



## IMPACTS OF WATER AVAILABILITY AND PLANT DENSITY ON MORPHO-PHYSIOLOGICAL CHARACTERISTICS OF FENUGREEK (*Trigonella foenum-graecum*)

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**ABSTRACT.** Plant density and water availability are the most important factors determining the yield of crops and medicinal plants. To investigate the effect of these two factors and their interaction on the yield and morpho-physiological characteristics of fenugreek, an experiment was conducted in the form of split plots based on a randomized complete block design (RCBD) with three replications in 2020. Water availability (favourable conditions, mild stress and severe stress) were placed in main plots and plant densities (22, 44 and 66 plants  $m^{-2}$ ) were placed in sub-plots. The results showed a three-fold increase in plant density under optimal moisture conditions increased grain yield from 998 to 1 380  $kg\ ha^{-1}$  and biological yield from 2 600 to 3 259  $kg\ ha^{-1}$ , respectively, while in mild and severe water stress, did not affect grain yield and biological yield. In all three moisture conditions, a 3-fold increase in plant density reduced the number of seeds per pod and a 2-fold increase in plant density reduced the number of pods per plant. Although in some crops, the increase in density under water stress conditions can compensate for the decrease in yield, in fenugreek, the increase in density under water stress conditions was not beneficial for the plant. Increasing the density to medium (44 plants  $m^{-2}$ ) reduces the source strength and applying high density through sink restriction causes a decrease in yield. On the other hand, moisture limitation by reducing the number of pods per plant, the number of seeds per pod and the number of seeds per plant reduced the size of the sink and the mass of 1 000 seeds, which indicates the strength of the source, was not affected.

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### Introduction

Fenugreek is a plant with the scientific name of *Trigonella foenum-graecum* L. whose leaves are used as a fresh vegetable or in some Iranian dishes. The ground fenugreek seeds are widely used in traditional medicine (Seghatoleslami, Ahmadi Bonakdar, 2010). In addition to grain yield, its biological yield is also important. Plant density is one of the most important factors determining the yield of crops and medicinal plants. (Postma *et al.*, 2021).

Numerous studies on the effect of plant density on fenugreek yield have been conducted mainly in the range of plant densities of 20 to 120 plants  $m^{-2}$ . Experiments conducted by Khosravi *et al.* (2014) showed that at an initial density of 22 plants  $m^{-2}$ , grain and biological yield, were 900 and 2 500  $kg\ ha^{-1}$ , respectively, and

increasing plant density had no effect on these two traits. In the experiment of Zandi *et al.* (2013), the highest grain yield was obtained from the density of 60 plants  $m^{-2}$  and increasing the density to 80 22 plants  $m^{-2}$  resulted in a 7.7% increase in biological yield.

The availability of water in the soil is also one of the most important factors determining the yield of crops and medicinal plants (Ahmed *et al.*, 2018; Pieczynski *et al.*, 2013). According to the report of Dadrasan *et al.* (2015), at full irrigation, the seed yield of fenugreek was 839  $kg\ ha^{-1}$ , which decreased by 27 and 42%, while the irrigation volume decreased by 25 and 50%, respectively. In their experiment, the reduction of biological yield of fenugreek in 25 and 50% irrigation treatments compared to full irrigation was 40 and 65%, respectively.

Few studies have focused on the interaction of water stress and plant density in fenugreek. In the experiment of Khosravi *et al.* (2014), the effect of irrigation intervals of 5 and 10 days was investigated in different densities. When the irrigation intervals were 5 days, the increase in density led to a 12% increase in grain yield, but when the irrigation intervals were 10 days, the increase in density, did not lead to an increase in grain yield. Research by Rahimi *et al.* (2009) in asparagus showed that in optimal irrigation, medium plant density, compared to low plant density, increased grain yield, but when water stress occurred, no difference in grain yield was observed between the two densities. According to their research, high plant density compared to low plant density also led to a significant reduction in grain yield in all irrigation treatments. Studies by Abdolahi-Mayvan *et al.* (2019) on European borage showed that increasing plant density from low to medium increased biological yield and high densities reduced grain yield at all irrigation treatments. The above researches indicate the ineffectiveness or negative effect of increasing plant density under water stress.

