, I1: no irrigation at emergence stage, I2: no irrigation at stem elongation stage, I3: no irrigation at flowering stage, I4: no irrigation at seed setting stage. Fertilizer treatments included control (no fertilizer application); chemical fertilizer application according to local practices; manure application of 10 tons; and manure application of 20 tons per hectare. In general, seed yield, percentage of protein and seed oil in Mashhad was higher than in Neishabour. I2 treatment had the least negative effect on relative leaf water content. Application of chemical fertilizers, 10 tons and 20 tons of animal manure increased the percent of seed protein by 1.43, 1.66 and 2.37 compared to the control, respectively. The highest percentage of seed oil (5.91%) was obtained for treatment I2 in Mashhad and the lowest percentage of seed oil (4.18%) was obtained for treatment I4 in Neishabour. The lowest seed yield due to I1 treatment was observed in Neishabour and the highest seed yield was related to I0 treatment with 20 tons of manure and was observed in Mashhad. The results showed that the yield and water stress tolerance potential of quinoa can be modified by irrigation, fertilizer source and location.

Keywords: quinoa seed quality, irrigation management, organic fertilizer, plant pigment, electrolyte leakage.

INTRODUCTION

Quinoa (*Chenopodium quinoa*) is native to the Andes Mountains in Bolivia, Chile and Peru, and Bolivia, Peru and Ecuador are the most important producers (Vega-Galvez et al., 2010). Quinoa belongs to the Chenopodiaceae family, a genus of *Chenopodium*, and is considered as similar to cere-

als. Quinoa is rich in protein, iron and calcium and is better than wheat and rice in terms of amino acid balance (James, 2009). It is a very high nutritional value has been compared to dry milk by the FAO, and has been called vegetable caviar (James, 2009). Global interest and attention to this plant are due to its high nutritional value (Garcia et al., 2015; Nowak et al., 2016). In general, quinoa can be less affected by frost (Jacobsen et al., 2005), salinity (Hariadi et al., 2011; Razzaghi et al., 2011; Ruiz-Carrasco et al., 2011), poor soils. (Jacobsen et al., 2009) and drought (Hirich and Choukr-Allah, 2014) than other crop species. It is believed that the presence of several drought resistance mechanisms in quinoa has made it suitable for arid and semi-arid regions (El youssfi et al., 2012; Hirich et al., 2012). In 2018, the cultivation area and production of quinoa in the world were estimated as 178313 hectares and 158920 tons, respectively (FAO, 2020). Quinoa is currently grown in twenty provinces in Iran.

Drought stress is one of the most common and destructive abiotic stresses across the world and limits the growth and yield of plants, especially in arid and semi-arid regions (Wang et al., 2014; Heuberger and Pfeiffer 2013). Drought stress affects a combination of physiological and biochemical processes of plants and negatively impact the growth and yield (Zulkarami et al., 2016). Deficit irrigation strategy is a good way to increase water productivity in crop production under drought conditions. This strategy is very effective in arid and semi-arid regions that suffer from water scarcity (Hirich and Choukr-Allah, 2014). Global warming has affected the climate of different parts of the world, including Iran, which has increased the salinity of the country's arable land. The Quinoa shows a high tolerance to drought, salinity and cold stress, which can be a suitable plant for achieving sustainable agriculture and proper nutrition according to the existing conditions in Iran. Quinoa yield varies according to climatic conditions, planting date and cultivar. Geerts et al. (2008) reported the maximum yield under full irrigation conditions as 2.04 t/ha, under deficit irrigation as 2.01 t/ha, and under rainfed conditions as 1.68 t/ha. Stikic et al. (2011) reported that quinoa has great agronomic and nutritional potential, even in dryland conditions and without fertilizer application. In their study, the grain yield was 1.72 t/ha and the grain quality was good with the protein content of in the range of 15.16 and 17.41 percent.

The Addition of organic matter increases the storage capacity of soil water under water deficit conditions. Studies on the addition of organic fertilizers to the soil in arid and semi-arid regions have shown that organic

matter improves soil fertility, soil moisture content and increases soil hydraulic conductivity (Wesseling et al., 2009). Organic matter to soil also has a positive effect on crop growth and yield (Gopinath et al., 2008; Ibrahim et al., 2008). There are few studies examining soil organic management on quinoa yield (Hirich et al., 2014), however other crops in other studies have responded positively to such treatments as well (Hartley et al., 2010; Dadkhah, 2014).

Although quinoa has the potential for growth in harsh environments with fewer resources availability and high stress tolerance compared to other crops, so far not many studies have been performed on the production and quality of quinoa under Iranian agricultural systems. Therefore, this study was conducted to determine the effect of deficit irrigation and organic fertilizer on quantitative and qualitative yield as well as some physiological traits of quinoa about drought stress in Mashhad and Neishabour.

MATERIALS AND METHODS

This experiment was performed in 2019 in two locations, the Agricultural Research Field of Toroq in Mashhad and the Agricultural Research Field of Ayesha in Neishabour. The climatic characteristics of these two locations are presented in (Table 1). The experiment was performed as split plots based on a randomized complete block design with three replications in both locations. The treatments of this experiment included different levels of irrigation allocated as the main plot and fertilizer treatments as the subplots.

Deficit irrigation levels included I0: full irrigation, I1: cessation of irrigation in emergence stage, I2: cessation of irrigation in elongation of the stem stage, I3: cessation of irrigation in the flowering stage, I4: cessation of irrigation in seed setting stage (Sosa-Zuniga et al., 2017). Fertilizer treatments also included control (no fertilizer application), chemical fertilizer based on local management fertilizer recommendation, 10 t/ha of livestock manure and 20 t/ha of livestock manure. It should be noted that the recommendation of quinoa fertilizer in Iran includes 100 kg/ha of phosphate fertilizer, 100 kg of potash fertilizer and 50 kg of nitrogen fertilizer. Before the experiment, the soil sample was randomly taken from a depth of 0-30 cm from the fields in both locations and transferred to the laboratory to determine the soil properties (Table 2).

Sub-plots were 3 m long and 2 m wide. The dimensions of the subplot were 0.6 m². The distance between the plots was 1.5 m and the distance between the blocks

Table 1. Physical and chemical characters of soil in two study locations, Neyshabour and Mashhad (Soil depth 0-30 cm).

Location	pH	EC (dS.m ⁻¹)	SAR	O.C (%)	Total N (%)	Available P (ppm)	Available K (ppm)	Lime (%)	Soil tissue
Neyshabur	7.93	0.899	4.95	0.742	0.07	3.4	217	17.73	Loam
Mashhad	7.74	2.34	8.23	0.362	0.03	3.5	145	13.75	Loam

Table 2. Climate and geographical conditions of two study locations (Neyshabour and Mashhad).

Location	Longitude	Latitude	Altitude above sea level (m)	Average annual rainfall (mm)	Annual mean temperature (°C)	Annual mean daily maximum temperatures (°C)	Annual mean daily minimum temperatures (°C)
Neyshabur	58° 50'E	36°10'N	1250	240	14.7	22.5	6.8
Mashhad	59° 35'E	36°20'N	950	222.3	16.5	23	9.4

Table 3. Summary of information and date of field operations tested at two locations.

