



Yield, Morphological traits and Nitrogen use Efficiency of *Eruca sativa* as affected by Irrigation, Plant density and Nitrogen Fertilization

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ABSTRACT: The effect of different levels of irrigation, plant density and N fertilization was studied on the yield, morphological traits and N use efficiency (NUE) of *Eruca sativa* in the research farm of Islamic Azad University of Birjand, Iran in 2013-2014 in a factorial split-plot experiment. The main plot was devoted to water deficit stress at two levels of no stress (optimum irrigation) and stress at reproductive stage, the sub-plot was devoted to plant density at two levels of 60 and 160 plants m⁻², and the sub-sub-plot was devoted to N fertilization at three rates of 0, 100 and 200 kg ha⁻¹. It was found that drought stress at reproductive stage resulted in 68.5% loss of seed yield, 66.7 and 73.1% loss of N use efficiency for biomass and seed production as compared to optimum irrigation. In addition, the increase in density from 60 to 160 plants m⁻² resulted in 29.5% higher seed yield, 11.5% higher plant height, 30.3 and 21.1% higher NUE for biomass and seed production, 20.3% higher number of auxiliary branches, and 44.9 and 35.7% higher NAE for biomass and seed production. The interaction between plant density and N was significant for NUE for biomass production. Also the interaction between irrigation, plant density and N was significant for seed yield, NUE for biomass and seed production. According to the results, it is recommended to use the density of 160 plants m⁻² optimally irrigated and fertilized with 100 kg N ha⁻¹ because of its optimum seed yield and NUE which takes environmental issues under consideration too.

Keywords: Low irrigation, plant density, N, morphological traits, NUE.

INTRODUCTION

Optimum water application in crop production as an important environmental factor affecting the growth and development of the plants is of crucial importance, particularly in arid and semi-arid regions like Iran (Mirzaei *et al.*, 2005). On the other hand, a growing focus is given to water deficit stress and its influences on crops owing to the increase in applied water costs and the decrease in its availability in these regions (Winter, 1990). Severe water stress reduces the growth and photosynthesis, disrupts physiological processes and finally, results in the death of the plants (Kafi and Damghani, 2002).

In a study on the effect of optimum and limited irrigation on the yield of rapeseed, Qushchi *et al.* (2010) reported that water deficit stress reduced plant height, auxiliary branches, seed yield and biological yield per ha. In another study on rapeseed cultivars, limited irrigation resulted in the greatest loss of plant height, auxiliary branch number and biological yield (Rezadost *et al.*, 2009).

In a study on the effect of the application of three irrigation regimes (no irrigation, irrigation at flowering, and irrigation at flowering and seed filling) on rapeseed, it was found that irrigation at flowering and seed filling significantly increased seed yield, whereas no statistically significant differences were observed in seed yield under the treatments of no irrigation and irrigation at flowering (Faraji *et al.*, 2005).

In a study on the impact of irrigation level (100, 75 and 50% of field capacity) on safflower seed yield, it was reported that seed yield was significantly influenced by irrigation so that the decrease in irrigation level from 75 to 50% of field capacity, seed yield was decreased by 63% (Norouzi *et al.*, 2012). Severe drought stress reportedly resulted in 44% loss of seed yield as compared to optimum irrigation in oil sunflower (*Helianthus annuus*), too (Gholynejad *et al.*, 2008). Daneshmand *et al.* (2006) stated that the highest N use efficiency for seed production in rapeseed was obtained under the treatment of irrigation after 80% soil water depletion.

Plant density can influence the yield and most other traits of the plants. In a comparison of the densities of 60, 80 and 100 plants m^{-2} , Matinfar *et al.* (2012) reported that the highest seed yield of rapeseed was obtained at the density of 80 plants m^{-2} but further increase in the density up to 100 plants m^{-2} reduced seed yield. Bagheri and Safahani (2010), too, compared rapeseed densities of 80 and 120 plants m^{-2} and found that seed yield was higher at the former density and higher density resulted in significantly lower yield, whereas after studying the densities of 50, 70, 90 and 110 plants m^{-2} , Fathi (2008) stated that the seed yield of rapeseed was increased with the increase in density up to 110 plants m^{-2} .