Therefore, the hypothesis of the present study is based on the idea that medicinal plants grow even in their richest natural habitats with much lower plant densities than field planting and even the lowest conventional planting densities are a kind of density increase imposition for the medicinal plant (Lemaire *et al.*, 2001; Ojija *et al.*, 2021; Trinder *et al.*, 2021;), therefore, increasing plant density will have little benefit to compensate for the decrease in the individual plant yield due to water stress (Rahimi *et al.*, 2009) and may even reduce yield (Abdolahi-Mayvan *et al.*, 2019).

Another purpose of this study is to determine the best plant density in different conditions of water availability to achieve acceptable yield with the least amount of water consumption. If the hypothesis of this study that the increase in plant density is not beneficial under water stress is correct. Then saving on seeds and other inputs will be beneficial.

## Materials and Methods

The experiment was conducted in the South Khorasan province of Iran (longitude N 58° 83' and latitude E 34° 1') and at an altitude of 1 500 meters above sea level. The average long-term annual temperature and precipitation are 14.3 °C and 134 mm, respectively. The Köppen-Geiger climate classification is BWk (desert or arid climate).

The soil was the montmorillonite clay loam (34% clay, 22% sand, 44% silt), low in organic matter (7.112 g kg<sup>-1</sup>) with a pH of 7.7, EC of 0.99 dS m<sup>-1</sup>, and bulk density of 1.45 g cm<sup>-3</sup>. The amount of nitrogen and absorbable phosphorus were 38.0 mg kg<sup>-1</sup> and 13.4 mg kg<sup>-1</sup> respectively.

This study was performed as split plots in a randomized complete block design with three replications. Water stress (100, 75 and 50% of the required water) in the main plot were considered as optimal conditions, mild stress and severe water stress, respectively. Plots

were irrigated through a drip irrigation system. Drippers were placed 50 cm apart, with a flow rate of 1.5 litres per hour. Irrigation frequency was based on total available soil water (TAW) and water soil depletion. Crop water requirement during the growing season was determined based on evapotranspiration (ETP). Reference ETP (ETP<sub>0</sub>) was measured using a class A evaporation pan. ETP<sub>0</sub> was then multiplied by the water stress coefficient (K<sub>s</sub>) and the crop coefficient (K<sub>c</sub>) to calculate the crop evapotranspiration (ETP<sub>c</sub>). To create mild and severe water stress, the plots were irrigated after depletion of 25% and 50% of available soil water content (AWC), respectively. Soil water content (SWC) was measured daily by using four granular matrix sensors (Watermark Soil Moisture Sensors, Irrometer Co. Inc., Riverside, CA), installed in the soil at 20 cm intervals, up to 80 cm deep.

Plant densities (66, 44 and 22 plants m<sup>-2</sup>) were assigned to sub-plots. Row space was 30 cm and in-row space was 5, 10 and 15 cm, respectively. Each plot consisted of 4 rows 5 m long.

60 kg of nitrogen (urea), 60 kg of phosphorus (P<sub>2</sub>O<sub>5</sub>) and 60 kg of potassium (K<sub>2</sub>O) per hectare were added to the soil during field preparation based on the field soil test. Irrigation and plant density treatments were applied after plant establishment (about 30 days after emergence).

Nitrogen was applied during the two more stages (the first stage after thinning of plants and the second stage at the beginning of flowering) at a rate of 50 kg urea ha<sup>-1</sup> in each stage. Seeds were disinfected with Tiram fungicide at a concentration of 2 g L<sup>-1</sup>. Sowing, in the form of a dry planting method, was done on both sides of the ridges. No specific plant pests and diseases were observed and weeding was done manually.

The number of pods per plant, number of seeds per pod and 1 000 seed mass were measured by sampling ten random plants from the middle rows of each plot.