Location	Planting date	Rainfall August (mm)	Rainfall September (mm)	Date of chemical fertilizer use *	Date of manure fertilizer use	Irrigation date of emergence stage	Irrigation date Stem elongation stage	Irrigation date of flowering stage	Irrigation date Seeding stage
Neyshabur	27-7-19	0	0	22/06/19	22/06/19	29/07/19	11/08/19	26/08/19	08/09/19
Mashhad	4-8-19	0	0	22/06/19	22/06/19	07/08/19	19/08/19	01/09/19	13/09/19

*Chemical fertilizers including 220 kg of potassium sulfate, 130 kg of triple superphosphate and 200 kg of urea per hectare were applied. Urea was given to two farms during the germination stage.

was 3 m. In order to prepare the ground, first reversible plowing and then disc were used. After leveling and plotting, the manure was spread on the plots based on specified amount and mixed thoroughly with the soil with a shovel. Then 6 rows were created in each plot with a distance of 0.25 m and quinoa seeds were planted. The employed seed in this experiment was Titicaca cultivar which was obtained from Agricultural Research Center of Yazd. After planting, in order to achieve uniformity of emergence, the first irrigation was applied equally for all plots. The second irrigation was applied three days later. Then, after ensuring the establishment of seedlings, deficit irrigation treatments were performed according to the mentioned developmental stages. Surface irrigation was applied to irrigate each plot and irrigation intervals were considered as 14 days. The water requirement of the plant was calculated using Cropwat software. The geographical, climatological characteristics and the date of planting, irrigation and fertilization operations of the two studied locations are presented in (Table 3).

Evaluation was performed by measuring traits including relative leaf water content, soluble sugar content, total chlorophyll, carotenoids, electrolyte leakage of ions, pro-

tein percentage, oil percentage and grain yield. For measurement of relative leaf water content, ten leaf discs were collected from the middle leaves of each experimental unit and after weighing with an accuracy of 0.0001 g, they were transferred to double-distilled petri dishes containing water and for 24 hours were stored in dark at 4 °C for complete water absorption. The leaves were then removed and were put between two filter papers to dry extra water and then leaves weight as turgid weight was measured. The leaves were dried in an oven at 70 °C for 48 hours and weighed again as dry weight. Then the relative water content of the fresh leaves was determined using (Equation 1) (Mahmood et al., 2003):

$$RWC = (Fw - Dw) / (Tw - Dw) \times 100 \quad (1)$$

Where Fw is the fresh weight of the leaf, Dw is the dry weight of the leaf and Tw is the turgid weight of the leaf. The amount of plant solution sugars was read using the sulfuric phenol method and was read by the UV-2100 model spectrophotometer at 485 nm wavelength (Irigoyen et al., 1992). Porra (2002) method was used to measure chlorophyll content. For this purpose,

500 mg of each selected leaf was homogenized in 5 ml of 80% acetone and after centrifugation at 4 °C for 15 minutes at 13000 rpm, the supernatant was removed and then its volume with 80% acetone was reduced to 10 ml. The concentration of the solution was measured by spectrophotometer at wavelengths of 663 nm for chlorophyll a, 645 nm for chlorophyll b and 470 nm for carotenoids. To measure total chlorophyll, the values of chlorophyll a and b were added together. Flint et al. (1967) method was used to measure ion leakage. The protein content of the samples was determined by a micro-kejl-dahl UK electrothermal equipment by using half a gram of plant samples after digestion and distillation (Helvich, 1990). In order to extract the oil and determine its percentage, Soxhlet and hexane solvent method was used (Carrillo et al., 2017). Sampling was performed randomly by (1×1) m quadrat from each plot, considering the removal of the margin effect, to determine the final grain yield.

Analysis of variance and comparison of mean data were performed by Duncan's multiple range test with SAS 9.4 software and figures were prepared by MS Excel. Before performing the combined analysis of variance, the homogeneity of variance of experimental errors was tested using Bartlett test.

RESULTS AND DISCUSSION

Relative leaf water content

One of the physiological parameters responding to dehydration is the relative leaf water content, which

shows a good correlation with drought tolerance. In other words, changes in leaf moisture content can be used as a short-term response to stress and a measure of the ability to maintain resource strength under drought stress conditions (Ahmadi et al., 2010). According to the analysis of variance, the effects of location, deficit irrigation and fertilizer as well as the interaction of location and fertilizer on the relative water content of the leaves were significant (Table 4). The highest relative water content was observed for full irrigation treatment (80.11%), then no irrigation (I₂) at the growth stage of stem elongation (77.28%) had the highest relative water content with complete irrigation treatment. In other words, among the deficit irrigation levels, no irrigation at stage I₂ had the least negative effect on the relative water content of leaves, so it can be said that by eliminating irrigation at the stage of the stem elongation in addition to saving limited water resources, the leaf moisture content change is not significant. In contrast, no irrigation at the flowering stage (I₃) resulted in the lowest relative water content of leaves (60.84%) (Table 5).

If drought stress persists, the relative water content of the leaf decreases, causing a change in the cell membrane and consequently an increase in electrolyte leakage from the cell (Fu et al., 2004). A similar relationship was observed between the relative leaf water content and ion electrolyte leakage in this study. It seems the plants under water stress, by increasing the osmotic pressure, reduce the spaces between cells and the water content of their tissue, allowing water to move from the soil to the cells, and as a result, their relative amount of water

Table 4. Compound analysis of various quinoa traits under deficit irrigation in different phenological stages and fertilizers (chemical and organic) in Mashhad and Neishbour.

SOV	df	Leaf relative water content	Soluble sugar content	Total chlorophyll content	Carotenoid content	Ion electrolyte leakage	Seed protein (%)	Seed oil (%)	Seed yield
Location	1	9490.79**	23.11**	0.46 ^{ns}	0.0000 ^{ns}	5967.26**	25.76**	5.09**	189210.21**
Rep (location)	4	80.89	0.70	0.25	0.1029	66.86	0.33	0.58	35545.08
Deficit irrigation	4	1357.51**	3.81**	5.47**	0.3463**	892.76**	6.28**	3.38**	152903.93**
Location × Deficit irrigation	4	33.40 ^{ns}	1.08**	0.21 ^{ns}	0.1992**	87.32**	6.93**	1.87**	20796.38**
Error a	16	81.43	0.23	0.72	0.0142	128.76	0.63	0.83	10972.53
Fertilizer	3	175.93**	14.41**	16.73**	0.3394**	954.64**	1.00*	0.24*	67648.28**
Fertilizer × Deficit irrigation	12	19.54 ^{ns}	0.18**	0.22 ^{ns}	0.0257**	30.96 ^{ns}	0.07 ^{ns}	0.21**	10650.94*
Location × Fertilizer	3	71.72*	1.99**	0.59*	0.0517**	89.41**	0.18 ^{ns}	0.14 ^{ns}	22080.36**
Fertilizer × Deficit irrigation × Location	12	9.59 ^{ns}	0.07*	0.16 ^{ns}	0.0351**	26.85 ^{ns}	0.01 ^{ns}	0.11 ^{ns}	9037.06*
Error b	60	25.56	0.032	0.16	0.0054	18.45	0.035	0.065	4470.06
CV		7.04	9.99	12.86	12.39	13.55	3.21	4.69	21.03

ns, * and **: Non-significant, significant at 5% and 1% probability level, respectively.