In a study on the effect of plant density on morphological traits of soybean, it was reported that higher planting densities resulted in considerably taller plants (Kebraei *et al.*, 2009).

On the other hand, the availability of nutrients, especially N, is another key factor for plants growth. Nonetheless, over-fertilization, particularly over-application of N, can result in the plants sensitivity to lodging, plant sensitivity to diseases, crop late-maturity, lower crop quality, higher chlorophyll growth, and the accumulation of nitrates in plant tissues (Malakouti *et al.*, 2009). One way to manage chemical fertilizer application is to increase their use efficiency. Higher fertilizer use efficiency is an important factor in cutting the production costs and avoiding nitrate contamination of water, soil and crop (Silespor *et al.*, 2009).

Siadat *et al.* (2010) studied the effect of N rate of 45, 90, 135 and 180 $kg\ ha^{-1}$ on rapeseed and found the highest seed yield at N rate of 180 $kg\ ha^{-1}$, whereas in a study on N rates of 0, 100 and 200 $kg\ ha^{-1}$, Mazloun *et al.* (2009) reported the highest rapeseed yield at N rate of 100 $kg\ ha^{-1}$.

Foroughi and Ebadi (2012) compared N fertilization rates of 0, 50 and 100 $kg\ ha^{-1}$ in spring rapeseed and reported that seed yield and number of auxiliary branches were strongly influenced by the increase in N application. In another study, it was reported that higher N rate resulted in higher seed yield, plant height and auxiliary branches in sesame (Garshasbi *et al.*, 2011).

In a study on the effect of different N rates of 0, 180, 240 and 300 $kg\ ha^{-1}$ on rapeseed, Rabie and Toosi (2011) reported the highest N use efficiency and N agronomical efficiency at N rates of 180 and 240 $kg\ ha^{-1}$, respectively. Also, Seyed Sharifi *et al.* (2011) reported about rapeseed that the highest N use efficiency was obtained at N rate of 100 $kg\ ha^{-1}$ among N rates of 50, 100 and 150 $kg\ ha^{-1}$.

Given the review of literature, the objective of the present study was to examine the influence of the variations of plant density and N application rate on

yield, morphological traits and N use efficiency of *Eruca sativa* under late-season water deficit stress in Birjand, Iran.

MATERIALS AND METHODS

The present study was carried out in research farm (Long 59°13' E., Lat. 32°52' N., Alt. 1480 N.) of Islamic Azad University of Birjand, Iran in 2013-2014 as a factorial split-plot experiment with three replications. The texture of the soil in research farm was loam with the pH of 7.79, electrical conductivity of 7.23 mms/cm whose organic carbon, total N, P, and K content was 0.17%, 0.04%, 15.4 ppm and 266 ppm at the depth of 0-30 cm, respectively. Mean long-term minimum and maximum temperature was 4.6 and 27.5°C in Birjand with mean annual precipitation of 169 mm and mean minimum and maximum relative humidity of 23.5 and 59.6%, respectively. The regional climate is arid and hot.

The main plot was devoted to water deficit stress at two levels of no stress (optimum irrigation) and stress at reproductive stage (irrigation withdrawal from flowering until harvest time), the sub-plot was devoted to plant density at two levels of 60 and 160 plants m^{-2} , and the sub-sub-plot was devoted to N fertilization at three rates of 0, 100 and 200 $kg\ ha^{-1}$. In optimum irrigation treatment, the farm was irrigation with the intervals of 9 days during whole growing season.

The study farm had been left fallow in the previous year. To prepare the field, it was plowed and then, it was leveled by two vertical discs in late-October. Afterwards, furrow and ridges were built by tractor and furrower.