To measure relative leaf water content (RWC), five whole leaves were removed from each plant. First, fresh mass (FM) was measured with the help of a precise digital scale (Atrorios Germany) with an accuracy of 0.0001 g, then to reach the state of turgescence, was placed in the refrigerator for four hours in distilled water. To remove excess moisture, after removing the leaves from distilled water, they were dried between two layers of filter paper and then their turgor mass (TM) was measured. To measure the dry mass (DM), the samples were placed in an oven for 48 hours at a temperature of 70 °C to dry, and their dry mass was measured, and then the relative water content of the leaf was calculated according to the Equation 1 (Maxwell, Johnson, 2000).

$$RWC = \frac{(FM - DM)}{(TM - DM)} \times 100 \quad (1)$$

The amount of chlorophyll and fluorescence parameters of chlorophyll (F<sub>0</sub>, F<sub>m</sub>, F<sub>v</sub>, F<sub>v</sub>/F<sub>m</sub>), were measured using OS1-FL Chlorophyll Fluorimeter 2 (Opti-Sciences, Inc., Hudson NH, USA). To measure the amount of leaf chlorophyll fluorescence parameters,

first, the youngest whole leaf from 5 plants of each plot, was chosen and after about 20 minutes and adapting to the dark, with the use of special receivers for the device, evaluation took, for this purpose, the flash of infrared light connected to the place of the clamp valve and immediately after opening the valve, the data corresponding to minimum fluorescence (F0), maximum fluorescence (Fm) and the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) reading and its fluorescence was recorded. Measurement of chlorophyll fluorescence was done in the early morning and near evening when the intensity of radiation was low.

Irrigation continued for up to 15 days before the pods were fully mature. Harvesting was carried out 180 days after planting when the pods were dry. Biological yield and seed yield were measured from the middle part of each plot and the harvest index was calculated by the ratio of grain yield to biological yield.

SAS 9.4 statistical software was used for the statistical analysis of data. The means were compared using Duncan's multiple range test at the level of 0.05 probability and the figures were drawn using the MS Excel 2019 software.

## Results and Discussion

### Water stress

Under optimal irrigation conditions, grain yield was 1173 kg ha<sup>-1</sup>, while in mild and severe water stress, 24.1 and 37.5% reduction in grain yield was observed, respectively, and the difference between all three stress levels was significant in terms of grain yield (Tables 1 and 2). The decrease in biological yield in mild and

severe stress compared to the favourable conditions, was 16.1 and 24.1%, respectively (Tables 1 and 2), and there was a significant difference between the water stress levels in terms of biological yield (Table 2). According to the report of Dadrasan *et al.* (2015), 25 and 50% irrigation treatments reduced the yield of fenugreek seeds by 27 and 42%, respectively, and their biological yield by 40 and 65%, respectively, compared to full irrigation. Whereas, contrary to this report, our results showed that the reduction in water availability reduced grain yield more severely than biological yield. Increasing the intensity of water stress led to a significant decrease in harvest index with a similar trend to grain yield (Table 2). The harvest index difference was significant in all three stress levels, similar to grain yield (Table 2). This result shows that the decrease in grain yield due to the increase in water stress has little to do with dry matter production and is more related to how dry matter is distributed. In an experiment by Meena *et al.* (2021), the decrease in grain yield and total biomass of fenugreek due to mild water stress was different and was 9.1 and 12.9%, respectively. However, in severe water stress conditions, it was the same and about 43.5%.

The response of the number of seeds per pod and 1 000 seed mass to water stress also showed that the number of seeds per pod decreased significantly under stress conditions, but 1 000 seed mass did not change contrary to expectations (Table 2). As in the research of Baradaran *et al.* (2013), increasing the irrigation interval from 4 to 12 days caused a significant decrease in the number of seeds per pod and the number of pods per plant and a significant increase in 1 000 seed mass (Table 2).

**Table 1.** Mean squares for the effect of irrigation regimes, plant density and their interaction on some studied traits in fenugreek