Table 5. Mean comparison of different quinoa traits under deficit irrigation in different phenological stages and fertilizer treatments (chemical and organic) in Mashhad and Neishabour.

	Relative leaf water content (%)	Chlorophyll content (mg/g)	Seed yield (g/m ²)		Protein (%)	Seed yield (g/m ²)
I ₀ : Full Irrigation	80.11 ^{a*}	3.59 ^a	343.9 ^a	Control	18.12 ^b	253.4 ^c
I ₁ : NO Irrigation at emergence	71.77 ^b	2.33 ^d	176.21 ^b	Chemical fertilizer	18.38 ^{ab}	322.5 ^b
I ₂ : NO Irrigation at stem elongation	77.28 ^a	3.38 ^{ab}	357.2 ^a	Manure 10 t/ha	18.42 ^{ab}	327.9 ^b
I ₃ : NO Irrigation at flowering	60.84 ^c	3.16 ^{bc}	344.6 ^a	Manure 20 t/ha	18.55 ^a	367.8 ^a
I ₄ NO:Irrigation at seed setting	69.14 ^b	3.07 ^c	367.7 ^a			

* Means with same letter(s) for each component indicates no significant difference based on Duncan test at 5% probability level.

Table 6. Mean comparison of interaction of location and deficit irrigation treatments effects on quinoa traits.

Location	Deficit irrigation	Electrolyte leakage (%)	Protein (%)	Oil (%)
Neishabour	I ₀ : Full Irrigation	17.88 ^{e*}	16.52 ^g	5.62 ^{cd}
	I ₁ : NO Irrigation at emergence	32.18 ^c	17.59 ^f	5.66 ^{bc}
	I ₂ : NO Irrigation at stem elongation	19.21 ^e	18.19 ^d	5.42 ^{de}
	I ₃ : NO Irrigation at flowering	27.30 ^d	18.14 ^e	5.31 ^e
	I ₄ NO:Irrigation at seed setting	26.65 ^d	19.09 ^b	4.18 ^f
Mashhad	I ₀ : Full Irrigation	27.28 ^d	18.59 ^{cde}	5.83 ^{ab}
	I ₁ : NO Irrigation at emergence	44.95 ^a	19.63 ^a	5.34 ^e
	I ₂ : NO Irrigation at stem elongation	39.00 ^b	18.63 ^{bcd}	5.91 ^a
	I ₃ : NO Irrigation at flowering	40.60 ^b	18.52 ^{cde}	5.76 ^{abc}
	I ₄ NO:Irrigation at seed setting	41.90 ^{ab}	18.79 ^{bc}	5.40 ^e

*Means with same letter(s) for each component indicates no significant difference based on Duncan test at 5% probability level.

decreases in water shortage conditions. According to (Ma et al., 2006), water stress reduces water potential, relative leaf water content, transpiration, stomatal conductance and ultimately yield.

Comparison of the mean of the interaction of location and fertilizer treatment showed that in general, due to differences in climatic parameters, the relative water content of leaves in Mashhad (62.93%) was lower than Neishabour (80.72%), because according to (Table 1), in Mashhad the average rainfall was lower and the average temperature was higher than Neishabour, which causes a higher evaporative demand of the atmosphere. In addition, the fertilizer treatments showed significantly different results at the two locations. In Neishabour, application of chemical fertilizer treatments, 10 tons and 20 tons of livestock manure increased the relative water content by 3.7%, 5.2% and 2.1% percent, respectively, while in Mashhad, the values of increasing the relative water content but 2% is lower, were higher by 11.4 percent for the organic fertilizer treatments, respectively. (Table 6).

Soluble sugar content

Soluble sugars have assimilated that increase under stress conditions and their accumulation causes osmotic regulation and cell turgescence, and on the other hand, protects and stabilizes membranes and proteins under stress conditions. According to the results of analysis of variance, all single effects, dual interactions as well as triple interactions of location, irrigation and fertilizer on the content of soluble sugars were significant (Table 4). Comparison of the mean of the interaction of location, irrigation and fertilizer in (Figure 1) showed that in general the amount of soluble sugars in Neishabour was higher than in Mashhad. But the response of this trait to fertilizer and deficit irrigation treatments in two study locations was not similar. In general, fertilizer treatments in all conditions increased the amount of soluble sugars. The highest increase in soluble sugars under no irrigation treatment was observed at the flowering stage (I₃) along with the application of 20 tons of manure in Neishabour and the lowest increase under no irrigation treatment was observed in stage (I₂) of stem elonga-