The experimental plots were composed of four planting rows 4 meters in length. According to soil analysis, the farm was fertilized with 100 $kg\ ha^{-1}$ potassium sulfate and 150 $kg\ ha^{-1}$ superphosphate triple before tillage and sowing. The seeds of *E. sativa* were manually sown on November 9, 2013 with inter-row spacing of 50 cm at the depth of 3 cm. In the treatment of 60 plants m^{-2} , the inter-plant spacing was adjusted to 5 cm after thinning. To ensure uniform emergence, all treatments were regularly irrigated once every 4-5 days until full emergence. The N demand of the plots in terms of N fertilization levels was supplied at the first irrigation after final thinning of the plants in late-March from urea source. To measure the yield and morphological traits, an area of 2 m^2 was harvested from the middle of the plots by taking care of marginal effects. After harvesting, the height of the plants and the length of husks were measured and the number of auxiliary branches was counted. Then, the seeds were winnowed to calculate seed yield and biological yield.

For measuring plant height and number of auxiliary branches, 10 plants were harvested from the middle of the plots. The length of husks was taken as the mean length of 30 husks randomly selected from the plants. N use efficiency for biomass (dry matter) and seed production per unit area was obtained by the following equations:

Nitrogen use efficiency for seed = Y_i/N_i

Nitrogen use efficiency for biomass = B_i/N_i

Where Y_i is the seed yield in fertilized plot, B_i is biological yield in fertilized plot; and N_i the nitrogen application rate in plot.

In the end, all data were statistically analyzed by MSTAT-C statistical software package. Means were compared by Duncan Multiple Range Test at 5% level and the graphs were drawn by MS-Excel software package.

RESULTS AND DISCUSSION

A. Morphological traits

Analysis of variance indicated that plant height is not only affected by plant density, but also the number of auxiliary branches was significantly influenced by plant density and the interaction between irrigation and N fertilization. But, none of the simple effects and interactions was significant for pod length (Table 1).

Table 1: Mean square for morphological traits and seed yield of *Eruca sativa* as affected by different levels of irrigation, plant density and nitrogen.

SOV	df	MS			
		Plant height	Branch number in main stem	Pod length	Seed yield
Replication	2	93.98 ^{ns}	5.52 ^{ns}	0.03 ^{ns}	521.44 ^{ns}
Irrigation (A)	1	453.69 ^{ns}	4.84 ^{ns}	0.09 ^{ns}	87596.27*
E _a	2	56.53	2.92	0.03	1668.80
Plant density (B)	1	201.64**	15.21**	0.001 ^{ns}	5329.00**
A × B	1	27.04 ^{ns}	0.75 ^{ns}	0.03 ^{ns}	665.64 ^{ns}
Nitrogen (C)	2	37.34 ^{ns}	2.28 ^{ns}	0.03 ^{ns}	7409.02**
A × C	2	71.83 ^{ns}	5.00*	0.03 ^{ns}	5519.70**
B × C	2	24.70 ^{ns}	4.24 ^{ns}	0.06 ^{ns}	1248.37 ^{ns}
A × B × C	2	32.18 ^{ns}	1.28 ^{ns}	0.01 ^{ns}	2169.61**
E _b	20	21.56	1.33	0.03	362.21
CV (%)	-	10.69	20.09	10.02	20.11

^{ns}Non Significant at 0.05 probability level and *, ** Significant at 0.05 and 0.01 probability levels, respectively

Table 2: Means comparison for morphological traits and seed yield of *Eruca sativa* as affected by different levels of irrigation, plant density and nitrogen.

Treatment	Plant height (cm)	Branch Number in main stem	Pod length (cm)	Seed yield (kg.ha ⁻¹)
Irrigation				
optimum irrigation	46.98a	6.12a	1.75a	143.97a
stress at reproductive stage	39.88a	5.38a	1.65a	45.31b
Density (plants.m ⁻²)				
50	41.06b	6.40a	1.71a	82.47b
150	45.79a	5.10b	1.69a	106.81a
Nitrogen rate (kg N.ha ⁻¹)				
0	41.63a	5.25a	1.65a	66.35b
100	43.51a	6.05a	1.71a	104.63a
200	45.15a	5.95a	1.74a	112.93a

Means followed by the same letters in each column-according to Duncan's multiple range test are not significantly (P<0.05)

According to means comparison, plant height under the density of 150 plants m^{-2} (45.79 cm) was 11.5% higher than that under the density of 50 plants m^{-2} (Table 2). It seems that this increase in plant height can be related to the greater competition between plants for intercepting radiation and shading the tangent plants under higher densities. The higher plant height under higher plant density is reported by Rajabian *et al.* (2009) for rapeseed and Kebraei *et al.* (2009) for soybean, too.