Sources of variation	df	Pod length, cm	Seed per pod	Pods per plant	Stem diameter, mm	Plant height, cm
Block	2	5.4**	8.2**	64.3**	0.04 <sup>NS</sup>	21.6 <sup>NS</sup>
Irrigation (I)	2	1.4 <sup>NS</sup>	7.4**	45.2**	0.11**	322.4**
Error <sub>a</sub>	4	0.3	2.5	7.0	0.01	6.1
Plant Density (D)	2	1.84*	14.9**	11.4*	0.07*	139.4**
I × D	4	0.2 <sup>NS</sup>	0.83 <sup>NS</sup>	6.3*	0.04 <sup>NS</sup>	81.9*
Error <sub>b</sub>	1	0.48	4.7	1.5	0.02	20.4
CV%		10.5	8.5	7.9	10.1	17.4
		Chlorophyll fluorescence	Relative water contents, %	Biological yield, kg ha <sup>-1</sup>	Grain yield, kg ha <sup>-1</sup>	1 000 seed mass, g
Block	2	0.42 <sup>NS</sup>	900.4 <sup>NS</sup>	477 284**	264 261**	7.2 <sup>NS</sup>
Irrigation (I)	2	0.97*	1270*	1 111 780**	448 290**	0.5 <sup>NS</sup>
Error <sub>a</sub>	4	0.02	313.3	30 172	33 815**	1.1
Plant Density (D)	2	0.52*	5218*	258 070**	90 074	0.3 <sup>NS</sup>
I × D	2	0.22**	2352**	15 576*	33 140*	0.01 <sup>NS</sup>
Error <sub>b</sub>	1	0.05	567.2	39 456	7 898	1.4
CV%		7.6	3.5	8.0	9.5	16.0

<sup>NS</sup> – non-significant, \* – significant at 0.05 level and \*\* – significant at 0.01 level

Despite the decrease in the number of seeds per pod due to the increase in stress intensity, the pod length was not affected by water stress (Tables 1 and 2), which shows that water stress reduces the availability of photosynthetic material for each seed and thus parts of the pod remains empty and this has also reduced grain yield. Also, increasing the stress decreased the stem reserves so that the stem height and stem diameter decreased with water stress (Table 2), which shows an

increase in the remobilization of stem dry matter to the seeds under conditions of reduced photosynthesis. In the experiment of Bazrkar-Khatibani and Fakheri (2018), water stress reduced plant height from 59.1 to 47.5 cm (19.5%), but unlike the present study, their results showed that the amount of leaf area per pod under optimal irrigation and water stress conditions was almost the same. In the experiment of Bazrkar-Khatibani and Fakheri (2018), water stress reduced

plant height from 59.1 to 47.5 (19.5%), but unlike the present study, their results showed that the number of pods and the amount of leaf area per pod under optimal irrigation and water stress conditions, was almost the same, indicating that the source strength-sink size relationship did not change in water stress condition.

Overall, the evidence of the present study shows that water stress mainly by reducing the sink size and partitioning efficiency of dry matter to grain (harvest index) has reduced grain yield in fenugreek and has less correlation with biological yield and dry matter production efficiency.

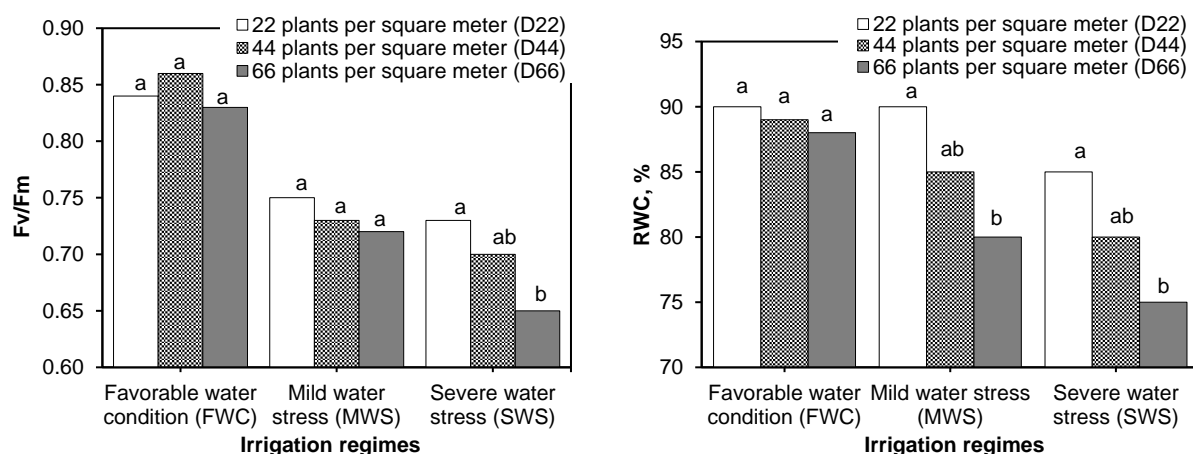
**Table 2.** Mean comparisons for simple effect of irrigation regimes and plant density on some studied traits in fenugreek