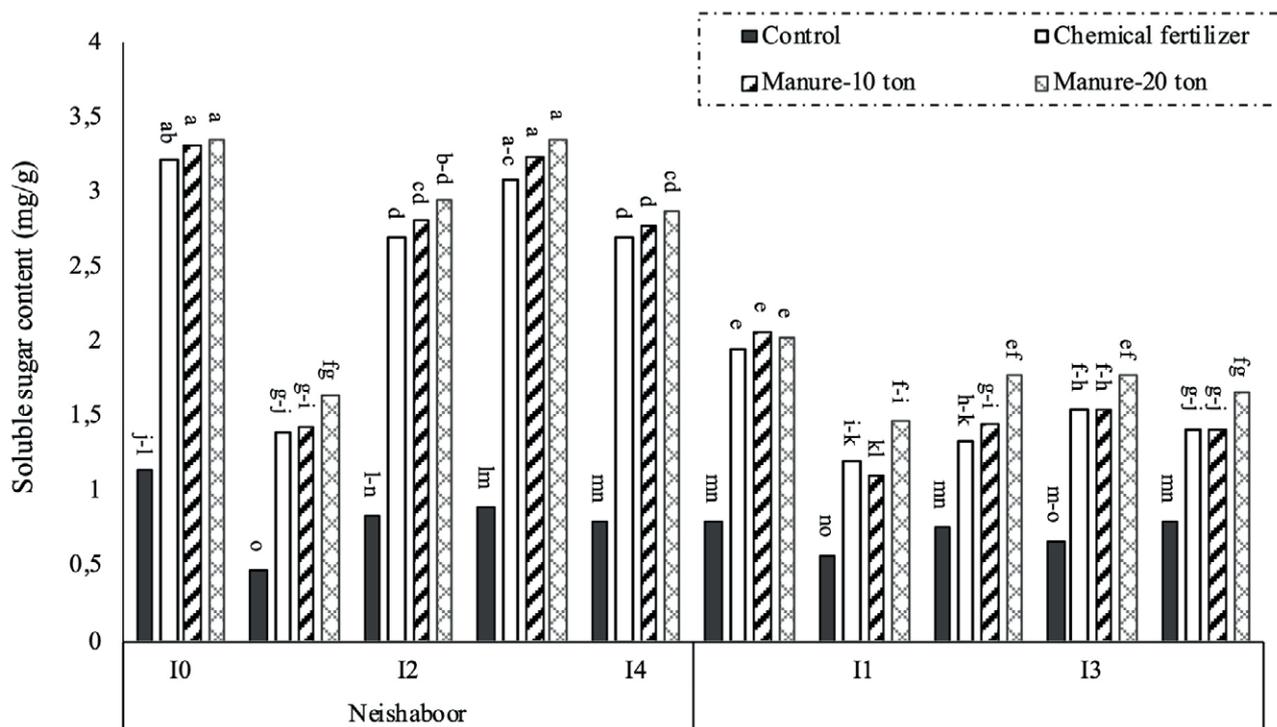


Fig. 1. Mean comparison of triple interaction of location, deficit irrigation and fertilizer effects on soluble sugars content. I₁: no irrigation at emergence stage, I₂: no irrigation at stem elongation stage, I₃: no irrigation at flowering stage, I₄: no irrigation at seed setting stage.

tion and application of chemical fertilizer in Mashhad (Figure 1). The lowest soluble sugars of 0.46 mg/g were observed under no irrigation treatment at emergence stage (I₁) and no fertilizer application in Neishabour and the highest soluble sugars were observed in treatments of 10 and 20 tons of manure and no irrigation levels of I₀ and I₃ in Neishabour (Figure 1). The increase of soluble sugar the cell, which creates an increasing osmotic pressure gradient between soil and plant and thus enables more water absorption from the soil. Accumulation of soluble sugars inside the cells plays an important role in osmotic regulation. It helps reduce cell water potential and keeps more water in the cell to maintain turbulence under dehydration (Turner, 2018). This mechanism promotes biological membrane stability, proteins, increased photosynthesis, and drought resistance. In tolerant plants compatible osmolytes attach to protein surfaces and maintain their natural structure under stress conditions, while in susceptible plants proteins are degraded (Hoekstra et al., 2001). Increased accumulation of soluble sugars in the cell under drought stress would regulate osmotic pressure in quinoa (Bascañán-Godoy et al., 2016; González et al., 2009; Muscolo et al., 2016) and corn (Johari, 2010). However, (Gámez et al., 2019) reported that some genotypes showed an increase

and others showed a decrease of soluble sugars under drought stress, which indicated the difference between genotypes in terms of response to drought stress.

Total chlorophyll content

One of the methods for evaluating and predicting crop tolerance to water stress is to study the amount of changes that occur in chlorophyll (a+b) leaf synthesis due to water scarcity. Decreased chlorophyll synthesis is one of the general reactions of plants to water deficiency (Gardner et al., 2017; Sadak, 2016). Analysis of variance showed that the single effects of irrigation and fertilizer as well as the interaction of location and fertilizer were significant on total chlorophyll content (Table 4). Comparison of the mean of the single effect of deficit irrigation treatment showed that the highest amount of leaf chlorophyll after control (full irrigation) was through the no irrigation (I₂) at the stage of stem elongation (Table 5). No irrigation (I₃) at the flowering stage showed the lowest chlorophyll content of leaves (Table 6). Chlorophyll content in living plants is one of the important factors for photosynthesis. Based on the severity, duration and crop growth stage, the drought effect on each of the chlorophyll levels in plants is different. In fact,

the decrease in chlorophyll due to water stress is related to the increase in the production of oxygen radicals in the cell, because these radicals cause peroxidation and thus decomposition of this pigment (Sheteawi and Tawfik, 2007). Water stress has a direct effect on reducing the chlorophyll index of plant leaves (Adebayo et al., 2014; Elewa et al., 2017). Reduction of photosynthetic pigments can be due to reduced synthesis of the main complex of chlorophyll pigments, optical degradation of the b-pigment protein complex that protects the photosynthetic apparatus, oxidative damage of chloroplast lipids of pigments and proteins or higher chlorophyllase enzyme activity and hormonal disorders (Pandey et al., 2012; Anjum et al., 2011). In addition, stress interferes with the absorption of some essential elements such as iron and magnesium, which are essential for chlorophyll synthesis (Neocleous and Vasilakakis, 2007). Lipoxigenase has also been reported to be one of the enzymes involved in chlorophyll catabolism. This enzyme is one of the enzymes involved in lipid peroxidation during stress (Farooq et al., 2009). Schmidhalter et al. (2006) reported that drought stress disrupts the chlorophyll-making process by limiting the plant's ability to absorb nitrogen. On the other hand, plant uptake and assimilation under drought stress conditions are largely controlled by the two main factors of leaf area and photosynthesis per unit of leaf area. Cell wall solubility, which is required for leaf elongation, prevents an increase in leaf and in turn strongly reduces the number of leaf chloroplasts (Bänziger et al., 2000). In the general reduction of photosynthesis pigments can be mainly due to chloroplast structure disruption and photosynthesis apparatus, photo oxidation of chlorophyll, pigments reaction with singlet oxygen, disruption of required materials for chlorophyll synthesis, prevention of biosynthesis of new chlorophyll, activating the chlorophyll degradation enzymes, and hormonal disorders.

Comparison of the mean of interaction of location and fertilizer treatment showed that 20 tons of livestock manure in Mashhad was associated with the highest chlorophyll content (3.81 mg/g) and non-fertilizer application in Mashhad had the lowest chlorophyll content (1.78 mg/g) (Table 6). Fertilizer application increased the total chlorophyll content of the leaves however the rate of increase was not the same in the two study locations. Treatment of 20 tons of manure in Mashhad had the highest increase and chemical fertilizer treatment in Neishabour had the lowest increase in total chlorophyll (Table 6). Because fertilizer treatments contain nitrogen and nitrogen is the main constituent of chlorophyll in the plant, the application of fertilizers increases the amount of chlorophyll. Livestock manure had a more

positive effect on chlorophyll content, probably due to the more gradual release of nutrients in the soil.