As the results of means comparison revealed, the highest number of auxiliary branches (6.40) was obtained under the density of 50 plants m^{-2} which was 25.5% higher than that under the density of 150 plants m^{-2} (Table 2).

The availability of more space around the plants and the lack of severe competition with the neighboring plants can be stated as the main reasons for the increase in the number of auxiliary branches at lower density. In this

sense, the plant attempts to use greater surface for utilizing solar radiation by producing more auxiliary branches. The higher number of auxiliary branches at lower plant density is in agreement with the results reported by Rajabian *et al.* (2009) for rapeseed and by Kebraei *et al.* (2009) for soybean.

Means comparison of the interaction between irrigation and N fertilization indicated that the highest number of auxiliary branches (6.92, on average) was obtained under optimum irrigation fertilized with 200 kg N ha^{-1} and the lowest one (4.933, on average) under optimum irrigation with no fertilization (Fig. 1). As is evident, water deficit stress (irrigation withdrawal at reproductive stage) significantly reduced branching potential only when 200 kg N ha^{-1} was applied. Under the application of 0 and 100 kg N ha^{-1} , no statistical differences were observed between optimum irrigation and stress at reproductive stage.

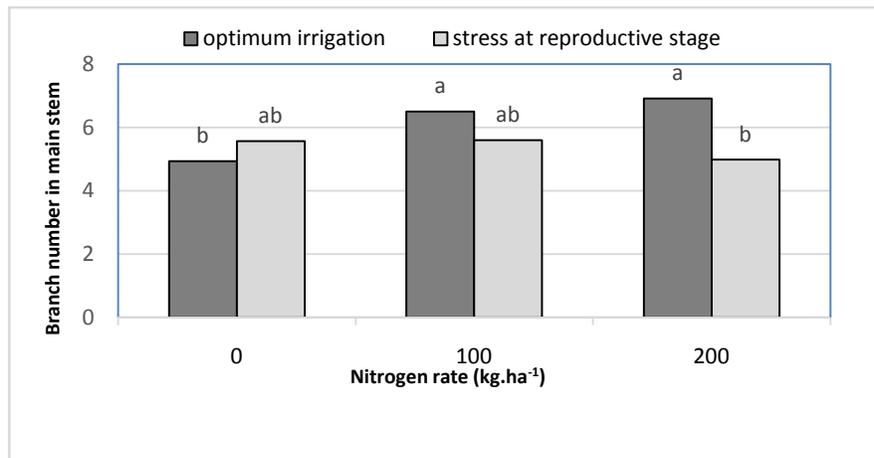


Fig. 1. Interaction effect of irrigation and nitrogen on branch number in main stem of *Eruca sativa*.

This can be associated to the effective use of increased N fertilization under optimum irrigation. In other words, the optimum level of moisture in root zone causes soil N to be readily available to the plant and to be used for vegetative growth and branching, whereas under water deficit stress at reproductive stage, the use of N could not be effective in increasing the branching mainly due to moisture deficit and its ineffective absorption. Given the fact that pod length was not influenced by irrigation, plant density and N level, it can be said that this morphological trait is possibly a trait that is mainly affected by genetic factors than by environment.

B. Seed yield

Analysis of variance revealed that seed yield was significantly influenced by irrigation, plant density, N fertilization and the interaction between irrigation and N and between irrigation, plant density and N (Table 1).