Traits	Plant height, cm	Stem diameter, mm	Pods per plant	Seeds per pod	Pod length, cm	1 000 grains mass, g	Grain yield, kg ha <sup>-1</sup>	Biological yield, kg ha <sup>-1</sup>	Harvest index, %	Chlorophyll fluorescence	Relative water contents, %
Irrigation regimes											
FWC	31.7 <sup>a</sup>	1.5 <sup>a</sup>	17.4 <sup>a</sup>	31.2 <sup>a</sup>	7.2 <sup>a</sup>	9.8 <sup>a</sup>	1173 <sup>a</sup>	2 859 <sup>a</sup>	41.0 <sup>a</sup>	0.88 <sup>a</sup>	85.1 <sup>a</sup>
MWS	26.0 <sup>b</sup>	1.3 <sup>b</sup>	16.4 <sup>a</sup>	12.7 <sup>b</sup>	6.9 <sup>a</sup>	9.7 <sup>a</sup>	890 <sup>b</sup>	2 398 <sup>b</sup>	37.1 <sup>b</sup>	0.84 <sup>ab</sup>	74.3 <sup>b</sup>
SWS	19.8 <sup>c</sup>	1.2 <sup>b</sup>	13.2 <sup>b</sup>	8.90 <sup>b</sup>	6.4 <sup>a</sup>	9.4 <sup>a</sup>	732 <sup>b</sup>	2 170 <sup>c</sup>	33.7 <sup>c</sup>	0.79 <sup>b</sup>	68.2 <sup>b</sup>
Plant density											
D22	29.1 <sup>a</sup>	1.4 <sup>a</sup>	17.2 <sup>a</sup>	22.5 <sup>a</sup>	7.2 <sup>a</sup>	9.8 <sup>a</sup>	837 <sup>b</sup>	2333 <sup>b</sup>	35.8 <sup>b</sup>	0.85 <sup>a</sup>	77.2 <sup>a</sup>
D44	26.9 <sup>a</sup>	1.3 <sup>ab</sup>	16.6 <sup>a</sup>	13.5 <sup>b</sup>	6.9 <sup>ab</sup>	9.6 <sup>a</sup>	922 <sup>b</sup>	2431 <sup>b</sup>	37.9 <sup>ab</sup>	0.84 <sup>a</sup>	74.0 <sup>a</sup>
D66	21.5 <sup>b</sup>	1.2 <sup>b</sup>	13.2 <sup>b</sup>	12.5 <sup>b</sup>	6.3 <sup>b</sup>	9.4 <sup>a</sup>	1037 <sup>a</sup>	2663 <sup>a</sup>	38.9 <sup>a</sup>	0.80 <sup>b</sup>	75.2 <sup>a</sup>

FWC – favourable water conditions; MWS – mild water stress; SWS – severe water stress.

D22, D44 and D66 – 22, 44 and 66 plants per m<sup>2</sup><sup>-1</sup>, respectively.

Means followed by the different letters in the same column differ statistically by the Tukey test (P <0.05).



**Figure 1.** The mean comparisons for the interaction effect of irrigation regimes × plant density for chlorophyll fluorescence and leaf relative water content in fenugreek (Fv/Fm – ratio of variable fluorescence to maximum fluorescence; RWC – relative leaf water content). Means followed by the different letters differ statistically by the Tukey test (P <0.05).

### Plant density

Increasing plant density from 22 to 44 plants m<sup>2</sup><sup>-1</sup> only reduced the number of seeds per pod and other traits, especially 1 000 seed mass did not change (Table 2), so it can be concluded that this increase in density by reducing the number of seeds per pod, reduces the yield of an individual plant, but the grain yield per unit area did not change (Table 2), which indicates that the yield loss of individuals was compensated by increasing the density, up to 44 plants m<sup>2</sup><sup>-1</sup>.