Carotenoid content

According to the results of analysis of variance, the single effects of irrigation and fertilizer, dual interactions as well as triple interaction of location, irrigation and fertilizer on carotenoid content were significant (Table 4). Comparison of the mean of the triple interaction showed that the levels of carotenoids in Neishabour were higher than in Mashhad (Figure 2). Because photosynthetic pigments strongly respond to water stress and radiation, there is a difference between the two study locations in this respect. Irrigation and fertilizer treatments showed different effects on carotenoid content. The highest amount of carotenoids (1.067 mg/g) was observed for 10 tons of manure and complete irrigation in Neishabour. The lowest amount of carotenoids as 0.187 mg/g, was obtained for no irrigation treatment at the emergence stage and no fertilizer application in Mashhad (Figure 2). For most of the fertilizer treatment, carotenoids increased, the only exceptions were the application of chemical fertilizers and 10 tons of livestock manure in Neishabour and no irrigation (I_4) at the grain setting stage. When 20 tons of livestock manure and no irrigation (I_1) at the emergence stage were applied, in Mashhad, the highest increase in carotenoid content was achieved (Figure 2). Elewa et al. (2017) also reported a decrease in carotenoid content in quinoa under water stress conditions. Animal manure application increased the carotenoid content in onions (Singh and Sharma, 2018) and cabbage leaves (Qureshi et al., 2014). Increasing the content of carotenoids as auxiliary pigments due to the application of animal manure can be due to increasing the amount and activity of chlorophyll (Qureshi et al., 2014). Carotenoids can transfer energy to chlorophyll a and increase the amplitude of wavelengths that affect photosynthesis, as well as reduce the number of ions. Carotenoids release a lot of energy from photosystems I and II in the form of heat, or harmless chemical reactions, and can maintain chloroplast membranes (Juan et al., 2005). Water stress by reducing the biosynthesis of photosynthetic pigments reduces the concentration of pigments and reduces the potential of photosynthesis and limits the initial production. Water stress, on the other hand, disrupts enzymatic systems that reduce the activity of reactive oxygen species and increases lipid peroxidation, resulting in damage to cell membranes and pigment degradation (Ruiz-Sánchez et al., 2011).

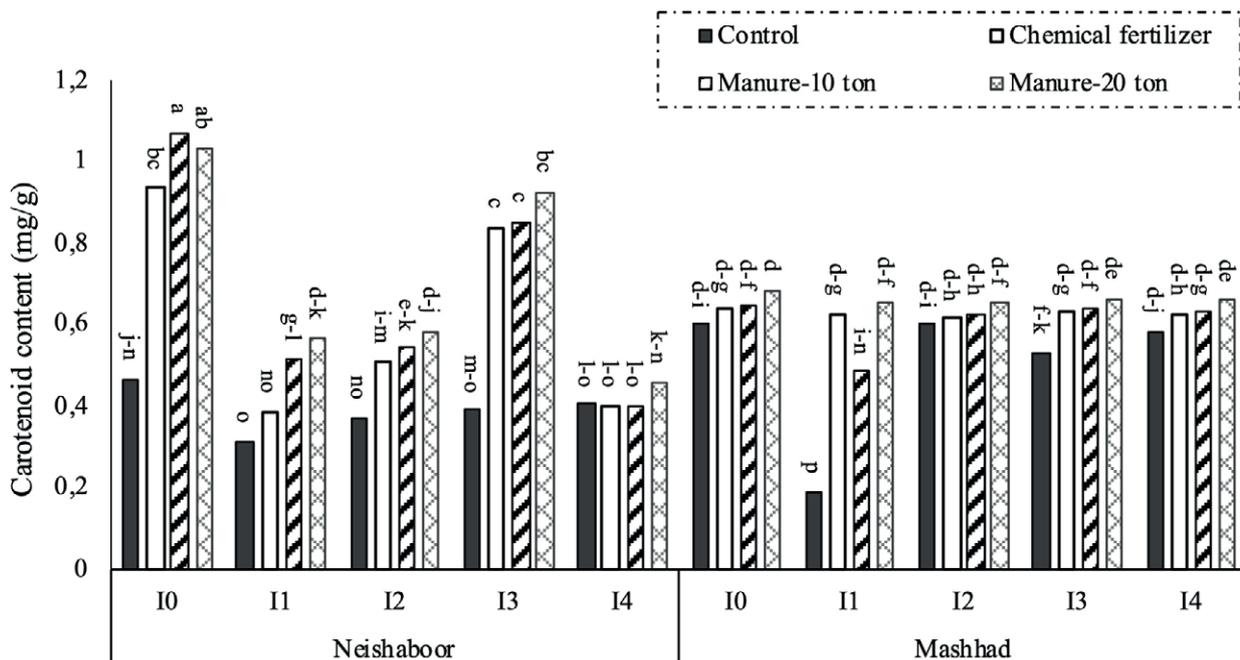


Fig. 2. Mean comparison of triple interaction of location, deficit irrigation and fertilizer on carotenoid content of quinoa crop. I₁: no irrigation at emergence stage, I₂: no irrigation at stem elongation stage, I₃: no irrigation at flowering stage, I₄: no irrigation at seed setting stage.

Electrolytic leakage of ions

The cell membrane is one of the first organs to be damaged under stress and its permeability is increased and electrolyte leakage from the cell eventually causes its death. Therefore, the stability of the membrane is assessed by evaluating the ion permeability (Sairam et al., 2002). According to the results of the analysis of variance, all single effects and dual interactions of applied treatments were significant on the rate of ion electrolyte leakage (Table 4). A comparison of the mean interaction of location and fertilizer treatment and the interaction of location and deficit irrigation is presented in (Tables 6 and 7). In general, the percentage of ion leakage in Mashhad was higher than Neishabour and no irrigation at the flowering and seeding stages showed the highest percentage of ion leakage (Table 7).

Due to the sensitivity of the flowering stage and seed setting to water stress, increasing ion electrolyte leakage at these stages also confirms the sensitivity of these stages to deficit irrigation. However, in no irrigation at the stem elongation stage, no increase in ion electrolyte leakage was observed. The response to fertilizer treatments was not similar in the two locations. Application of fertilizer treatments increased the percentage of electrolyte leakage of ions (Table 6), but the rate of increase was not the same in two locations and at deficit irrigation levels (Table 7). Application of fertilizer treatments

at all levels of irrigation increased the electrolyte leakage of ions. The highest increase in ion leakage (118.7%) was obtained in the treatment of 20 tons of manure and complete irrigation and the lowest increase (10.06%) was observed in the treatment of chemical fertilizer and no irrigation at the stem elongation stage (Figure 3). Application of manure increased the relative water content of leaves thus internal cell turgescence required for cell growth. Such a situation decreases the cell membrane stability to enable the cell growth conditions. As cell growth is conditioned to maximum turgor pressure, loosening of the cell wall, and sedimentation of at cell wall so it seems that by increasing the application of manure and soil physical and chemical improvement including water holding capacity, plants less face the drought and reduce investment to increase the membrane stability (Saneoka et al., 2004).

Percentage of grain protein

Analysis of variance showed that the single effects of location, deficit irrigation and fertilizer as well as the dual interaction of location and deficit irrigation on the percentage of quinoa seed protein were significant (Table 4). Comparison of the average effect of single fertilizer treatment showed that the use of chemical fertilizers, 10 tons and 20 tons of animal manure, increased the

Table 7. Mean comparison of interaction of location and fertilizer (chemical and organic) on quinoa traits.