Means comparison showed that drought stress severely decreased economical yield so that seed yield under optimum conditions (no stress) (143.97 g m^{-2}) was 3.2 times greater than that under irrigation withdrawal at reproductive stage (Table 2). Since the results for drought stress conditions (irrigation withdrawal at reproductive stage) shows no significant difference in the number of seeds per pod, it can be said that the decrease in seed yield under these conditions was mainly caused by the decrease in pod number and 1000-seed weight. In other words, it seems that drought stress affected the plant height and number of branches per plant through which it adversely influenced pod number per plant as an important seed yield component on the one hand and reduced photosynthesis activity and the production and mobilization of assimilates during seed filling period resulting in lower 1000-seed weight on the other hand.

Consequently, seed yield was significantly decreased under water deficit stress at reproductive stage. Lower seed yield under water deficit stress is reported by Qushchi *et al.* (2010) and Ghaderi *et al.* (2010) for rapeseed, Rahimi (2014) for medicinal flax, Soghani *et al.* (2014) for soybean and by Norouzi *et al.* (2012) for safflower, too.

Means comparison showed that seed yield was 106.81 g m⁻² under the density of 150 plants m⁻² which was 1.3 times higher than that under the density of 50 plants m⁻² (Table 2). These results are in agreement with the studies on rapeseed (Farnia and Arasteh, 2012; Matinfar *et al.*, 2012) and on sesame (Rezvani Moghaddam *et al.*, 2005). Also, means comparison for seed yield at different N rate showed the positive effect of fertilization on this trait so that the increase in N rate from 0 to 100 and 200 kg ha⁻¹ resulted in 57.7 and 70.2% higher seed yield of *E. sativa*, respectively. Nonetheless, no statistically significant difference was observed between the treatments of 100 and 200 kg N ha⁻¹ (Table 2). It appears that N fertilization improved the use of solar radiation and other resources by *E. sativa* through increasing leaf area index and duration and the stimulation of vegetative growth. So, it paved the way for increasing assimilation and consequently, seed yield. The higher seed yield under higher N rate is reported by Faraji *et al.* (2005), Jafarnejadi (2005), Mazloum *et al.* (2009) and Siadat *et al.* (2010) for rapeseed, by Foroughi and Ebadi (2012), Rasooli *et al.* (2012) and Soleimanzadeh *et al.* (2013) for safflower, Moosavi and Mohamad (2014) for rice and by Ahmadi and Bohrani (2009), Shakeri *et al.*

(2012) and Garshasbi *et al.* (2011) for sesame. Means comparison for the interaction between irrigation and N revealed that the highest seed yield (174.27 g m⁻², on average) was produced under the treatment of optimum irrigation fertilized with 200 kg N ha⁻¹ and the lowest one (41.78 g m⁻², on average) under the treatment of water deficit stress at reproductive stage with no N fertilization. In other words, it shows that under optimum irrigation, *E. sativa* was able to use N more effectively for increasing seed yield (Fig. 2). It implies that the necessary condition for appropriate efficiency of N use is the availability of enough moisture in root zone so that soil N content can readily be available to plant and is used for inducing vegetative growth, photosynthesis and seed yield enhancement. Furthermore, means comparison for the interaction between irrigation, plant density and N showed that the highest seed yield (193.30 g m⁻², on average) was produced under the treatment of optimum irrigation at the density of 160 plants m⁻² fertilized with 100 kg N ha⁻¹ and the lowest one (33.67 g m⁻², on average) under the treatment of water deficit stress at reproductive stage at the density of 60 plants m⁻² with no N fertilization (Fig. 3). As can be seen in Fig. 3, seed yield variation under optimum irrigation is tangibly affected by the variations of plant density and N, while the treatment of water deficit stress at reproductive stage did not result in significant changes in seed yield between various levels of density and N rate, so that all density levels and N rates were ranked in the same statistical group.