This result implies that doubling the plant density from 22 to 44 plants m<sup>2</sup><sup>-1</sup> and consequently increasing competition between plants, had no effect on the availability of photosynthetic material at the beginning of flowering, which determines the number of pods per plant as well as, the availability of photosynthetic materials for each grain during grain filling, which determines the mass of a thousand grains. However, in the interval after the beginning of pod formation until the maximum pod length (beginning of seed filling),

which almost coincides with the maximum leaf area index, the reduction of photosynthetic material due to shading of leaves on top of each other, causes the loss of several newly-formed seeds and producing pods with fewer seeds or hollow pods. Further increase in plant density from 44 to 66 plants m<sup>2</sup><sup>-1</sup> showed a different result so that increasing plant density in this range did not reduce the number of seeds per pod but reducing the number of pods per plant (Table 2), led to a decrease in the yield of individuals. However, by increasing the density in this range, grain yield per unit area increased. These results may suggest that increasing plant density, first by reducing source strength due to shading, and then by further increasing plant density through sink size restriction, leads to reduced individual seed yield. Some experiments performed in Iran on the effect of plant density on fenugreek yield indicated that this factor does not have a significant effect on yield and yield components. Increasing plant density from 10 to 40 (Seghatoleslami, Ahmadi

Bonakdar, 2010) and from 60 to 120 plants  $m^{-2}$  (Zandi *et al.*, 2013), had no effect on 1 000 seed mass, number of seeds per pod, number of pods per plant, and grain yield, and only in some cases did it increase total biomass, for example increasing the density from 60 to 80 plants  $m^{-2}$  led to a 7.4 % increase in biomass (Zandi *et al.*, 2013).

### Water stress and density interactions

The interaction effects of plant density and water stress on relative leaf water content and chlorophyll fluorescence were significant (Table 1). Under optimal irrigation conditions and mild stress, there was no difference in chlorophyll fluorescence between different densities, while at severe water stress, 66 plants had a significantly lower chlorophyll fluorescence than 22 plants  $m^{-2}$  (Fig. 1). Also, under favourable water conditions, there was no difference in relative leaf water content between different densities, but in mild and severe water stress conditions, the relative leaf water content at a density of 66 plants compared to 22 plants  $m^{-2}$ , was significantly lower (Fig. 1). The interaction effect of plant density with water stress was significant for several pods per plant, plant height, grain yield, biological yield, but for 1 000 seed mass, number of seeds per pod and stem diameter was not significant (Table 1).

As mentioned earlier, the increase in plant density, probably due to shading and decrease in canopy photosynthesis and, consequently the need to increase the remobilization of photosynthetic material from stem to seeds, has reduced plant height. However, the decrease in plant height due to increasing plant density was observed only in optimal irrigation conditions and mild stress and only after a 3-fold increase in plant density from 22 to 66 plants  $m^{-2}$ , but in severe water stress, plant height did not differ significantly at different densities (Fig. 2). This result confirms that in optimal irrigation conditions or mild water stress where there is no sharp decrease in the number of pods per plant, the size of the sink is large and requires a lot of current photosynthesis. Under these conditions, increasing plant density causes leaf shade and reduces the penetration of light into the canopy and consequently, sufficient photosynthetic material for the seeds does not provide. As a result, stem reserves are transferred to the seed, which reduces the height and diameter of the stem. However, in severe water stress, the number of seeds per pod and the number of pods per plant is drastically reduced (Table 2), and due to the small size of the sink, the current photosynthetic material meets the grain requirements and the stem reserves are not used much. Therefore, in severe water stress, increasing plant density did not affect plant height (Fig. 2).

In the experiment of Ghobadi and Fattahi (2016) on coriander, the interaction effect of plant density with irrigation on plant height and harvest index was insignificant and on all yield components (number of umbels per plant, number of seeds per umbel and seed mass) and grain yield and biological yield were