Location	Fertilizer treatment	Relative leaf water content (%)	Chlorophyll content (mg/g)	Electrolyte leakage (%)
Neishabour	Control	78.55 ^{b*}	2.25 ^d	18.98 ^e
	Chemical fertilizer	81.46 ^{ab}	3.36 ^{bc}	24.96 ^d
	Manure 10 t/ha	82.66 ^a	3.48 ^{bc}	26.52 ^{cd}
	Manure 20 t/ha	80.22 ^{ab}	3.59 ^{ab}	28.11 ^c
Mashhad	Control	59.20 ^d	1.78 ^e	28.24 ^c
	Chemical fertilizer	60.39 ^d	3.26 ^c	40.72 ^b
	Manure 10 t/ha	65.93 ^c	3.33 ^{bc}	40.72 ^b
	Manure 20 t/ha	66.22 ^c	3.81 ^a	45.30 ^a

* Means with same letter(s) for each component indicates no significant difference based on Duncan test at 5% probability level.

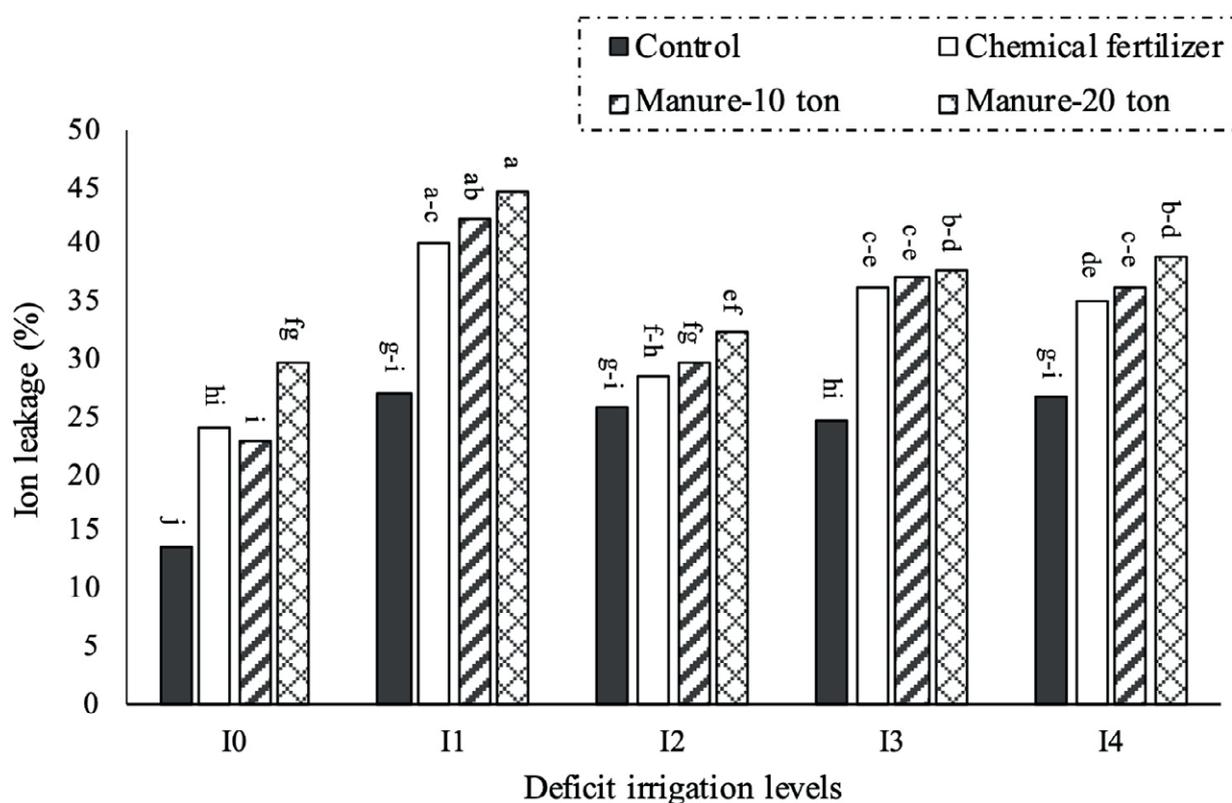


Fig. 3. Mean comparison of interaction of deficit irrigation and fertilizer on ion leakage of quinoa crop. I₁: no irrigation at emergence stage, I₂: no irrigation at stem elongation stage, I₃: no irrigation at flowering stage, I₄: no irrigation at seed setting stage. (This analysis comprises both locations together).

grain protein compared to the control (18.12%) by 1.43%, 1.66% and 2.37% (Table 5). Quinoa seed protein content has been reported to be between 8% and 22% (Jancurová et al., 2009). Studies have indicated that the application of nitrogen fertilizer not only increases the growth and yield of quinoa, but also increases grain quality (Geren, 2015). Thanapornponpong (2004) reported the positive

effect of nitrogen fertilizer on grain yield, grain protein content and amino acid profile of quinoa. Kakabouki et al. (2014) showed that manure and nitrogen fertilizer treatments had higher values of crude protein content compared to the control treatment. Animal manure can provide the conditions for further growth and expansion of the root system by enhancing the soil structure, soil

temperature and aeration, and as a result, the expansion and distribution of the root system increases the likelihood of nitrogen uptake (Mirzakhani et al., 2009). In fact, nitrogen availability determines the grain protein content and quinoa responds strongly to nitrogen fertilizer (Iqbal and Afzal, 2014). Most of nitrogen uptake would be used to produce the amino acids, amides, enzymes including those enzymes that are involved in photosynthesis. Nitrogen fertilizer increases the amount of nitrogen entering the seed from vegetative parts compared to carbohydrates, thus increases the concentration of nitrogen in the grain and its protein percentage (El Gendy et al., 2015). Other studies have shown that application of nitrogen fertilizer has increased the amount of nitrogen in the soil and therefore the total nitrogen in the grain and consequently the percentage of grain protein in wheat (Limon-Ortega et al., 2008) and soybeans (Devi et al., 2013) increased.

Average percentage of grain protein in Mashhad was higher than Neishabour, but different levels of deficit irrigation showed different effects on grain protein (Table 7). As mentioned earlier, the average rainfall was lower and the average temperature was higher in Mashhad than Neishabour, which indicates that the plant faced more water stress in Mashhad. In Neishabour, the highest percentage of grain protein was recorded under no irrigation treatment at seed setting stage (I_4) and in Mashhad, no irrigation (I_1) at the emergence stage showed the highest percentage of grain protein (Table 7). Protein accumulation changes in response to environmental stresses. In addition to proteins that play an active role in biosynthesis and metabolism, stored proteins and proteins that play a protective role against biotic and abiotic stresses accumulate in the grain, as well (Balla et al., 2011). For example, according to (Esmaeilian et al., 2012), water stress at the flowering stage of sunflower increased the percentage of grain protein. Some studies have also reported an increase in wheat grain protein under late-season heat stress (Labuschagne et al., 2009).