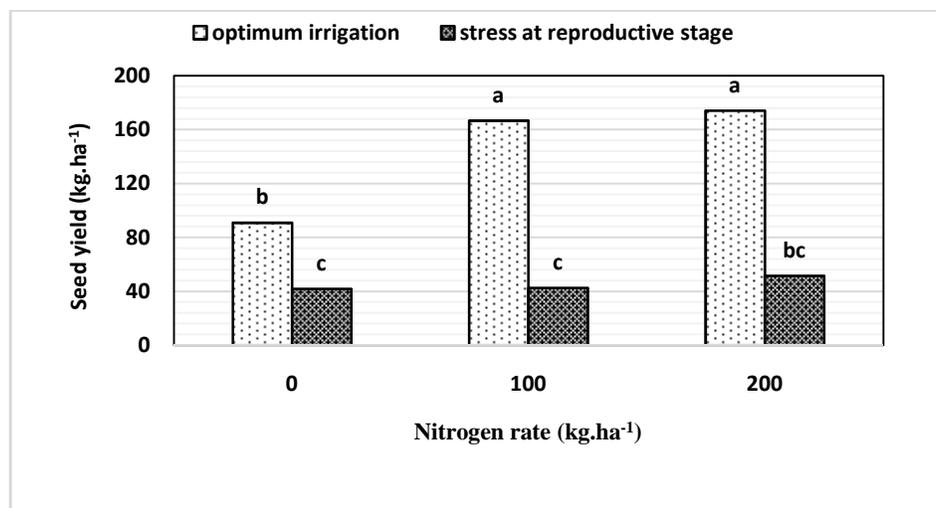


Fig. 2. Interaction effect of irrigation and nitrogen on seed yield of *Eruca sativa*.

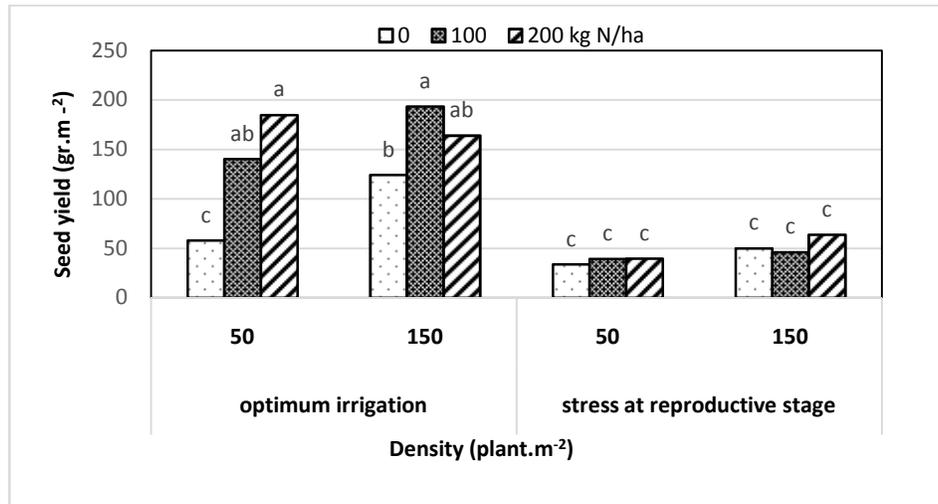


Fig. 3. Interaction effect of irrigation, plant density and nitrogen rate on seed yield of *Eruca sativa*.

C. N use efficiency

As the results of analysis of variance showed, the simple effects of irrigation, plant density and N and the interaction between irrigation and N were significant for N use efficiency (NUE) for biomass and seed production, but the interaction between plant density

and N was significant only for NUE for biomass production at 5% probability level and the interaction between irrigation, plant density and N was significant for NUE of biomass and seed production at 5% probability level (Table 3).

Table 3: Mean square for nitrogen use efficiency (NUE) of biomass and seed of *Eruca sativa* as affected by different levels of irrigation, plant density and nitrogen.