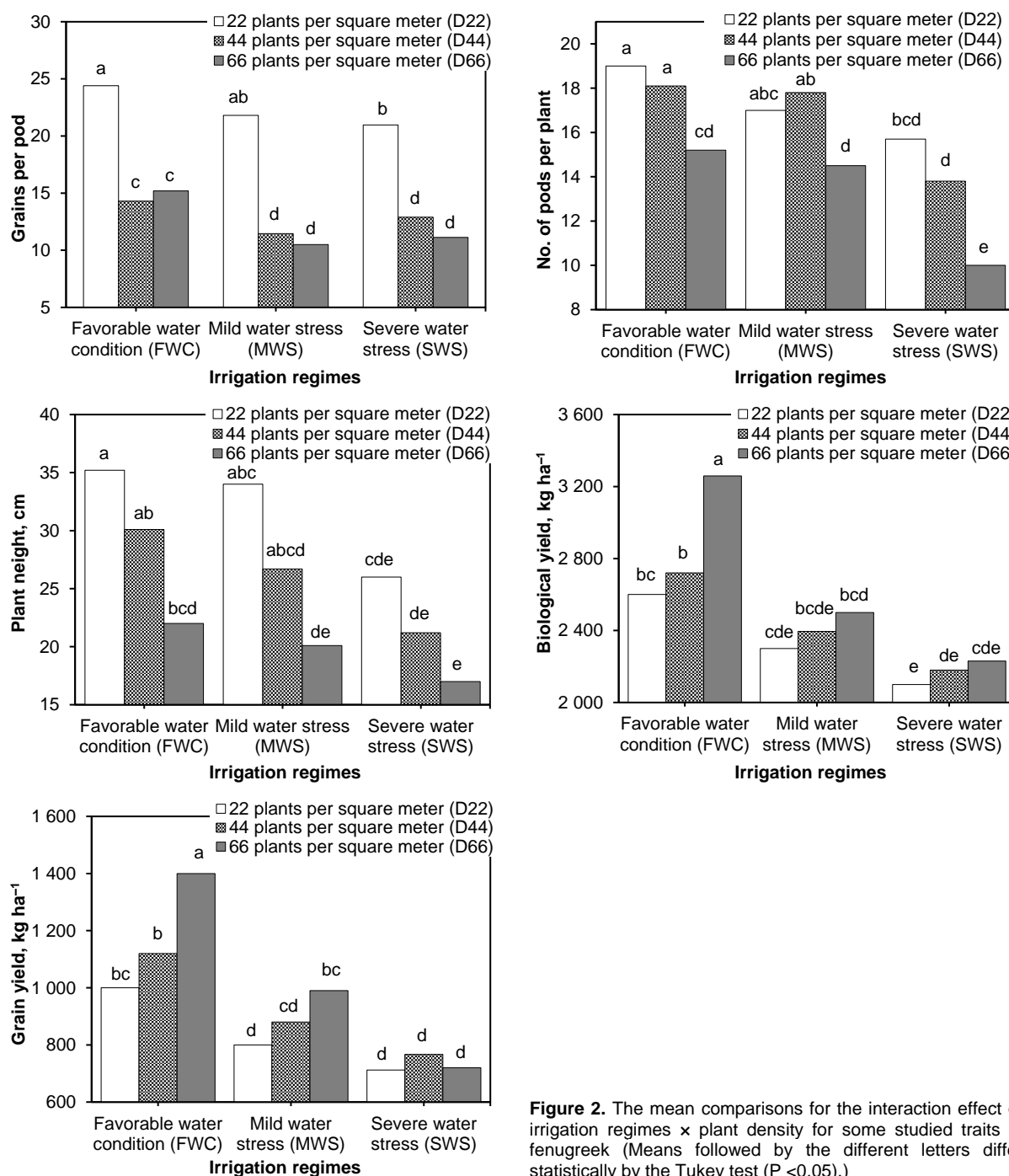
significant. This indicates the small role of stem reserves in the formation of yield and its small impact on plant regeneration efforts. In their experiment, increasing plant density in both optimal irrigation and water stress conditions in the shoot formation stage did not affect yield, but when water stress was in the reproductive stage, increasing the density from 10 to 50 plants  $m^{-2}$  increased yield. However, increasing the density from 50 to 70 plants  $m^{-2}$  reduced the yield.

At all levels of water stress, there was no difference in the number of pods per plant between densities of 22 and 44 plants  $m^{-2}$ , while the density of 66 plants  $m^{-2}$  compared to lower plant densities, significantly reduced the number of pods per plant (Fig. 2).