Percentage of seed oil

Analysis of variance showed that the single effects of location and deficit irrigation as well as the dual interaction effects of location and deficit irrigation interaction between location and fertilizer showed a significant effect on the percentage of quinoa seed oil (Table 4). Application of deficit irrigation reduced the percentage of seed oil, although no similar responses were observed at different levels of deficit irrigation in two locations (Table 7). No irrigation at the seedling stage in Neishabour

showed the highest decrease in the percentage of seed oil, while no irrigation treatment at the stem elongation stage (I_2) showed an increase in the percentage of seed oil compared to the control (full irrigation) (Table 7). The highest percentage of seed oil (5.91%) under deficit irrigation treatment at the stem elongation stage (I_2) was obtained in Mashhad and the lowest percentage of seed oil (4.18%) was obtained in Neishabour under no irrigation treatment at the seed setting stage (I_4) (Table 7). The comparison of the mean interaction of low irrigation and fertilizer treatment showed that in general, deficit irrigation treatments reduced the percentage of seed oil compared to full irrigation treatment (Figure 4). Studies have shown that quinoa seed oil is between 2% to 10% and is similar to soybean oil in terms of fatty acid composition (Jancurová et al., 2009; Altuna et al., 2018). Elewa et al. (2017) also reported a decrease in the percentage of quinoa seed oil under water stress conditions.

Application of fertilizer treatments only in full and no irrigation treatments at stem elongation stage increased the percentage of seed oil, but in other levels of deficit irrigation such a positive response was not observed and even a decrease in oil percentage was recorded due to fertilizer application (Figure 4). In this study, there was an inverse relationship between the percentage of seed oil and the percentage of seed protein. Other studies also found that with increasing nitrogen, grain yield increased but grain oil content decreased. Because with increasing nitrogen, nitrogenous substances increase and the materials available for fatty acid synthesis decrease and thus the percentage of oil decreases (Khan et al., 2002). Nitrogen fertilizers also delay the maturation of the plant and lead to longer seed development, as a result of which the seed does not reach full maturity and the oil content decreases.

Seed yield

Water stress and fertilizer and their dual interactions as well as triple interaction of location, water stress and fertilizer on seed yield were significant (Table 4). The response of quinoa seed yield to deficit irrigation and different levels of fertilizer applied at the two study locations was not similar. Comparison of the mean simple effect of low irrigation levels showed that grain yield had the greatest decrease only in treatment I_1 (cessation of irrigation in the emergence stage). At other levels, no significant difference was observed between treatments in terms of response to irrigation deficiency (Table 5). Also, the comparison of the mean of the simple effect of fertilizer treatments showed that the treatment of 20 tons of manure per hectare had the highest grain yield

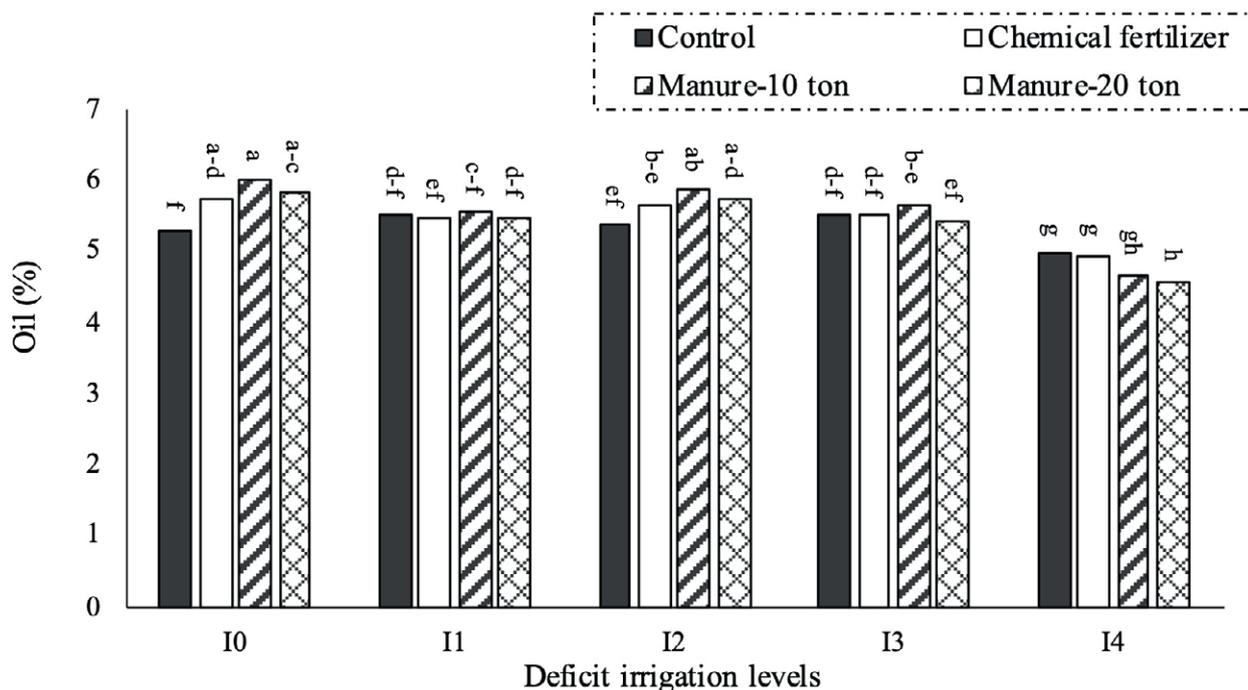


Fig. 4. Mean comparison of interaction of deficit irrigation and fertilizer on oil percentage of quinoa crop. I₁: no irrigation at emergence stage, I₂: no irrigation at stem elongation stage, I₃: no irrigation at flowering stage, I₄: no irrigation at seed setting stage. (This analysis comprises both locations together).

and the control treatment had the lowest grain yield (Table 5). The mean of the triple interaction indicated that the lowest seed yield was achieved for the no irrigation treatment at the emergence stage (I₁) in Neishabour and the highest seed yield was observed for the full irrigation treatment using 20 tons of manure in Mashhad (Figure 5). Deficit irrigation treatments reduced seed yield, but the rate of reduction was not the same at all levels. The highest decrease in seed yield (71.7%) compared to full irrigation was observed in no irrigation treatment at the emergence stage in Neishabour (Figure 5). Studies have shown that high photosynthesis and a specific quinoa leaf area in the early stages of growth allow water to be absorbed by the larger root system and help the plant to avoid water stress at later growth stages (Geerts et al., 2008). Therefore, no irrigation at the emergence stage was not able to prevent the reduction of seed yield and resulted in the lowest yield compare to other levels of deficit irrigation. According to (Hirich et al., 2014), the yield potential of quinoa varies under optimal conditions and depends on climate, soil, planting date and cultivar. In this study, performance fluctuations in the two test sites and between the applied treatments were relatively high (Figure 5). One of the reasons for quinoa response can be the difference in soil salinity in

the two places tested. In agricultural systems of arid and semi-arid regions, water and salinity stress are among the main abiotic stresses that affect potential yield and cause yield instability in quinoa (Fuentes and Bhargava, 2011; Razzaghi et al., 2012). Geerts et al. (2008) reported the maximum yield of quinoa in full irrigation conditions of 2.04 t/ha and in low irrigation conditions of 2.01 t/ha, while in dryland conditions quinoa seed yield decreased to 1.68 t/ha. Razzaghi (2011) also reported quinoa seed yield under optimal conditions of 2.3 tons per hectare. Lack of irrigation and water stress reduced quinoa seed yield shoot dry weight and harvest index (Razzaghi et al., 2012; González et al., 2009; Hirich et al., 2014). During seed filling, water deficiency reduces seed yield per unit area by reducing photosynthesis. Water stress in the seed filling stage, especially if accompanied by an increase in temperature, accelerates leaf aging, reduces the seed filling period, average seed weight and yield. This is done by reducing the transfer of photosynthetic material to the developing seeds (Saeidi et al., 2017).