SOV	df	MS	
		NUE for biomass	NUE for seed
Replication	2	0.47 ^{ns}	1.26 ^{ns}
Irrigation (A)	1	3341.52 ^{**}	516.15 [*]
E _a	2	30.23	5.99
Plant density (B)	1	230.33 ^{**}	14.26 [*]
A × B	1	3.64 ^{ns}	2.14 ^{ns}
Nitrogen (C)	1	1119.71 ^{**}	139.39 ^{**}
A × C	1	330.12 ^{**}	59.22 ^{**}
B × C	1	80.12 [*]	12.76 ^{ns}
A × B × C	1	83.66 [*]	17.75 [*]
E _b	12	8.99	2.96
CV (%)	-	12.73	21.37

^{ns}Non significant at 0.05 probability level and *, ** significant at 0.05 and 0.01 probability levels, respectively

Results indicated that NUE were significantly higher under optimum irrigation than under water deficit stress at reproductive stage (Table 4) implying that it is higher when the soil and space in root zone is moist and the plant can better use applied N under optimum moisture conditions.

According to means comparison, NUE for biomass and seed production under optimum irrigation (35.36 and 12.69 kg kg⁻¹, respectively) was about 3 and 3.7 times higher than that under water deficit stress at reproductive stage, respectively (Table 4).

According to means comparison, NUE for biomass and seed production at the density of 150 plants m⁻² was 30.3 and 21.1% higher than that at the density of 50 plants m⁻², respectively (Table 4). Higher NUE for biomass and seed production at higher density can be related to the higher biological and seed yield.

Means comparison indicated that as N rate was increased from 100 to 200 kg ha⁻¹, NUE for biomass and seed production were significantly decreased (Table 4).

Table 4: Means comparison for nitrogen use efficiency (NUE) of biomass and seed of *Eruca sativa* as affected by different levels of irrigation, plant density and nitrogen.

Treatment	NUE for biomass (kg kg ⁻¹)	NUE for seed (kg kg ⁻¹)
Irrigation		
optimum irrigation	35.36a	12.69a
stress at reproductive stage	11.76b	3.42b
Density (plants.m ⁻²)		
50	20.46a	7.28a
150	26.65b	8.82b
Nitrogen rate (kg N.ha ⁻¹)		
100	30.39a	10.46a
200	16.73b	5.64b

^{ns}Non significant at 0.05 probability level and *, ** significant at 0.05 and 0.01 probability levels, respectively

Table 5: Interaction effect of irrigation and plant density on nitrogen use efficiency (NUE) of biomass and seed of *Eruca sativa*.

Irrigation	Density (plants.m ⁻²)	NUE for biomass (kg kg ⁻¹)	NUE for seed (kg kg ⁻¹)
Optimum irrigation	50	31.87 a	11.62 a
	150	38.84 a	13.76 a
Stress at reproductive stage	50	9.05 b	2.94 b
	150	14.47 b	3.89 b

Means with similar letter(s) in each column did not show significant differences

Table 6: Interaction effect of irrigation and nitrogen rate on nitrogen use efficiency (NUE) of biomass and seed of *Eruca sativa*.

Irrigation	Nitrogen rate (kg N.ha ⁻¹)	NUE for biomass (kg kg ⁻¹)	NUE for seed (kg kg ⁻¹)
optimum irrigation	100	45.90 a	16.67 a
	200	24.82 b	8.71 b
stress at reproductive stage	100	14.88 c	4.260
	200	8.64 c	2.58 c

Means with similar letter(s) in each column did not show significant differences

It is consistent with the results of some studies according to which the highest fertilizer use efficiency is obtained in the first stages of their application. It seems that higher fertilization level gradually meets the nutrient deficiency of the plant since then the response of the plant to the applied fertilizer starts to disappear resulting in the loss of fertilizer use efficiency (law of diminishing return). In a study on the effect of different N rates on rapeseed, Adriana *et al.* (2002) found that NAE was significantly lower at higher N rates. Additionally, they reported that NAE had a high correlation with NUE. These results are consistent with the results reported by Seyed Sharifi *et al.* (2011) and Rabie and Toosi (2011) about rapeseed. In total, the higher NUE under the treatment of 100 kg N ha⁻¹ as compared to the treatment of 200 kg N ha⁻¹ can be

related to the higher availability and uptake of N at growth stages and the stimulation of the plant for producing more number of auxiliary branches per plant and more number of pods per plant, higher photosynthesizing area and assimilate production, and its mobilization to reproductive parts as well as lower losses of leaching, denitrification, sublimation and finally, seed and biomass yield.