This decrease in severe, mild stress and optimal conditions was 36%, 20% and 15%, respectively, *i.e.* in severe stress conditions, it decreased more than twice as much as optimal (Fig. 2). Our result shows that in optimal irrigation conditions, due to sufficient photosynthetic material in the fertilization stage of flowers and the initial formation of pods, competition due to increased plant density did not affect the number of pods per plant. Water stress reduced grain yield mainly due to the decrease in the number of pods per plant and the role of the number of seeds per pod was small. Hence, in water stress conditions, also increase in plant density due to an increase in leaf area during the conversion of flowers into pods has reduced the penetration of light into the canopy and reduced current photosynthesis which led to the formation of fewer pods. This result is more pronounced in mild water stress than in severe water stress (Fig. 2).

The reason for this difference may be related to the greater possibility of maintaining and developing leaves and more shading and more severe loss of current photosynthesis in the flower-to-pod conversion stage under mild stress conditions compared to severe stress. As mentioned earlier, the results of Ghobadi and Fattahi (2016) research indicated the usefulness of medium plant densities in water stress conditions compared to low densities for coriander, while the results of Rahimian *et al.* (2019) were different and similar to the results of the present study. In their experiment, increasing plant density increased grain yield of asparagus and psyllium, but under water stress, increasing plant density led to reduced yield.

The interaction of plant density and water stress was significant for the number of grains per pod (Table 1 and 2). In all three levels of stress, increasing the density from 22 to 44 plants  $m^{-2}$ , caused a significant decrease in the number of seeds per pod, adding more plants (density of 66 plants  $m^{-2}$ ), in none of the stress levels, did not affect the number of seeds per pod (Fig. 2). As mentioned earlier, increasing plant density first by reducing the number of seeds per pod reduces grain yield and by increasing the density, grain yield decreases by reducing the number of pods per plant, which can be the reason for not reducing the number of seeds per pod from 44 to 66 plants  $m^{-2}$ , at all levels of stress.



**Figure 2.** The mean comparisons for the interaction effect of irrigation regimes × plant density for some studied traits in fenugreek (Means followed by the different letters differ statistically by the Tukey test ( $P < 0.05$ )).

The interaction effects of water stress and density on grain yield and biological yield were significant (Table 1), and the pattern of interactions of these two traits is the same (Fig. 2). At mild and severe stress, no difference was observed between different plant densities in terms of biological yield and grain yield (Fig. 2). While in optimal irrigation conditions, increasing the density from 22 to 66 plants  $m^{-2}$  caused a significant increase in both grain yield and biological yield (Fig. 2). Therefore, it seems that increasing plant density cannot compensate for the decrease in grain yield or biological yield due to water stress, however, in optimal irrigation conditions, a 3-fold increase in plant density could significantly increase grain yield and biological yield (Fig. 2).

## Conclusion

The yield of each plant is the result of the inter-relationship between the source and the sink. One of the most important factors affecting the size of the source and sink is plant density. Plant density can affect yield by changing yield components, changing source strength as well as allocation and partitioning of assimilates. The results of this experiment confirm that water stress reduced the size of the sink by reducing the number of pods per plant, number of seeds per pod and number of seeds per plant, while the mass of 1 000 seeds, which is the most indicative of source strength, did not change. On the other hand, increasing the density led to a decrease in single plant yield, but this

decrease was compensated by increasing the density so that the yield per unit area did not change. It seems that, at medium densities, single plant yield is reduced by reducing source strength, and at higher densities due to sinking restriction. The reason for this is that at high densities the number of pods per plant decreases. Under optimal water conditions, increasing density limits the current photosynthesis. On the other hand, in the absence of water stress, due to the larger size of the sink and the need for more photosynthetic materials, the remobilization ratio increased. However, in severe water stress due to a reduction in sink size, current photosynthesis met the plant needs and, as a result, remobilization did not change. The research hypothesis was based on the idea that medicinal plants, even in the richest and most suitable natural habitats, grow with a much lower density than the common plantings in agriculture. For this reason, planting the fenugreek medicinal plant in the agricultural system at a density higher than the natural habitat can be a kind of imposition of density on the plant, which is aggravated in the conditions of drought stress and it is necessary to pay more attention to this issue in research.

#### Conflict of interest

The authors have declared that no competing interest exists.

#### Author contributions

MHD – guidance and monitoring of experiment, designed the study, wrote the protocol, critical revision on the initial draft and approved of the final manuscript

AM – wrote the first draft of the manuscript, reviewed the experimental design, analyzed the data

AB – Cooperation in farm implementation.

All authors read and approved the final manuscript.

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