Fertilizer treatments also had different responses. Under no irrigation treatment at the emergence stage in Neishabour, the use of chemical and livestock fertilizers reduced seed yield compared to no fertilizer application.

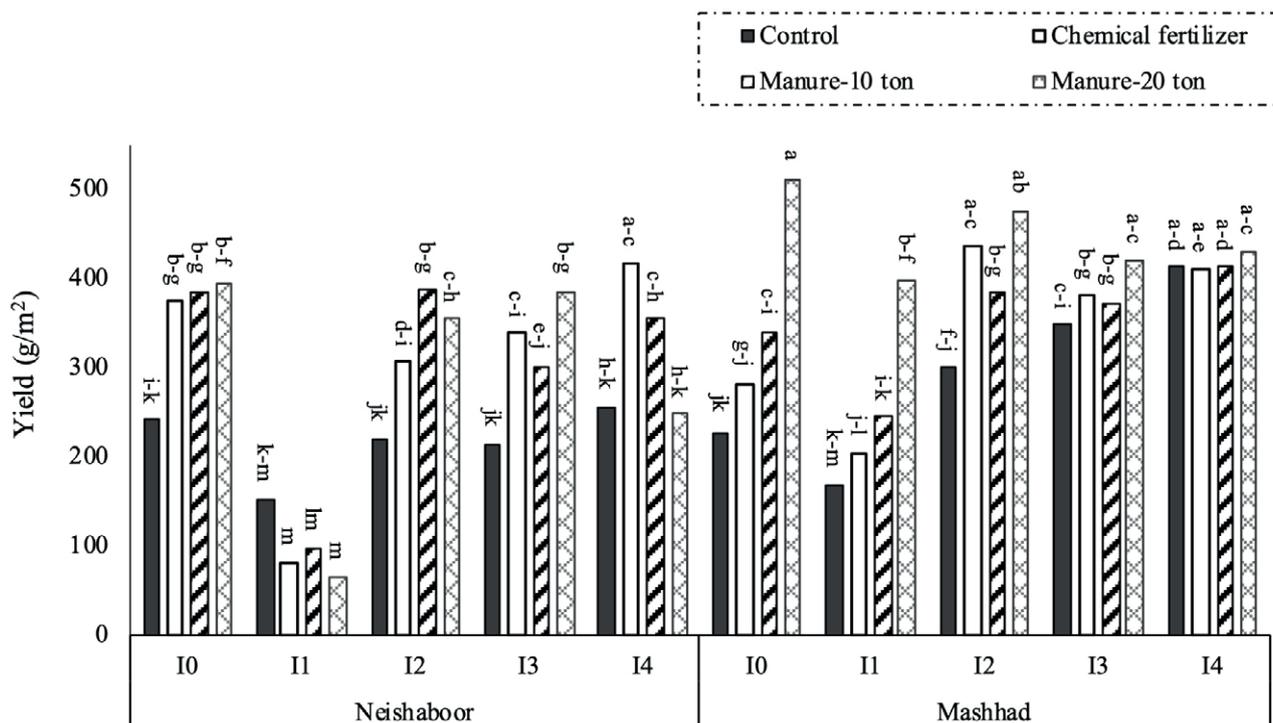


Fig. 5. Mean comparison of triple interaction of location, deficit irrigation and fertilizer on seed yield of quinoa crop. I₁: no irrigation at emergence stage, I₂: no irrigation at stem elongation stage, I₃: no irrigation at flowering stage, I₄: no irrigation at seed setting stage.

It may be the effect of too high salinity at the beginning due to reduced leaching at that site. In Mashhad, under no irrigation at the seed setting stage, the application of fertilizer treatments had no significant effect on seed yield. However, in other deficit irrigation treatments in both locations, the use of chemical and livestock fertilizers increased seed yield (Figure 5). Because the use of animal manure can increase the amount of available nitrogen and because nitrogen is an important component of the chlorophyll molecule, the more it is supplied, the larger the leaves and higher the level of carbonation so increasing the production of hydrocarbons leads to higher yields. Kaul et al. (2005) reported that quinoa strongly reacts to nitrogen fertilizer application. Nitrogen fertilizer increases vegetative growth, plant metabolism and dry matter accumulation (Gomaa, 2013). Shams (2012) pointed to the role of nitrogen in stimulating the metabolic activity of plants and reported that nitrogen increases the amount of metabolites that play an essential role in yield and yield components (Shams, 2012). Shams (2012) also reported an increase in grain yield and biological yield of quinoa using nitrogen fertilizer. The study of the effect of organic matter on soil water holding capacity, especially in arid and semi-arid conditions, on several crops showed that the addition

of organic fertilizers increases field capacity, soil water content and soil hydraulic conductivity (Wesseling et al., 2009; Hirich et al., 2014). Adding organic fertilizer to the soil also has a positive effect on growth, and yield (Gopinath et al., 2008; Ibrahim et al., 2008). Manure in the soil after mineralization of organic matter, improves the soil in terms of nutrients and increase the availability of nutrients as a result, nutrient uptake, plant growth and production would be improved (Hartley et al., 2010).

CONCLUSION

The quantitative and qualitative yield potential of quinoa seeds was affected by irrigation, fertilizer source and climatic conditions of study location. In this study, although the average rainfall was lower in Mashhad, overall, the yield of quinoa in Mashhad was higher than Neishabour. This may be the inherent potential of quinoa to tolerate water stress. This feature makes it possible to adopt a deficit irrigation strategy to save water. Application of deficit irrigation at different crop growth stages induced water stress to the crop and negatively impacted the seed quality and physiological traits of quinoa including chlorophyll, carotenoids, ion electrolyte leakage and

relative leaf water content. However, water stress induced by no irrigation at the stem elongation stage was less effective than other levels of deficit irrigation. Because it seems that at this stage the plant has a higher tolerance to water stress. Also, the application of manure compared to chemical fertilizers, increased the water holding capacity in the soil and provided more water to the plant due to the modification of soil organic matter. As a result, seed yield and quality traits did not show a sharp decrease with deficit irrigation. Finally, it can be said that the combination of deficit irrigation, because it seems treatment at the stem elongation stage along with the treatment of 10 tons of manure in both locations, in addition to saving water, showed high performance.

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