As the means comparison for the interaction between irrigation and N fertilization showed, the highest NUE for biomass and seed production (45.90 and 16.67 kg kg⁻¹, respectively) were obtained under optimum irrigation fertilized with 100 kg N ha⁻¹ which were ranked in the same statistical group with the treatment of optimum irrigation fertilized with 200 kg N ha⁻¹.

It should be noted that under the application of 100 and 200 kg N ha⁻¹ applied with water deficit stress at reproductive stage, the variation of N level had no significant impact on these traits and both N rates were ranked in the same statistical group in terms of these traits under water deficit stress at reproductive stage (Table 6). According to means comparison for the interaction between plant density and N rate, under the

application of 100 kg N ha⁻¹ the increase in density from 50 to 150 plants m⁻² significantly resulted in 38.7% higher NUE while under the application of 200 kg N ha⁻¹, this trait was insignificantly increased only by 16.4% (Table 7). It implies that as the density was increased, the amount of dry matter produced was much higher under the application of 100 kg N ha⁻¹ than under the application of 200 kg N ha⁻¹.

Table 7: Interaction effect of plant density and nitrogen rate on nitrogen use efficiency (NUE) of biomass and seed of *Eruca sativa*.

Density (plants.m ⁻²)	Nitrogen rate (kg N.ha ⁻¹)	NUE for biomass (kg kg ⁻¹)	NUE for seed (kg kg ⁻¹)
50	100	25.46 b	8.96 b
	200	15.46 c	5.60 c
150	100	35.31 a	11.96 a
	200	18.00 c	5.69 c

Means with similar letter(s) in each column did not show significant differences

Table 8: Interaction effect of irrigation, plant density and nitrogen rate on nitrogen use efficiency (NUE) of biomass and seed of *Eruca sativa*.

Irrigation	Density (plants.m ⁻²)	Nitrogen rate (kg N.ha ⁻¹)	NUE for biomass (kg kg ⁻¹)	NUE for seed (kg kg ⁻¹)
Optimum irrigation	50	100	38.71 b	14.01 b
		200	25.02 c	9.23 c
	150	100	53.08 a	19.33 a
		200	24.61 c	8.19 cd
Stress at reproductive stage	50	100	12.21 de	3.91 e
		200	5.89 e	1.97 e
	150	100	17.55 cd	4.60 de
		200	11.38 de	3.18 e

Means with similar letter(s) in each column did not show significant differences

Means comparison for the interaction between irrigation, plant density and N fertilization revealed that the highest NUE for biomass and seed production (53.08 and 19.33 kg kg⁻¹, respectively) was related to the treatment of optimum irrigation at the density of 150 plants m⁻² fertilized with 100 kg N ha⁻¹ and the lowest ones (5.89 and 1.97 kg kg⁻¹, respectively) were related to water deficit stress at reproductive stage at the density of 50 plants m⁻² fertilized with 200 kg N ha⁻¹ (Table 8).

These results show that although density was high, the plants were able to absorb N even when low rates of N were applied thanks to the presence of enough moisture in soil resulting the highest NUE for biomass and seed production.

CONCLUSION

According to the results it can be concluded that water deficit at reproductive stage severely reduced NUE for biomass and seed production of *E. sativa* due to the severe loss of vegetative and reproductive growth and seed yield on the one hand and the loss of the condition required for the appropriate performance of N fertilizing and its use efficiency, i.e. the presence of adequate moisture in soil, on the other hand. In total, it can be said that the best yield and NUE was obtained from the treatment of optimum irrigation at the density of 150 plants m⁻² fertilized with 100 kg N ha⁻¹ if the environmental issues are paid attention.

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